IEF for two mouse monoclonal antibodies IgG2a (κ) and IgG1 (κ).[17] A mature monoclonal antibody, OKT3 (IgG2a), contain cyclized N-terminus (pyroglutamic acid, −17 D) in both H and L chains, processed C-terminus (no Lys, −128 D) of the H chains, and a small amount of deamidated form.[10] Similar observation was also reported for a humanized IgG1 (κ).[18] In rare cases, gene cross-over may lead to formation of abnormal heavy chains. For example, a purified monoclonal anti-IgE antibody contains a small amount of a variant H chain, which had 16 fewer amino acid residues than the normal H chain (position is between Arg108 of the L chain and Ala124 of the H chain).[19]

Secondary and Higher-Order Structure

The basic secondary and higher-order structural features of IgGs have been reviewed.[6] Only a small portion of the three-dimensional structures of IgGs has been solved.[20] The antibody's secondary structure is formed as the polypeptide chains form anti-parallel β-sheets. The major type of secondary structure in IgGs is these β-sheets and its content is roughly 70% as measured by FTIR.[21] The light chain consists of two and the heavy chain contains four domains, each about 110 amino acid long.[6,20] All these domains have similar folded structures—β barrel, also called immunoglobulin fold, which is stabilized by a disulfide bond and hydrophobic interaction (primary). These individual domains (~12 kDa in size) interact with one another (V_H and V_L; C_H1 and C_L; and between two C_H3 domains except the carbohydrate-containing C_H2 domain) and fold into three equal-sized spherical shape linked by a flexible hinge region. These three spheres form a Y shape (mostly) and/or a T shape.[22]

The less globular shape of IgGs is maintained both by disulfide bonds and by strong noncovalent interactions between the two heavy chains and between each of the heavy-chain/light-chain pairs.[23] Through noncovalent interactions, a less stable domain becomes more stable, and thus, the whole molecule can be stabilized.[24] A detailed study indicates that the interaction between two CH3 domains are dominated by six contact residues, five of these residues (T366, L368, F405, Y407, and K409) forming a patch at the center of the interface.[25] These noncovalent interactions are spatially oriented such that variable domain exchange (switching V_H and V_L; inside-out IgG; ioIgG) induces noncovalent multimerization.[26]

The six hypervariable regions in CDR (L1, L2, L3, H1, H2, and H3) form loops of a few predictable main-chain conformations (or canonical forms), except H3 loop, which has too many variations in conformation to be predicted accurately.[27,28] There is a slight difference in the loop composition and shape between the two types of light chains.[20] However, no functional difference was found in antibodies having λ or κ chain.[6]

Basic Functions of Antibodies

The basic functions of antibodies have been reviewed.[6] There are two functional areas in IgGs—the V and C regions. The V regions of the two heavy and light chains offer two identical antigen-binding sites. The binding of the two sites (bivalent) can be independent of each other and does not seem to depend on the C region.[29] The exact antigen-binding sites are the CDR regions with participation of the frame work regions.[30] Binding of antigens seems through the induced-fit mechanism.[31,32] The induced-fit mechanism allows multispecificity and polyreactivity. It has been suggested that about 5–10 residues usually contribute significantly to the binding energy.[32]

The C regions of antibodies have three main effector functions (1) being recognized by receptors on immune effector cells, initiating antibody-dependent cell cytotoxicities (ADCC), (2) binding to complement, helping to recruit activated phagocytes, and (3) being transported to a variety of places, such as tears and milk.[6] In addition, C domains also modulate *in vivo* stability.[23,29,33] The function of Fc is affected by the structure of Fab. Variable domain exchange (switching V_H and V_L; inside-out IgG; ioIgG) affected Fc-associated functions such as serum half-life and binding to protein G and FcγRI.[26]

The hinge region provides flexibility in bivalent antigen binding and activation of Fc effector functions.[26] Two chimeric IgG3 antibodies lacking a genetic hinge but with Cys residues in CH2 regions was found to be deficient in their intermolecular assembly, and both IgG3 ΔH + Cys and IgG3 ΔH + 2Cys lost greatly their ability to bind FcγRI and failed to bind C1q and activate the complement cascade.[34]

Alternative Forms of Antibodies

In addition to species-specific antibodies, other antibody forms are generated to meet various needs. In the early development of antibody therapies, antibodies were made from murine

sources. However, these antibodies easily elicit formation of human anti-mouse antibody (HAMA). Therefore, humanized chimeric antibodies were generated. Chimeric monoclonal antibodies (60–70% human) are made of mouse variable regions and human constant regions.[2] Such antibodies can still induce formation of human anti-chimeric antibody (HACA). Highly humanized antibodies, CDR-grafted antibodies, are made by replacing only the human CDR with mouse CDR regions (90–95% human).[2] These antibodies are almost the same in immunogenicity potential as completely human antibodies, which may illicit formation of human anti-human antibody (HAHA).

Other alternative forms of antibodies have also been generated and these different forms have been reviewed.[35] Treatment with papain would cleave the N-terminal side of the disulfide bonds and generate two identical Fab fragments and one Fc fragment. Fab's are 50 kDa ($V_H + C_H1$)/($V_L + C_L$) heterodimers linked by a single disulfide bond. Treatment with pepsin cleaves the C-terminal side of the disulfide bonds and produces a F(ab)'$_2$ fragment. The remaining H chains were cut into several small fragments.[6] Cleavage by papain occurs at the C-terminal side of His-H228[36] or His-H227.[37] Reduction of F(ab')$_2$ will produce two Fab'.[23]

Fv fragments are noncovalent heterodimers of V_H and V_L. Stabilization of the fragment by a hydrophilic flexible peptide linker generates single-chain Fv (scFvs).[2] Fragments without constant domains can also be made into domain antibodies (dAbs). These scFvs are 25–30 kDa variable domain ($V_H + V_L$) dimers joined by polypeptide linkers of at least 12 residues. Shorter linkers (5–10 residues) do not allow pairing of the variable domains but allow association with another scFv form a bivalent dimer (diabody) (about 60 kDa, or trimer: triabody about 90 kDa).[38] Two diabodies can be further linked together to generate bispecific tandem diabody (tandab).[39] Disulfide-free scFv molecules are relatively stable and useful for intracellular applications of antibodies—"intrabodies."[38] The smallest of the antibody fragments is the minimal recognition unit (MRU) that can be derived from the peptide sequences of a single CDR.[2]

ANTIBODY INSTABILITY

Antibodies, like other proteins, are prone to a variety of physical and chemical degradation pathways, although antibodies, on the average, seem to be more stable than other proteins. Antibody instabilities can be observed in liquid, frozen, and lyophilized states. The glycosylation state of an antibody can significantly affect its degradation rate.[40] In many cases, multiple degradation pathways can occur at the same time and the degradation mechanism may change depending on the stress conditions.[41] These degradation pathways are divided into two major categories—physical and chemical instabilities. This section will explore the possible degradation pathways of antibodies and their influencing factors.

Physical Instability

Antibodies can show physical instability via two major pathways—denaturation and aggregation.

Denaturation

Antibodies can denature under a variety of conditions. These conditions include temperature change, shear, and various processing steps. Compared with other proteins, antibodies seem to be more resistant to thermal stress. They may not melt completely until temperature is raised above 70°C,[21,42,43] while most other mesophilic proteins seem to melt below 70°C.[44] Shear may cause antibody denaturation. For example, the antigen-binding activity of a recombinant scFv antibody fragment was reduced with a first-order rate constant of 0.83/h in a buffer solution at a shear of approximately 20,000/s.[45]

Lyophilization can denature a protein to various extents. An anti-idiotypic antibody (MMA 383) in a formulation containing mannitol, saccharose, NaCl, and phosphate was found to loose its *in vivo* immunogenic properties (only 10–20% of normal response rate) upon lyophilization.[46] Since the protein showed no evidence of degradation after lyophilization, no change in secondary structure by CD (29% β-sheet, 14% α-helix, and 57% "other"), the loss of activity was attributed to the conformational change. Indeed, tryptophan fluorescence properties were different between the lyophilized and unlyophilized antibodies.[46]

Aggregation

Antibody aggregation is a more common manifestation of physical instability. The concentration-dependent antibody aggregation was considered the greatest challenge to developing protein formulations at higher concentrations.[47] This is

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because protein aggregates generally have reduced activity and more importantly, greater immunogenicity potential because of the multiplicity of epitopes and/or conformational changes.[14,48] Immunoglobulin aggregates have been shown to cause serious renal failure[49] and anaphylactoid reactions such as headache, fever, and chills.[50] Therefore, the aggregate level in commercial intravenous immunoglobulin products is limited to less than 5% based on the WHO standards.

Aggregates can form easily both in liquid and solid states under a variety of conditions (Tab. 2). Since protein aggregation is often the consequence of protein–protein interactions, a process influenced by diffusion rate and geometric constraints of the interaction sites, such factors would influence significantly the aggregation rate, including protein concentration change, viscosity, ionic strength, pH, and temperature.[51] Other conditions may also affect protein aggregation, including shaking, long-term storage, freeze-thaw process, lyophilization process, etc.

Increasing the concentration of antibodies often increases the aggregation tendency of the protein. It was demonstrated that increasing the IgG1 concentration (in 10 mM citric acid, 100 mM NaCl pH 5.5) from 2.7 mg/mL to 50 mg/mL almost linearly increased the Nephelometric Unit (NU) from 2 to 40.[52] Since EP defines a clear solution as having equal or less than three NU, most antibody solutions in the study are opalescent except those at 5 mg/mL or less. However, as the weight average molecular weight was found to be 0.9–1.3 times that expected of a monomer (about 149 kDa), minimal protein–protein association (and readily reversible) is suggested.[52] Shaking can accelerate antibody precipitation and the shape of the precipitates may depend on the sample volume in a container.[53] It appears that protein solutions exhibiting lower surface tension are more susceptible to protein denaturation and precipitation.[53]

Low-temperature treatment may induce aggregation of antibodies. The reversible low-temperature-induced aggregation (below 37°C) of serum cryoglobulins is well known.[54] Human IgM cryoglobulin preparations easily precipitate or gel at temperatures below 10–12°C and the process is reversible at a higher temperature.[20] In a recent study, aggregation of IgG1 at low temperature at above 18 mg/mL was reversible as measured by light scattering.[52] The low-temperature-induced aggregation of cryoglobulins is poorly understood and was thought to involve sites within both Fab and Fc.[20] The authors of this article believe that low temperature reduces the hydrophobic interaction, which is the major force in protein folding. Without enough hydrophobic interaction at low temperature, hydrophobic regions of antibodies become more exposed to solvent and lead to increased intermolecular hydrophobic interaction, leading to aggregation.

In close relation to the low-temperature effect, freeze-thaw process often induces protein aggregation. However, freeze-thaw-induced antibody aggregation does not seem to be a major issue, partly due to the reversibility of antibody aggregates. Freeze-thawing of a chimeric (L6) antibody solution at pH 7.2 for as many as 35 times led to formation of dimers (not larger aggregates).[41] Maximum amount of dimers (about 20%) was observed at pH 6.5 and minimum was below 5.5 or above 8.5 (less than 2%). The freeze-thaw-induced aggregation is apparently reversible after treatment at 37°C for a few hours.[41] Freeze-thawing of rhuMAb anti-CD20 three times (freezing to either −20 or −70°C, thawing to 5°C) did not lead to significant change in aggregation, no significant loss of monomer by RP-HPLC or SEC-HPLC.[55] On the other hand, isolated Fabs seems to be more prone to freeze-thaw-induced aggregation relative to full-length antibodies. For example, Lee[56] found that freeze-thawing of a scF_v (MW 27,000 d) at 1.45 mg/mL in sodium phosphate buffered saline (PBS) at pH 7.3 dropped the %monomer content (due to aggregation) from 95% to 84%, 59%, 44%, and 37%, respectively after 1–4 cycles.

Lyophilization may induce antibody aggregation to a variable degree. It was demonstrated that lyophilization of a recombinant humanized monoclonal antibody (rhuMAb HER2), in the absence of sugars, led to a slight increase in aggregate (less than 1.2%).[57] Lyophilization caused detectable aggregation of a human monoclonal antibody in PBS or physiological saline as measured by OD600,[58] and aggregation of a mouse monoclonal antibody (MN12) [IgG2a (κ)] in the absence or presence of 5% sucrose, dextran, or hydroxypropyl-beta-cyclodextrin (HP-β-CD), as measured by visual examination.[59] In a different study, lyophilization of a monoclonal antibody-vinca conjugate in PBS at pH 7.4 caused 6.2% aggregation.[60] The lyophilization-induced aggregates could be non-covalent-linked monomers and/or even disulfide-linked.[61,62]

Antibody aggregation can easily occur during storage in a liquid state. Storing of a fully human anti-IL8 monoclonal antibody (ABX-IL8; IgG2)

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Table 2. Instabilities of Antibodies

Instability	Antibody	Formulation	Condition	Effect	References
Denaturation	scFv	3 μg/mL in 10 mM phosphate-buffered saline containing 0.02% sodium azide	Shaking at a mean velocity gradient approximately 20,000/s	Loss of activity at a first-order rate of 0.83/h	45
Aggregation	Chimeric mouse/ human IgG1	2 mg/mL in PBS at pH 7.2	Stirring for 48 h with Teflon bar at 600 RPM	Increased turbidity Formation of >15 k particles (>10 μ)	91
	Human Anti-IL-8 (IgG2)	55 mg/mL, 5 mM His, pH 6	Freeze/thaw three times (room/−70°C)	About 0.6% aggregates (by SEC-HPLC)	21
	Anti-IL-8 (IgG2)	100 mg/mL, 200 mM Sucrose, 15 mM Arg, 15 mM His, pH 6	Incubation at 40°C for 6 months	About 11% aggregates (by SEC-HPLC)	21
	rhuMAbE25	10 mg/mL in excipient-free solution	Spray drying Tin = 105°C, Tout = 50–55°C	5–6% aggregate (by SEC-HPLC)	65
	MAb (type not specified)	MAb at 40 mg/mL, 85 mM sucrose, 5 mM His at pH 6, 0.01% Tween 20	Lyophilization	Cloudy (OD350 > 0.2)	1
	Human Anti-IL-8 (IgG2)	50 mg/mL, 20 mM Gly, 4 mM His, 0.2% mannitol, 16 mM Glu, 0.03% Tween 20 pH 5.7	Incubation of lyophilized form at 37°C for 1 month	4.8% aggregates (by SEC-HPLC)	21
	RhuMAb HER2	50 mg/mL in 100 mM trehalose, 5 mM His at pH 6.0	Incubation of lyophilized form at 40°C for 23 months	9.7% aggregates (by SEC-HPLC)	57
Deamidation	OKT3 (IgG2a)	1 mg/mL, PBS at pH 7.0 + Tween80	Incubation at 37°C for 2 months	90% deamidation of Asn386	10
	LA298 (IgG1)	0.5 mg/mL in 10 mM citrate at pH 7	Incubation at 25°C for 6 weeks	~12% increase in deamidation at Asn55	78
Isomerization	MAb	Lyophilized at 40 mg/mL, 85 mM sucrose, 5 mM His, 0.01% Tween 20, pH 6.0, containing 8.4% moisture	Incubation at 5°C	First-order rate constant $\sim 0.33 \times 10^{-4}$/day	1
Oxidation	rhuMAb HER2	5 mg/mL, 147 mM NaCl, 0.01% polysorbate 20, 5 mM sodium acetate at pH 5.0	5, 30, and 40°C for 2 weeks	10, 17, and 52% (total peak area)	81

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in aqueous solutions at 2–8°C or 25°C led to formation of high-molecular-weight (HMW) species.[21] Incubation of two mouse monoclonal antibodies IgG2a (κ) and IgG1 (κ) at 37°C for 32 days caused precipitation in pH 3 and 4 solutions for both antibodies.[17] Aggregation can also occur to an antibody conjugate. Storing a monoclonal antibody-vinca alkaloid conjugate (murine IgG2a) in phosphate-buffered saline solutions at 5°C between pH 4.5 and 7.4 for 1.5 months caused precipitation regardless of solution pH and aggregation was accelerated at 30°C throughout the pH range.[63]

Antibody aggregation is a common phenomenon during storage in a lyophilized state. Aggregation (OD350 > 0.2) was observed of a high concentration, lyophilized, monoclonal antibody (type not specified) by SEC under accelerated conditions.[1] So did a lyophilized chimeric monoclonal antibody during storage at 5°C (0.4% after 2 months).[64] In the absence of sugars, the extent of rhuMAb HER2 aggregation increased from about 1% to >10% after 3 months at 40°C and the rate of aggregation for these formulations was about 0.2% per month at 40°C.[57]

Antibody aggregation can readily occur in other solid forms during storage.[5] It was found that the primary degradation upon storage of a spray-dried anti-IgE (protein:mannitol = 80:20) is aggregation, and the amount of aggregates increased with increasing temperature and relative humidity (Maa et al., 1998a).[65] As much as 52% aggregate was observed by SEC-HPLC of a spray-dried anti-IgE (protein:mannitol = 80:20) after 1 year at 40°C and 38% RH (Maa et al., 1998b).[66]

Antibody aggregation is usually accelerated upon thermal treatment. In the study of chimeric (human/mouse) monoclonal antibody (BR96), Alexander and Hughes demonstrated that thermal treatment at 60°C for 166 h generated noncovalently linked aggregates.[67] In the evaluation of the relative stability of murine IgG2a (κ), chimeric IgG1(κ), trioma IgG1 (λ), and murine IgG1 (κ), Hartmann et al. demonstrated that IgG2a (κ) aggregated and precipitated after heating at 55°C for 8 h and the half-life for the loss of monomer for those four IgGs is 1, 18.1, 3.4, and 8.2 h with different aggregate species (dimer, trimer. . .) by SEC-HPLC.[68]

Surface Adsorption

Antibodies, like other proteins, can easily adsorb to a variety of surfaces. Surface adsorption can significantly reduce the antibody concentration in a solution. For example, a mouse monoclonal IgG1 has been shown to be adsorbed on the surface of glass shake flasks, causing a loss of protein in plant culture media.[69] Such a loss was minimized by coating the glass vessels or by addition of Pluronic F127.

Chemical Instability

A variety of chemical degradation pathways have been observed. These chemical degradants may or may not loose protein activity depending on the site of changes. For examples, deamidation of a monoclonal antibody, OKT3 (IgG2a) as measured by change in IEF pattern does not correlate with loss of antigen binding potency (90% deamidation of Asn386 vs. 80% activity in the potency assay).[10] In comparison, the activity of light chain deamidated recombinant humanized monoclonal antibody HER2 (rhuMAb HER2, Herceptin, trastuzuMAb) at Asn30 (to aspartate) is only 70% of the main species and that of the isomerized (heavy chain) asp102 species is only 9–21% of major species.[37] The major chemical degradation pathways in an antibody include cross-linking, deamidation, isomerization, oxidation, and fragmentation.

Disulfide Formation/Exchange

Disulfide bond formation/exchange is probably the most common cross-linking pathway, leading to chemical aggregation. This degradation pathway can occur readily in various processing steps. For example, lyophilization of a murine monoclonal antibody (IgG2a) without additives reduced the average number of –SH groups per molecule from 6.65 to 6.28, suggesting disulfide formation.[70] Since the thiolate ions are often the initiating species for this chemical degradation, increasing formulation pH usually leads to increased formation of disulfide formation. This is the case for an antifebrin T2G1s Fab′ (IgG1 (κ)), containing free –SH groups, where the percentage of dimers increased with increasing the pH from 5.8 to 9.5.[61]

Storage often leads to disulfide-based aggregation. Storage of a lyophilized excipient-free antibody (IgG1, containing 12 intra and 4 interchain disulfides) formulation for 1 year at 30°C resulted in formation of reducible dimer and trimers.[62] Similarly, reducible aggregates of a monoclonal antibody (type not specified) were detectable

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by SDS–PAGE after storage of the lyophilized formulation at 50°C for 6 months.[1] Such disulfide-linked covalent dimers and trimers were also observed during storage of spray-dried anti-IgE humanized monoclonal antibody.[71]

Nonreducible Cross-Linking

Nonreducible cross-linking was observed in several studies. Incubation of a human monoclonal antibody (C23) in solution at a basic pH at 37°C for 14 days resulted in formation of nonreducible bands above the light chains at pH 4 and 10.[72] Incubation of two mouse monoclonal antibodies—IgG2a (κ) and IgG1 (κ), between pH 3.0 and 10 at 37°C for 32 days led to formation of a nonreducible 88 kDa species by SDS–PAGE.[17] A species with the same molecular weight was observed after incubation of another monoclonal antibody, OKT3 (IgG2a), at 37°C for 2 months.[10,73] Further examination suggests that this species is a cross-linked product of H and L chains between the L46-52 and H99-121 regions. Although the exact chemical nature was not determined, an oxidative step is apparently involved as inhibition of oxidation delayed the formation of this species.[10,73]

A recent study shows that a nonreducible thioether bond can be formed between C223 of the heavy chain and the C-terminal Cys residue of the light chain in humanized IgG1 antibodies. This cross-linked product has a molecular weight of 75 kDa by MS but shows an apparent molecular weight of 92 kDa by SDS–PAGE.[74]

Nonreducible cross-linked products were also observed in solid dosage forms. Incubation of a lyophilized mouse monoclonal antibody [IgG2a (κ)] without lyoprotectant or in the presence of 5% sucrose, dextran, or hydroxypropyl-beta-cyclodextrin (HP-β-CD) at 56°C for 18 days led to formation of 100-kDa band under reduced conditions by SDS–PAGE.[59]

Deamidation

Antibody deamidation has been extensively reported in the literature. This common protein degradation pathway is so prevalent that purified antibody preparations may contain many deamidated forms. For example, several deamidated forms of recombinant humanized monoclonal antibody HER2 (rhuMAb HER2, Herceptin, trastuzuMAb) were found by cation-exchange chromatography, including (1) deamidated Asn30 (to aspartate) on one light chain, (2) deamidated Asn30 (to aspartate) on two light chains, and (3) deamidated Asn55 on the heavy chain (to isoaspartate).[37] The sole conversion of Asn30 to aspartate (not isoaspartate) suggests that the deamidation mechanism in cell culture may be different from that during storage.

Storage can easily generate large amounts of deamidated products. Incubation of a monoclonal antibody, OKT3 (IgG2a) in a PBS solution at pH 7 containing Tween 80 at 37°C for 2 months led to more than 90% deamidation at Asn H386.[10] By monitoring the ammonia generation, Poborji et al.[41] estimated that 4% of the total number of Asn and Gln residues in a chimeric antibody (L6) was deamidated after storing the solution at pH 7.2 at 50°C for 3 weeks.

Deamidation also occurs in solid state. In the evaluation of storage stability of a model recombinant humanized monoclonal antibody (rhuMAb HER2), Cleland et al. reported that incubation of a high-moisture formulation (8.4% moisture) for 12 months at 40°C resulted in a 19% drop in the main peak by IEX due to deamidation.[57]

Deamidation in proteins goes mostly through the intermediate of succinimide at Asn (more readily) and Gln (see reviews[75,76]).This seems to be the case for antibodies, even though alternative mechanisms were reported, such as deamidation of Asn30 on the light chain of recombinant humanized monoclonal antibody HER2 (rhuMAb HER2, Herceptin, trastuzuMAb).[37] A method has been developed to detect the formation of succinimide.[77]

The rate of deamidation via succinimidation in proteins can be affected by a variety of factors, such as pH, sequence, and steric effect (see review[44]). A recent report demonstrated a strong pH-dependency of deamidation at Asn55 (pH 7 > pH 6 > pH 5 > pH 4) in a fully human IgG1 in the pH range of 4–7 during storage at 25°C.[78] Using small peptide as a model, Tyler-Cross and Schirch[79] found that deamidation of Asn residues depends on the amino acid residue on the carboxyl side of Asn in neutral and alkaline solutions. Increasing the size and branching of these amino acids decreased the rate of deamidation by as much as 70-fold compared with the N–G sequence (greatest rate of deamidation) but under acidic conditions, these amino acids lost their protective effect.

Isomerization

The most common isomerization in antibodies is iso-aspartic acid formation, which results not only

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from direct isomerization of Asp but also, mostly, from hydrolysis of succinimide intermediate. The pH-dependent formation of succinimide intermediate can occur either from Asn deamidation or Asp dehydration.[36] In neutral and alkaline condition, the isoAsp and Asp products formed in a peptide were at a ratio of about 3:1.[79]

Isomerization is also prevalent and difficult to control. Again, many purified proteins contain significant amount of isomerized products, either from deamidation or direct isomerization. A typical example is recombinant humanized monoclonal antibody HER2 (rhuMAb HER2, Herceptin, trastuzuMAb).[37] In addition, since isomerization occurred at Asp H102 in CDR regions, the activity of the isomerized species is only 9–21% of the major species.[37] Similarly, isomerization of Asp L32 on one light chain in a recombinant monoclonal anti-IgE antibody (E25) reduced the relative binding activity of $F(ab)'_2$ to 42% and the activity of isomerized form on both chains (iso-Asp-iso-Asp) to 15%.[36]

Since formation of the succinimide intermediate does not need participation of water, it can readily occur in solid state. Asp isomerization is one of the major degradation pathways for a lyophilized, monoclonal antibody (type not specified), and increases with increasing storage temperatures both above and below their Tg values.[1]

Like deamidation, the relative rate of isomerization can be influenced strongly by steric effect. For example, a recombinant monoclonal anti-IgE antibody (E25) contains two Asp-Gly sequences within its CDRs, but only one site was found to be labile to isomerization (Asp L32). The unreactivity of one Asp residue is suggested to be due to hydrogen bonding.[36]

Oxidation

The oxidizable residues in proteins include Met, Tyr, Trp, His, and Cys. Although oxidation is not as prevalent as deamidation and isomerization in antibodies, it can easily occur during storage of antibodies. For example, the major route of degradation of a monoclonal antibody, OKT3 (IgG2a), in solution during storage at 5°C is oxidation of a nondisulfide Cys and several Met residues.[10] Oxidation was also observed in rhuMAb anti-CD20 in solution during storage at 40°C for 2 months.[55]

Oxidation of CDP870, a pegylated one-half antibody (one light and one heavy chain) at a Met residue (M3) was also observed after incubation of the protein at 25°C or 40°C for 12 weeks (14% at pH 4.1 and 4.2% at pH 5.5).[80] Light exposure can accelerate baseline oxidation. For example, light exposure (20,000 lux for 2 weeks at 27°C) of recombinant humanized monoclonal antibody HER2, rhuMAb HER2 caused 5–10% increase in oxidation in various liquid formulations at Met H255 (primary site) and Met H431 at temperatures 30 and 40°C.[81]

Formation of Acidic or Basic Species

Antibodies can form acidic or basic species, which are often monitored by IEF or ion exchange chromatography. Among all the possibilities, deamidation is the most likely degradation pathway, leading to formation of acidic species. Therefore, formation of such species is used loosely as an indication of deamidation in the absence of other confirmatory studies. Strictly speaking, formation of such species can only be considered as an indication of chemical instability (see below). A pH-dependency is almost certain for this kind of degradation. In the stabilization of CDP870, a one-half antibody (one light and one heavy chain), which is linked through Cys to a branched (20 kDa each) PEG, Johnson et al.[80] found that the protein generated more acidic species (up to 17% at pH 4.1 and 41% at pH 5.5) by IEF as the solution pH was increased from 4.1, 4.5, 5.0, to 5.5 during storage at 25°C for 12 weeks.[80]

Another possible mechanism for generating acidic species in antibodies is the Maillard reaction, where reducing sugars can react with lysine residues. Such a reation will result in loss of positive charges and formation of apparently more acidic species. This is the case for an spray-dried anti-IgE humanized monoclonal antibody with lactose during storage.[71]

Formation of basic species was also observed in antibodies. Its formation can result from several possibilities, most likely as a result of succinimide formation, removal of sialic acids, or pyroGlu formation. Such a basic species was observed after incubation of a human monoclonal antibody (C23) at 37°C for 14 days in a solution at an acidic pH by IEF.[72]

C-Terminal Clipping

Terminal cleavages can easily occur during normal production of antibodies. For example, the majority of a purified chimeric protein rCD4-IgG lacks the heavy chain C-terminal Lys.[82]

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This is also the case for recombinant humanized monoclonal antibody HER2 (rhuMAb HER2, Herceptin, trastuzuMAb)[37] and a recombinant monoclonal anti-IgE antibody (E25).[36] It was suggested that the cleavage was due to the action of basic carboxypeptidases during production.[37]

Fragmentation

The most likely sequences leading to fragmentation in proteins are Asp-Gly and Asp-Pro,[83] although other sequences can also experience cleavages, such as Asn-Ser.[79] Fragmentation can easily occur in antibodies even during production processes. A small amount of fragment (MW < LC) was detectable under reduced condition in a purified recombinant MAb IgG4 produced in Chinese hamster ovary (CHO) cells.[7] Half molecules should be monitored routinely for IgG4s.[84]

A variety of processing conditions may accelerate fragmentation of antibodies, such as acidic or basic treatment,[72] thermal treatment,[41,67] freeze-thaw,[41] and storage.[17,73,78] Fragments can simply include masses with a loss of a light chain (M-LC), a loss of a Fab arm (M-Fab), and separated heavy-chain (HC) and light-chain (LC), as well as species less than common antibody subunits, resulting from both peptide and/or disulfide bond cleavage.[67] Antibodies are also subject to radiation-induced cleavages.[85]

Maillard Reaction

Proteins may form adducts with sugars. In an investigative study, mouse MAbs were found to form glucose adducts after incubation with 0.5 M glucose at pH 7.4 for 14–21 days at 37°C and the glycated antibodies significantly increased the dissociation of the antibody-antigen binding complex.[86]

ANTIBODY FORMULATION

As mentioned above, antibodies, like other proteins, may generate a variety of degradants during production, processing, and storage both in liquid and solid states. Their tendency to generate such degradants depends very much on their individual sequence, pI, hydrophobicity, and carbohydrate content.[23] The antibody degradation products may have reduced activity and more importantly, increased immunogenicity.[14,87] A strong immune response (antibody formation) may lead to severe neutralization of the protein drug product and even endogenous proteins.

The major purpose of formulating antibodies as therapeutic agents is to control the rate of antibody degradation to provide an acceptable shelf life for worldwide shipping and storage. This can be done by choosing proper formulation excipients and other formulation conditions. It should be stressed that one formulation excipient stabilizing a specific antibody may not be suitable for another because of the differences in their sequence. A proper formulation composition should not only maximally preserve the activity of the antibody but also minimize the potential immune response, as excipients could alter the immune response by changing the presentation of the protein (such as the spacing of epitopes).[14] The following section will discuss critical factors in formulating both liquid and lyophilized antibody products. Advanced formulations will also be briefly described.

Liquid Formulation

Liquid dosage form is usually preferable to lyophilized products as it is easier to administer and less expensive to manufacture. Among all the commercial antibody products, about half are stable enough to be formulated in a liquid form (Tab. 1). Formulating a successful liquid product needs consideration of at least the following aspects.

Protein Concentration

Since the EC50 values of antibodies are often 10–1,000 times higher than for other proteins such as hormones and cytokines,[35] a relatively large amount of antibodies needs to be dosed to achieve any therapeutic effect. A large dose requires that an antibody product needs to be formulated/reconstituted at a high concentration to reduce dose volume, especially for subcutaneous injections; otherwise, a large volume of antibody preparation has to be infused. Since the aggregation process is usually concentration-dependent, antibodies at high concentrations have at least two potential issues: high tendency to aggregate during storage and likely high viscosity, leading to more difficulty during injection (injection time correlated with viscosity).[47] It has been suggested that the concentration-dependent protein aggregation is the greatest challenge to developing high-concentration

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protein formulations.[47] On the other hand, moderate increase in protein concentration may not have a significant effect. In fact, increasing the concentration of antifebrin T2G1s Fab' (IgG1 (κ)), from 0.05 to 5.0 mg/mL in solution resulted in a decrease in dimer formation during storage (73.2% to 22.9% after 13 weeks at 22°C).[61]

An effective way to reduce the viscosity of an antibody solution is to add sufficient amount of NaCl.[5,88] Adjustment of formulation pH could potentially reduce the viscosity of an antibody solution.[88] Although not very effective, histidine has been shown to reduce the solution viscosity of a fully human anti-IL8 monoclonal antibody (ABX-IL8; IgG2) from 20 to about 9 centistokes when its concentration was increased from 0 to 60 mM.[21] However, use of such excipients in reducing viscosity can be limited, as the excipient can adversely affect the stability of the antibody, such as MAb anti-CD20.[55]

Another potential issue in formulating high-concentration antibody products is their solubility limits, as presence of undissolved proteins are generally not acceptable for intravenous injection. Fortunately, the solubility of antibodies seems to be relatively high and solubility-limited formulation difficulty has rarely been reported to our knowledge. To increase the solubility of antibodies, excipients such as surfactants may be used. In a recent report, Golovanov[89] found that a combination of L-Arg and L-Glu at an equimolar concentration (50 mM) significantly increased the solubility of several proteins (either alone does not have a significant effect). In addition, the room-temperature storage stability (fragmentation) of proteins was significantly improved as monitored by SDS–PAGE.

One potential advantage of formulating a protein product at higher concentrations is the greater freeze-thaw stability. Often, proteins at high concentrations are more resistant to freeze-thaw-induced aggregation, as the interface-induced protein denaturation can easily reach a saturation point. However, it does not seem to be the case for antibodies. Increasing the concentration of a chimeric antibody (L6) did not inhibit freeze-thaw-induced aggregation.[41] In addition, surfactants such as Pluronic F68 and Tween 80 did not appreciably prevent antibody aggregation. In a different study, Tween 80 alone (or Pluoronic F68) at 0.1% failed to offer any protection against freeze-thaw-induced aggregation of a single-chain antigen-binding protein CC49/218 sF_v (MW 27,000 d) at 1.45 mg/rnL in sodium PBS at pH 7.3.[56] These results suggest that freeze-thaw-induced antibody aggregation may not be a surface phenomenon.

Effect of Formulation pH

Formulation development often starts with determination of a stable formulation pH. Like other proteins, the pH effect on the stability of antibodies depends on the formulation composition,[55] stress conditions,[41] and even antibody concentration.[84] Formulation pH may affect the physical stability of antibodies as it alters the number and distribution of charges on the protein surface. For example, low pH (below 3) caused a loss of tertiary structure in rhuMAb anti-CD20.[55] Low pH (pH 3 and 4) is also responsible for the precipitation of two mouse monoclonal antibodies IgG2a (κ) and IgG1 (κ) during incubation at 37°C for 32 days.[17] Susceptibility to pH stress varies among different IgG molecules.[68]

Regarding chemical stability, formulation pH may play a critical role in controlling many degradation pathways, such as disulfide bond formation/exchange, deamidation, fragmentation, isomerization, etc. Due to variable effects on different degradation pathways, the optimum pH value varies depending on both the sequence of an antibody and the analytical methods used in monitoring the stability. By IEF, Lam et al. found that rhuMAb anti-CD20 is most stable at pH 5 within the pH range of 3–6 irrespective of protein concentration in formulations containing 10 mM citrate, 8% trehalose, and 0.05% Tween 20.[55] By SEC-HPLC, Usami et al. found that pH 6 was the most stable condition during incubation of a human monoclonal antibody (C23) at 37°C for 14 days in a solution containing isotonic sodium chloride between pH 4 and 10.[72] Sometimes, the optimum pH values reside outside a certain range. In the study of the stability of a chimeric antibody (L6), minimum amount of dimmer formation was observed at pH below 5.5 or above 8.5 (less than 2%) during freeze-thaw.[41]

It needs to be mentioned that oxidation could be inhibited by adjusting formulation pH. In a formulation containing 40 mg/mL MAb anti-CD20, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% Tween 20, and 50 mM histidine, reducing the pH from 7.5 to 5 virtually eliminated the oxidation during storage at 40°C for 2 months.[55] In a different study, oxidation (M3) of PEG-CDP870, a one-half antibody, in a formulation containing 160 mg/mL CDP870, 10 mM sodium acetate, and

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125 mM NaCl was slower at pH 5.5 than at pH 4.1 (4.2% vs. 14% after 12 weeks at 40°C).[80]

Due to the simultaneous presence of multiple antibody degradation pathways, the formulation pH has to be adjusted to balance all the potential degradations. Generally, a weakly acidic condition seems to be the optimum for most antibodies (see Tab. 1).

Effect of Buffering Agents

It has been long recognized that both the type and concentration of a buffering agent may affect the stability of proteins (see review[44]). For example, phosphate buffer accelerated deamidation of an IgG1 relative to citrate buffer at both pH 6.5 and 7.0 during storage at 25°C.[78] Therefore, proper choice of a buffering agent can potentially achieve both pH control and stabilization of antibodies. In rare cases, a buffering agent good for one antibody may be harmful to another. One of these agents is histidine, which has a pKa of pH 6 and potentially good in controlling a weakly acidic condition. It has been shown to provide better protection against precipitation for a fully human anti-IL8 monoclonal antibody (ABX-IL8; IgG2) upon thermal incubation both at 50 and 40°C than citrate at pH 6.[21] Addition of a small amount of histidine (0.4 mM) also increased the freeze-thaw (4×) recovery of a single-chain antigen-binding protein CC49/218 sF_v (MW 27,000 d) at 1.45 mg/rnL in PBS solution at pH 7.3 from 37% to 90%.[56] On the contrary, histidine was found not only to increase the rate of aggregation of rhuMAb anti-CD20 during storage but also to induce coloration of the solution compared to a acetate-buffered antibody solution at pH 5.[55] In addition, oxidation of Fc was also faster in histidine solution.

The concentration effect of a buffering agent was demonstrated in a study where freeze-thaw-induced aggregation of a chimeric antibody (L6) was higher with increasing phosphate buffer concentration (0.01 vs. 0.05).[41] Therefore, both the type and concentration of buffering agents need to be examined.

Effect of Formulation Excipients

Use of formulation excipients remains a major and convenient approach in stabilizing antibodies in solutions. It is so at least in reducing antibody aggregation.[47] A variety of formulation excipients have been shown to stabilize antibodies under different processing conditions and during storage, including sugars,[41] polyols,[41] amino acids,[41,84] surfactants,[84] and polymers.[45,84]

Formulation excipients are effective generally in protecting the physical stability of an antibody. Among these excipients, sugars are the most commonly used. A variety of sugars, such as sucrose, trehalose, glucose, have been shown to reduce the freeze/thaw-induced aggregation of rhuMAb anti-CD20,[55] a chimeric antibody (L6),[41] a human anti-IL8 monoclonal antibody (ABX-IL8; IgG2),[21] and a single-chain antigen-binding protein CC49/218 sF_v (27 kDa).[56] Other excipients can be equally or even more effective in protecting antibodies.. Histidine was shown to be equally effective as sucrose in reducing freeze-thaw induced aggregation of a human anti-IL8 monoclonal antibody (ABX-IL8; IgG2) and combination with Arg resulted in a better protection than sucrose in solution stability at different incubation temperatures.[21] Sugars are not always effective. In the evaluation of freeze-thaw stability of a single-chain antigen-binding protein CC49/218 sF_v (MW 27 kDa) at 1.45 mg/mL in sodium phosphate buffered saline (PBS) at pH 7.3, addition of 10 mM trehalose, lactose, glucose, or mannitol did not offer any protection.[56] In a rare case, sucrose has been shown to promote agitation-induced aggregation of an IgG1 antibody.[90]

Formulation excipients may inhibit chemical degradation of antibodies. Replacing 0.14 M NaCl with 8% trehalose or 4% mannitol in a rhuMAb anti-CD20 increased the chemical stability as measured by IEX during storage at 40°C.[55] Such an effect is likely a result of stabilization of antibody conformation. Some excipients may protect antibodies against oxidation. Replacing NaCl with 4% mannitol and 1% benzyl alcohol protected recombinant humanized monoclonal antibody HER2, rhuMAb HER2, against oxidation (primarily Met-255) at 30 or 40°C.[81] This effect is likely due to a function of mannitol as a free radical scavenger. Some commonly used antioxidants, such as thiosulfate and methionine, are effective in inhibiting antibody oxidation. The minimum effective levels (molar ratios of antibody to antioxidant) required to inhibit temperature-induced oxidation of rhuMAb HER2 were 1:5 and 1:25 for methionine and thiosulfate, respectively.[81] However, use of thiosulfate has led to formation of a thiosulfate adduct of rhuMAb HER2.[81] The safety of such a thio-containing adduct has not been reported.

One of the main excipients often used in antibody formulation is surfactants. They are usually

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effective in reducing shaking/stirring-induced aggregation[91] but may have negative effect during long-term storage.[92] One obvious concern is that such compounds may contain a residual level of peroxides, which have been shown to cause protein oxidation.[93] Therefore, use of surfactants should be kept to a minimum in an antibody formulation if possible. For example, removing Tween 20 in a recombinant humanized monoclonal antibody HER2, rhuMAb HER2 solution reduced the oxidized antibody from 52% to <10% at 40°C.[81]

Effect of Shaking/Shearing

Both shaking and shearing can denature antibodies. It was found that shaking murine antibody-based products caused antibody precipitation and the shape of the precipitates are different depending on the fill volume.[53] In the evaluation of shear effect on the antigen-binding activity of a recombinant scFv antibody fragment in a buffer solution, Harrison et al.[45] found that the binding activity decayed at a mean rate constant of 0.83/h at a shear of approximately 20,000/s.

Effect of Preservatives

A limited number of studies have been conducted on the compatibility of preservatives with antibodies. Compatibility depends both on the antibody and the preservative. In the evaluation of the effect of several preservatives, including benzyl alcohol, chlorobutanol, methylparaben, propylparaben, phenol, and m-cresol, on the stability of a humanized monoclonal antibody, Gupta and Kaisheva[94] found that the antibody was most stable in the presence of methylparaben and propylparaben, compatible with benzyl alcohol and chlorobutanol at low concentrations, and not compatible with phenol and m-cresol, as measured by a variety of assays, including SEC-HPLC, DSC, light scattering, UV, and potency assay.

Effect of Processing Equipment

Another important issue in antibody formulation is the choice of proper processing equipment/containers. A few studies have shown that antibody stability can be significantly affected if an inappropriate choice is made. In the study of the stability of recombinant humanized monoclonal antibody HER2, rhuMAb HER2, in liquid formulations, Lam et al.[81] found that replacing the stainless steel filler with a nonstainless steel filler reduced the oxidized antibody (primarily Met-255) from 52% to 18% after storage for 2 weeks at 40°C. It was suggested that Fe ions, released from erosion of stainless steel by chloride ion at low pH, catalyzed the oxidation. In a similar case, Chen et al.[21] demonstrated that a fully human anti-IL8 monoclonal antibody (ABX-IL8) in aqueous formulations containing 60 mM His had a higher level of aggregates after freeze-thaw in a stainless steel vessel and subsequent storage at 40°C, possibly due to the effect of Fe ions generated from a similar mechanism. In addition, proteins have been shown to facilitate metal corrosion.[95]

Effect of Product Containers

The effect of product containers was clearly demonstrated in a recent study. McCormick et al.[96] reported that Tween 80, intended to replace human albumin in the original formulation, interacted with uncoated rubber stoppers in single-use Eprex syringes. The stoppers leached a small amount of plasticizers in the drug solution, which acted as an adjuvant, and caused a strong IgG response to the recombinant human erythropoietin. The immune response was significantly minimized later by switching to PTFE-coated rubber stoppers. In addition, headspace oxygen can accelerate oxidation of antibodies and replacement with an inert gas could minimize such a reaction.[73]

Accelerated Stability Studies

To accelerate formulation development process, accelerated stability studies are usually conducted. However, estimation of room temperature or 5°C stability based on accelerated stability studies for antibodies, like other proteins, may or may not be possible or accurate. The unpredictability is due to the presence of complex and multiple degradation pathways, which may have different degree of temperature dependencies.[10] Kroon et al.[10] found that relative to other degradation pathways of a monoclonal antibody, OKT3 (IgG2a), Asn deamidation was preferentially accelerated with increasing temperature. Therefore, caution needs to be applied in interpreting such high-temperature-induced deamidation. On the other hand, Poborji et al.[41] found that loss of monomeric chimeric antibody (L6) at pH 7.2 followed Arrhenius behavior in the temperature range of 30–50°C and extrapolation of the high temperature SEC data to 2–8°C at pH 7 and

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5.5 resulted in comparable stability estimates with the available real-time data.

Lyophilized Formulation

Preparation of lyophilized protein formulations has been extensively reviewed.[97–101] Like most proteins, some antibodies are not stable enough in a liquid form and lyophilized dosage forms will have to be considered. Lyophilization was suggested to be one of the two approaches in solving antibody aggregation.[47] This section will discuss some critical issues in formulating a lyophilized antibody product.

Amorphous Versus Crystalline State

It has been commonly believed that proteins are more stable in an amorphous state.[103] This is mainly because proteins need another excipient(s) to replace water molecules to from the required H-bonds as water molecules are removed during drying and a crystalline matrix does not provide intimate H-bonding between the excipient and the protein.[104,105] It appears that if the protein itself can be crystallized during the lyophilization process (may be difficult practically), the protein could be more stable and might not need as much stabilizing agent as in the amorphous state. In a recent report, Shenoy et al.[106] compared the stability of two model proteins, glucose oxidase (GO) and lipase, and demonstrated that dry crystalline formulations are significantly more stable than their amorphous counterparts as monitored by size-exclusion chromatography. It remains to be seen whether this can be applicable to antibodies.

Effect of Formulation Excipients

To make a lyophilized antibody product, at least one formulation excipient, a bulking agent, is needed to confer an acceptable cake. If the antibody is not stable enough in the presence of a bulking agent during lyophilization or storage, a stabilizer(s) is included. The relative amount of formulation excipients (bulking and stabilizing agents) should be carefully chosen as these agents may affect each other's behavior, the tonicity, and stability of the lyophilized products.

Commonly used bulking agents include mannitol and glycine. Crystallization of these agents during lyophilization makes them wonderful bulking agents but poor stabilizing agents.[57] Although mannitol could stabilize an antibody as in the case for a monoclonal antibody-vinca conjugate in a PBS solution at pH 7.4 upon lyophilization,[60] many studies showed that mannitol could not stabilize effectively or even destabilizes antibodies. Such examples include the aggregation of a lyophilized model recombinant humanized monoclonal antibody (rhuMAb HER2) in the presence of mannitol during storage.[3,57] In the lyophilization of a human monoclonal antibody at 1 mg/mL, it was demonstrated that addition of 5% mannitol has no effect on the lyophilization-induced aggregation in PBS or physiological saline as measured by OD600 and addition of 10% mannitol actually accelerated aggregation during the lyophilization process.[58] On the other hand, when used in combination with sucrose or trehalose, similar degree of stabilization to that for sugar alone may be achieved such as the case for model recombinant humanized monoclonal antibody (rhuMAb HER2).[57] The disaccharide/mannitol formulations also inhibited deamidation of this antibody during storage to a greater extent than the lyoprotectant formulations alone.[57]

Sugars are the most common stabilizing agents used in lyophilized formulations. Among these, two nonreducing sugars, sucrose and trehalose, are usually the first choice. Both sugars protected the stability of a few lyophilized antibodies equally well. These antibodies include lyophilized recombinant humanized monoclonal antibody, rhuMAb HER2,[57] IgG1 (containing 12 intra and 4 interchain disulfides),[62] anti-IgG,[107] and chimeric monoclonal antibody.[64] However, sucrose seemed to be more effective in protecting a lyophilized IgG1 against aggregation during storage at 40°C at a sugar:protein weight ratio of 1:1 (Chang et al. 2005b).[102] In another study, sucrose at 5% failed to offer any protection to a lyophilized mouse monoclonal antibody (MN12) (IgG2a (κ)) during storage at 56°C for 18 days.[59] Again, as mentioned in the liquid formulation section, even the most commonly used stabilizers may not stabilize all antibodies in the solid state and antibodies need to be treated as individually different molecules.

When sugars are used as stabilizing agents (lyoprotectants), a sufficient quantity needs to be used to achieve a significant effect depending on the protein concentration. It has been suggested that a molar lyoprotectant:protein ratio of 300:1 or greater should be used.[47] Indeed, the available literature data indicate a ratio higher than 300 is needed for better stabilization. It was shown that sugars protect the stability (aggregation and Asp isomerization) of a lyophilized, monoclonal

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antibody (type not specified) to a significant level when the sugar to protein molar ratio was increased to 500:1, which happens to the theoretical number of water binding sites in the molecule.[1] Similarly, Andya et al.[62] demonstrated that use of sucrose or trehalose inhibited the aggregation of a lyophilized antibody (IgG1—containing 12 intra and 4 interchain disulfides) during storage at 30°C to a maximum degree when the sucrose/trehalose:protein ratio was 500 or higher, which is roughly equivalent to the number of water-binding sites on the surface of the protein (550 estimated). Increasing the trehalose/anti-HER2 ratio from 60 mM (360 molar ratio) to 200 mM (1,200 molar ratio) significantly increased the stability (monomer by SEC-HPLC) of lyophilized formulation at 25 mg/mL at 40°C.[107] In a recent report, it was found that at least a weight ratio of 2:1 (molar ratio of about 800) was needed to achieve a maximum protection of a lyophilized sucrose:IgG1 formulation against both aggregation and chemical degradation during storage at 40°C (Chang et al. 2005a).[101]

Other formulation excipients were also used and some of these are effective in stabilizing certain antibodies. A recent study demonstrated that addition of sorbitol to a sucrose or trehalose formulation resulted in further enhancement of storage stability of a lyophilized IgG1 antibody (Chang et al. 2005b).[102] Either dextran or HB-β-CD (better) protected the activity of a lyophilized mouse monoclonal antibody (MN12) (IgG2a (κ)) during storage.[59] Glucose was shown to inhibit gamma irradiation-induced fragmentation of humanized monoclonal antibodies (hr3).[85]

Effect of Formulation "pH" and Buffering Agents

The pH of the liquid formulation can have a potential effect on the stability of the lyophilized formulation, even though the solid-state "pH" could not be well defined. In the evaluation of the stability of a lyophilized monoclonal antibody-vinca conjugate, aggregation seems to be lower at a neutral pH 7.1 than at either pH 8.5 or 6.1 when the lyophilized formulation was stored at 25°C.[60] In adjusting the formulation pH, use of a buffering agent may have effect on the antibody stability both during lyophilization and storage of the lyophilized products. For example, in the stability study of a fully human anti-IL8 monoclonal antibody (ABX-IL8; IgG2), Chen et al.[21] demonstrated that addition of histidine inhibited the formation of HMW species upon lyophilization and during storage at 37°C in a concentration-dependent manner. Among several excipients including His, Gly, mannitol, Glu, and polysorbate 20, His is the most critical in protecting solid-state stability of the antibody (minimizing aggregation). In a different study, the aggregation rate of a lyophilized anti-IgE at 5°C at 5 mg/mL in 10 mM buffer is affected both by pH and buffers in the following order: Na Phosphate, pH 7 > K Phosphate, pH 7 > Na Succinate, pH 6 or 5 > Histidine, pH 7.[107] It should be noted that some buffering agents may induce significant pH change due to selective crystallization during lyophilization, such as sodium phosphate. Such buffering agents should be avoided in preparing pH-sensitive antibody formulations.

Effect of Protein Concentration

In many cases, increasing the protein concentration increases the stability of the protein during lyophilization (see review).[98] However, antibodies do not seem to follow this trend and many antibodies have been shown to be less stable both during lyophilization and storage at high concentrations. For example, lyophilization of a human monoclonal antibody caused significant aggregation of MAb formulated in PBS or physiological saline as measured by OD600 and increasing the protein concentration from 1 to 2.5 or 5 mg/mL increased the OD600 from 0.194 to 0.311 or 0.427, respectively.[58] Similar trend was also observed during storage of lyophilized formulation. Increasing the concentration of a chimeric monoclonal antibody from 2.5 mg/mL to 25 mg/mL in a lyophilized formulation, containing 31 mg/mL sucrose, 15 mM NaCl, 0.02% Tween 80, 10 mM citrate, increased the aggregate level from 0.4% to 0.6% after 2 months at 5°C.[64] In the evaluation of the stability of a model recombinant humanized monoclonal antibody (rhuMAb HER2), Cleland et al.[57] demonstrated that increasing the protein concentration from 25 mg/mL to 50 mg/mL in a lyophilized formulation (containing 60 mM trehalose, 5 mM His at pH 6) increased the aggregate level from 8.5% to over 30% by SEC-after storage at 40°C for 44 months. Such behavior was also observed of anti-HER2 from 5 to 21 mg/mL containing 250 mannitol.[107]

Effect of Moisture Content

The effect of moisture on the stability of lyophilized antibodies has not been consistent and seem

to depend on the antibody and the degradation pathways being monitored.[57] Generally, increasing moisture content of a lyophilized antibody will gradually increase the rate of degradation during storage. Such negative effect of moisture was clearly demonstrated in a variety of degradation pathways, including dimerization of T2G1s Fab' (IgG1 (κ)) in a moisture content range of 1.5–12.3%,[61] aggregation of TNF-MAb between 0.68% and 8%,[108] formation of acidic/basic species of rhuMAb HER2 between 1% and 8.4%[57] and fragmentation of a mouse monoclonal antibody (MN12) with and without secondary drying.[59]

In other cases, the effect of moisture is either complex or not significant. For example, Breen et al.[1] found that high-moisture formulations (8%) of a lyophilized monoclonal antibody (type not specified) showed a higher rate of aggregation, fragmentation, and Asp isomerization during storage than those containing 2% or 5% moisture but formulations containing intermediate moisture levels (2–3%) were less prone to aggregation than those containing a lower moisture levels (1%) as measured by SEC under accelerated storage conditions. Similarly, the aggregation of a lyophilized IgG1 was found to be minimal at an intermediate moisture level around 3% at different storage temperatures. (Chang et al. 2005b).[102] In a different study, Cleland et al.[57] found that using a model recombinant humanized monoclonal antibody (rhuMAb HER2), a residual moisture between 1 and 8.4% had no impact on the rate of aggregation during storage at 2–8 or 40°C for 12 months.

Accelerated Stability Studies

Like antibodies in a liquid state, those in a solid state may or may not undergo degradation that is characteristic of Arrhenius behavior. It is generally believed that stability studies should be conducted at least below the glass transition temperature of the formulation. Duddu et al.[64] found that accelerated stability data generated in the glassy state (40°C) seems to be a better predictor of the relative stability of formulations than the data generated at a higher temperature (60°C). In the study of the stability of a high-concentration, lyophilized, monoclonal antibody (type not specified), Breen et al.[1] demonstrated that chemical and physical degradation pathways followed Arrhenius kinetics during storage in the glassy state. Only Asp isomerization followed the Arrhenius model above the Tg value.

Advanced Formulations

In addition to the liquid and lyophilized dosage forms on the market, other dosage forms have been tried and limited success has been reported.

Spray-Dried Formulations

Spray drying is an efficient way of preparing solid dosage forms because of the rapid removal of a solvent(s) from a drug solution. It is a very attractive alternative to lyophilization in preparing solid protein formulations. So far, a number of studies have been conducted for proteins and partly because of the high-temperature exposure in the process, albeit very short, limited success has been achieved. Protein aggregation during spray drying seems to be the major challenge.[65,66,109,110]

Spray drying of antibodies has also been tried. As for other proteins, process-induced protein aggregation seems to be the major issue.[71,110] Careful selection of proper excipients could minimize this problem. In the evaluation of a spray-dried formulation for a rhuMAbE25, it was demonstrated that trehalose or lactose minimized the aggregation during spray drying (aggregate dropped from 5–6% to ≤1%). For trehalose, the molar ratio for significant inhibition of aggregation is at or above 300:1 to 500:1 (excipient: protein).[71] Such a ratio seems to be comparable with that used in the lyophilized formation. The addition of mannitol also reduced aggregation, but only up to a molar ratio of 200:1 and further increase in mannitol content resulted in crystallization, which had a detrimental effect on protein stability and aerosol performance.[71] In a different study, either trehalose or sorbitol could inhibit IgG aggregation during spray drying.[110]

Stabilized Antibodies via Mutagenesis

Antibody fragments, such as scFv, are alternative antibody therapeutics. However, since their stability is not as good as full-length antibodies, mutagenesis, or chemical modifications have been tried to improve its stability. For example, R71A mutagenesis in the heavy chain of an unstable scFv dramatically increased its stability when incubated in human serum while having only a minor effect on the binding affinity of the molecule.[111] A more stable heavy chain, made through mutation, can increase the stability of

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the light chain, and thereby confer more stability to the whole scFv.[24]

Stabilized Antibodies via Chemical Modifications

The clinical performance of an antibody can be desirably changed by conjugation of the antibody to another entity. For example, McIntosh et al.[112] demonstrated that conjugation of carboxymethyl dextran to a tumor-antigen-specific monoclonal antibody (A5B7) increased the clearance of the antibody in mice but conjugation of cisplatin (*cis*-dichlorodiammine platinum II) with the antibody complex restricted its tissue distribution and reduced its systemic clearance.

PEGylated Antibodies

A common method of increasing plasma half-life of proteins is to increase the size of the protein by attaching a PEG moiety. Currently, there are at least four PEGylated commercial protein products on the market. However, the long plasma half-lives (as high as 7–23 days) of antibodies made much less appealing the development of such an antibody product. Since the long half-life was due to the binding of Fc to the Fc-receptor (neonatal Fc receptor, FcRn) in various tissues,[33,35] antibody fragments, such as Fab′ lacking the Fc portion have a much shorter half-life and could be good candidates for PEGylation. Such a modification was made on a humanized IgG Fab′ fragment via site-specific attachment to an additionally engineered Cys residue, as modification via the lysine residues led to substantial losses in binding activity.[29]

Controlled Release Systems

Controlled release systems have been extensively investigated for proteins in the past two decades.[113,114] However, limited work has been conducted in this area for antibodies, partly due to their relatively long plasma half-lives compared with other proteins. To achieve long-term effect, a controlled released system consisting of biodegradable poly (lactic-glycolic) acid (PLGA) was prepared for an anti-cocaine catalytic monoclonal antibody and an *in vivo* release of the antibody for up to 10 days was demonstrated in mice following subcutaneous injection.[115] It is anticipated that such systems could be useful if a minimum of 1-week duration of release is achieved.

SUMMARY

Antibodies have similar tertiary structures. The presence of a significant number of disulfide bonds and intimate domain–domain interactions in antibodies make them relatively stable and more resistant to moderate thermal stress compared to other proteins. Nevertheless, antibodies do experience a variety of instabilities similar to most proteins. These physical and chemical instabilities include denaturation, aggregation, surface adsorption, deamidation, oxidation, isomerization, fragmentation, etc. Due to the significant difference in the primary sequence among different antibodies, the relative severity of these degradation pathways can be significantly different. On the average, aggregation, deamidation, and isomerization seem to be more prevalent in antibodies.

Partly due to the stability difference, some antibody products are made into a liquid form while others are lyophilized (Tab. 1). The solution pH of these commercial products is either neutral or weakly acidic, suggesting that such a pH range is probably optimal for most antibodies. Surfactants have been used in most of these antibody products, presumably to inhibit antibody aggregation, and will likely be used in other antibody drug candidates. Two most commonly used formulation excipients in these antibody formulations are sucrose and NaCl. For liquid formulations, either one can be used if they offer the same level of stability protection. For lyophilized formulations, sucrose is preferable as NaCl does not form intimate H-bonds with the antibody and also reduce the glass transition temperature of formulation, making lyophilization less efficient. All the formulation excipients and buffering agents used in commercial antibody products or discussed in the article should be evaluated individually for each antibody drug candidate through stability studies before they are chosen as part of the product, as antibodies are structurally different.

The available stability data in the literature and the information on the commercial antibody products suggest that antibody formulation seems challenging but relatively manageable, compared to other protein drugs. Three major issues in antibody formulation are apparently challenging and need significant attention in the coming years: (1) development of stable high-concentration formulations, (2) management of the high-concentration-associated viscosity of these formulations,

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and (3) minimization of antibody-formulation-induced immune response.

REFERENCES

1. Breen ED, Curley JG, Overcashier DE, Hsu CC, Shire SJ. 2001. Effect of moisture on the stability of a lyophilized humanized monoclonal antibody formulation. Pharm Res 18:1345–1353.
2. Roque ACA, Lowe CR, Taipa MA. 2004. Antibodies and genetically engineered related molecules: Production and purification. Biotechnol Prog 20: 639–654.
3. Pavlou AK, Belsey MJ. 2005. The therapeutic antibodies market to 2008. Eur J Pharm Biopharm 59:389–396.
4. Martsev SP, Tsybovsky YI, Stremovskiy OA, Odintsov SG, Balandin TG, Arosio P, Kravchuk ZI, Deyev SM. 2004. Fusion of the antiferritin antibody VL domain to barnase results in enhanced solubility and altered pH stability. Protein Eng Des Sel 17:85–93.
5. Harris RJ, Shire SJ, Winter C. 2004. Commercial manufacturing scale formulation and analytical characterization of therapeutic recombinant antibodies. Drug Dev Res 61:137–154.
6. Janeway CA, Travers PA, Walport M, Shlomchik MJ. 2001. Immunobiology. New York: Garland Publishing.
7. Zhang W, Czupryn MJ. 2002. Free sulfhydryl in recombinant monoclonal antibodies. Biotechnol Prog 18:509–513.
8. Ruzheinikov SN, Muranova TA, Sedelnikova SE, Partridge LJ, Blackburn GM, Murray IA, Kakinuma H, Takahashi-Ando N, Shimazaki K, Sun J, Nishi Y, Rice DW. 2003. High-resolution crystal structure of the Fab-fragments of a family of mouse catalytic antibodies with esterase activity. J Mol Biol 332:423–435.
9. Glockshuber R, Schmidt T, Pluckthun A. 1992. The disulfide bonds in antibody variable domains: Effects on stability, folding in vitro, and functional expression in Escherichia coli. Biochemistry 31: 1270–1279.
10. Kroon DJ, Baldwin-Ferro A, Lalan P. 1992. Identification of sites of degradation in a therapeutic monoclonal antibody by peptide mapping. Pharm Res 9:1386–1393.
11. Wright A, Morrison SL. 1997. Effect of glycosylation on antibody function: Implications for genetic engineering. Trends Biotechnol 15:26–32.
12. Tao MH, Morrison SL. 1989. Studies of aglycosylated chimeric mouse-human IgG. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. J Immunol 143:2595–2601.
13. Leibiger H, Wustner D, Stigler RD, Marx U. 1999. Variable domain-linked oligosaccharides of a human monoclonal IgG: Structure and influence on antigen binding. Biochem J 338:529–538.
14. Hermeling S, Crommelin DJA, Schellekens H, Jiskoot W. 2004. Structure-immunogenicity relationships of therapeutic proteins. Pharm Res 21: 897–903.
15. Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Satoh M, Yamasaki M, Hanai N, Shitara K. 2003. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466–3473.
16. Hunt G, Moorhouse KG, Chen AB. 1996. Capillary isoelectric focusing and sodium dodecyl sulfate-capillary gel electrophoresis of recombinant humanized monoclonal antibody HER2. J Chromatogr A 744:295–301.
17. Jiskoot W, Beuvery EC, de Koning AAM, Herron JN, Crommelin DJA. 1990. Analytical approaches to the study of monoclonal antibody stability. Pharm Res 7:1234–1241.
18. Wang L, Amphlett G, Lambert JM, Blattler W, Zhang W. 2005. Structural characterization of a recombinant monoclonal antibody by electrospray time-of-flight mass spectrometry. Pharm Res 22: 1338–1349.
19. Wan M, Shiau FY, Gordon W, Wang GY. 1999. Variant antibody identification by peptide mapping. Biotechnol Bioeng 62:485–488.
20. Ramsland PA, Farrugia W. 2002. Crystal structures of human antibodies: A detailed and unfinished tapestry of immunoglobulin gene products. J Mol Recognit 15:248–259.
21. Chen B, Bautista R, Yu K, Zapata GA, Chamow SM, et al. 2003. Influence of histidine on the stability and physical properties of a fully human antibody in aqueous and solid forms. Pharm Res 20:1952–1960.
22. Furtado PB, Whitty PW, Robertson A, Eaton JT, Almogren A, Kerr MA, Woof JM, Perkins SJ. 2004. Solution structure determination of monomeric human IgA2 by X-ray and neutron scattering, analytical ultracentrifugation and constrained modelling: A comparison with monomeric human IgA1. J Mol Biol 338:921–941.
23. Bogard WC, Jr., Dean RT, Deo Y, Fuchs R, Mattis JA, McLean AA, Berger HJ. 1989. Practical considerations in the production, purification, and formulation of monoclonal antibodies for immunoscintigraphy and immunotherapy. Semin Nucl Med 19:202–220.
24. Worn A, Pluckthun A. 1998. Mutual stabilization of VL and VH in single-chain antibody fragments,

Samsung Bioepis Exhibit No. 1002
Page 6395

investigated with mutants engineered for stability. Biochemistry 37:13120–13127.
25. Dall'Acqua W, Simon AL, Mulkerrin MG, Carter P. 1998. Contribution of domain interface residues to the stability of antibody CH3 domain homodimers. Biochemistry 37:9266–9273.
26. Chan LA, Phillips ML, Wims LA, Trinh KR, Denham J, Morrison SL. 2004. Variable region domain exchange in human IgGs promotes antibody complex formation with accompanying structural changes and altered effector functions. Mol Immunol 41:527–538.
27. Morea V, Tramontano A, Rustici M, Chothia C, Lesk AM. 1997. Antibody structure, prediction and redesign. Biophys Chem 68:9–16.
28. Oliva B, Bates PA, Querol E, Aviles FX, Sternberg MJE. 1998. Automated classification of antibody complementarity determining region 3 of the heavy chain (h3) loops into canonical forms and its application to protein structure prediction. J Mol Biol 279:1193–1210.
29. Weir ANC, Nesbitt A, Chapman AP, Popplewell AG, Antoniw P, Lawson ADG. 2002. Formatting antibody fragments to mediate specific therapeutic functions. Biochem Soc Trans 30:512–516.
30. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, Jardieu PM. 1993. Humanization of an antibody directed against IgE. J Immunol 151:2623–2632.
31. Sheriff S, Chang CYY, Jeffrey PD, Bajorath J. 1996. X-ray structure of the uncomplexed antitumor antibody br96 and comparison with its antigen-bound form. J Mol Biol 259:938–946.
32. Van Regenmortel MHV. 2002. Reductionism and the search for structure-function relationships in antibody molecules. J Mol Recognit 15:240–247.
33. Lobo ED, Hansen RJ, Balthasar JP. 2004. Antibody pharmacokinetics and pharmacodynamics. J Pharm Sci 93:2645–2668.
34. Coloma MJ, Trinh KR, Wims LA, Morrison SL. 1997. The hinge as a spacer contributes to covalent assembly and is required for function of IgG. J Immunol 158:733–740.
35. Stockwin LH, Holmes S. 2003. Antibodies as therapeutic agents: Vive la renaissance! Expert Opin Biol Ther 3:1133–1152.
36. Cacia J, Keck R, Presta LG, Frenz J. 1996. Isomerization of an aspartic acid residue in the complementarity-determining regions of a recombinant antibody to human IgE: Identification and effect on binding affinity. Biochemistry 35:1897–1903.
37. Harris RJ, Kabakoff B, Macchi FD, Shen FJ, Kwong M, Andya JD, Shire SJ, Bjork N, Totpal K, Chen AB. 2001. Identification of multiple sources of charge heterogeneity in a recombinant antibody. J Chromatogr B Biomed Sci Appl 752: 233–245.
38. Dall'Acqua W, Carter P. 1998. Antibody engineering. Curr Opin Struct Biol 8:443–450.
39. Le Gall F, Reusch U, Little M, Kipriyanov SM. 2004. Effect of linker sequences between the antibody variable domains on the formation, stability and biological activity of a bispecific tandem diabody. Protein Eng Des Sel 17:85–93.
40. Chu GC, Coulibaly SB, Raibekas A, Deechongkit S. 2005. Protein formulation of an IGG1 antibody with atypical glycosylation: A study on the stability of its glycoforms. Abstr Pap Am Chem Soc 229:U210–U211.
41. Paborji M, Pochopin NL, Coppola WP, Bogardus JB. 1994. Chemical and physical stability of chimeric L6, a mouse-human monoclonal antibody. Pharm Res 11:764–771.
42. Welfle K, Misselwitz R, Hausdorf G, Hohne W, Welfle H. 1999. Conformation, pH-induced conformational changes, and thermal unfolding of anti-p24 (HIV-1) monoclonal antibody CB4-1 and its Fab and Fc fragments. Biochim Biophys Acta 1431:120–131.
43. Li SQ, Bomser JA, Zhang QH. 2005. Effects of pulsed electric fields and heat treatment on stability and secondary structure of bovine immunoglobulin G. J Agric Food Chem 53:663–670.
44. Wang W. 1999. Instability, stabilization, and formulation of liquid protein pharmaceuticals. Int J Pharm 185:129–188.
45. Harrison JS, Gill A, Hoare M. 1998. Stability of a single-chain Fv antibody fragment when exposed to a high shear environment combined with air-liquid interfaces. Biotechnol Bioeng 59:517–519.
46. Taschner N, Muller SA, Alumella VR, Goldie KN, Drake AF, Aebi U, Arvinte T. 2001. Modulation of antigenicity related to changes in antibody flexibility upon lyophilization. J Mol Biol 310:169–179.
47. Shire SJ, Shahrokh Z, Liu J. 2004. Challenges in the development of high protein concentration formulations. J Pharm Sci 93:1390–1402.
48. Braun A, Kwee L, Labow MA, Alsenz J. 1997. Protein aggregates seem to play a key role among the parameters influencing the antigenicity of interferon alpha (IFN-alpha) in normal and transgenic mice. Pharm Res 14:1472–1478.
49. Demeule B, Gurny R, Arvinte T. 2005. Where disease pathogenesis meets protein formulation: Renal deposition of immunoglobulin aggregates. Eur J Pharm Biopharm.
50. Ryan ME, Webster ML, Statler JD. 1996. Adverse effects of intravenous immunoglobuin therapy. Clin Pediatr 35:23–31.
51. Schreiber G. 2002. Kinetic studies of protein-protein interactions. Curr Opin Struct Biol 12: 41–47.
52. Sukumar M, Doyle BL, Combs JL, Pekar AH. 2004. Opalescent appearance of an IgG1 antibody

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Page 6396

at high concentrations and its relationship to noncovalent association. Pharm Res 21:1087–1093.
53. Levine HL, Ransohoff TC, Kawahata RT, McGregor WC. 1991. Use of surface tension measurements in the design of antibody-based product formulations. J Parenter Sci Technol 45:160–165.
54. Ferri C, Zignego AL, Pileri SA. 2002. Cryoglobulins. J Clin Pathol 55:4–13.
55. Lam XM, Oeswein JQ, Ongpipattanakul B, Shahrokh Z, Wang SX, Weissburg RP, Wong RL. 2000. Stabilized Antibody Formulation. Genentech, Inc., US Patent US 6991790 B1.
56. Lee LS. 1997. Stabilized monomeric protein compositions. Enzon, Inc., US Patent US 5656730.
57. Cleland JL, Lam X, Kendrick B, Yang J, Yang TH, Overcashier D, Brooks D, Hsu C, Carpenter JF. 2001. A specific molar ratio of stabilizer to protein is required for storage stability of a lyophilized monoclonal antibody. J Pharm Sci 90:310–321.
58. Hagiwara H, Yuasa H, Yamamoto Y. 2000. Stabilized human monoclonal antibody preparation, Yoshihide Hagiwara.
59. Ressing ME, Jiskoot W, Talsma H, Van Ingen CW, Crommelin DJ, et al. 1992. Influence of sucrose, dextran, and hydroxypropyl-beta-cyclodextrin as lyoprotectants for a freeze-dried mouse IgG2a monoclonal antibody (MN12). Pharm Res 9:266–270.
60. Roy ML, Pikal MJ, Rickard EC, Maloney AM. 1992. The effects of formulation and moisture on the stability of a freeze-dried monoclonal antibody-vinca conjugate: A test of the WLF glass transition theory. Dev Biol Stand 74:323–339; discussion 340.
61. Kamat MS, Tolman GL, Brown JM. 1996. Formulation development of an antifibrin monoclonal antibody radiopharmaceutical. Pharm Biotechnol 9:343–364.
62. Andya JD, Hsu CC, Shire SJ. 2003. Mechanisms of aggregate formation and carbohydrate excipient stabilization of lyophilized humanized monoclonal antibody formulations. AAPS PharmSci 5:E10.
63. Riggin A, Clodfelter D, Maloney A, Rickard E, Massey E. 1991. Solution stability of the monoclonal antibody-vinca alkaloid conjugate, KS1/4-DAVLB. Pharm Res 8:1264–1269.
64. Duddu SP, Dal Monte PR. 1997. Effect of glass transition temperature on the stability of lyophilized formulations containing a chimeric therapeutic monoclonal antibody. Pharm Res 14:591–595.
65. Maa YF, Nguyen PA, Andya JD, Dasovich N, Hsu CC, et al. 1998. Effect of spray drying and subsequent processing conditions on residual moisture content and physical/biochemical stability of protein inhalation powders. Pharm Res 15:768–775.
66. Maa YF, Nguyen PA, Hsu SW. 1998. Spray-drying of air-liquid interface sensitive recombinant human growth hormone. J Pharm Sci 87:152–159.
67. Alexander AJ, Hughes DE. 1995. Monitoring of IgG antibody thermal stability by micellar electrokinetic capillary chromatography and matrix-assisted laser desorption/ionization mass spectrometry. Anal Chem 67:3626–3632.
68. Hartmann WK, Saptharishi N, Yang XY, Mitra G, Soman G. 2004. Characterization and analysis of thermal denaturation of antibodies by size exclusion high-performance liquid chromatography with quadruple detection. Anal Biochem 325: 227–239.
69. Doran PM. 2006. Loss of secreted antibody from transgenic plant tissue cultures due to surface adsorption. J Biotechnol 122:39–54.
70. Morales AA, Nunez-Gandolff G, Perez NP, Veliz BC, Caballero-Torres I, Duconge J, Fernandez E, Crespo FZ, Veloso A, Iznaga-Escobar N. 1999. Freeze-dried formulation for direct 99mTc-labeling ior-egf/r3 MAb: Additives, biodistribution, and stability. Nucl Med Biol 26:717–723.
71. Andya JD, Maa YF, Costantino HR, Nguyen PA, Dasovich N, Sweeney TD, Hsu CC, Shire SJ. 1999. The effect of formulation excipients on protein stability and aerosol performance of spray-dried powders of a recombinant humanized anti-IgE monoclonal antibody. Pharm Res 16:350–358.
72. Usami A, Ohtsu A, Takahama S, Fujii T. 1996. Effect of pH, hydrogen peroxide and temperature on the stability of human monoclonal antibody. J Pharm Biomed Anal 14:1133–1140.
73. Rao PE, Kroon DJ. 1993. Orthoclone OKT3 chemical mechanims and functional effects of degradation of a therapeutic monoclonal antibody. In: Pearlman R, Wan YJ, editors. Stability and characterization of protein and peptide drugs case histories. New York: Plenum Press. p. 135–158.
74. Tous GI, Wei Z, Feng J, Bilbulian S, Bowen S, Smith J, Strouse R, McGeehan P, Casas-Finet J, Schenerman MA. 2005. Characterization of a novel modification to monoclonal antibodies: Thioether cross-link of heavy and light chains. Anal Chem 77:2675–2682.
75. Cleland JL, Powell MF, Shire SJ. 1993. The development of stable protein formulations: A close look at protein aggregation, deamidation, and oxidation. Crit Rev Ther Drug Carrier Syst 10:307–377.
76. Xie M, Schowen RL. 1999. Secondary structure and protein deamidation. J Pharm Sci 88:8–13.
77. Kwong MY, Harris RJ. 1994. Identification of succinimide sites in proteins by N-terminal sequence analysis after alkaline hydroxylamine cleavage. Protein Sci 3:147–149.
78. Zheng JY, Janis LJ. 2005. Influence of pH, buffer species, and storage temperature on physicochem-

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Page 6397

ical stability of a humanized monoclonal antibody LA298. Int J Pharm 308:46–51.
79. Tyler-Cross R, Schirch V. 1991. Effects of amino acid sequence, buffers, and ionic strength on the rate and mechanism of deamidation of asparagine residues in small peptides. J Biol Chem 266: 22549–22556.
80. Johnson RE, Qi H, Borgmeyer JR, Kessler RK, Zeng DL. 2004. Stable pH optimized formulation of a modified antibody. Pharmacia Corporation. International patent Wo2004/019861 A2.
81. Lam XM, Yang JY, Cleland JL. 1997. Antioxidants for prevention of methionine oxidation in recombinant monoclonal antibody HER2. J Pharm Sci 86:1250–1255.
82. Harris RJ. 1995. Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture. J Chromatogr A 705:129–134.
83. Powell MF. 1996. A compendium and hydropathy/flexibility analysis of common reactive sites in proteins: Reactivity at Asn, Asp, Gln, and Met motifs in neutral pH solution. In: Pearlman R, Wang YJ, editors. Formulation, characterization, and stability of protein drugs. New York: Plenum Press. p. 1–140.
84. Sarno MEC, Vasquez RA, Yung S-G, Graf CR. 1999. Stable intravenously-administrable immune globulin preparation. Baxter International Inc., US patent US5945098.
85. Caballero I, Altanes S, Castillo A, Deridder V, Gomez T, Miralles Y, Naydenov V, Prieto E, Tilquin B. 2004. Radiosensitivity study of freeze-dried antibodies to Gamma irradiation. Ame Pharm Rev 7:40–46, 81.
86. Kennedy DM, Skillen AW, Self CH. 1994. Glycation of monoclonal antibodies impairs their ability to bind antigen. Clin Exp Immunol 98:245–251.
87. Patten PA, Schellekens H. 2003. The immunogenicity of biopharmaceuticals. Lessons learned and consequences for protein drug development. Dev Biol (Basel) 112:81–97.
88. Liu J, Nguyen MD, Andya JD, Shire SJ. 2005. Reversible self-association increases the viscosity of a concentrated monoclonal antibody in aqueous solution. J Pharm Sci 94:1928–1940.
89. Golovanov AP, Hautbergue GM, Wilson SA, Lian LY. 2004. A simple method for improving protein solubility and long-term stability. J Am Chem Soc 126:8933–8939.
90. Kueltzo LA. 2004. Aggregation of monoclonal antibodies in aqueous solution with and without agitation. Formulation Strategies for Protein Therapeutics. Boston, MA: IBC Life Sciences.
91. Mahler HC, Muller R, Friebeta W, Delille A, Matheus S. 2005. Induction and analysis of aggregates in a liquid IgG1-antibody formulation. Eur J Pharm Biopharm 59:407–417.
92. Treuheit MJ, Kosky AA, Brems DN. 2002. Inverse relationship of protein concentration and aggregation. Pharm Res 19:511–516.
93. Ha E, Wang W, Wang YJ. 2002. Peroxide formation in polysorbate 80 and protein stability. J Pharm Sci 91:2252–2264.
94. Gupta S, Kaisheva E. 2003. Development of a multidose formulation for a humanized monoclonal antibody using experimental design techniques. AAPS PharmSci 5:E8.
95. Kocijan A, Milosev I, Pihlar B. 2003. The influence of complexing agent and proteins on the corrosion of stainless steels and their metal components. J Mater Sci Mater Med 14:69–77.
96. McCormick D. 2004. Small changes, big effects in biological manufacturing. Pharm Technol 28:16.
97. Carpenter JF, Pikal MJ, Chang BS, Randolph TW. 1997. Rational design of stable lyophilized protein formulations: Some practical advice. Pharm Res 14:969–975.
98. Wang W. 2000. Lyophilization and development of solid protein pharmaceuticals. Int J Pharm 203: 1–60.
99. Akers MJ, Vasudevan V, Stickelmeyer M. 2002. Formulation development of protein dosage forms. Pharm Biotechnol 14:47–127.
100. Chang BS, Hershenson S. 2002. Practical approaches to protein formulation development. Pharm Biotechnol 13:1–25.
101. Chang LL, Shepherd D, Sun J, Ouellette D, Grant KL, Tang XC, Pikal MJ. 2005. Mechanism of protein stabilization by sugars during freeze-drying and storage: Native structure preservation, specific interaction, and/or immobilization in a glassy matrix? J Pharm Sci 94:1427–1444.
102. Chang LL, Shepherd D, Sun J, Tang XC, Pikal MJ. 2005. Effect of sorbitol and residual moisture on the stability of lyophilized antibodies: Implications for the mechanism of protein stabilization in the solid state. J Pharm Sci 94:1445–1455.
103. Pikal MJ, Rigsbee DR. 1997. The stability of insulin in crystalline and amorphous solids: Observation of greater stability for the amorphous form. Pharm Res 14:1379–1387.
104. Crowe JH, Crowe LM, Carpenter JF. 1993. Preserving dry biomaterials: The water replacement hypothesis, part 1. Biopharm 6:28–37.
105. Crowe JH, Crowe LM, Carpenter JF. 1993. Preserving dry biomaterials: The water replacement hypothesis, part 2. Biopharm 6:40–43.
106. Shenoy B, Wang Y, Shan W, Margolin AL. 2001. Stability of crystalline proteins. Biotechnol Bioeng 73:358–369.
107. Andya J, Cleland JL, Hsu CC, Lam XM, Overcashier DE, Shire SJ, Yang JY-F, Wu SS-Y. 2004. Protein formulation. Genentech, Inc.
108. Ma X, Wang DQ, Bouffard R, MacKenzie A. 2001. Characterization of murine monoclonal antibody

to tumor necrosis factor (TNF-MAb) formulation for freeze drying cycle development. Pharm Res 18:196–202.
109. Maa YF, Costantino HR, Nguyen PA, Hsu CC. 1997. The effect of operating and formulation variables on the morphology of spray-dried protein particles. Pharm Dev Technol 2:213–223.
110. Maury M, Murphy K, Kumar S, Mauerer A, Lee G. 2005. Spray-drying of proteins: Effects of sorbitol and trehalose on aggregation and FT-IR amide I spectrum of an immunoglobulin G. Eur J Pharm Biopharm 59:251–261.
111. Krauss J, Arndt MA, Zhu Z, Newton DL, Vu BK, Choudhry V, Darbha R, Ji X, Courtenay-Luck NS, Deonarain MP, Richards J, Rybak SM. 2004. Impact of antibody framework residue VH-71 on the stability of a humanised anti-MUC1 scFv and derived immunoenzyme. Br J Cancer 90:1863–1870.
112. McIntosh DP, Cooke RJ, McLachlan AJ, Daley-Yates PT, Rowland M. 1997. Pharmacokinetics and tissue distribution of cisplatin and conjugates of cisplatin with carboxymethyldextran and A5B7 monoclonal antibody in CD1 mice. J Pharm Sci 86:1478–1483.
113. Yewey GL, Duysen EG, Cox SM, Dunn RL. 1997. Delivery of proteins from a controlled release injectable implant. Pharm Biotechnol 10:93–117.
114. Porjazoska A, Goracinova K, Mladenovska K, Glavas M, Simonovska M, Janjevic EI, Cvetkovska M. 2004. Poly(lactide-co-glycolide) microparticles as systems for controlled release of proteins—preparation and characterization. Acta Pharm 54:215–229.
115. Homayoun P, Mandal T, Landry D, Komiskey H. 2003. Controlled release of anti-cocaine catalytic antibody from biodegradable polymer microspheres. J Pharm Pharmacol 55:933–938.

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RAPID COMMUNICATION

IDEC-C2B8 (Rituximab) Anti-CD20 Monoclonal Antibody Therapy in Patients With Relapsed Low-Grade Non-Hodgkin's Lymphoma

By David G. Maloney, Antonio J. Grillo-López, Christine A. White, David Bodkin, Russell J. Schilder, James A. Neidhart, Nalini Janakiraman, Kenneth A. Foon, Tina-Marie Liles, Brian K. Dallaire, Ken Wey, Ivor Royston, Thomas Davis, and Ronald Levy

IDEC-C2B8 is a chimeric monoclonal antibody (MoAb) directed against the B-cell–specific antigen CD20 expressed on non-Hodgkin's lymphomas (NHL). The MoAb mediates complement and antibody-dependent cell-mediated cytotoxicity and has direct antiproliferative effects against malignant B-cell lines in vitro. Phase I trials of single doses up to 500 mg/m^2 and 4 weekly doses of 375 mg/m^2 showed clinical responses with no dose-limiting toxicity. We conducted a phase II, multicenter study evaluating four weekly infusions of 375 mg/m^2 IDEC-C2B8 in patients with relapsed low-grade or follicular NHL (Working Formulation groups A-D). Patients were monitored for adverse events, antibody pharmacokinetics, and clinical response. Thirty-seven patients with a median age of 58 years (range, 29 to 81 years) were treated. All patients had relapsed after chemotherapy (median of 2 prior regimens) and 54% had failed aggressive chemotherapy. Infusional side effects (grade 1-2) consisting of mild fever, chills, respiratory symptoms, and occasionally hypotension were observed mostly with the initial antibody infusion and were rare with subsequent doses. Peripheral blood B-cell depletion occurred rapidly, with recovery beginning 6 months posttreatment. There were no significant changes in mean IgG levels and infections were not increased over what would be expected in this population. Clinical remissions were observed in 17 patients (3 complete remissions and 14 partial remissions), yielding an intent to treat response rate of 46%. The onset of these tumor responses was as soon as 1 month posttreatment and reached a maximum by 4 months posttreatment. In the 17 responders, the median time to progression was 10.2 months (5 patients exceeding 20 months). Likelihood of tumor response was associated with a follicular histology, with the ability to sustain a high serum level of antibody after the first infusion, and with a longer duration of remission to prior chemotherapy. One patient developed a detectable but not quantifiable immune response to the antibody that had no clinical significance. IDEC-C2B8 in a dose of 375 mg/m^2 weekly for 4 weeks has antitumor activity in patients with relapsed low-grade or follicular NHL. Results with this brief, outpatient treatment compare favorably with results with standard chemotherapy, and IDEC-C2B8 has a better safety profile. Further studies evaluating IDEC-C2B8 in other types of lymphoma either alone or combined with chemotherapy are warranted.
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THE TREATMENT OF cancer with agents specific for the tumor cell with sparing of the host has long been a goal of oncology. Unfortunately, treatment approaches using chemotherapy and radiotherapy have increased host toxicity to obtain antitumor activity. The development of monoclonal antibodies (MoAbs) provided hope that tumor-targeted therapy would one day play a role in the treatment of cancer. Indeed, promising results have been presented in several areas; however, these treatments have often proved technically difficult, produced disappointing efficacy, and were often not broadly applicable to patients with a given malignancy. The treatment of patients with lymphoma is an exception. Patients with advanced stage or relapsed low-grade non-Hodgkin's lymphoma (NHL) are not curable using conventional approaches and are usually treated with combination chemotherapy regimens of increasing intensity as needed to reduce disease and palliate symptoms.[1] Aggressive approaches using high-dose therapy and autologous marrow support may provide maximal tumor reduction; however, there is currently no plateau on the failure-free survival curve.[2] New treatment modalities and approaches that provide tumor reduction with less toxicity are needed.

MoAbs against a variety of tumor surface antigens have shown activity in the treatment of patients with low-grade NHL. Unmodified murine MoAbs have included custom, patient-specific anti-idiotype MoAbs,[3-6] anti-CD20 MoAbs,[7] and antibodies against CD19 and CD22 armed with immunotoxins.[8-10] Anti-CD20 MoAbs radiolabeled with ^{131}-I or ^{90}-Y in low dose[11-13] and in high dose with stem cell support[14,15] have also demonstrated significant antitumor activity in the treatment of patients with relapsed disease, but have encountered dose-limiting toxicities and are limited by bone marrow involvement.

IDEC-C2B8 is a chimeric anti-CD20 MoAb containing human IgG1 and κ constant regions with murine variable regions.[16] It binds the CD20 antigen with high affinity (5×10^{-9} mol/L). Because of the human constant Ig regions, the chimeric antibody efficiently kills CD20$^+$ cells in vitro by augmented complement-mediated lysis and participates in antibody-dependent cell-mediated cytotoxicity (ADCC) using human complement and immune effector cells.[16] In some

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NHL cell lines, the binding of the antibody inhibits proliferation and directly induces apoptosis.[17] In patients with lymphoma, the administration of single doses up to 500 mg/m^2 was not associated with any dose limiting toxicity. Rapid binding to and depletion of CD20$^+$ normal B cells and tumor cells in the peripheral blood and bone marrow was observed, and tumor cells in lymph node biopsies obtained 2 weeks after antibody therapy showed that the chimeric MoAb bound to CD20 antigen sites.[18] The treatment was well tolerated, causing only minimal infusion-related symptoms. Tumor responses (2 partial remissions [PR] lasting 8.1 and 8.6 months and 5 minor responses lasting 0.9 to 6 months) were observed in 7 of 9 patients treated with single doses of at least 100 mg/m^2. Repeated weekly infusion for 4 weeks of doses of 125, 250, or 375 mg/m^2 in 20 patients with relapsed lymphoma has also recently been reported.[19] The treatment was well tolerated and 6 of 18 evaluable patients had a PR with no dose-limiting toxicity identified. The 375 mg/m^2 for 4 weeks dose was selected for phase II evaluation. We report here on the clinical results obtained in the treatment of 37 patients with relapsed low-grade or follicular lymphoma.

MATERIALS AND METHODS

Study design. This was a phase II, open-label, single-arm, multicenter trial evaluating four intravenous infusions of the chimeric monoclonal anti-CD20 antibody IDEC-C2B8 at a dose of 375 mg/m^2 in patients with relapsed low-grade NHL. Study objectives included the evaluation of safety, dose-limiting toxicities, pharmacokinetics, immune response, and clinical activity. Patients received a single infusion weekly, and completed the 4 antibody infusions in 22 days.

Antibody. IDEC-C2B8 is a chimeric MoAb containing human IgG1 (κ) heavy and light chain constant regions and murine variable regions from the murine antihuman CD20 MoAb IDEC-2B8 (murine IgG1, κ). The chimeric antibody was produced in vitro from a transfected Chinese hamster ovary (CHO) cell line.[16] The antibody is specific for the human CD20 antigen, a 35-kD phosphoprotein present on normal and malignant B cells, but not on other tissues.[20] The antibody was produced and supplied by IDEC Pharmaceuticals Corp (San Diego, CA).

Patient population and sample size. Adults with histologically confirmed, relapsed B-cell lymphoma expressing the CD20 antigen were eligible, as were nonresponders to first chemotherapy. All patients met the following criteria: expected survival of $\geq$3 months with no serious nonmalignant disease; prestudy performance status of 0, 1, or 2 on the World Health Organization (WHO) scale; seronegative for human immunodeficiency virus (HIV) and hepatitis B surface antigen; serum IgG level of $\geq$600 mg/dL; hemoglobin level of $\geq$8.0 g/dL; white blood cell count of $\geq 3.0 \times 10^3/\mu L$; absolute granulocyte count of $\geq 1.5 \times 10^3/\mu L$; platelet count of $\geq 75 \times 10^3/\mu L$; bilirubin level of less than 1.5 mg/dL; alkaline phosphatase level of less than 2$\times$ normal; aspartata amino transferase (AST) level of less than 2$\times$ normal; and serum creatinine level of less than 2.0 mg/dL. Patients tested negative for human antimurine antibody (HAMA).

The 375 mg/m^2 dose was identified from a phase I dose-escalation trial evaluating 125, 250, and 375 mg/m^2 infusions for 4 weeks.[19] To determine clinical efficacy, a total of 37 patients were treated at the 375 mg/m^2 dose and form the basis of this report.

Study sites. Patients were treated at seven sites in the United States. All patients signed an informed consent approved by the local Institutional Review Boards at Stanford University Medical Center (Palo Alto, CA), San Diego Regional Cancer Center (San Diego, CA), Scripps Memorial Hospitals (La Jolla and Encinitas, CA), Fox Chase Cancer Center (Philadelphia, PA), University of New Mexico Cancer Center (Albuquerque, NM), Henry Ford Hospital (Detroit, MI), or Markey Cancer Center (Lexington, KY). Patients were enrolled and treated between August 12, 1993 and September 13, 1994.

Pharmacokinetic sampling and HAMA/HACA evaluation. Serum samples were obtained pretreatment and at scheduled follow-up evaluations to determine anti-CD20 MoAb levels and to analyze for evidence of human antichimeric antibody (HACA) and HAMA immune response. Extended pharmacokinetic sampling was performed during the treatment course in selected patients whose schedules permitted. This included samples during the weeks 1 and 4 of treatment, including before infusion, postinfusion, and 24, 48, 72, and 96 hours postinfusion, as well as preinfusion and postinfusion samples from weeks 2 and 3. Serum pharmacokinetics were determined using frozen serum samples on microtiter plates coated with polyclonal goat anti–IDEC-2B8 antibody. Serum samples were serially diluted and washed, and bound IDEC-C2B8 was detected using labeled goat antihuman IgG and compared with a standard curve obtained using IDEC-C2B8 diluted into normal human serum. The assay has a lower limit of quantification of 0.5 μg/mL of the chimeric antibody.

HAMA was detected using microtiter plates coated with murine IgG. Serum samples were serially diluted on the plate and washed. Any bound human Ig was then detected using labeled goat antihuman Ig. HACA was detected by incubating serum samples on plates coated with IDEC-C2B8. Human antibody bound to the microtiter wells was then detected using biotin–IDEC-C2B8 followed by avidin-horseradish peroxidase and the substrate 2, 2-azinobis (3-ethylbenzthiazoline sulfonic acid) (ABTS). The minimum quantifiable level for these assays was 100 ng/mL (detectable to 5 ng/mL).

Patient monitoring and efficacy evaluation. Patients were monitored for safety and for clinical antitumor effect with regular medical histories, physical examinations, and laboratory studies including complete blood count and differential, chemistry panel, quantitative serum Igs, serum complement, serum anti-CD20 levels (HACA and HAMA), and urinalysis performed at baseline; at weeks 1, 2, 3, and 4; and at specified intervals to a maximum of 4 years. Toxicity was evaluated using the National Cancer Institute's Adult Toxicity Criteria. Peripheral blood was analyzed by flow cytometry to determine lymphocyte subsets at baseline, week 1, months 1, and 3 and at 3-month intervals for 1 year. Chest x-ray was performed at baseline and at 1 and 3 months after the completion of IDEC-C2B8 therapy. Computed tomography scans of the neck, chest, abdomen, and pelvis, as well as physical examination and measurement of palpable tumor masses or lymph nodes was conducted at baseline, at 1 and 3 months after treatment, and at 3-month intervals for 1 year and then yearly for 4 years. Tumor lesions were serially measured by physical exam and radiologic procedures. PR were defined as a decrease of greater than 50% in the total sum of the products of the bidimensional measurements. Complete remissions (CR) were defined as the disappearance of all disease. Stable disease was defined by no significant change in tumor measurements without progression over the period of observation. Remissions (PR and CR) were confirmed on a second complete evaluation at least 28 days later. Disease progression was determined by a 25% increase in measurable disease or the appearance of any new lesion. Time to progression was measured from date of first antibody infusion to first date when progressive disease was documented.

RESULTS

Patient characteristics. Thirty-seven patients were assigned to receive treatment at the phase II dose level of 375

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Table 1. Patient Characteristics (N = 37)

	No.	% or Range
Age	58	29-81
Sex		
Female	16	43
Male	21	57
Histologic grade*		
Low	34	92
A	4	11
B	21	57
C	9	24
Intermediate	3	8
D	2	5
G	1	3
Stage at diagnosis		
I	5	14
II	2	5
III	10	27
IV	18	49
Unknown	2	5
Prior Therapy		
Chemotherapy	37	100
Median no. of regimens	2	1-5
Time from last Rx median (mo)	10.2	0.9-70.4
Radiotherapy	13	35
ABMT	3	8
All therapy	37	100
Median no.	2	1-8
Time from last Rx, median (mo)	7.2	0.9-67.9
First chemotherapy		
Single agent	9	25
COP or CP	11	31
Aggressive combination	17	44
Response to first chemotherapy		
CR or PR	29	81
Duration, median (mo)	12	
No response	5	14
Unknown	2	5
Response to last therapy		
CP or PR	25	86
Duration, median (mo)	12	
Unknown	2	5

* Histologic group based on Working Formulation.

mg/m^2 for 4 weeks, the highest dose used in the dose escalation phase I portion of the trial. Patient characteristics at diagnosis and prior lymphoma therapy for all 37 patients are detailed in Table 1. The median age was 58 years, with a range of 29 to 81 years. All but 1 patient had an initial diagnosis of a low-grade or follicular histology (Working Formulation [WF] groups A-D), and 76% were diagnosed with stage III or IV disease. At the time of antibody therapy, 17 patients (46%) had bone marrow involvement, 10 patients (27%) had an elevated lactate dehydrogenase (LDH) level, and 10 patients (27%) had at least one lesion greater than or equal to 7 cm. All patients had relapsed after standard chemotherapy with a median of 2 prior regimens (range, 1 to 5). Forty-four percent of patients had failed to respond to an initial aggressive combination chemotherapy regimen such as CHOP, PROMACE-CYTABOM, or MACOP-B. Fifty-six percent of patients were initially treated with a single agent (25%) or combination therapy with an alkylating agent and prednisone. Most patients (81%) had a CR or PR to initial chemotherapy with a median duration of 12 months. Relapsed disease was treated with a variety of agents including radiotherapy, single-agent, and aggressive combination chemotherapy including high-dose therapy supported with autologous stem cells (3 patients). Eighty-six percent of patients had responded to their most recent treatment with a CR (41%) or PR (45%) with a median duration of 12 months (20 patients with duration of <6 months). Treatment was administered at a median of 4.5 years from initial diagnosis (range, 0.8 to 13.3 years) and a median of 10.2 months from last therapy. Two patients had previously been treated in a phase I single-dose study using IDEC-C2B8, one of whom achieved a PR lasting 8 months.

Antibody infusions. IDEC-C2B8 infusions were administered in an outpatient setting, usually through peripheral intravenous catheters. Patients were often premedicated for the first antibody infusion with oral diphenhydramine and acetaminophen. The total dose of antibody was diluted into normal saline (generally 1 mg/mL) and administered intravenously by infusion at an initial rate of 50 mg/h, escalating every 30 minutes in the absence of side effects to a maximum of 200 mg/h.

The average dose of antibody administered during each infusion was 711 mg (range, 562 to 825 mg), with a cumulative dose over 4 infusions of 2,730 mg (maximum cumulative dose of 3,300 mg). The initial antibody infusion duration averaged 5.6 hours (range, 1.5 to 12.7 hours). In the absence of symptoms, subsequent infusions started at 200 mg/h with an average duration of infusion of 4.5, 4.4, and 4.2 hours for doses number 2, 3, and 4, respectively.

Adverse events. The most common adverse events thought to be related to treatment are detailed in Table 2. Thirty-two of the 37 patients experienced adverse events during the course of the trial; 6 patients experienced 12 grade 3 or 4 adverse events (9 reported as related to treatment). The most frequent infusion-related events included grade 1 or 2 fever (73% of patients), chills (38%), nausea (19%), asthenia (16%), headache (16%), and a sensation of tongue or throat swelling (11%). Hypotension occurred in 6 patients and was limited to grade 1 in 3 cases and grade 2 in 3 cases. Treatment-related side effects were seen most frequently with the initial antibody infusion (129 of 192 [67%]) and dramatically decreased during the second, third, and fourth infusions. Adverse events were managed by the administration of diphenhydramine or acetaminophen as indicated and by temporarily halting the antibody infusions until the symptoms abated. The infusions could in general then be restarted with minimal further toxicity. Two patients were withdrawn from study after receiving only the first of 4 planned antibody infusions (described in detail below).

Grade 3 and grade 4 adverse events included thrombocytopenia and anemia in 1 patient, abdominal pain and constipation in 1 patient, transient neutropenia at 4 months and 9 months in 2 patients, myocardial infarction in 1 patient, and malignant pleural effusion with dyspnea and traumatic hip fracture 3 weeks after antibody therapy in 1 patient. Two of the 37 patients did not complete the 4 planned antibody

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Table 2. Observed Adverse Events

	N (%)	Events (%)
Any adverse event	32 (86.5)	192 (100)
Body as a whole		
Fever	27 (73.0)	55 (28.6)
Chills	14 (37.8)	14 (7.3)
Asthenia	6 (16.2)	6 (3.1)
Headache	6 (16.2)	11 (5.7)
Cardiovascular system		
Hypotension	6 (16.2)	6 (3.1)
Digestive system		
Nausea	7 (18.9)	7 (3.6)
Vomiting	4 (10.8)	4 (2.1)
Dyspepsia	2 (5.4)	2 (1.0)
Blood and lymphatics		
Thrombocytopenia	7 (18.9)	7 (3.6)
Neutropenia	3 (8.1)	5 (2.6)
Anemia	2 (5.4)	3 (1.6)
Coagulation disorder	2 (5.4)	2 (1.0)
Leukopenia	1 (2.7)	4 (2.1)
Nervous system		
Vasodilation	1 (2.7)	3 (1.6)
Respiratory system		
Rhinitis	6 (16.2)	6 (3.1)
Laryngismus	4 (10.8)	4 (2.1)
Chest pain	2 (5.4)	2 (1.0)
Skin and appendages		
Rash	5 (13.5)	5 (2.6)
Night sweats	3 (8.1)	3 (1.6)
Urticaria	3 (8.1)	4 (2.1)
Pruritus	2 (5.4)	3 (1.6)
Special senses		
Lacrimation disorder	5 (13.5)	5 (2.6)

Included are events reported as possibly or probably related or of unknown relationship to study treatment.

Abbreviations: N, number of patients; Events, number of events.

infusions. The first patient, who had small lymphocytic lymphoma, had previously been treated with a single infusion of IDEC-C2B8 in an earlier trial and had experienced transient grade 3 thrombocytopenia. Within 24 hours of the first infusion of 375 mg/m^2 antibody, the platelet count again decreased from a baseline value of 93,000/μL to a nadir of 19,000/μL. This was associated with marked elevation in LDH level (629 to 2660 U/L), a decrease in hemoglobin level from 12.6 to 7.8 g/dL, and an elevation in uric acid to 10.5 mg/dL. She received outpatient treatment with allopurinol, prednisone, and a single platelet transfusion. Evaluation for disseminated intravascular coagulation was nondiagnostic and the patient improved (counts returned to above baseline) by day 31. The second patient, a 63-year-old man with follicular small cleaved cell NHL, had a prior history of hypertension, hypercholesterolemia, and atherosclerotic cardiovascular disease, including a prior myocardial infarction (4 months earlier); developed anginal pain within 30 minutes of initiation of his first IDEC-C2B8 infusion; and received nitroglycerin with resolution of symptoms. The antibody infusion was then completed with stable vital signs. However, the patient was hospitalized 5 days later with a myocardial infarction and was removed from study. An occluded right coronary artery was found and the patient underwent a surgical coronary artery bypass procedure.

Hematologic and chemistry laboratory effects. A total of 18 patients had some clinically significant hematologic nadir after antibody therapy and 11 of these patients had bone marrow involvement by their lymphoma. Eleven patients sustained decreases in hemoglobin or hematocrit of greater than 1.5 g or 3%, respectively. Eight patients had decreases in platelet counts to less than 100,000/μL and 10 patients had transient reductions in white blood cell count or absolute granulocyte count of less than 3,000/μL or 1,500/μL, respectively. In general, these effects were mild and patients spontaneously recovered without intervention. Two patients developed late onset isolated neutropenia of unclear etiology at 4 and 10 months after therapy. Both instances were transient and resolved within 1 month.

No clinically significant changes in chemistry laboratory values were noted.

Reported infections. A total of 9 patients (11 infections) were reported to have acquired an infection during the course of the treatment or follow-up. Infections during the first 60 days of antibody therapy included viral upper respiratory infections in 3 patients, localized skin infections in 3 patients, and herpes zoster and otitis media in 1 patient each. Reported late infections, occurring 7 to 12 months after antibody therapy, included pneumonia, impetigo, and sinusitis. All infections were grade 1 or 2 and resolved without complications after appropriate therapy.

Depletion of peripheral blood B cells. Peripheral blood lymphocytes were evaluated by flow cytometry at baseline, 72 hours after the first infusion, and at 1, 2, 6, 9, and 12 months posttreatment. The percentage and absolute number of cells expressing the CD19 and CD20 antigens was determined. Treatment with the chimeric anti-CD20 antibody rapidly and effectively depleted B cells from the peripheral blood circulation as detected by the unrelated anti-CD19 B-cell antibody. In some patients, baseline CD20 cell counts were elevated (due to the presence of circulating lymphoma cells). Most patients had a rapid decrease in the relative percentage and absolute number of CD19$^+$ cells by day 4. This level remained nearly undetectable until approximately 6 months posttreatment, followed by slow gradual recovery.

Serum Ig and complement levels. The CD20 antigen is expressed on early B cells but not upon differentiation to antibody secreting plasma cell. Because of the possibility that the elimination of normal B cells and plasma cell progenitors could affect antibody production, serum Ig (IgG, IgA, and IgM) levels were quantitated at baseline and throughout the study. Mean serum Ig levels remained stable; however, individual patients experienced transient decreases in some subsets. Among these 37 patients, 4, 4, and 5 patients experienced more than a 20% decline in IgG, IgA, or IgM levels, respectively (total of 10 patients with 3 patients having decreases in both IgG and IgA levels).

No clinically significant change in serum complement levels was observed.

Evaluation of immune response (HAMA/HACA). Of the 37 patients evaluated, there were no quantifiable HAMA or HACA titers identified. One patient (with a PR) had a

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Table 3. Summary of Clinical Response

Patient Group	N	CR (%)	PR (%)	CR and PR (%)	95% CI (CR and PR)
Intent to treat	37	3 (8)	14 (38)	17 (46)	30-62%
Evaluable*	34	3 (9)	14 (41)	17 (50)	33-67%

Abbreviation: CI, confidence interval.

* Patients had low-grade or follicular lymphoma and completed four antibody infusions.

detectable, but not a quantifiable (<100 ng/mL) HACA titer 7 months posttreatment. There was no toxicity related to this immune response.

Clinical response. Clinical responses (CR or PR) were observed in 17 of the 37 patients (46%) and are detailed in Table 3. Two patients were withdrawn from the study for adverse events after receiving a single infusion of the antibody and 1 patient had a high-grade B-cell malignancy. Thus, 34 patients completed all four antibody infusions and were considered evaluable for response. All 34 had a low-grade or follicular histology. Three of the 34 patients (9%) had a CR and 14 of 34 (41%) had a PR for an overall response rate for evaluable patients of 50%. An additional 8 patients had some evidence of tumor response (>20% reduction), but did not qualify as responders (PR or CR). Thus, 25 of the 34 evaluable patients (74%) had evidence of antitumor effect. Eleven patients (32%) had stable disease (SD) and 6 patients (18%) were judged to have progressive disease (PD). The median time to onset of clinical response for the 17 responders was 50 days (range, 7 to 112 days). Measured tumor masses continued to regress, with the maximum response in sentinel tumor lesions occurring 3 to 4 months after antibody therapy. The median time to progression for the clinical responders was 10.2 months, with 5 patients exceeding 20 months and 2 patients exceeding 30 months. The median duration of response was 8.6 months. A Kaplan-Meier survival curve for the time to progression is shown in Fig 1 for the clinical responders.

The majority of the patients had follicular histologies. Of 21 patients with follicular small cleaved histology (WF group B), there were 11 PR and 2 CR for a response rate of 62%. Of 9 patients with follicular mixed small and large cell histology (WF group C), there was 1 CR and 3 PR (44%). One of 2 patients with follicular large cell lymphoma (group D) had a PR. The response rate appears similar among the follicular histologies. In contrast, none of 4 patients with small lymphocytic lymphoma (1 with mantle cell histology) had a clinical remission.

There was no clear influence of type of prior therapy on the likelihood of clinical response to antibody treatment. Two of three patients who had been treated previously with high-dose therapy and autologous stem cell rescue achieved a PR to antibody therapy with times to disease progression of 4.2 and 21.7 months. Twenty patients had extranodal disease and two showed a CR and seven showed a PR to antibody therapy. There was a correlation observed between the duration of response to last chemotherapy and response to treatment with IDEC-C2B8 ($P = .05$; Fisher's exact test). Twenty of the 37 patients had a duration of response to last chemotherapy of 6 months or less. Of these 20, 6 (30%) responded to chimeric MoAb therapy. Seventeen of the 37 patients had a duration of response to last chemotherapy of more than 6 months. Of these 17, 11 (65%) responded to IDEC-C2B8.

Antibody pharmacokinetics. At the 375 mg/m^2 dose used in this trial, all studied patients exhibited detectable circulating antibody throughout the treatment period. Preinfusion (doses 2 through 4) antibody concentrations ranged from 0.8 to 517.7 μg/mL and postinfusion levels ranged from 107.8 to 962.7 μg/mL. Five patients consistently had preinfusion antibody levels at or less than 10 μg/mL, suggesting that they did not reach steady state or cleared the antibody at a faster rate. Most patients exhibited increasing preinfusion antibody concentrations with each subsequent infusion.

The Wilcoxon rank sum test was used to examine the relationship between the preinfusion and postinfusion chime-

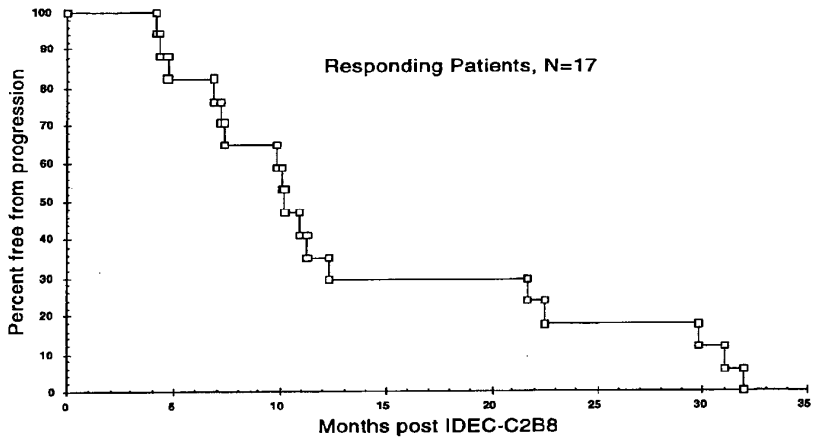


Fig 1. Kaplan-Meier graph of the time to progression from the date of initial treatment with the first dose of IDEC-C2B8 until documented tumor relapse or progression in the 17 clinical responders.

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ric antibody concentrations and the clinical response. A correlation was observed between clinical response and the median values of IDEC-C2B8 serum levels before the second infusion ($P = .029$), with responders having a median of 82.7 μg/mL (range, 1.2 to 125.3 μg/mL) versus nonresponders with a median of 21.9 μg/mL (range, 1.1 to 99.0 μg/mL). Detailed pharmacokinetic sampling was performed on 9 patients, with a mean C_{max} of 500.5 ± 135.2 μg/mL (range, 201.4 to 663.0 μg/mL) and serum antibody half-life values from 12.7 to 370.8 hours (mean $T_{1/2}$ of 225.9 ± 102.7 hours).

DISCUSSION

IDEC-C2B8 represents a new treatment for patients with recurrent low-grade or follicular NHL. This therapy has a response rate in evaluable patients of 50% and a time to progression of 10.2 months. The treatment consists of four intravenous infusions administered over a 22-day period in the outpatient setting with minimal toxicity. This compares favorably with other agents currently in use for the treatment of patients with relapsed disease. These results have recently been confirmed in preliminary reports in a separate phase II/III multicenter trial in more than 150 patients.[21]

The adverse events observed in this trial were predominately infusion related and generally consisted of mild grade 1 or 2 symptoms of fever, chills, rigor, rash, nausea, and, rarely, mild respiratory symptoms or hypotension. All of these side effects were transient and did not interfere with the completion of the planned antibody infusion. Two patients were removed from study after a single dose of the antibody; 1 patient developed thrombocytopenia and an elevated LDH and a second patient with known cardiac disease experienced chest pain and was admitted 1 week later with a myocardial infarction. All other patients completed the planned 4 weekly infusions. The etiology of the observed infusion related symptoms is not completely understood, but may represent the rapid depletion of B cells and lysis in the reticuloendothelial systems of the lung, liver, and spleen. This constellation of symptoms has been seen in other studies using murine MoAbs.[22] The marked decrease in infusional side effects noted with subsequent infusions likely reflects the elimination of most circulating B lymphocytes, the saturation of antigenic sites with the first infusion, and persistent antibody serum levels present at the time of the subsequent antibody infusions.

As expected, normal B cells were rapidly depleted from the peripheral blood of nearly all patients and remained depleted until nearly 6 months posttreatment, followed by a slow recovery. Despite this depletion of B cells, there was minimal change in serum Ig levels and no increase in the frequency or severity of infectious complications. The infections noted in this trial are common to patients with relapsed B-cell lymphoma and are not clearly related to treatment with the antibody. Prolonged B-cell depletion is common to other chemotherapy treatments and after BMT. The type and risk of infection with MoAb treatment appears less than that reported with administration of some of the newer purine analogues (fludarabine and 2-CDA) that are associated with significant depletion of the $CD4^+$ T-cell population.

The patients included in this trial are typical of those represented in the general population and of low-grade patients studied in the Working Formulation Lymphoma Classification Project.[23] The International Prognostic Index (IPI) originally developed for aggressive NHL uses 5 prognostic factors, including age greater than 60 years; stage III/IV disease; performance status greater than 1; elevated LDH level; and extranodal sites greater than 1.[24] It has been applied to patients with low-grade NHL and permits prognostic stratification into risk groups.[25] Using data from presentation, we were able to determine the IPI classification in 32 of the 37 patients included in this trial. As expected, in patients with low-grade NHL, the majority of patients were in the low (41%) and low-intermediate (27%) prognostic groups, followed by those in intermediate/high (16%), high (3%), and unknown (14%) groups. There was no statistically significant correlation (Fisher's exact test, $P = .31$) between the risk groups and response to treatment with IDEC-C2B8. However, there was a correlation observed between the duration of response to last chemotherapy and the response to IDEC-C2B8. Patients with less than a 6-month response to last chemotherapy had a 30% response rate, compared with a 65% response rate for patients with last duration of remission of greater than 6 months. In contrast with the experience with some MoAbs, antitumor activity was observed in patients with bulky disease and in poor prognostic patients who had relapsed after anthracycline-containing regimens or after high-dose therapy with stem cell support. Tumor responses were noted in patients with bulky lymph nodes, splenomegaly, extranodal masses, and lymphoma in blood and bone marrow.

A correlation was also observed between the level of IDEC-C2B8 present in the serum before infusion number 2 and the response to MoAb therapy. Whether this is due to differences in tumor burden or to differences in intrinsic clearance of the MoAb is unknown. All 17 clinical remissions occurred in patients with a follicular histology (WF groups B-D), although the number of patients with other histologies was small. In contrast, none of the 4 patients with small lymphocytic lymphoma (WF group A) responded. These patients had heterogenous disease, including 1 patient with mantle cell histology. Although patients with chronic lymphocytic leukemia (CLL) were excluded from this trial (based on the presence of >5,000 lymphocytes/μL for this histologic subgroup), it is possible that the decreased response rate in this subgroup was due to a lower expression of the CD20 surface antigen that has been observed in cases of CLL.

The method of tumor cell killing by the MoAb is not completely understood and likely involves several mechanisms. The chimeric MoAb was engineered to contain human IgG1 constant regions to augment immune-mediated antitumor activity using human complement and human ADCC effector cells.[16] In vitro, the antibody clearly exhibits much greater activity than the parent murine IgG1 MoAb in these assays. However, in this clinical trial, there were no significant changes in serum complement levels observed after the MoAb treatments. In addition, in earlier phase I trials, tumor biopsies 2 weeks posttreatment showed antibody bound to tumor cells in patients thought to be immunocompetent who

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did not have a clinical remission.[18] The CD20 molecule is thought to aggregate on the cell surface and create or control a Ca^{2+} channel that is important in initiation and progression through the cell cycle.[26] Antibodies binding to CD20 may directly inhibit the cell cycle.[27] Recent data using IDEC-C2B8 have shown that the antibody inhibits proliferation and directly induces apoptosis in some B-cell NHL lines in vitro.[17] The degree to which this additional antitumor mechanism contributes to the observed clinical remissions is unknown and is actively being evaluated in the laboratory.

The optimal treatment for patients with advanced-stage or relapsed low-grade NHL remains unsettled. Diverse options, from watch and wait to treatment with aggressive chemotherapy, are frequently used.[1] Although most patients initially respond to a variety of chemotherapeutic regimens with a PR or CR, all will eventually relapse and require additional treatment with decreasing response rates and decreasing durations of remission. Aggressive combination chemotherapy produces higher CR rates, and high-dose therapy with stem cell or bone marrow support may result in longer durations of remission. Unfortunately, most trials of such aggressive therapies have shown continued risk of relapse without a convincing plateau on the failure-free survival curve.[2] An exception may be allogenic BMT, but this treatment is restricted to younger patients with an appropriate donor and carries a high risk of morbidity and mortality.[28] Most patients undergo a series of treatments with increasing toxicity and decreasing bone marrow reserve until they ultimately become refractory to treatment and die of disease progression or from complications of treatment.

IDEC-C2B8 chimeric anti-CD20 MoAb presents the opportunity to obtain meaningful tumor reductions with minimal toxicity in patients with relapsed low-grade NHL. Retreatment is possible and early experience suggests that a majority of patients with a prior response may respond to retreatment. Fludarabine has also been studied in patients with relapsed disease, with an overall response rate of approximately 40%.[29,30] Time to treatment failure was 4.6 months in one study.[31] Treatment complications from fludarabine included infections or fever of unknown origin in 62% of patients, bone marrow suppression, and opportunistic infections. Similar responses have been observed with cladribine; however, marked immunosuppression and hematologic toxicity was noted.[32,33]

To date, experience using IDEC-C2B8 has been predominately in patients with relapsed measurable disease. A clinical trial combining IDEC-C2B8 with 6 cycles of CHOP chemotherapy in newly diagnosed patients has recently been completed. Early evaluation of this experience suggests that this combination resulted in a PR or CR in all patients and in the clearance of polymerase chain reaction-detectable cells from the peripheral blood and bone marrow in 7 of 8 patients with a positive pretreatment assay.[34] It remains to be determined if this will ultimately decrease the risk of relapse or prolong disease-free survival. Additional areas that should be investigated using this new agent include (1) extended and repeated dosing regimens, (2) combination with or after standard chemotherapy, (3) use as an in vivo B-cell purging agent before collection of bone marrow or stem cells for high-dose therapies, (4) evaluation in other B-cell histologies, and (5) lymphomas arising in association with immunodeficiency such as acquired immunodeficiency syndrome or organ transplantation.

In conclusion, this experience using an engineered chimeric MoAb finally provides practical substance to the promise of antibody therapy of cancer that was envisioned nearly 100 years ago by Ehrlich.[35]

REFERENCES

1. Horning SJ: Treatment approaches to the low-grade lymphomas. Blood 83:881, 1994

2. Bierman PJ, Vose JM, Anderson JR, Bishop MR, Kessinger A, Armitage JO: High-dose therapy with autologous hematopoietic rescue for follicular low-grade non-Hodgkin's lymphoma. J Clin Oncol 15:445, 1997

3. Miller RA, Maloney DG, Warnke R, Levy R: Treatment of B cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med 306:517, 1982

4. Meeker TC, Lowder J, Maloney DG, Miller RA, Thielemans K, Warnke R, Levy R: A clinical trial of anti-idiotype therapy for B cell malignancy. Blood 65:1349, 1985

5. Brown SL, Miller RA, Horning SJ, Czerwinski D, Hart SM, McElderry R, Basham T, Warnke RA, Merigan TC, Levy R: Treatment of B-cell lymphomas with anti-idiotype antibodies alone and in combination with alpha interferon. Blood 73:651, 1989

6. Maloney DG, Brown S, Czerwinski DK, Liles TM, Hart SM, Miller RA, Levy R: Monoclonal anti-idiotype antibody therapy of B-cell lymphoma: The addition of a short course of chemotherapy does not interfere with the antitumor effect nor prevent the emergence of idiotype-negative variant cells. Blood 80:1502, 1992

7. Press OW, Appelbaum F, Ledbetter JA, Martin PJ, Zarling J, Kidd P, Thomas ED: Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood 69:584, 1987

8. Amlot PL, Stone MJ, Cunningham D, Fay J, Newman J, Collins R, May R, McCarthy M, Richardson J, Ghetie V, Ramilo O, Thorpe PE, Uhr JW, Vitetta ES: A phase I study of an anti-CD22-deglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy. Blood 82:2624, 1993

9. Conry RM, Khazaeli MB, Saleh MN, Ghetie V, Vitetta ES, Liu T, LoBuglio AF: Phase I trial of an anti-CD19 deglycosylated ricin A chain immunotoxin in non-Hodgkin's lymphoma: Effect of an intensive schedule of administration. J Immunother Emphasis Tumor Immunol 18:231, 1995

10. Stone MJ, Sausville EA, Fay JW, Headlee D, Collins RH, Figg WD, Stetler-Stevenson M, Jain V, Jaffe ES, Solomon D, Lush RM, Senderowicz A, Ghetie V, Schindler J, Uhr JW, Vitetta ES: A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma. Blood 88:1188, 1996

11. Kaminski MS, Zasadny KR, Francis IR, Milik AW, Ross CW, Moon SD, Crawford SM, Burgess JM, Petry NA, Butchko GM, Glenn SD: Radioimmunotherapy of B-cell lymphoma with [131I]anti-B1 (anti-CD20) antibody. N Engl J Med 329:459, 1993

12. Kaminski MS, Zasadny KR, Francis IR, Fenner MC, Ross CW, Milik AW, Estes J, Tuck M, Regan D, Fisher S, Glenn SD, Wahl RL: Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. J Clin Oncol 14:1974, 1996

13. Knox SJ, Goris ML, Trisler K, Negrin R, Davis T, Liles T-M, Grillo-López AJ, Chinn P, Varns C, Ning S-C, Fowler S, Deb N, Becker M, Marquez C, Levy R: Yttrium-90-labeled anti-CD20 monoclonal antibody therapy of recurrent B-cell lymphoma. Clin Cancer Res 2:457, 1996

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14. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, Glenn S, Butchko G, Fisher D, Porter B, Matthews DC, Fisher LD, Bernstein ID: Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. N Engl J Med 329:1219, 1993

15. Press OW, Eary JF, Appelbaum FR, Martin PJ, Nelp WB, Glenn S, Fisher DR, Porter B, Matthews DC, Gooley T, Bernstein ID: Phase II trial of 131I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. Lancet 346:336, 1995

16. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Rabb R, Newman RA, Hanna N, Anderson DR: Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 83:435, 1994

17. Maloney DG, Smith B, Appelbaum FR: The anti-tumor effect of monoclonal anti-CD20 antibody therapy includes direct anti-proliferative activity and induction of apoptosis in CD20 positive non-Hodgkin's lymphoma cell lines. Blood 88:637a, 1996 (abstr, suppl 1)

18. Maloney DG, Liles TM, Czerwinski DK, Waldichuk C, Rosenberg J, Grillo-López A, Levy R: Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood 84:2457, 1994

19. Maloney DG, Grillo-López AJ, Bodkin DJ, White CA, Liles TM, Royston I, Varns C, Rosenberg J, Levy R: IDEC-C2B8: Results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma J Clin Oncol (in press)

20. Anderson KC, Bates MP, Slaughenhoupt BL, Pinkus GS, Schlossman SF, Nadler LM: Expression of human B cell-associated antigens on leukemias and lymphomas: A model of human B cell differentiation. Blood 63:1424, 1984

21. McLaughlin P, Cabanillas F, Grillo-López AJ, Link BK, Levy R, Czuczman M, Heyman MR, Williams M, Jain V, Bence-Bruckler I, Ho AD, Lister J, Rosenberg J, Dallaire BK, Shen D: IDEC-C2B8 anti-CD20 antibody: Final report on a phase III pivotal trial in patients with relapsed low-grade or follicular lymphoma. Blood 88:90a, 1996 (abstr, suppl 1)

22. Dillman RO, Beauregard J, Shawler DL, Halpern SE, Markman M, Ryan KP, Baird SM, Clutter M: Continuous infusion of T101 monoclonal antibody in chronic lymphocytic leukemia and cutaneous T-cell lymphoma. J Biol Resp Mod 5:394, 1986

23. Writing Committee for the Non-Hodgkin's Lymphoma Pathologic Classification Project: National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a working formulation for clinical usage. Cancer 49:2112, 1982

24. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 329:987, 1993

25. López-Guillermo A, Montserrat E, Bosch F, Terol MJ, Campo E, Rozman C: Applicability of the International Index for aggressive lymphomas to patients with low-grade lymphoma [see comments]. J Clin Oncol 12:1343, 1994

26. Tedder TF, Engel P: CD20: A regulator of cell-cycle progression of B lymphocytes. Immunol Today 15:450, 1994

27. Tedder TF, Forsgren A, Boyd AW, Nadler LM, Schlossman SF: Antibodies reactive with the B1 molecule inhibit cell cycle progression but not activation of human B lymphocytes. Eur J Immunol 16:881, 1986

28. van Besien KW, Khouri IF, Giralt SA, McCarthy P, Mehra R, Andersson BS, Przepiorka D, Gajewski JL, Bellare N, Nath R, Romaguera JE, McLaughlin P, Korbling M, Deisseroth AB, Cabanillas FF, Champlin RE: Allogeneic bone marrow transplantation for refractory and recurrent low-grade lymphoma: The case for aggressive management. J Clin Oncol 13:1096, 1995

29. Redman JR, Cabanillas F, Valasquez WS, McLaughlin P, Hagemeister FB, Swan F, Jr., Rodriguez MA, Plunkett WK, Keating MJ: Phase II trial of fludarabine phosphate in lymphoma: An effective new agent in low-grade lymphoma. J Clin Oncol 10:790, 1002

30. Hochster HS, Kim KM, Green MD, Mann RB, Neiman RS, Oken MM, Cassileth PA, Stott P, Ritch P, O'Connell MJ: Activity of fludarabine in previously treated non-Hodgkin's low-grade lymphoma: Results of an Eastern Cooperative Oncology Group study. J Clin Oncol 10:28, 1992

31. Falkson CI: Fludarabine: A phase II trial in patients with previously treated low-grade lymphoma. Am J Clin Oncol 19:268, 1996

32. Hoffman M, Tallman MS, Hakimian D, Janson D, Hogan D, Variakogis D, Kuzel T, Gordon LI, Rai K: 2-Chlorodeoxyadenosine is an active salvage therapy in advanced indolent non-Hodgkin's lymphoma. J Clin Oncol 12:788, 1994

33. Kay AC, Saven A, Carrera CJ, Carson DA, Thurston D, Beutler E, Piro LD: 2-Chlorodeoxyadenosine treatment of low-grade lymphomas. J Clin Oncol 10:371, 1992

34. Czuczman MS, Grillo-López AJ, Saleh M, Gordon L, Jonas C, White CA, LoBuglio AF, Varns C, Rogers J: IDEC-C2B8/CHOP chemoimmunotherapy in patients with low-grade lymphoma: Interim clinical and bcl-2 (PCR) results. Ann Oncol 7:56, 1996 (suppl 1)

35. Ehrlich P: On immunity with specific reference to cell life. Proc R Soc Lond 66:424, 1900

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IDEC-C2B8 (Rituximab) Anti-CD20 Monoclonal Antibody Therapy in Patients With Relapsed Low-Grade Non-Hodgkin's Lymphoma

David G. Maloney, Antonio J. Grillo-López, Christine A. White, David Bodkin, Russell J. Schilder, James A. Neidhart, Nalini Janakiraman, Kenneth A. Foon, Tina-Marie Liles, Brian K. Dallaire, Ken Wey, Ivor Royston, Thomas Davis and Ronald Levy

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ANNEX I: Commercial monoclonal antibodies 2006 (all modes of administration)

Antibody	Trade name	Year	Concentration [mg/ml] *	Concentration range [mg/ml]			
				0.1 - 50	0.5 - 25	2 - 10	10
Gemtuzumab ozogamicin	Mylotarg	2000	0.25	X			
Indium-111 capromab pendetide	ProstaScint	1996	0.5	X	X		
Satumomab pendetide	OncoScint	1992	0.5	X	X		
Muromomab-CD3	Orthoclone OKT	1986	1	X	X		
Acrituomab; Tc-99	CEA-Scan	1996	1.25	X	X		
Ibritumomab-Tiuxetan	Zevalin		1.6	X	X		
Abciximab	ReoPro	1994	2	X	X	X	
Cetuximab	Erbitux	2004	2	X	X	X	
Basiliximab	Simulect	1998	4	X	X	X	
Daclizumab	Zenapax	1997	5	X	X	X	
Alemtuzumab	Campath	2001	10	X	X	X	X
Infliximab	Remicade	1998	10	X	X	X	X
Nofetumomab	Verluma	1996	10	X	X	X	X
Ranibizumab	Lucentis	2006	10	X	X	X	X
Rituximab	Rituxan	1997	10	X	X	X	X
Tositumomab and I-131 Tositumomab	Bexxar	2003	14	X	X		
Natalizumab	Tysabri	2004	20	X	X		
Trastuzumab	Herceptin	1998	21	X	X		
Bevacizumab	Avastin	2004	25	X	X		
Adalimumab	Humira	2002	50	X			
Efalizumab	Raptiva	2003	100				
Palivizumab	Synagis	1998	100				
Omalizumab	Xolair		125				
Number of antibodies (total number: 23)				**20**	**18**	**9**	**5**
Proportion of all antibodies				**87 %**	**78 %**	**39 %**	**22 %**

* Liquid form or after reconstitution

Exhibit no. ANNEX I
of the letter / report / expert opinion / plaint / defense
dated 09.08.2017
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81925 München, Arabellastr. 30

ANNEX II: Commercial monoclonal antibodies 2006 (for intravenous administration)

Antibody	Trade name	Year	Concentration [mg/ml] *	Concentration range [mg/ml]			
				0.1 - 50	0.5 - 25	2 - 10	10
Gemtuzumab ozogamicin	Mylotarg	2000	0.25	X			
Indium-111 capromab pendetide	ProstaScint	1996	0.5	X	X		
Satumomab pendetide	OncoScint	1992	0.5	X	X		
Muromomab-CD3	Orthoclone OKT	1986	1	X	X		
Acrituomab; Tc-99	CEA-Scan	1996	1.25	X	X		
Ibritumomab-Tiuxetan	Zevalin		1.6	X	X		
Abciximab	ReoPro	1994	2	X	X	X	
Cetuximab	Erbitux	2004	2	X	X	X	
Basiliximab	Simulect	1998	4	X	X	X	
Daclizumab	Zenapax	1997	5	X	X	X	
Alemtuzumab	Campath	2001	10	X	X	X	X
Infliximab	Remicade	1998	10	X	X	X	X
Nofetumomab	Verluma	1996	10	X	X	X	X
Rituximab	Rituxan	1997	10	X	X	X	X
Tositumomab and I-131 Tositumomab	Bexxar	2003	14	X	X		
Natalizumab	Tysabri	2004	20	X	X		
Trastuzumab	Herceptin	1998	21	X	X		
Bevacizumab	Avastin	2004	25	X	X		
Number of antibodies (total number: 18)				**18**	**17**	**8**	**4**
Proportion of all antibodies				**100 %**	**94 %**	**44 %**	**22 %**

* Liquid form or after reconstitution

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Date
13.09.2017

Reference	Application No./Patent No.
P2913 EP/1	11001632.6 - 1466 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

Communication of notices of opposition (R. 79(1) EPC)

Notices of opposition have been filed within the opposition period by:

01.
Amgen Inc.
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ETATS-UNIS D'AMERIQUE
02.
Hoffmann Eitle
Patent- und Rechtsanwälte PartmbB
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81925 München
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The notices of opposition indicated above have already been communicated to you.

You are requested to file your observations within a period of **four months** from notification of this communication.

You may also file amendments, where appropriate, to the description, claims and drawings within the period specified. One set of these documents is to be filed.

If you introduced documents which have not yet been mentioned during the proceedings, your attention is drawn to Rule 83 EPC.

Enclosures:

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15. Sep. 2017

Munich, September 15, 2017

Our Ref.:	**198 441 u6/ne** **Eculizumab Composition / ALEXION**
Opposition to:	**EP 2 359 834 B1 (11001632.6)**
Proprietor:	**Alexion Pharmaceuticals, Inc.** **Cheshire, CT 06410 (US)**
Opponent:	**Hoffmann Eitle** **Patent- und Rechtsanwälte PartmbB** **Arabellastr. 30** **81925 Munich (DE)**

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Spanish Patent Attorneys
Dutch Patent Attorneys
Belgian Patent Attorneys
European Trademark Attorneys
European Design Attorneys

Submission in respect to request for documents pursuant Rule 83 EPC

In response to the communication dated August 17, 2017 we understand that documents D2, D4, D5 and D6 have already been filed by Opponent Amgen. Thus, there is no need for us anymore to resubmit these documents.

Leo Polz

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Date: 25.09.2017

Reference	Application No./Patent No.
198 441 u6/ne OPPO 02	11001632.6 - 1466 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

BRIEF COMMUNICATION

Subject:
- ☒ Your letter of 15.09.2017
- ☐ Our telephone conversation of
- ☐ Communication of
- ☒ Request of 17.08.2017

Enclosure(s):
- ☐ Letter from the proprietor of the patent of
- ☐ Letter from the opponent of
- ☐ Copy (copies)

Communication:

☐ A letter from the opponent /proprietor was received on ..... . The documents specified as patent documents in this letter are available via the European Patent Register at **www.epo.org/register.** Should you wish to receive these documents in paper format, they will be supplied to you free of charge if specifically requested on receipt of this communication (OJ EPO 2009, 434).

☒ With the notice of opposition, opponents should file all relevant documents, even those available in the EPO documentation, translations of any documents not in an EPO official language and, wherever possible, a copy of any further evidence indicated in the notice.

Amtsblatt EPA Official Journal EPO Journal officiel OEB 2016, A42

For the Opposition Division

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☒ **Registered letter**

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(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0221382 A1**

Rother (43) **Pub. Date: Oct. 6, 2005**

(54) **STRATIFICATION OF PATIENT POPULATIONS HAVING OR SUSPECTED OF HAVING RHEUMATOID ARTHRITIS**

(76) Inventor: **Russell P. Rother**, Prospect, CT (US)

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(21) Appl. No.: **10/508,174**

(22) PCT Filed: **Mar. 17, 2003**

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(52) **U.S. Cl.** **435/7.1**

(57) **ABSTRACT**

The present disclosure is directed to screening, diagnosing and treating patients having, or suspected of having, rheumatoid arthritis. Levels of glucose-6-phophate isomerase or antibodies to glucose-6-phophate isomerase are assayed in test subjects or populations to determine susceptibility to, or existence of, an antibody mediated form of rheumatoid arthritis in such test subjects or populations. The results of the assays provide guidelines for therapeutic intervention with complement inhibiting agents.



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STRATIFICATION OF PATIENT POPULATIONS HAVING OR SUSPECTED OF HAVING RHEUMATOID ARTHRITIS

BACKGROUND

[0001] 1. Technical Field

[0002] The present disclosure relates to stratifying patients having, or suspected of having rheumatoid arthritis, and selection of therapeutic agents useful in the treatment thereof.

[0003] 2. Background of Related Art

[0004] Rheumatoid arthritis (RA) is a chronic disease which can exhibit a variety of systemic manifestations. This disease has an unknown etiology and characteristically exhibits a persistent inflammatory synovitis which usually involves peripheral joints in a symmetric distribution. Despite the destructive potential of RA, its course can be quite variable. In many patients, a chronic polyarthritis begins insidiously with fatigue, anorexia, generalized weakness and vague musculoskeletal symptoms until the appearance of synovitis becomes apparent. In about 10% of patients, the onset can be more acute with rapid development of polyarthritis. In about one-third of patients, symptoms may initially be confined to one or a few joints. Overall, most patients will experience an intermediate course of the disease. The potential of inflammation to cause cartilage destruction, bone erosions and, ultimately, joint deformities is the most important feature of this disease.

[0005] Many drugs have been used to treat rheumatoid arthritis without complete relief of the symptoms. Conventional drugs include non-steroidal anti-inflammatory drugs (NSAIDs, aspirin, ibuprofen), gold salt, penicillamine, TNFα inhibitors, IL-1 receptor antagonists, and steroidal hormones. The steroidal hormones, which are considered to be the most potent and effective, have side effects when taken for long periods. NSAIDs also have side effects such as stomach irritation and can be ulcerigenic. Recently, a compound which blocks the C5 component of the complement cascade (h5G1.1-mAb) has been shown to be an effective therapy in a murine model of RA. This compound is now in human clinical trials for the treatment of RA.

[0006] Although the etiologic stimulus for RA has not been identified, established rheumatoid synovitis is characterized by persistent immunologic activity. The presence of rheumatoid factor, which is an autoantibody reactive with IgG, suggests that there may be an underlying immune dysfunction in association with RA. This factor was initially described by Waaler in 1940 and later described by Rose and co-workers in 1948. Rheumatoid factors consist of IgA, IgG and/or IgM immunoglobulin classes. The idiotypic nature of rheumatoid factor autoantibodies has been described and partially characterized. This suggests that there may be a perturbation in the idiotype/anti-idiotype humoral immune network of patients with RA. In addition, the presence of rheumatoid factor can be of prognostic significance because patients with high titers tend to have more severe and progressive disease with extraarticular manifestations. Rheumatoid factor can also be employed to confirm the diagnosis of RA in patients with a clinical presentation of RA and, if at high titer, to designate patients at risk for severe systemic disease.

[0007] The examination of established rheumatoid synovitis reveals a characteristic constellation of features which include hyperplasia and hypertrophy of the synovial lining cells, focal or segmental vascular changes and infiltration with mononuclear cells often collected into aggregates or follicles around small blood vessels. The predominant infiltrating cells are T lymphocytes with T4 (helper-inducer) cells predominating over T8 (suppressor-cytotoxic) cells. Evidence of B cell activation can also be found in the inflamed synovium and plasma cells producing immunoglobulin and rheumatoid factor are characteristic features of rheumatoid synovitis. The resultant production of immunoglobulin and rheumatoid factor can lead to the formation of immune complexes with subsequent complement activation and exacerbation of the inflammatory process through the production of anaphylatoxins and chemotactic factors. Indeed, levels of total hemolytic complement, C3 and C4 are markedly diminished in synovial fluid relative to total protein concentration as a result of activation of the classic complement pathway by locally produced immune complexes. All these findings suggest that progression of RA is an immunologically mediated event.

[0008] The most widely used immunodiagnostic assay of RA is the so-called Waaler-Rose assay discussed above. This assay is based upon the antibody (rheumatoid factor) to the Fc region of IgG. Rheumatoid factor (RF) is present in about 60% to 70% of those individuals afflicted with RA. However, the test may not always be accurate because it has been found to give false positives or negatives, and it does not assess the response to therapy or predict activation or reactivation of the disease process.

[0009] Since there is no unambiguous test distinguishing RA from other acute or chronic inflammatory diseases, differentiating RA from other arthritides, such as systemic lupus erythematosus (SLE), ankylosing spondylitis (AS), polyarticular gout (PAG), or psoriatic arthritis (PsA) is often difficult. Diagnosis of RA is usually made according to American Rheumatism Association (ARA) criteria, i.e.:

[0010] (1) morning stiffness;

[0011] (2) joint tenderness or pain on motion;

[0012] (3) soft-tissue swelling of the joint;

[0013] (4) soft-tissue swelling of a second joint (within three months);

[0014] (5) soft-tissue swelling of symmetrical joints (excludes distal interphalangeal joint);

[0015] (6) subcutaneous nodules;

[0016] (7) X-ray changes;

[0017] (8) serum positive for rheumatoid factors;

[0018] wherein diagnosis of 3 or 4 of these factors is considered representative of probable RA and diagnosis of 5 or more of the factors is considered representative of definite RA.

[0019] Recently, a transgenic mouse model (K/BxNT cell receptor) was found to exhibit spontaneous inflammatory arthritis resembling many of the features of human RA. See, Matsumoto et al., Science, 286, 1732-1735 (1999) and Ji et al. J. Exp. Med., 194 (3) 321-330 (2001). The inflammatory arthritis was traced to the presence of glucose-6-phosphate

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isomerase as an antigen targeted by T-cells and IgG immunoglobulins. Glucose-6-phosphate isomerase ("GPI") is a dimeric enzyme that catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate. The protein functions in different capacities inside and outside the cell. In the cytoplasm, the gene product is involved in glycolysis and gluconeogenesis, while outside the cell it functions as a neurotrophic factor for spinal and sensory neurons. The enzyme has also been known as glucosephosphate isomerase, phosphohexose isomerase (PHI), phosphoglucose isomerase (PGI), autocrine motility factor (AMF) and neuroleukin (NLK). In addition, it has recently been shown that a majority of humans with RA have elevated levels of anti-GPI IgG immunoglobulin in sera and elevated levels of soluble GPI in the sera and synovial fluid. See, e.g., Schaller et al., Nature Immunology, Vol. 2, No. 6, 746-753 (2001).

[0020] In light of the ambiguity and relative subjectivity of existing tests for RA there is a need for diagnostic procedures that allow non-subjective determination of the existence of RA and which provide a concrete indication of a course of therapeutics.

SUMMARY

[0021] In one aspect, a method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent is provided which includes conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of a patient to determine the level of GPI in the patient's serum or synovial fluid, and comparing the level of GPI in the serum or synovial of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The baseline serum GPI levels may encompass a mean concentration of 0.069+/−0.048 U/ml, P<0.0001. The baseline synovial fluid GPI levels may encompass a mean concentration of 0.060+/−0.052 U/ml P<0001. The patient may be a mammal such as a human.

[0022] In another aspect, a method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent is provided which includes conducting an assay for glucose-6-phosphate isomerase antibody (anti-GPI) in the serum or synovial fluid of a patient to determine the level of anti-GPI in the patient's serum or synovial fluid, and comparing the level of anti-GPI in the serum or synovial of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The baseline serum anti-GPI levels may encompass a mean concentration of (A_{405}) 0.059+/−0.037, P<0.0001. The baseline synovial fluid anti-GPI levels may encompass a mean concentration of (A_{405}) 0.645+/−0.209 P<0001. The patient may be a mammal such as a human.

[0023] In another aspect, a method for screening a population of subjects for antibody mediated rheumatoid arthritis and determining treatment thereof is provided which includes conducting a glucose-6-phophate isomerase (GPI) assay on the serum or synovial fluid of a population of subjects, and identifying subjects having GPI assay results which are statistically significantly greater than (P<0.05) the respective mean GPI serum or synovial fluid assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent. The subjects so identified may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G 1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0024] In another aspect, a method for screening a population of subjects for rheumatoid arthritis and determining treatment thereof is provided which includes conducting an assay for glucose-6-phophate isomerase antibody (anti-GPI) on the serum or synovial fluid of a population of subjects, and identifying subjects having anti-GPI assay results which are statistically significantly greater than (P<0.05) the respective mean anti-GPI serum or synovial fluid assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent. The subjects so identified may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0025] In another aspect, a method for predicting susceptibility of a patient to rheumatoid arthritis and to treatment with a complement inhibiting agent is provided which includes conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid, and comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of susceptibility to rheumatoid arthritis in the patient which is further susceptible to treatment with a complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement

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inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0026] In another aspect, a method for predicting susceptibility of a patient to rheumatoid arthritis and to treatment with a complement inhibiting agent is provided which includes conducting an assay for glucose-6-phosphate isomerase antibody (anti-GPI) of the serum or synovial fluid of the patient to determine the level of anti-GPI in the serum or synovial fluid, and comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of susceptibility to rheumatoid arthritis in the patient which is further susceptible to treatment with a complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0027] The present disclosure provides convenient, reliable methods to screen and treat patients having, or suspected of having, rheumatoid arthritis (RA). Recognition that glucose-6-phosphate isomerase (GPI) is an antigen which triggers an autoimmune response in a significant portion of the population afflicted with RA provides the basis for these methods. Detection of elevated levels of GPI or antibodies to GPI (anti-GPI) in the sera or synovial fluid of such individuals is indicative of antibody mediated complement activation and provides a signpost for therapeutic intervention through complement inhibition. Thus, by checking for elevated levels of serum or synovial GPI or anti-GPI antibodies in a population of subjects, a determination can be made as to whether members of that population will respond to therapeutic agents that inhibit complement.

[0028] In one aspect, patients already diagnosed with RA are stratified with respect to susceptibility to treatment with complement inhibitors. By determining the level of GPI or anti-GPI in serum or synovial fluid, a decision can be made by medical practitioners to treat the RA with a complement inhibitor or some other therapeutic approach. For example, a patient with RA who exhibits elevated GPI or anti-GPI levels may be treated with any pharmaceutically acceptable complement inhibitor. Those RA patients who do not exhibit elevated levels of GPI or anti-GPI would be directed to treatments which are not based on complement inhibition.

[0029] It is contemplated that any technique known to those skilled in the art can be utilized to determine the level of GPI or anti-GPI in the serum or synovial fluid. While enzyme-linked immunosorbent assay (ELISA) is preferred for detection of GPI or anti-GPI levels, other assays, e.g., enzyme activity assays, fluid phase assays, radioimmunoassay, precipitation, agglutination, direct and indirect immunofluorescence and complement fixation can be used. These assays may employ any protocol such as competitive, inhibition or sandwich.

[0030] Once measured, the GPI or anti-GPI concentrations in serum and/or synovial fluid of non-rheumatoid arthritis patients can be calculated to create a baseline GPI test standard. For example, an assay involving spectrophotometric analysis of enzyme activity to determine the level of GPI in the sera of normal individuals provided a human mean concentration baseline standard of 0.069+/−0.048 U/ml. See, Schaller et al., Nature Immunology, Vol. 2, No. 6, 746-753 (2001). An example of a baseline level of GPI in synovial fluid is 0.060+/−0.052 U/ml, P<0.001. Id. An example of baseline levels of anti-GPI in sera and synovial fluid of healthy individuals was determined by ELISA to be (A_{405}) 0.059+/−0.037, P<0.001, and (A_{405}) 0.645+/−0.209 P<0001, respectively. Id. Baseline levels may otherwise be determined in accordance with established principles and various techniques known to those skilled in the art.

[0031] One can then numerically compare the GPI or anti-GPI assay results of a test subject to the respective GPI or anti-GPI assay results of a rheumatoid arthritis-free population to determine whether the GPI or anti-GPI assay results of the test subject exceed the numerical range of the test results of the rheumatoid arthritis-free population. The numerical comparison involves comparing one or more of the above referenced GPI or anti-GPI results from the test subject to determine if one or more of the results is outside of the numerical range of the test results of the rheumatoid arthritis-free population. Optionally, statistical analysis can be performed to determine if the GPI or anti-GPI test results of the test subject exceed about two standard deviations or more above the mean test results of the rheumatoid arthritis-free population. In another option, statistical analysis can be performed to determine if the GPI or anti-GPI test results of the test subject exceed about one standard deviation or more above the mean test results of the rheumatoid arthritis-free population. These findings are used to support a diagnosis in the test subject of a predisposition to a positive response to complement inhibition therapies.

[0032] It should be understood by those of ordinary skill in the art that, in one aspect, this method is useful to diagnose a predisposition for the subsequent development of RA in a test subject or population of test subjects so that an early therapeutic regimen of complement inhibiting agents may be instituted. It should also be understood that not all test subjects with elevated level test results will always develop RA (i.e., there may be false positives). The test results from normal (NL) and RA populations may have overlapping ranges, with RA patients generally having the higher numerical range. The strength of the diagnosis relates to the test results obtained as follows. In a case in which the test results of a test subject lies above, that is, exceeds the numerical range of, the test results obtained from a rheumatoid arthritis-free population, it is said that the test subject is likely to develop RA and that serious consideration of therapy involving complement inhibiting agents should be given. However, not all test subjects that will develop RA will have test results exceeding the numerical range of test results from a rheumatoid arthritis-free population. Therefore, one can perform a statistical analysis to determine if a test result of the test subject is more than two standard deviations above the mean of the test results from a rheumatoid arthritis-free population. The test result of the test subject can be greater than two standard deviations or more above the mean and also be within the absolute numerical range of the results from a rheumatoid arthritis-free popu-

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lation. In this case, it is said that the test subject is likely to develop RA and that complement inhibiting agents should be given consideration. In a final case, the test result of a test subject can be more than one standard deviation above the mean of the test results from a rheumatoid arthritis-free population and also be within the absolute numerical range of test results for RA confirmed patients. The diagnosis of a predisposition for RA and complement inhibiting agent therapy based on these results is less clear. In this case, a test subject may develop RA. However, the percentage of individuals that ultimately develop RA will be smaller than those with test results about two standard deviations or more above normal or those exceeding the numerical range of normal (i.e., an increase in the incidence of false positives).

[0033] As mentioned above, it is possible to determine the susceptibility of an entire population of subjects to RA and to determine whether complement inhibiting agents should be given prophylactically in accordance with the present disclosure. The method of screening a population of subjects for the presence or absence of a predisposition toward developing RA includes: (1) performing a GPI or anti-GPI assay on a population of subjects; (2) calculating the results of the GPI or anti-GPI assay for the population; and (3) diagnosing in the test subjects the presence or absence of a predisposition toward developing RA by statistical analysis of the GPI or anti-GPI assay results. If there is a predisposition toward developing RA, prophylactic administration of complement inhibiting compounds should be considered.

[0034] The GPI or anti-GPI assay is performed on the population to be screened essentially as described above. Of course, the larger the population of subjects screened, the greater the potential accuracy of the diagnosis. Preferably, the population includes at least about 20 subjects. The GPI or anti-GPI test results for the population of subjects are calculated as described above. The test results are then analyzed statistically to identify subjects having GPI or anti-GPI assay results that are significantly greater than the mean GPI or anti-GPI assay results of the entire population of subjects. For example, a linear regression outlier analysis can be performed on the GPI or anti-GPI assay results for the entire population. Those subjects having one or more GPI or anti-GPI assay results that are statistically significantly greater than the mean values for the population (i.e., the test results is an outlier) are diagnosed as having a predisposition toward developing RA. Although the confidence level for the statistical analysis can be as low as $p<0.001$, typically, a $p<0.05$ is used. Linear regression outlier analysis is a known statistical analysis that identifies a data point that differs significantly (i.e., at a given p value) from the mean of a pool of data points from which the data point has been selected. The difference between the data point and the mean of the pool of data points being statistically significantly greater than that which is consistent with the inherent variability of the variable which the data point represents. "p value" or "P<" refers to the probability of observing a sample at least as unlikely as the one observed if the null hypothesis is true. For example, a sample result is statistically significant at the 5% level if, and only if, it leads to a rejection of the null hypothesis when the type I error probability alpha (i.e., the significance level) is 0.05.

[0035] Once a determination is made that a subject has elevated GPI or anti-GPI levels, a decision can be made regarding therapeutic intervention. The fact that soluble GPI is present in the synovial fluid of inflamed RA joints indicates that anti-GPI IgG forms immune complexes with the soluble GPI, which precipitate on the surface of the synovial lining with subsequent activation of the complement cascade. See Schaller et al., supra. Accordingly, compounds that interfere with the complement cascade can be utilized to treat subjects having GPI associated RA.

[0036] The complement cascade has been studied in depth and is fairly well understood. The complement system is an important means by which a host defends itself against infection. The complement cascade system is a component of the immune system that helps provide a natural immunity against invading microbes and is also an effector arm of antibody mediated humoral immunity. Complement is responsible for activating cells and other molecules involved in the inflammatory process as well as being directly related to the destruction of microbial invaders. The activation of complement involves a cascade of proteolytic reactions that lead to the release of inflammatory mediators and result in the assembly of the terminal complement complex. This cascade system has been characterized as containing at least thirty serum and membrane proteins that are activated by antibody-antigen complexes or by the invasion, in a host or experimentally in culture, by a microorganism, or other antigenic molecules.

[0037] The complement cascade consists of two major branches, the classical and alternative pathways. Though these pathways are initiated differently, they converge at the step of complement protein C3 activation. The complement cascade can mediate undesirable cellular damage in inflammatory, immune or autoimmune (auto-antibody-mediated) conditions. A series of regulatory proteins are involved in the control of the complement cascade. These proteins are considered part of the complement system and act to block endogenous complement activity at various stages of the complement cascade.

[0038] The classical pathway of the complement system is initialized by complement protein C1 binding to antigen bound IgG or IgM. C4 and C2 are then activated in the formation of C3 convertase at which point an amplification takes place that generates literally thousands of C3a and C3b fragments. C3b fragments can bind to complement protein complex C4b2a to form C4b2a C3b which is called C5 convertase and generates thousands of C5a and C5b fragments. C3b can also be used to regenerate C3 convertase which causes a greater amplification of complement activation. One approach to inhibit complement mediated effects is by depleting complement.

[0039] Any compounds that bind to or otherwise block the generation and/or activity of any of the human complement components, such as, for example, antibodies specific to a human complement component are useful herein and are referred to as complement inhibitors or complement inhibiting agents. Some complement inhibiting agents include 1) antibodies or antibody fragments directed against complement components C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2 and 2) naturally occurring or soluble forms of complement inhibitory compounds such as CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, serine protease inhibitors and K76 COOH.

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[0040] Particularly suitable complement inhibiting agents for use herein are antibodies or fragments thereof that reduce, directly or indirectly, the conversion of complement component C5 into complement components C5a and C5b. One class of useful antibodies are those having at least one antibody-antigen binding site and exhibiting specific binding to human complement component C5, wherein the specific binding is targeted to the alpha chain of human complement component C5. Such an antibody 1) inhibits complement activation in a human body fluid; 2) inhibits the binding of purified human complement component C5 to either human complement component C3 or human complement component C4; and 3) does not specifically bind to the human complement activation product for C5a. Particularly useful complement inhibitors are compounds which reduce the generation of C5a and/or C5b-9 by greater than about 30%. A particularly useful anti-C5 antibody is 5G1.1-mAb. Also particularly useful is a single chain Fv version of 5G1.1-mAb referred to as 5G1.1-scFv. In a preferred embodiment, humanized versions of these antibodies or scFv referred to as h5g1.1-mAb and h5G1.1-scFv are utilized. Methods for the preparation of these antibodies including h5G1.1-mAb and h5G1.1-scFv are described in U.S. patent application Ser. No. 08/487,283 filed Jun. 7, 1995, now U.S. Pat. No. 6,355,245, and "Inhibition of Complement Activity by Humanized Anti-C5 Antibody and Single Chain Fv", Thomas et al., *Molecular Immunology*, Vol. 33, No. 17/18, pages 1389-1401, 1996, the disclosures of which are incorporated herein in their entirety by reference. It is contemplated that Fab fragments, Fab' fragments, $F(ab')_2$ fragments, diabodies, and other forms of antibody fragments directed to the C5 component are suitable as well.

[0041] It is intended that the methods of the present disclosure are applicable to various animals such as mammals, e.g., humans, dogs, cats, horses, etc., which can all be benefited by screening and treatment herein. The complement inhibitors can be utilized in pharmaceutical compositions by adding an effective amount of one or more complement inhibitors to a suitable pharmaceutically acceptable diluent or carrier. Those skilled in the art are familiar with numerous techniques and formulations utilized to compound pharmaceutical compositions. The pharmaceutical compositions herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, (pulmonary, e.g., by inhalation or insufflation of liquids, powders or aerosols, including by nebulizer; intratracheal, intranasal, enteral, epidermal and transdermal), oral, sublingual, buccal or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intramedullary or intracranial, e.g., intrathecal or intraventricular, administration. Direct injection into affected joints is contemplated as well.

[0042] Pharmaceutical compositions for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, troches or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients. Pharmaceutical compositions herein include, but are not limited to, solutions, emulsions, suspensions, foams and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids, according to conventional methods, by one of skill in the art.

[0043] The pharmaceutical formulations herein, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product. Further details on techniques for formulation and administration of numerous dosage forms may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.). The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

[0044] Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0045] Pharmaceutical compositions suitable for use herein include compositions wherein the complement inhibitors are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically

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effective dose refers to that amount of active ingredient, which ameliorates, partially or completely, the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for animal or human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0046] The exact dosage will be determined by the practitioner in light of factors related to the subject that require treatment. Dosage and administration are adjusted to provide sufficient levels of the complement inhibitors to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation. Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g per kilogram, depending upon the route of administration. For example, anti-C5 monoclonal antibodies may be administered at about 8 mg/kg or at a 600 mg dose. Anti-C5 scFv may be administered at about 2 mg/kg. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art.

[0047] Although preferred and other embodiments have been described herein, modifications of the embodiments and further embodiments may be envisioned by those skilled in the art without departing from the scope of the invention as defined by the following claims.

What is claimed is:

1. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent comprising:

conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid; and

comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis patient to treatment with the complement inhibiting agent.

2. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 1 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2

3. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 2 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

4. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 3 wherein the antibody is 5G1.1-mAb or h5G1.1-mAb.

5. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 3 wherein the functional antibody fragment is 5G1.1-scFv or hG51.1-scFv

6. A method for determining the eligibility of a patient having rheumatoid arthritis to treatment with a complement inhibiting agent according to claim 1 wherein the baseline serum GPI levels encompass a mean concentration of 0.069+/−0.048 U/ml, P<0.0001 and the baseline synovial fluid GPI levels encompass a mean concentration of 0.060+/−0.052 U/ml P<0001.

7. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 1 wherein the patient is a mammal.

8. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 7 wherein the patient is a human.

9. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent comprising:

conducting an assay for antibodies to glucose-6-phosphate isomerase (anti-GPI) in the serum or synovial fluid of the patient to determine the level of ant-GPI in the serum or synovial fluid; and

comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent.

10. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 9 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

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11. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 10 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

12. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 11 wherein the antibody is 5G1.1-mAb or h5G1.1-mAb.

13. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 11 wherein the functional antibody fragment is 5G1.1-scFv or hG51.1-scFv.

14. A method for determining the eligibility of a patient having rheumatoid arthritis to treatment with a complement inhibiting agent according to claim 9 wherein the baseline serum anti-GPI levels encompass a mean concentration of (A_{405}) 0.059+/−0.037, P<0.0001 and the baseline synovial fluid anti-GPI levels encompass a mean concentration of (A_{405}) 0.645+/−0.209 P<0001.

15. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 9 wherein the patient is a mammal.

16. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 15 wherein the patient is a human.

17. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof comprising:

conducting a glucose-6-phophate isomerase (GPI) assay on the serum or synovial fluid of a population of subjects;

identifying subjects having GPI assay results which are statistically significantly greater than (P<0.05) the mean GPI assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent.

18. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 17 further comprising treating the subjects so identified with a complement inhibiting agent.

19. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 18 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

20. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 19 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

21. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 20 wherein the antibody is 5G1.1 or h5G1.1.

22. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 20 wherein the C5 complement inhibiting antibody wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

23. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 17 wherein the subjects are mammals.

24. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis according to claim 23 wherein the subjects are humans.

25. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof comprising:

conducting an assay for antibodies to glucose-6-phophate isomerase (anti-GPI) in the serum or synovial fluid of a population of subjects;

identifying subjects having anti-GPI assay results which are statistically significantly greater than (P<0.05) the mean anti-GPI assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent.

26. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 25 further comprising treating the subjects so identified with a complement inhibiting agent.

27. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 26 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

28. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 27 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

29. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 28 wherein the antibody is 5G1.1 or h5G1.1.

30. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 28 wherein the C5 complement inhibiting antibody wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

31. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 25 wherein the subjects are mammals.

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32. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis according to claim 31 wherein the subjects are humans.

33. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent comprising: conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid; and comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of a predisposition for antibody mediated rheumatoid arthritis in the patient which is susceptible to treatment with a complement inhibiting agent.

34. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 further comprising treating the patient exceeding the numerical range with a complement inhibiting agent.

35. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

36. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 35 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

37. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 36 wherein the wherein the antibody is 5G1.1 or h5G1.1.

38. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 36 wherein the antibody fragment is 5G1.1-scFV or h5G10.1-scFv.

39. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 wherein the patient is a mammal.

40. A method for predicting susceptibility of a patient to rheumatoid arthritis according to claim 39 wherein the patient is a human.

41. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent comprising:

conducting an assay for antibodies to glucose-6-phosphate isomerase (anti-GPI) in the serum or synovial fluid of the patient to determine the level of anti-GPI in the serum or synovial fluid; and

comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of a predisposition for antibody mediated rheumatoid arthritis in the patient which is susceptible to treatment with a complement inhibiting agent.

42. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 41 further comprising treating the patient exceeding the numerical range with a complement inhibiting agent.

43. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 42 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

44. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 43 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

45. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 44 wherein the wherein the antibody is 5G1.1 or h5G1.1.

46. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 44 wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

47. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 41 wherein the patient is a mammal.

48. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 47 wherein the patient is a human.

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ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

Section Editor: Craig M. Kessler, MD

Eculizumab for the Treatment of Paroxysmal Nocturnal Hemoglobinuria

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H&O Can you give a short background on the standard of care for paroxysmal nocturnal hemoglobinuria before the advent of eculizumab?

AH Until recently, the treatment for paroxysmal nocturnal hemoglobinuria (PNH) was predominantly supportive. Red blood cell (RBC) transfusions were administered when appropriate, and warfarin or other anticoagulation therapy was given following a thrombosis. In the UK we practice primary prophylaxis with anticoagulant therapy depending on the neutrophil PNH clone size. Other therapies, such as corticosteroids or androgen therapy, have also been used, but less commonly and with efficacy in only a few patients; the mechanism of action behind these treatments is unclear. Stem cell transplantation has also been used in the past, but of course this approach is associated with serious complications. It has been difficult to develop an evidence base for treatment because of the heterogeneous nature and rarity of this disease. Before eculizumab (Alexion), treatment for PNH was limited to alleviating symptoms and improving patient quality of life. Eculizumab is a monoclonal antibody that blocks the complement system at protein C5.

H&O What is the rationale for the development of eculizumab for the treatment of PNH?

AH We know the hemolysis in PNH is complement-mediated, thus this drug should be effective in preventing hemolysis. The pathogenesis of PNH is known to be due to complement sensitivity of RBCs against complement attack. RBCs in PNH have lost their protective proteins (eg, CD55 and CD59) that would normally prevent complement-mediated lysis. It was hypothesized that if the complement attack on the RBCs could be blocked, the hemolysis in PNH and its associated symptoms could be prevented. Based on patients who have inherited complement deficiencies, it appears that patients with deficiencies in complement proteins C5–C9 suffer few clinical consequences compared to those with deficiencies in the complement proteins before C5. Therefore, C5 seemed to be a logical target for blocking the complement cascade. Eculizumab was developed for the purpose of blocking the complement system safely and without severe complications.

H&O Eculizumab has been evaluated for the treatment of rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune conditions. Is there a similarity between these conditions and PNH that spurred investigators to evaluate eculizumab in PNH?

AH The research strategy has been to develop therapeutic approaches that block complement activity, which contributes to a variety of disorders. There is some evidence that some components of autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus are complement-mediated. In PNH, however, almost all of the clinical symptoms are due to the uncontrolled activity of complement. Therefore, whereas there are many possible disorders in which complement blockade might have some efficacy, PNH is the disorder in which we had little doubt that eculizumab would be effective.

H&O What were some of the initial findings with eculizumab in PNH?

AH In May 2002, we began the pilot study of eculizumab in PNH. Patients enrolled had been having regular transfusions, and most exhibited dark urine (a sign of intravascular hemolysis) and severe symptoms of PNH such as difficulty swallowing (dysphagia) and abdominal pain. Men complained of erectile dysfunction. Additionally, they had biochemical parameters indicative of active, ongoing intravascular hemolysis. We found that just 1 infusion of the drug caused a resolution of the

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dark urine by the next morning. Other symptoms, such as abdominal pain and difficulty in swallowing, resolved quickly. Of the 6 male patients who had experienced erectile dysfunction, 5 experienced complete resolution of this symptom. The patients felt better quickly and, in terms of biochemical parameters, we found quick improvements in lactate dehydrogenase levels as well.

H&O What were the next steps after this initial study?

AH The pilot study was initially planned to be 12 weeks in duration, but at the end of the 12 weeks, all patients wanted to continue with eculizumab therapy. The extension phase of the trial commenced and is still continuing with 10 of the 11 patients. Patients have experienced a dramatic improvement in their quality of life and generally prefer 2 weekly infusions of eculizumab rather than their previous treatment, which might have been, for example, 4 weekly blood transfusions. Some patients have become transfusion-independent.

H&O What side effects are associated with eculizumab?

AH The side effects associated with eculizumab have been very minimal. One patient in the pilot study had a reaction to the initial infusion, consisting of shivering and fever. She required observation only and has tolerated every infusion after that extremely well with no further reactions; she is still on the drug more than 3 years later. No other patients experienced any infusion-related reactions. The only minor side effect has been headache after the first or second infusions in some of the patients, but otherwise toxicity has not been a problem. There is a theoretical risk of meningitis, but this has not been a major issue to date.

H&O Is there any sense about whether eculizumab treats all patients equally or are there variations between patients that might change the efficacy of the agent?

AH We found that patients who have the classic purely hemolytic PNH respond better to the drug than those who have a hypoplastic bone marrow. The reason for this difference is simply that marrow failure can sometimes contribute more significantly than hemolysis to anemia, in which case, although the hemolysis will be blocked by eculizumab, marrow failure will most likely still lead to anemia and therefore continuing transfusions will be required. Certainly any patient with classical PNH who is hemolyzing frequently is a good candidate for eculizumab therapy. This agent would also be appropriate for patients with biochemical and clinical evidence of hemolysis because their quality of life can be improved.

H&O What is the clinical trial status of eculizumab?

AH Two phase III trials opened worldwide in the last year: TRIUMPH and SHEPHERD. The first opened about a year ago and the second opened earlier this year. The trials are now closed to recruitment, and we are eagerly awaiting the results. If the results of this trial are positive, I would expect that applications for product licenses would be made relatively quickly.

H&O Does eculizumab appear to have any effect on thrombosis, the most significant morbidity associated with PNH?

AH Although at the moment we have no evidence that eculizumab will prevent thrombosis, it is reasonable to hypothesize that thrombosis may be prevented by this terminal complement blockade through its mechanism of action. Theories for the causes of thrombosis in PNH include activation of PNH platelets by complement attack and nitric oxide depletion through free hemoglobin scavenging. Eculizumab should prevent both consequences. This possibility will be very interesting to examine in the future. In the pilot study there was no evidence of thromboses in any of the patients.

Suggested Reading

Hill A, Hillmen P, Richards SJ, et al. Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. *Blood*. 2005;106:2559-2565.

Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2004;350:552-559.

Parker C, Omine M, Richards S, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. *Blood*. 2005; [Epub ahead of print].

Poggi A, Negrini S, Zocchi MR, et al. Patients with paroxysmal nocturnal hemoglobinuria have a high frequency of peripheral-blood T cells expressing activating isoforms of inhibiting superfamily receptors. *Blood*. 2005;106:2399-2408.

Young NS. Paroxysmal nocturnal hemoglobinuria: current issues in pathophysiology and treatment. *Curr Hematol Rep*. 2005;4:103-109.

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WEDNESDAY, 9 APRIL 2003

Oral Communication

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Eculizumab, a C5 complement blocking antibody, is the first therapy to reduce transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria (PNH)

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Paroxysmal nocturnal haemoglobinuria (PNH) is characterized by intravascular haemolysis and venous thrombosis due to the unopposed effect of complement on PNH red cells and platelets, respectively. Eculizumab is a humanized monoclonal antibody that binds C5 and inhibits terminal complement activation. Eleven transfusion-dependent PNH patients have completed a 3-month pilot study receiving 600 mg eculizumab by intravenous infusion over 30 min weekly for four doses and then 900 mg every other week. Adverse events were relatively minor and self-limiting. The biochemical parameters of haemolysis dramatically improved for all patients within a week. Mean LDH decreased from 3089 ± 599 U/L before to 597 ± 32 U/L after treatment ($P = 0.002$) and AST from 77 ± 20 to 26 ± 3 U/L ($P = 0.04$). Episodes of haemoglobinuria reduced by 90% ($P < 0.001$). Quality of life (EORTC QLC30) improved significantly post-eculizumab compared to pre-eculizumab. One patient began treatment during a severe acute haemolytic episode with almost black urine and within 24 h there was a complete resolution of her haemoglobinuria and symptoms. Transfusions for all patients were significantly reduced in the 3 months of eculizumab compared with the previous 12 months from 2.2 to 0.7 units/month ($P = 0.003$). The reduction in transfusions was most dramatic in the seven patients without thrombocytopenia from 2.3 to 0.2 units/month ($P = 0.001$). There was an increase in PNH type III red cells from a mean of 35.6% pre-eculizumab to 58.7% post-eculizumab ($P = 0.005$) due to their protection from complement-mediated lysis and the reduction in transfusions. There was no change in the proportion of PNH neutrophils, monocytes or platelets. All patients have continued on eculizumab in an extension study. In summary, eculizumab is well tolerated, decreases haemolysis, improves symptoms and significantly reduces transfusions in PNH patients. Eculizumab appears to be the first effective therapy for haemolysis in PNH and merits further development. This is the first trial of an anti-complement antibody therapy in PNH. The responses were remarkable both in reduction in haemolysis and transfusions and in the improvement in quality of life. Thus it is the first effective therapy for haemolysis in PNH and would also be expected to reduce the risk of thrombosis (this study could not address this). The trial was performed at two centres in the UK only.

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The induction of tolerance to RhD in HLA DR15 transgenic mice

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The RhD protein is a clinically important red cell alloantigen that induces the production of anti-D in RhD-negative individuals. Because of the immunogenicity of RhD, all blood donations are typed and matched for this antigen, and RhD-negative women are managed for the prevention of haemolytic disease of the newborn with prophylactic anti-D immunoglobulin. Understanding the mechanisms by which helper T-cells control the antibody response to RhD would enable the design of alternative strategies such as active toleration. We have shown that CD4+ helper T cells from alloimmunized donors can proliferate and produce both IFN-gamma and IL-4 in response to synthetic peptides derived from the RhD sequence, indicating a Th0 balanced cytokine response. The responses are HLA class II restricted, with HLA-DR15 being over-represented in good responders. Certain peptides are immunodominant, and induce proliferative responses in most alloimmunized donors. These peptides are candidates for the induction of tolerance to the RhD protein by the mucosal route. On the other hand, some peptides from the RhD protein preferentially induce IL-10 production by putative Tr1 regulatory cells *in vitro*, and these peptides inhibit T-cell proliferation in response to stimulation with purified RhD protein *in vitro*. These peptides may be used to induce tolerance by the parenteral route. We have used a HLA-DR15 transgenic mouse model to show that T-cells from mice immunized with the RhD protein proliferate in response to purified RhD protein and to RhD peptides, and serum antibodies specific for human erythrocytes are also produced. Mucosal administration of immunodominant RhD peptides, or parenteral administration of IL-10 inducing peptides prior to immunization with RhD protein, inhibits both T-cell proliferation and antibody production. These results suggest that synthetic RhD peptides can induce tolerance to the RhD protein and may be of value in the prevention of immunization in RhD-negative individuals.

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A humanised monoclonal antibody against vWF A1 domain inhibits vWF:RiCof activity and platelet adhesion in human volunteers

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AJW200 (Ajinomoto), a humanized IgG4 monoclonal antibody directed against the A1 domain of von Willebrand factor (vWF), specifically inhibits vWF : platelet GPIb receptor interactions. At

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high shear, it inhibits platelet adhesion, aggregation and activation, and in a dog model, inhibits arterial thrombus formation, without prolonging the bleeding time. In a randomized, double blind study, placebo, 0.01, 0.03, or 0.05 mg/kg AJW200, were infused i.v., into 24 healthy men. No clinically significant adverse events were recorded and there was no evidence of immunogenicity. Mean PK results were: C_{max} 204.8, 586.2, 833.2 ng/ml; T_{max} 1.0, 0.92, 0.57 h, T/2 23.5, 24.3, 27.2 h, for ascending doses. The maximum vWF occupancy by AJW200 was 19.4, 51.0 and 62.4%, showing correlation with plasma AJW200 concentration. Ristocetin cofactor (RiCof) was reduced 1 h post-infusion compared to baseline: mean (SD) 58 (22) and 110 (25)%, $P < 0.0005$ and 34 (16) and 116 (41)%, $P < 0.005$, at 0.03 and 0.05 mg/kg respectively, for up to 12 h. vWF : Ag, FVIII, vWF multimers and cleaving protease activity were unchanged. Template bleeding time (BT) was normal at all AJW200 doses, but the PFA-100 showed prolonged closure times 1 h post-infusion, with both CADP: 146 (22) and 82 (10) and 202 (78) and 80 (19) s, $P < 0.01$; and CEPI cartridges: 182 (28) and 108 (16) and 233 (56) and 107 (19) s, $P < 0.01$, at 0.03 and 0.05 mg/kg AJW200, respectively. This effect lasted for 3–6 h with the lower and up to 12 h for the higher dose. The RiCof and PFA-100 responses showed dose dependent inhibition and the magnitude of the effect appeared to be related to the baseline vWF level. AJW200 showed inhibition of vWF : RiCof activity and PFA-100 responses, without prolonging the skin bleeding time, and merits further clinical studies for thrombotic disorders. This is the first study demonstrating the effect of a monoclonal antibody that blocks GPIb/vWF in humans.

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Phase I study of the adoptive transfer of tetramer selected cytomegalovirus-specific cytotoxic T cells after allogeneic stem cell transplantation

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Cytomegalovirus (CMV) remains an important cause of morbidity and mortality following stem cell transplantation (SCT). Adoptive transfer of donor CMV-specific CD8+ T cell clones has been shown to protect against CMV reactivation in previous studies. However, this procedure has not been widely used due to the need for extensive *ex vivo* culture of T cells prior to infusion. We have combined HLA-peptide tetramer staining with the use of magnetic beads against phycoerythrin to facilitate the rapid selection of CMV-specific CTL from donor peripheral blood using a CliniMACS machine. In this study we infused tetramer selected donor CMV-CTL to six allogeneic stem cell transplant (SCT) recipients following CMV reactivation. An additional patient had undergone transplantation from a matched unrelated donor and CTL were infused for treatment of CMV reactivation refractory to Foscarnet, Ganciclovir and Cidofovir. In the 6 sibling allografts between 10^5 and 1.6×10^6 (mean 6×10^5) CMV-specific CD8+ T cells were infused from 250 mL of donor peripheral blood. The unrelated donor transplant patient received 2×10^6 CMV-CTL isolated from a cryopreserved lymphopheresis product. No infusion reactions were noted. One patient developed grade II GvHD which responded to treatment. CMV-CTL were not detectable prior to infusion in three of six patients but were detected in all patients following infusion. CMV-CTL expanded to represent up to 22.1% of the CD8 compartment. This expansion occurred in the first 14 days followed by a contraction to a basal level. Four patients did not require antiviral therapy. In the unrelated transplant recipient CMV-specific CTL reached a peak of 0.6% of the CD8 compartment and CMV antigenaemia became negative 6 days following CTL infusion. This novel approach to the adoptive transfer of antigen-specific CTL is rapid and relatively straightforward. It has considerable potential in the development of antigen-specific T cell therapy after allogeneic transplantation.

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Bone marrow mesenchymal stromal cells protect chronic lymphocytic leukaemia (CLL) from spontaneous and drug-induced apoptosis

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CLL cells are able to survive for prolonged periods *in vitro* and yet in most instances undergo rapid spontaneous apoptosis when cultured *in vitro*. Sustained viability *in vitro* is probably maintained by contact with 'nurse-like' cells in the peripheral blood (PB) and the bone marrow (BM) microenvironment. An *in vitro* model to mimic *in vitro* conditions was developed using adherent BM mesenchymal stromal cells (MSCs) from the iliac crest of normal donors. This model was used to test protection of CLL PB mononuclear cells both from apoptosis and from the effects of CD20 (Rituximab) and CD52 (CAMPATH-1H) therapeutic monoclonal antibodies, the proteasomal inhibitor MG132 and Bis9, an inhibitor of protein kinase C. Levels of apoptosis were determined by flow cytometry, using AnnexinV-FITC and propidium iodide. BM MSCs abrogated spontaneous apoptosis of CLL cells over a 5-day period of culture (80–90% viability, $n = 9$). Protection required CLL cells to be in close contact with the MSCs; separation by a microporous membrane induced apoptosis, comparable to that seen in control samples cultured without MSCs (30–40% viability). Rituximab treatment did not have any obvious apoptotic effect on CLL cells *in vitro* but CAMPATH-1H was able to induce apoptosis in a subpopulation of cells even in the presence of MSCs. Treatment with Bis9 and MG132 caused substantial and rapid apoptosis of CLL cells (0–15% viability $n = 3$). At lower doses of both drugs (200 nM), this apoptosis was prevented by MSC co-culture (70–90% viability). MSCs can therefore maintain CLL viability *in vitro* and can influence drug-induced apoptosis. The critical cell–cell interactions remain unknown, but are likely to include the beta-integrin, TNFSF13B and the CXCR4/CXCRL12 pathways. The expression of these molecules and their possible interactions in maintaining CLL viability will be investigated to assess if any might be targeted therapeutically.

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Targeting FGFR3 in multiple myeloma: the use of SU5402 and PD173074 to inhibit t(4;14) positive cells

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The t(4;14)(p16.3;q32), associated with 10–20% of cases of multiple myeloma (MM), deregulates the expression of two genes, MMSET and FGFR3. Most t(4;14) positive myeloma patients express FGFR3 and constitutive activation of this receptor is seen in some cases. To assess the potential of FGFR3 as a drug target we evaluated the effects of three FGFR inhibitors (SU6668, SU5402 and PD173074) on control and MM cell lines. Activity was assessed initially against Ba/F3 cells transformed to IL-3 independence by ZNF198-FGFR1, a constitutively active fusion gene associated with the 8p11 myeloproliferative syndrome (EMS), and compared to activity against control BaF3 cells grown with IL-3. Three methods were employed: MTS to assess proliferation/metabolic activity, trypan blue staining to determine cell viability and TUNEL to assess apoptosis. SU6668 inhibited the growth of both transformed and control cells lines at concentrations of >20 μM and therefore was not pursued further. SU5402 and PD173074 specifically inhibited the growth of ZNF198-FGFR1 transformed cells in a dose-dependent fashion with an IC50 of 5 μM and 5 nM, respectively. Both compounds also caused decreased cell viability and increased apoptosis. These compounds were then tested against two t(4;14) positive MM lines, KMS-11 and OPM-2, which express FGFR3 and harbour activating mutations of this gene. Dose-dependent growth inhibition was observed in both lines with IC50s similar to those in ZNF198-FGFR1 transformed cells. Importantly, inhibition was still observed in the presence of IL-6. Both compounds induced a dose-dependent reduction in cell viability and an increase in apoptosis, accompanied by a decrease in FGFR3 and ERK phosphorylation. In contrast, no inhibition was seen with either compound against the t(4;14) negative lines U266 and HS-Sultan, or the t(4;14) positive, FGFR3 negative cell line KMS-18. FGFR3 is thus a plausible candidate for targeted therapy in a subset of MM patients.

Oral Communication

Myeloid Malignancy

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Decreased expression of a novel growth regulatory protein, Nov, in chronic myeloid leukaemia

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Chronic myeloid leukaemia (CML) is characterized by the constitutively active Bcr-Abl tyrosine kinase. We have used a murine multipotent haematopoietic stem cell line, FDCP-MIX, expressing a temperature-sensitive mutant Bcr-Abl (ts Bcr-Abl FDCP-MIX), to investigate changes in gene expression using DNA microarray analysis. RNA was prepared from cells expressing the Bcr-Abl tyrosine kinase and from control cells, labelled with biotin and hybridized to an Affymetrix Mu6500 array (Affymetrix, USA). The Bcr-Abl kinase activity resulted in differential expression of 300 genes, the most significant being a 25-fold decrease in expression of a novel gene called nephroblastoma overexpressed (Nov). Northern blot analysis detected a 4-fold reduction in Nov expression in the Bcr-Abl positive cells in comparison with the control cells. Western blot analysis of immunoaffinity-purified Nov detected a two-fold reduction in expression as a result of the Bcr-Abl kinase activity. Confocal microscopy revealed strong vesicular cytoplasmic staining for Nov in FDCP-MIX control cells whilst fluorescence was barely detectable in cells expressing Bcr-Abl. A similar pattern of Nov expression was observed in primary human cells (five CML, five normal controls). Normal CD34+ progenitor cells uniformly expressed an intense fluorescence signal for Nov expression. In contrast, the majority of CD34+ cells within the CML patient samples were negative ($P < 0.001$). Nov expression has previously been associated with cell differentiation, quiescence and has been shown to promote cell adhesion. Loss of these properties is consistent with the CML phenotype.

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Reduced hTR expression in CML CD34+ cells at diagnosis: a novel mechanism for telomere loss in leukaemia

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CML is characterized by massive myeloid expansion, and significant telomere loss has been documented at diagnosis and during disease progression. Critical telomere shortening results in chromosomal instability, which can lead to disease progression. The regulatory mechanisms and expression levels of the key telomerase components (hTERT and hTR, the reverse transcriptase and RNA template respectively) in the CML stem cell compartment have not been described; however studies in solid tumours and cell lines generally show increased expression of both components. We selected CD34+ cells from 16 CML diagnostic leucapheresis specimens and 12 non-CML mobilized PBSC (control population) (median purity 94.1, 96.3% respectively; BCR-ABL positive in CML group). High-resolution cell cycle analysis demonstrated increased cycling in the CML CD34+ fraction. Q-RT-PCR for full-length (functional) hTERT showed an approximately two-fold rise in CML expression compared with control cells. RT-PCR with primers designed to detect the full-length hTERT transcript plus the three non-functional splice variants (with quantitative analysis) demonstrated a shift away from splicing in the CML samples. By combining these two complementary techniques we determined that expression of all (i.e. full length plus splice variants) transcripts are not different between CML and control cells: the increased levels of functional hTERT mRNA appear to be accounted for mainly by a reduction in the splicing out of the active sites. Surprisingly, Q-RT-PCR for hTR demonstrated a five-fold reduction in hTR levels in CML compared with control cells. Even more striking was the difference in hTERT/hTR ratio: 1 : 180 in CML cells versus 1 : 1800 in non-CML cells. We therefore hypothesize that despite increased hTERT levels there is a disassociated reduction in hTR, which in conjunction with increased cell cycling and replication

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induced telomere loss, could alter telomere homeostasis in CML. This could contribute to disease evolution via chromosomal instability.

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Reduced-intensity allogeneic haematopoietic stem cell transplantation (HSCT) using fludarabine, busulphan and alemtuzumab conditioning in 63 patients with myelodysplastic syndromes (MDS)

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We report the largest series of patients with MDS who have received a reduced-intensity HSCT using Fludarabine 30 mg/m^2 i.v. (day −9 to −5); busulphan 4 mg/kg/day orally (day −3 to −2) and alemtuzumab 20 mg i.v. (day −5 to −1). Immunosuppression comprised ciclosporin from day −1 titrated to plasma troughs of 150–200 ng/L. 63 patients with MDS (RA = 16, RAEB-I = 9, RAEB-II = 11, CMML = 5, TLD-AML = 21, aCML = 1) have received transplants from matched sibling (MSD) (24) or volunteer unrelated donors (VUD) (39). Except for RA, all had received at least one course of chemotherapy. Twenty-two of 24 MSD utilized PBSCs compared with 19 of 39 VUD HSCTs, with a median CD34+ cells of 4.6×10^6/kg (1.83–17.5) and 3.9×10^6/kg (0.44–16.7) respectively. There was no difference in engraftment latency, with the median time to neutrophil engraftment of 15 days (4–57) and platelets 18 days (7–105). 85% achieved full donor chimerism with a 6% incidence of grade III/IV aGvHD (43% grade I–IV) and 3% extensive cGvHD. At a median follow-up of 343 days (50–1213) for MSD, and 248 days (37–1316) for VUD recipients, 63 and 62% were alive respectively. The median age of sibling allografts recipients was 54 years (41–70). At day +200, the TRM was 5% and DFS 68%. The median age for VUD recipients was 49 years (25–58). The TRM at day +200 was 21% with DFS 65%. At 1 year, the estimated OS was 66% for VUD and 77% for MSD, with a DFS of 57 and 53% respectively. Twenty-one received DLI at a median of day +273 (126–1290); 11 for relapse and 10 for declining donor chimerism. A graft versus leukaemia effect was demonstrable. Although longer follow-up is required, RIC allogeneic HSCT appears to be safe and feasible in this high-risk group of patients.

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Combined clofibric acid and medroxyprogesterone acetate exert multiple anti-leukaemic actions against acute myeloid leukaemia cells and cell lines

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Poor survival rates in acute myeloid leukaemia (AML) relate to both the failure of cytotoxic chemotherapy and inability of older patients to tolerate that therapy. Thus non-toxic drugs that specifically target the differentiation and apoptotic pathways of AML cells are urgently required. We and others have demonstrated that fibrates such as clofibric acid (CA) and inhibitors of the aldo-keto reductase AKR1C3, including medroxyprogesterone acetate (MPA), potentiate all trans-retinoic acid (ATRA) induced HL-60 cell differentiation. Fibrates are pharmacological PPARalpha ligands commonly used in the treatment of hyperlipidaemia whereas MPA is used to treat breast carcinoma. We recently demonstrated that, potentiation of HL-60 differentiation by AKR1C3 inhibitors occurs via the consequent activation of PPARgamma. Thus combined CA and MPA (CA/MPA) provide the means to simultaneously target PPARalpha and gamma using established drugs with low systemic toxicities. Here we demonstrate that CA/MPA killed cultured primary AML blasts in the presence or absence of IL3 and SCF ($P = 0.0001$) but not normal CD34+ve myeloid blasts. CA/MPA also sensitized AML cell killing by suboptimal doses of daunorubicin and Ara-C ($P < 0.003$) highlighting the drugs' potential as chemosensitizing agents. Although ATRA did not enhance AML cell killing by CA/MPA, all three drugs together resulted in the differentiation of the surviving cells from three of seven non-acute promyelocytic AML samples. Finally, high serum Nm23-H1 protein concentrations have been associated with the poorest prognosis in AML. Here we provide the rationale for this by demonstrating that Nm23-H1 acts as an autocrine survival factor. Importantly we also demonstrate that CA/MPA rapidly down-regulated Nm23-H1 expression in those AMLs that had highest initial expression ($P = 0.001$). We conclude that CA/MPA exert multiple antileukemic actions against AML cells and should be considered as adjuncts in the treatment of elderly and relapsed AML.

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The utility of *in vitro* testing for imatinib sensitivity in BCR-ABL negative myeloproliferative disorders

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Imatinib mesylate (STI571, Glivec) inhibits the tyrosine kinase activity of the protein tyrosine kinases ABL, ARG, PDGFRA, PDGFRB and KIT, and has proven clinical efficacy in the management of patients with BCR-ABL positive CML and MPDs with PDGFRB rearrangements. However, it is not known to what extent patients with BCR-ABL negative MPDs may harbour activating rearrangements or point mutations of imatinib-responsive genes. To test for imatinib responsiveness we have employed two *in vitro* assays. First, blood or marrow mononuclear cells (MNC) were grown in methylcellulose supplemented with growth factors and treated with 0, 1 or 5 μM imatinib. Colonies were counted on days 7 and 14. Secondly, blood or marrow MNCs were placed in liquid culture with 0, 1 and 5 μM imatinib and cell numbers were enumerated over 2–3 weeks. Imatinib-treated cells from CML patients showed a reduction in colony and cell numbers over time compared with untreated cells and treated cells from normal individuals. We have obtained results using one or both methods in 38 BCR-ABL, TEL-PDGFRB negative patients who had been diagnosed with atypical CML, BCR-ABL negative MPD, CMML, mastocytosis, myelofibrosis or hypereosinophilic syndrome (HES). Eleven patients showed a positive response, four of whom had a PDGFRB rearrangement as determined by FISH and one had TEL-ABL. The remaining six patients had HES ($n = 2$), secondary MDS with eosinophilia following treatment for AML ($n = 1$), aCML ($n = 1$), MF ($n = 1$) or mastocytosis ($n = 1$). Six

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patients (five with PDGFRB or ABL rearranged MPD and one with HES) were treated and all showed a cytogenetic and/or haematological response. We conclude that *in vitro* cultures are useful for predicting patients who might respond clinically to imatinib. Evidence to date suggests that most BCR-ABL negative patients who are imatinib-responsive have elevated eosinophil counts.

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Identification of a novel gene, GEMS, that is fused to FGFR1 in the 8p11 myeloproliferative syndrome

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The 8p11 myeloproliferative syndrome (EMS) is an atypical stem-cell myeloproliferative disorder which is caused by activation of the transmembrane receptor tyrosine kinase FGFR1. This typically occurs as a result of fusion of the tyrosine kinase domain of FGFR1 to an unrelated partner gene, which promotes dimerisation and consequent activation of the kinase in the absence of an appropriate ligand. To date, five FGFR1 partner genes have been identified: ZNF198 at 13q12, FOP at 6q27, CEP110 at 9q33, BCR at 22q11 and HERV-K at 19q13. In this study, we describe a patient with a clinical picture typical of EMS, who was found to have an acquired ins(12;8)(p11;?p11p22) by cytogenetics. Southern blotting and FISH were used to confirm that FGFR1 was disrupted and RT-PCR analysis indicated that the patient did not harbour any of the known FGFR1 fusion mRNAs or BCR-ABL. To identify the partner gene in this patient we employed 5′-RACE PCR, exploiting the fact that all known FGFR1 translocations generate mRNA fusions in which the partner gene is spliced to FGFR1 exon 9. A 629-bp product was obtained which was found to consist of sequence derived from chromosome band 12p11 fused to exon 9 of FGFR1. Analysis of ESTs and other clones indicated that the sequence was derived from a widely expressed novel gene that we have called GEMS (gene disrupted in eight p11 myeloproliferative syndrome). GEMS is predicted to be translated into a 29-kDa protein that contains an N-terminal coiled-coil domain but no other recognisable motifs. RT-PCR confirmed the presence of the GEMS-FGFR1 fusion but the reciprocal chimaeric mRNA was not detected. Experiments to determine the transforming ability of this novel fusion gene are underway.

Oral Communication

Haemostasis and Thrombosis 2

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Venous thromboembolism (VTE) associated with the management of acute thrombotic thrombocytopenic purpura (TTP)

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VTE is reported to occur in 3% of TTP/HUS cases following plasma exchange (PEX) therapy. From May 1997 to May 2002, 68 patients with acute TTP were treated. Eight documented VTE episodes (six DVTs and three PEs) were identified in seven patients (all female, mean age 31 years) during therapy for acute TTP (five primary presentation and three relapses). All six DVTs were associated with central lines at the site of thrombosis. Other precipitating factors included pregnancy (one), immobility (eight), obesity (three) and FV Leiden heterozygosity (one). VTE occurred at a mean of 53 days following the first PEX. Solvent detergent plasma (Octaplas, Octapharma, Austria) was the last plasma to be used in PEX prior to the VTE in seven of eight events. Octaplas was used as the sole plasma for three TTP episodes and in the other episodes was substituted for cryosupernatant or FFP owing to poor response (four) or allergy (one). The mean volume of Octaplas replacement prior to the VTE was 100 L (range 22–206). The mean platelet count at VTE was 161 (range 12–232) $\times 10^9$/L. The platelet count had been in the normal range for a mean of 18 days prior to the VTE in those with a normal platelet count (six of eight). One patient received prophylactic LMW heparin from initial TTP diagnosis and seven of eight received aspirin. In conclusion, we identified VTE in 12% of TTP cases and 88% of these were associated with Octaplas replacement in PEX. VTE is a multifactorial disease and several known precipitating factors are present in TTP patients. The use of large volumes of Octaplas in PEX may be an additional risk factor, possibly associated with protein S (PS) deficiency, particularly as the median functional PS in Octaplas is 58 IU/dL compared with 91 IU/dL and 102 IU/dL in FFP and cryosupernatant respectively.

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Results of D-dimer in the UK NEQAS (Blood Coagulation) proficiency testing programme: urgent need for improvements in standardisation of testing and reporting

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We report the proficiency testing of D-dimer in diagnostic departments. Two lyophilised samples were tested for D-dimer by over 240 centres using different methods. Around 30% used semi-quantitative tile-based latex agglutination assays and 70% used automated techniques including 20% who reported in fibrinogen equivalent units (FEU). Six automated methods were used in 10 or more centres and the median and range of results obtained with these methods are shown below in ng/mL. For sample 1, 70% of centres reported a result above the cut-off for DVT. For one method the median test result was within the normal range, whereas for other methods the median was 40–80% above the normal range depending on the technique. It was a major cause for concern that 24% reported a normal result for this sample. There appeared to be a poor understanding of the difference between the upper limit of normal and the cut-off for DVT diagnosis. For the 103 centres who reported using a cut-off for DVT diagnosis the value in use ranged from 80 to 1000 ng/mL. For sample 2, a pooled normal plasma, six centres reported their result as positive, abnormal or consistent with DVT. As reconstituted lyophilised plasma is unsuitable for the Nycocard system a different test sample was tested by 67 users of the Nycocard system who obtained results in the range of 80 to 1300 ng/mL for

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a liquid control sample having a median result of 500 ng/mL. We conclude that standardization of D-dimer testing and reporting is currently poor and that there is an urgent need for international reference preparations and recommendations.

		Sample 1		Sample 2		
	n	Median	Range	Median	Range	Median upper limit normal
Biomerieux VIDAS*	10	872	798–983	169	149–250	500
Dade-Behring DD Plus	10	170	120–219	100	60–170	190
Instrumentation Lab	68	352	240–848	103	10–280	260
Organon (FEU)*	18	915	770–4320	200	80–830	500
Organon (other units)	10	433	400–850	80	30–100	300
Stago*	15	890	800–1360	220	130–640	500

* FEU, fibrinogen equivalent units.

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Progression of liver disease in hepatitis C infected haemophiliacs assessed by serial liver biopsy

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More than 90% of haemophiliacs treated with plasma-derived factor concentrates prior to 1984 are infected with the hepatitis C virus. Although the timescale for progression to cirrhosis is many years, most patients will develop chronic liver disease. It has been suggested that liver biopsy in haemophiliacs is particularly hazardous and should be avoided. However, current treatment guidelines use liver biopsy to determine those who require therapy with ribavirin/interferon. We have previously reported our experience of liver biopsies in 35 patients with haemophilia/von Willebrand's disease and hepatitis C. We now report follow-up data on repeat biopsies on the same patient group 3–5 years following initial biopsy to assess progression of liver disease in this group. Patients who were HCV-PCR negative and those with inhibitors were excluded. To date, 25 patients have undergone repeat biopsy. Biopsies were graded according to fibrosis and inflammatory scores. Ten patients have progressed by one fibrosis grade. Three patients have progressed from grade 2 or less to grade 3 thereby qualifying for combination therapy. One patient has progressed from grade 0 to grade 3 and another progressed from grade 0 to grade 5. Both these patients had a history of excessive alcohol consumption. Thirteen patients showed no evidence of progression. Over a time course of at least 18 years only four patients have developed cirrhosis. There have been no bleeding complications related to the biopsies. We have performed 70 biopsies in patients with haemophilia/severe VWD and shown it to be a safe procedure. We have demonstrated the slow progression of liver disease in this group. The majority of our patients do not qualify for combination therapy according to national guidelines.

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UK surveillance of acquired haemophilia A: May 2001–April 2002

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Reports on acquired haemophilia (AH) are exclusively from referral centres which may have lead to publication bias. This study aims to establish the incidence and clinical characteristics of AH in all UK patients. In the first 12 months 256 of 257 UK Trusts circularised reported 98 cases (incidence 1.7/million/year). Clinical details are available on 68 patients. Median age is 80 (range 32–97). 39% had died at 6 months: 10% infection (pneumonia/sepsis), 4% bleeding, 7% malignancy, 7% cardiac and 11% others. Clinical associations are malignancy 29%, inflammation 20%, pregnancy 3%, pemphigoid 3% and none 59%. Median (range) factor VIII and anti-human inhibitor levels were 3 IU/dL (<1–25) and 13 BU/mL (<0.4–717). Bleeding manifestations are subcutaneous 78%, muscular 37%, gastrointestinal 22%, retroperitoneal 9%, haemarthrosis 7%, haematuria 7% and intracranial 3%. Subcutaneous bleeding was the only manifestation in 28%. Bleeding manifestations did not vary between CCC, haemophilia centres (HC) and other centres. Haemostatic treatment: none 38%, FEIBA 34%, rFVIIa 24%, human FVIII 22% and porcine FVIII 4%. No haemostatic treatment was required in 26% of patients at CCC, 55% at HC and 45% at other centres. Immunosuppression induced remission in 68% but only 44% of the cohort was alive and inhibitor-free at 6 months. There was no difference in remission rate between steroids alone 73% or cytotoxics and steroids 69% (IVIG made no significant difference); however, these results must be interpreted with caution as they are retrospective and not randomized. The remission rate at CCC, HC and other centres was 63, 65 and 91% respectively. These interim data suggest that overall survival is worse than previously reported, more patients died of infection than bleeding and many patients required no haemostatic treatment. Patients treated at non-haemophilia centres require less haemostatic treatment and are more likely to achieve remission suggesting that previously published data may be biased to a more severe phenotype.

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Procoagulant changes in human macrophages induced by environmental microparticles [PM10]: a possible pathogenetic mechanism in cardiovascular disease

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In epidemiological studies a strong relationship has been confirmed between increasing concentrations of environmental particles [PM10 and PM25] and acute cardiovascular events including myocardial infarction and stroke. No plausible pathogenetic mechanism

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has been demonstrated to explain this association. Recently it has been demonstrated that inhaled particles pass into the systemic circulation in humans (1). Therefore we hypothesized that vascular events may be triggered by coagulation activation on cell surfaces induced by contact with environmental particles. Human peripheral blood monocyte-derived macrophages and a human bronchial epithelial cell line were exposed to PM10 and supernatants examined for procoagulant activity in a plasma recalcification time assay. Control experiments employed culture medium only or hydrogen peroxide. Both PM10 and hydrogen peroxide induced an increase of procoagulant activity in culture supernatants from both cell types [shortening of clotting time versus control with PM10 was 31% with macrophages and 19% with epithelial cells; mean of three experiments, $P < 0.001$ and < 0.01 respectively]. In order to characterise the procoagulant activity from macrophages induced by PM10 we went on to determine the effects of these particles on surface expression of negatively charged phospholipid by annexin V binding, and tissue factor mRNA expression by RT-PCR. Finally we measured macrophage interleukin 8 (IL8) mRNA expression and release in response to PM10. Environmental particles induced macrophage tissue factor mRNA expression and significantly increased bound annexin V. Furthermore IL8 message and release were significantly enhanced also. We have demonstrated, for the first time, that environmental particles associated with increased cardiovascular risk can activate macrophages *in vitro* with transformation to a procoagulant phenotype which may be mediated through apoptosis/negatively charged phospholipid exposure and tissue factor expression. This may represent a novel prothrombotic mechanism triggered when environmental particles reach the systemic circulation.

Reference

1 Nemmar *et al. Circulation* 2002; **105**: 411–4.

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Is early activation of the clotting cascade involved in the pathogenesis of verotoxin-producing *Escerichia coli*-associated haemolytic uraemic syndrome?

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In most cases, haemolytic uraemic syndrome (HUS) is a thrombotic complication of infection with *Escherichia coli* O157 : H7. It usually follows bloody diarrhoea caused by verotoxin secreted by the bacteria, and comprises acute renal failure, non-immune, microangiopathic haemolytic anaemia and thrombocytopaenia. HUS is primarily a disorder of the vascular endothelium, with platelet aggregation at sites of endothelial cell damage in the renal microvasculature. Several *in vitro* studies demonstrate changes in endothelial cell anticoagulant properties when exposed to cytokines and under apoptotic conditions. Of interest are changes in tissue factor (TF) expression and activity, TF being crucial in initiating the coagulation cascade. Additionally, the expression of antithrombotic membrane components, notably thrombomodulin, has been shown to be downgraded. We examined the changes in TF and thrombomodulin on endothelial cells exposed to the *E. coli* products, verotoxin and lipopolysaccharide (LPS), and the inflammatory cytokines, interferon-gamma (IFNgamma) and tumour necrosis factor-alpha (TNFalpha), and their relationship to apoptosis. Macrovascular [primary culture of human umbilical vein endothelial cells (HUVEC)] and microvascular [human microvascular endothelial cells (HMEC)] endothelial cells were cultured with LPS and verotoxin in the presence and absence of cytokines. Surface TF and thrombomodulin expression and apoptosis were assessed using flow cytometry. Increases in TF expression were observed in activated HUVECs; changes were less apparent in HMECs which constitutively express TF. Thrombomodulin was consistently reduced on HMECs under similar conditions. It appeared not to be the apoptotic population that changed their expression of membrane proteins. HUVECs cultured with LPS and TNFalpha demonstrated the greatest increase in TF. Decreases in thrombomodulin were greatest on HMECs exposed to verotoxin. We demonstrate an up-regulation of TF and a down-regulation of thrombomodulin on endothelial cells stimulated with LPS, verotoxin and inflammatory cytokines. These cells are rendered more thrombogenic, mechanisms that may be involved in the pathogenesis of HUS.

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Paediatric and General Haematology

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Revealed: D-dimers are being inappropriately requested. Results of audit to influence subsequent requesting

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D-dimers, cross-linked fibrin degradation products, are extremely sensitive, but non-specific markers for coagulation activation; a process that complicates many conditions. They have been shown to be useful in negatively predicting for deep vein thrombosis (DVT), particularly in combination with a clinical assessment score for DVT. Junior medical staff and GPs are increasingly using the D-dimer test to screen for the presence of DVT. Radiology departments use a positive D-dimer to 'gate-keep' their Doppler service. Haematology departments have therefore identified a dramatic increase in D-dimer, requesting and associated cost (£26,000 in 2002 at Derriford Hospital). Over-investigation may lead to unnecessary, and potentially dangerous, further investigation and anticoagulation. This audit was conducted to address these concerns. The case-notes were reviewed from 50 consecutive patients presenting to/within Derriford Hospital with suspected DVT (34%) or pulmonary embolism (66%) in 2002. Not surprisingly, junior-most of the doctors requested the bulk of these investigations, 11% by registrars and none by the consultants. The reasons for requesting were documented in all but seven patients. The Biomericux MDA D-dimer assay kit was previously evaluated locally, giving a positive cut-off level of 0.5 mg/mL for DVT with >95% sensitivity. In the current group of patients, 28% fell below the cut-off value. Of the positive results, only 61% were investigated further. The management was not affected by the D-dimer result in 45% of cases. Of the original cohort, only four DVTs and two PEs were confirmed. One PE was diagnosed in spite of negative D-dimer. These data support the theory regarding over-requesting of D-dimers. The low specificity is highlighted by only six confirmed DVT/PEs of 72% patients having a raised D-dimer. Locally, the co-importance of validated clinical assessment score is emphasized and the futility/danger of performing D-dimers on

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in-patients and those with suspected PE until a specific cut-off value has been established.

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Anti-D immunoglobulin in the treatment of immune thrombocytopenic purpura in adults in the United Kingdom: an evaluation of clinical outcomes and resource utilization

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Immune thrombocytopenic purpura [ITP] is an autoimmune disorder distinguished by a low platelet count and mucocutaneous bleeding. Estimated incidence is 10 per 100,000 persons per year. ITP is classified as primary, or secondary to an underlying disorder, and acute (of 6 months or less in duration), or chronic. In 70% of children, resolution occurs in 6 months. By contrast, ITP in adults has an insidious onset, and is chronic in nature. Given comparable efficacy of anti-D immunoglobulin and IVIg in the treatment of ITP, this inquiry was designed to contrast health resource utilization patterns stemming from the use of each agent in the treatment of ITP in adults. Stemming from the schematic developed by Cines and Blanchette (1), and information contained in the report of the British Committee for Standards in Haematology, General Haematology Task Force (Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children, and in pregnancy) we employed Monte Carlo simulation to evaluate the utility (cost implications) of various treatment schema (intent-to-treat format; steroids; IVIg; anti-D immunoglobulin) for the treatment of ITP. Utilizing 100,000 replicate simulations per year of age examined [15 through 80 years (5-year intervals); quadratic regression equations] we discerned a significant ($P < 0.05$) efficacy/health service utilization-based saving (percent reduction) for the 'steroids to anti-D immunoglobulin' arm, relative to the 'steroids to IVIg' arm [34.0% (SE = 0.1%) for males; 33.4% (SE = 0.1%) for females]. In the United Kingdom, use of anti-D immunoglobulin in the management of ITP is estimated to result in reduced health service expenditures relative to IVIg. Specifically, use of anti-D immunoglobulin reduces the time required (cost) for medication administration, patient monitoring, and occupancy of health care facilities. The effect on patient's quality-of-life warrants further research.

Reference
1 Cines and Blanchett. *NEJM* 2002; 346: 995–1008.

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Reduced numbers and antigen unresponsiveness of natural killer T-cells in idiopathic (autoimmune) thrombocytopenia (ITP)

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Natural killer T cells (NKT cells) are a rare cell subset implicated in the control of autoimmunity. Here, untreated ITP patients and healthy controls were compared for the number of circulating NKT cells and their proliferative response to the NKT cell antigen alpha-galactosylceramide. The cells were identified as Valpha24 Vbeta11 double-positive (CD4 positive or CD4 CD8 double-negative) lymphocytes using flow cytometry. In controls, NKT cell frequency ranged from 0.008 to 0.351% of total lymphocytes (mean 0.13%, $n = 9$). In the ITP group, a low NKT cell frequency was much more common, resulting in a mean of 0.04% (range 0.002–0.336%, $n = 14$). The capacity for alpha-galactosylceramide-driven NKT cell expansion was variable in the ITP group, which could be divided into responders (55% of tested donors, $n = 13$) and non-responders (45%). In comparison, 92% of controls ($n = 13$) responded. Interestingly, repeated testing of a limited number of ITP patients showed that proliferative response to the antigen or unresponsiveness was not constant over time, thus one and the same patient may respond at one time but not another. Furthermore, while IL-2 increased antigen-induced NKT cell proliferation in the majority of controls, this was not observed in the ITP group. In conclusion, the number of circulating NKT cells is reduced in ITP and a proportion of ITP patients have both antigen and IL-2 unresponsive NKT cells. The lack of proliferative response after antigen exposure and the IL-2 unresponsiveness may be due to adaptive tolerance, or *in vivo* anergy. This is believed to be caused by a prior incomplete antigen activation *in vivo*. These observations therefore suggest that NKT cells from ITP patients are anergic and this may contribute to the autoimmune process. Future research is planned to assess NKT cell function and involvement in autoimmune thrombocytopenia.

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Outcome heterogeneity in childhood high hyperdiploid acute lymphoblastic leukaemia

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High hyperdiploid (HeH) karyotypes (51–65 chromosomes) are found in approximately one-third of children with acute lymphoblastic leukaemia (ALL). Generally, these patients respond well to standard therapy and enjoy a superior outcome compared with other children. It has been suggested that specific cytogenetic features might further refine this prognosis and provide a method for identifying those with a poorer outlook, however no consensual picture has yet emerged. We analysed outcome data from 700 HeH ALL children enrolled on two MRC trials (UKALLXI and ALL97) to examine the effect of sex, age, white blood cell (WBC) count, individual trisomics, modal number and structural abnormalities on survival. Univariate analysis showed that age (1–9 years), sex (female), trisomies of chromosomes 4, 10 and 18, and a modal number of 54–65 chromosomes were all associated with improved survival, whereas the presence or absence of structural abnormalities and WBC had no effect on outcome. Multivariate analysis revealed that only age, sex, trisomy 4 (+4) and trisomy 18 (+18) were independent indicators of outcome. These results enabled the construction of two hazard scores for predicting relapse and mortality. Three risk groups were found using age, sex and +18, with event-free survival (EFS) rates of 86, 75 and 50% at 5 years ($P < 0.0001$). The group with the highest risk of relapse comprised:

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boys aged >9 years, boys aged 1–9 years without +18 and girls >9 years without +18. While girls aged 1–9 years with +18 made up the group with the best EFS. In terms of mortality, those aged <10 years with both +4 and +18 had a significantly improved survival (96 versus 84% at 5 years, $P < 0.0001$). These findings confirm that the outcome of children with HeH ALL is heterogeneous and information regarding specific trisomies can help identify those with a greater risk of treatment failure.

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Correction of the anaemia of chronic disease (ACD) with small doses of recombinant erythropoietin

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The anaemia associated with chronic disease such as infectious, inflammatory or neoplastic conditions, contributes significantly to morbidity. It has been recognized recently that a similar anaemia is also seen in congestive cardiac failure (CCF) and diabetes without significant renal failure. Pharmacological doses of recombinant erythropoietin (EPO) correct ACD associated with rheumatoid arthritis, inflammatory bowel disease, and AIDS. Good responses to EPO have also been seen in cardiac failure, and type 1 diabetes. Large doses of EPO are used in some centres to treat anaemia associated with cancer and its treatment. We corrected ACD with small doses of EPO in 10 patients: six males and four females, age range 58–88 years. Three patients had inflammatory disease, three were diabetic, and four had CCF. Six had either normal or reactive bone marrow histology, normal cytogenetics, and normal or increased iron stores. Six were transfusion dependent prior to EPO therapy. The dose of subcutaneous recombinant human erythropoietin (Eprex) was titrated according to the haemoglobin. Nine patients were evaluated, one having died from his underlying disease. The mean dose required was 9000 units/week (range 3000–14,000). The average maximum increase in haemoglobin was 4.1 g/dL (range 1.3–6.8). Few side effects were encountered. One patient suffered transient cerebro-vascular ischaemia, possibly related to a rapidly rising haemoglobin. All patients became transfusion independent. All reported improved general health without the peak and trough effect associated with blood transfusion. Avoidance of red cell transfusion is desirable in view of the possible infective and non-infective complications of transfusion and concern over the availability and cost of blood products. Although EPO is expensive, the doses required were comparable with those used in chronic renal failure, and much smaller than usually required to treat anaemia associated with cancer and its treatment. The cost therefore, compared favourably with blood transfusion.

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Epidemiological evidence for an infectious origin for childhood leukaemia

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The aim of the study was to interpret the results of several analyses of childhood leukaemia data from the Manchester Children's Tumour Registry (MCTR), in view of current hypotheses regarding an infectious origin. The MCTR is population-based and has been collecting case data from a geographically defined region of North West England since 1954. Trend tests, space-time clustering tests (Knox and *K*-function), ecological regression and geographical mapping were used to study the data on the leukaemias, broken down by sub-type (ALL, AML). For the most recent period (since 1980), ALL was further examined by immunophenotypic sub-type. There was an increasing incidence of ALL, due mainly to an increase of the precursor B-cell sub-type in the childhood peak. Space-time clustering was evident for cases of ALL, aged 0–4 years, for the time period 1954–1985, mainly based on date of diagnosis and place of birth. For the time period 1980–2001, space-time clustering was confined to cases of precursor B-cell ALL, aged 18–54 months, based on date and place of birth. The incidence of ALL was greater in more densely populated wards, largely because of cases of non-precursor B-cell ALL. The studies present a complex picture, but are supportive of an infectious aetiology, particularly for precursor B-cell ALL. It is concluded that there may be at least two infectious mechanisms operating, with a different mechanism predominating in the earlier years from that in the later years.

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Eculizumab

Prop Inn; USAN

[redacted]

Humanized Anti-C5 Monoclonal Antibody

h5G1.1
5G1.1

Immunoglobulin, anti-(human complement C5 α-chain) (human-mouse monoclonal 5G1.1 heavy chain), disulfide with human-mouse monoclonal 5G1.1 light chain, dimer

CAS: 219685-50-4

EN: 239304

Abstract

Eculizumab (5G1.1) is a recombinant, fully humanized monoclonal antibody that inhibits the activation of terminal complement components. Eculizumab binds specifically to the terminal human complement protein C5, preventing the release of the inflammatory mediator C5a and the formation of C5b-9 by inhibiting the cleavage of human complement C5 to C5a and C5b. Preclinical studies have demonstrated that the antibody has high binding affinity for C5, blocks the formation of C5a and C5b-9, and protects mammalian cells from C5b-9-mediated damage. Favorable clinical results have been reported in patients with membranous nephritis, rheumatoid arthritis and paroxysmal nocturnal hemoglobinuria (PNH) and the antibody is in phase II clinical development for these disorders; phase I trials have also been conducted in dermatomyositis.

Introduction

There is increasing evidence that activated components of the complement system contribute significantly to the pathogenesis of a large number of acute and chronic inflammatory diseases including rheumatoid arthritis, atherosclerosis, acute myocardial infarction and vascular injury after cardiopulmonary bypass (1-5). Furthermore, complement plays a significant role in the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH), characterized by intravascular hemolysis and venous thrombosis, and associated with aplastic anemia. Patients with PNH have a deficiency in CD59 and CD55 proteins on the surface of erythrocytes, platelets and other blood cells, which normally protect cells from complement-mediated injury (6-8). Patients with PNH experience fatigue, dysphagia, pain and hemoglobinuria, and the disease is associated with about 50% mortality (9).

Strategies that inhibit the damaging effects of complement on disease pathogenesis have therefore become a major focus in pharmacological research. In particular, inhibition of the complement cascade at C5, the cleavage of which results in the generation of the potent inflammatory mediators C5a and C5b-9 (10), while allowing the upstream generation of C3b, essential for combatting infections and preventing the development of autoimmune diseases (11), would appear to represent a promising approach.

Eculizumab is a recombinant, fully humanized monoclonal antibody that inhibits the activation of terminal complement components. Eculizumab binds specifically to the terminal complement protein C5 and prevents the release of the inflammatory mediator C5a and the formation of C5b-9 by inhibiting the cleavage of human complement C5 to C5a and C5b (12). The antibody is being developed for chronic indications including rheumatoid arthritis, membranous nephritis, dermatomyositis, lupus nephritis and paroxysmal nocturnal hemoglobinuria (13).

Pharmacological Actions

Eculizumab, as well as Fab and scFv fragments thereof, was evaluated for binding to complement and inhibition of complement activation *in vitro*. Eculizumab had similar binding affinity for human C5 compared to the murine antibody (IC_{50} ~ 2 nM), as determined in an ELISA, and it inhibited human serum complement-induced lysis of porcine aortic endothelial cells (PAEC) at the same concentration as the murine antibody (12).

Pharmacokinetics

A double-blind, randomized, placebo-controlled study was conducted with eculizumab (8 mg/kg i.v. once or

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twice monthly) to evaluate the pharmacokinetics/pharmacodynamics in 117 patients with idiopathic membranous nephropathy (mean creatinine clearance = 111 ml/min; 24-h proteinuria = 9 g; protein/creatinine ratio = 5; serum cholesterol = 270 mg/dl; hemoglobin = 12.0 g/dl; serum albumin = 2.8 g/dl). Preliminary results were reported for 11 patients. Mean steady-state trough serum eculizumab levels were 18 μg/ml in the twice-monthly treatment group and 8 μg/ml in the monthly treatment group. Mean steady-state mid-dose interval levels (1 week postdose on day 119 of the study) were 47 μg/ml for patients treated twice monthly and 35 μg/ml for those treated once monthly. Urine levels of eculizumab at midtrial and at the end of the trial were below the level of quantification in 8 patients, and 3 patients excreted 65-400 μg/24 h of eculizumab. All eculizumab-treated patients demonstrated < 10% of the hemolytic activity of placebo-treated patients at 1 h postinfusion. Serum eculizumab levels > 38 μg/ml inhibited hemolysis in all patients. Treatment with eculizumab was found to be safe and well tolerated, with no hypersensitivity reactions noted (14).

Pharmacokinetics and pharmacodynamics were also examined in an open-label pilot study in 11 transfusion-dependent patients with PNH who received treatment with eculizumab (600 mg weekly for 4 weeks, then 900 mg every 2 weeks for 2 months). Peak and trough serum levels of eculizumab were measured 1 h after the initial dose and then at weeks 3, 4, 6, 10 and 12, and in 10 patients eculizumab levels were > 35 μg/ml from 1 h after the first dose to the end of the study period. In 1 patient, eculizumab levels were > 35 μg/ml for the initial 10 weeks but < 35 μg/ml at week 12. In 10 patients, serum hemolytic activity was 20% or less for the entire treatment period, representing complete blockade, with only 1 patient showing increased hemolytic activity at week 12, which corresponded with a decline in eculizumab levels to < 35 μg/ml (15).

Clinical Studies

The safety and biological activity of a single i.v. dose of eculizumab (0.1-8 mg/kg) were investigated in 40 patients with mild to moderate rheumatoid arthritis. Mean C-reactive protein (CRP) levels were significantly reduced at day 7 compared to baseline after the highest dose and returned to baseline by day 14. Patients treated at this dose showed a trend for reductions in the signs and symptoms of rheumatoid arthritis. Doses of 2 mg/kg and above resulted in > 80% inhibition of human complement C5 for at least 10 h, and a single dose of 8 mg/kg provided > 80% inhibition for 10 days, with a return to baseline levels after 17 days. Eculizumab was safe and well tolerated across patient groups (16).

Another study examined the safety, efficacy and pharmacokinetics of eculizumab following multiple doses in 209 patients with rheumatoid arthritis. Patients were randomized to receive placebo, eculizumab 8 mg/kg every 2 weeks, eculizumab 8 mg/kg every week x 5 weeks, then every 2 weeks, or eculizumab 8 mg/kg every week x 5 weeks, then monthly, and evaluated both after 3 months of treatment and 3 months after terminating treatment. After 3 months of treatment, the induction/monthly group met the primary endpoint of improvement in ACR20 score (44% *vs.* 18% on placebo), and both induction groups met the secondary endpoint of decrease in CRP. Baseline C5b-9 levels were correlated with increased CRP levels and therefore systemic inflammation. Baseline C5b-9 levels > 200 mg/ml were associated with clinical response to eculizumab. In cases of elevated C5b-9, an ACR20 response was obtained in 9% of patients in the placebo group, 33% of patients in the twice-monthly eculizumab treatment group, 57% of patients in the induction/monthly treatment group and 50% of patients in the induction/twice-monthly treatment group. The induction regimens completely suppressed complement-mediated serum hemolytic activity up to week 4. The half-life of eculizumab was 10-14 days (17-20).

A randomized, double-blind, placebo-controlled phase IIb clinical trial evaluated the efficacy and safety of eculizumab in 368 adult patients with mild to moderate active rheumatoid arthritis undergoing treatment with methotrexate and/or leflunomide. The patients were enrolled at 69 sites in the U.S. and Canada and were randomized to receive placebo, twice-monthly eculizumab (600 mg i.v. weekly for 5 weeks, then 600 mg once every 2 weeks) or monthly eculizumab (600 mg i.v. weekly for 5 weeks, then 600 mg monthly). The primary endpoint was improvement in ACR20 score after 6 months of treatment. Monthly administration of eculizumab following an induction period was associated with a statistically significant, moderate improvement in the ACR20 score compared to placebo at 6 months. The percentage of patients who achieved an ACR20 response was 34%, 24% and 22%, respectively, on monthly eculizumab, twice-monthly eculizumab and placebo. Monthly therapy was also associated with evidence of a long-term antiinflammatory effect, with a significant reduction in erythrocyte sedimentation rate at 6 months. The incidence of adverse events was slightly higher among patients given eculizumab every 2 weeks, although the incidence of injection-site reactions remained low (21).

A double-blind, placebo-controlled phase I pilot study was performed to determine the safety and tolerability of eculizumab (8 mg/kg weekly for 5 weeks, then once every 2 weeks) in 10 patients with dermatomyositis compared with placebo (n=3). After 2 months of treatment, results showed moderate improvements in skin score, physician global score and manual muscle test in the eculizumab group compared with controls. The placebo group showed deterioration of physician global score, but improvements in skin score and manual muscle test compared to baseline. Disease intensity measured by magnetic resonance imaging (MRI) and various parameters on skin biopsy were also improved in the treatment group compared with placebo. Eculizumab was safe and well

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tolerated, with no significant differences in adverse events between treatment groups (22).

Alexion has sponsored phase II trials in patients with membranous nephritis, with results showing good tolerance. One trial did not reach the primary clinical efficacy endpoint of reduction in proteinuria after 4 months, while in the other trial an increase in remission rate and significant improvements in proteinuria, hyperlipidemia, hypertriglyceridemia and hypoalbuminemia were obtained after 12 months. The company also began a phase II study in lupus nephritis several years ago following the observation of positive effects on proteinuria in a study in patients with systemic lupus erythematosus (13, 23).

Further results regarding efficacy and safety were reported from the open-label pilot study in 11 transfusion-dependent patients with PNH treated with eculizumab (600 mg weekly for 4 weeks, then 900 mg every 2 weeks for 2 months). After 3 months of initial treatment, all patients were enrolled into an open-label extension study and went on to receive eculizumab 900 mg every 2 weeks for over a year. In the 12 months prior to entrance into the study, patients had received over 4 red blood cell transfusions, had detectable glycosylphosphatidylinositol (GPI)-deficient hematopoietic clone, and had a negative throat culture for *Neisseria meningitidis* and *Neisseria gonorrhoeae*. After the first 3 months of treatment, serum hemolytic activity was completely inhibited in the 10 patients who showed serum trough levels of eculizumab above 35 µg/ml; quality-of-life markers were also significantly improved, transfusion rates decreased and serum levels of lactate dehydrogenase (LDH), an indicator of hemolysis, were significantly reduced. Increased levels of PNH type III erythrocytes were thought to be due to prolongation of PNH red cell survival and confirmed that treatment with eculizumab inhibits complement-mediated hemolysis. The proportion of PNH neutrophils, monocytes and platelets did not change. The reduction in transfusions was greatest for patients (n=7) without thrombocytopenia. Biochemical markers of hemolysis showed dramatic improvement 1 week after beginning treatment with eculizumab. The most common adverse events were headache and upper respiratory tract infections, followed by influenza-like symptoms, rigors, dizziness, nausea, nasal congestion and joint aches. No episodes of thrombosis were reported. No adverse events were considered to be drug-related and although 2 patients had serious adverse events, all patients completed the 12-week study (15, 24-26).

Reports of the cumulative results after 6 and 12 months of eculizumab treatment showed that patients with purely hemolytic disease (macroscopic hemoglobinuria with a normal platelet count; n=6) had the best response to treatment, with type III erythrocytes increased from 39% to 67%, median transfusions reduced from 2.0 units/patient/month to 0 units/patient/month, and suppression of hemolysis. Four patients who did not become transfusion-independent were treated with additional subcutaneous erythropoietin with some improvement. Two patients reported breakthroughs in complement activity, demonstrated by increased lactate dehydrogenase levels, hemoglobinuria and symptoms. These symptoms resolved immediately after the next dose of eculizumab and patients were managed by reducing the dosing interval. The most commonly reported adverse events after 12 months of eculizumab treatment were flu-like symptoms, sore throat, headache and upper respiratory infection. Four serious adverse events were reported, but there were no study discontinuations as a result of adverse events and all patients elected to continue eculizumab treatment. It was concluded that eculizumab was well tolerated, decreased hemolysis and transfusion requirements, and improved symptoms of PNH for over 12 months (27, 28).

Conclusions

Eculizumab is a promising new monoclonal antibody that binds to human complement C5 and inhibits its cleavage by C5 convertase. Eculizumab has been granted orphan drug status for the treatment of PNH in both the U.S. and Europe. A pilot program has been completed in patients with PNH and plans are being finalized with the FDA for the next stage of development. Pilot programs have also been completed in patients with dermatomyositis and eculizumab is in phase II trials for rheumatoid arthritis and membranous nephritis.

Source

Alexion Pharmaceuticals, Inc. (US).

References

1. Morgan, B.P. *Clinical complementology: Recent progress and future trends.* Eur J Clin Invest 1994, 24: 219-28.

2. Morgan, B.P. *Complement and rheumatic disease.* In: Complement, Clinical Aspects and Relevance to Disease. B.P. Morgan (Ed.), Academic Press, New York, 1990, 130.

3. Torzewski, M., Klouche, M., Hock, J., Messner, M., Dorweiler, B., Torzewski, J., Gabbert, H.E., Bhakdi, S. *Immunohistochemical demonstration of enzymatically modified human LDL and its colocalization with the terminal complement complex in the early atherosclerotic lesion.* Arterioscler Thromb Vasc Biol 1998, 18: 369-78.

4. Rus, H.G., Niculescu, F., Vlaicu, R. *The relationship between macrophages and C5b-9 complement complexes in human atherosclerosis.* Clin Immunol Immunopathol 1988, 48: 307-16.

5. Kilgore, K.S., Friedrichs, G.S., Homeister, J.W., Lucchesi, B.R. *The complement system in myocardial ischaemia/reperfusion injury.* Cardiovasc Res 1994, 28: 437-44.

6. Holguin, M.H., Fredrick, L.R., Bernshaw, N.J., Wilcox, L.A., Parker, C.J. *Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of*

Samsung Bioepis Exhibit No. 1002
Page 6436

the erythrocytes of paroxysmal nocturnal hemoglobinuria. J Clin Invest 1989, 84: 7-17.

7. Rollins, S.A., Sims, P.J. *The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9.* J Immunol 1990, 144: 3478-83.

8. Morgan, B.P. *Isolation and characterization of the complement-inhibiting protein CD59 antigen from platelet membranes.* Biochem J 1992, 282: 409-13.

9. Hillmen, P., Lewis, S.M., Bessler, M., Luzzatto, L., Dacie, J.V. *Natural history of paroxysmal nocturnal hemoglobinuria.* New Engl J Med 1995, 333: 1253-8.

10. Matis, L.A., Rollins, S.A. *Complement-specific antibodies: Designing novel anti-inflammatories.* Nat Med 1995, 1: 839-42.

11 Liszewski, M.K., Atkinson, J.P. *The complement system.* In: Fundamental Immunology, W.E. Paul (Ed.), Raven Press, New York, 1993, 917-39.

12. Thomas, T.C., Rollins, S.A., Rother, R.P., Giannoni, M.A., Hartman, S.L., Elliott, E.A., Nye, S.H., Matis, L.A., Squinto, S.P., Evans, M.J. *Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv.* Mol Immunol 1996, 33: 1389-401.

13. *Product profiles: Autoimmune.* Alexion Pharmaceuticals Web Site July 13, 2004.

14. Appel, G., Nachman, P., Hogan, S. et al. *Eculizumab (C5 complement inhibitor) in the treatment of idiopathic membranous nephropathy (IMN): Preliminary baseline and pharmacokinetic (PK)/pharmcodynamic (PD) data.* J Am Soc Nephrol 2002, 13: Abst SU-P0970.

15. Hillmen, P., Hall, C., Marsh, J.C.W. et al. *Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria.* New Engl J Med 2004, 350: 552-9.

16. Jain, R.I., Moreland, L.W., Caldwell, J.R., Rollins, S.A., Mojcik, C.F. *A single dose, placebo controlled, double-blind, phase I study of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* 63rd Annu Meet Am Coll Rheumatol (Nov 13-17, Boston) 1999, Abst 42.

17. Kivitz, A., Burch, F., Birbara, C., Rollins, S., Tesser, J. *Pharmacokinetics (PK) and pharmacodynamics (PD) of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S214.

18. Burch, F., Tesser, J., Bell, L. *Baseline C5b-9 level correlates with CRP and ACR 20 response to the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S214.

19. Tesser, J., Kivitz, A., Fleischmann, R., Mojcik, C.F., Bombara, M., Burch, F. *Safety and efficacy of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S274.

20. *Phase II clinical results for 5G1.1 anticomplement antibody support further testing in RA.* DailyDrugNews.com (Daily Essentials) Nov 19, 2001.

21. Mojcik, C.F., Kremer, J., Bingham, C., Burch, F., Vitiello, S., McCroskery, E., Bookbinder, S. *Results of a phase 2B study of the humanized anti-C5 antibody eculizumab in patients with rheumatoid arthritis.* Ann Rheum Dis 2004, 63(Suppl. 1): Abst FRI0170.

22. Takada, K., Bookbinder, S., Furie, R., Oddis, C., Mojcik, C., Bombara, M., Plotz, P., Kissel, J. *A pilot study of eculizumab in patients with dermatomyositis.* 66th Annu Meet Am Coll Rheumatol (Oct 25-29, New Orleans) 2002, Abst 1300.

23. *Alexion reports presentation of membranous nephritis clinical trials.* Alexion Pharmaceuticals Press Release November 4, 2002.

24. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Cullen, M., Richards, S.J., Rother, R.P. *Eculizumab, a C5 complement blocking antibody, is the first therapy to reduce transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria (PNH).* Br J Haematol 2003, 121(Suppl. 1): Abst 257.

25. Rother, R.P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Richards, S., Cullen, M., Hillmen, P. *Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and reduces transfusion dependency in patients with paroxysmal nocturnal hemoglobinuria (PNH).* Mol Immunol 2003, 40(2-4): Abst 54.

26. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Richards, S., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and renders hemolytic patients with paroxysmal nocturnal hemoglobinuria (PNH) transfusion independent.* Blood 2002, 100(11, Part 1): Abst 154.

27. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Richards, S., Rollins, S.A., Mojcik, C.F., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, controls hemolysis in paroxysmal nocturnal hemoglobinuria (PNH) with responses maintained over a prolonged period of therapy.* Blood 2003, 102(11, Part 1): Abst 1858.

28. Hillmen, P., Hall, C., Marsh, J.C.W., Elebute, M., Richards, S.J., Rollins, S.A., Mojcik, C.F., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, controls haemolysis in paroxysmal nocturnal haemoglobinuria (PNH) with responses maintained over a prolonged period of therapy.* Br J Haematol 2004, 125(Suppl. 1): Abst 5.

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Eculizumab Composition / ALEXION

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This is in response to the Request for Documents pursuant Rule 83 EPC dated August 17, 2017, and the Brief Communication dated September 25, 2017.

We herewith submit the documents cited as D2, D4, D5 and D6 in our Notice of Opposition dated August 9, 2017.

Leo Polz

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Applicant/Proprietor: Alexion Pharmaceuticals, Inc.

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Oral Communication

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Eculizumab, a C5 complement blocking antibody, is the first therapy to reduce transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria (PNH)

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Paroxysmal nocturnal haemoglobinuria (PNH) is characterized by intravascular haemolysis and venous thrombosis due to the unopposed effect of complement on PNH red cells and platelets, respectively. Eculizumab is a humanized monoclonal antibody that binds C5 and inhibits terminal complement activation. Eleven transfusion-dependent PNH patients have completed a 3-month pilot study receiving 600 mg eculizumab by intravenous infusion over 30 min weekly for four doses and then 900 mg every other week. Adverse events were relatively minor and self-limiting. The biochemical parameters of haemolysis dramatically improved for all patients within a week. Mean LDH decreased from 3089 ± 599 U/L before to 597 ± 32 U/L after treatment ($P = 0.002$) and AST from 77 ± 20 to 26 ± 3 U/L ($P = 0.04$). Episodes of haemoglobinuria reduced by 90% ($P < 0.001$). Quality of life (EORTC QLC30) improved significantly post-eculizumab compared to pre-eculizumab. One patient began treatment during a severe acute haemolytic episode with almost black urine and within 24 h there was a complete resolution of her haemoglobinuria and symptoms. Transfusions for all patients were significantly reduced in the 3 months of eculizumab compared with the previous 12 months from 2.2 to 0.7 units/month ($P = 0.003$). The reduction in transfusions was most dramatic in the seven patients without thrombocytopenia from 2.3 to 0.2 units/month ($P = 0.001$). There was an increase in PNH type III red cells from a mean of 35.6% pre-eculizumab to 58.7% post-eculizumab ($P = 0.005$) due to their protection from complement-mediated lysis and the reduction in transfusions. There was no change in the proportion of PNH neutrophils, monocytes or platelets. All patients have continued on eculizumab in an extension study. In summary, eculizumab is well tolerated, decreases haemolysis, improves symptoms and significantly reduces transfusions in PNH patients. Eculizumab appears to be the first effective therapy for haemolysis in PNH and merits further development. This is the first trial of an anti-complement antibody therapy in PNH. The responses were remarkable both in reduction in haemolysis and transfusions and in the improvement in quality of life. Thus it is the first effective therapy for haemolysis in PNH and would also be expected to reduce the risk of thrombosis (this study could not address this). The trial was performed at two centres in the UK only.

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The induction of tolerance to RhD in HLA DR15 transgenic mice

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The RhD protein is a clinically important red cell alloantigen that induces the production of anti-D in RhD-negative individuals. Because of the immunogenicity of RhD, all blood donations are typed and matched for this antigen, and RhD-negative women are managed for the prevention of haemolytic disease of the newborn with prophylactic anti-D immunoglobulin. Understanding the mechanisms by which helper T-cells control the antibody response to RhD would enable the design of alternative strategies such as active toleration. We have shown that CD4+ helper T cells from alloimmunized donors can proliferate and produce both IFN-gamma and IL-4 in response to synthetic peptides derived from the RhD sequence, indicating a Th0 balanced cytokine response. The responses are HLA class II restricted, with HLA-DR15 being over-represented in good responders. Certain peptides are immunodominant, and induce proliferative responses in most alloimmunized donors. These peptides are candidates for the induction of tolerance to the RhD protein by the mucosal route. On the other hand, some peptides from the RhD protein preferentially induce IL-10 production by putative Tr1 regulatory cells *in vitro*, and these peptides inhibit T-cell proliferation in response to stimulation with purified RhD protein *in vitro*. These peptides may be used to induce tolerance by the parenteral route. We have used a HLA-DR15 transgenic mouse model to show that T-cells from mice immunized with the RhD protein proliferate in response to purified RhD protein and to RhD peptides, and serum antibodies specific for human erythrocytes are also produced. Mucosal administration of immunodominant RhD peptides, or parenteral administration of IL-10 inducing peptides prior to immunization with RhD protein, inhibits both T-cell proliferation and antibody production. These results suggest that synthetic RhD peptides can induce tolerance to the RhD protein and may be of value in the prevention of immunization in RhD-negative individuals.

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A humanised monoclonal antibody against vWF A1 domain inhibits vWF:RiCof activity and platelet adhesion in human volunteers

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AJW200 (Ajinomoto), a humanized IgG4 monoclonal antibody directed against the A1 domain of von Willebrand factor (vWF), specifically inhibits vWF : platelet GPIb receptor interactions. At

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high shear, it inhibits platelet adhesion, aggregation and activation, and in a dog model, inhibits arterial thrombus formation, without prolonging the bleeding time. In a randomized, double blind study, placebo, 0.01, 0.03, or 0.05 mg/kg AJW200, were infused i.v., into 24 healthy men. No clinically significant adverse events were recorded and there was no evidence of immunogenicity. Mean PK results were: C_{max} 204.8, 586.2, 833.2 ng/ml; T_{max} 1.0, 0.92, 0.57 h, T/2 23.5, 24.3, 27.2 h, for ascending doses. The maximum vWF occupancy by AJW200 was 19.4, 51.0 and 62.4%, showing correlation with plasma AJW200 concentration. Ristocetin cofactor (RiCof) was reduced 1 h post-infusion compared to baseline: mean (SD) 58 (22) and 110 (25)%, $P < 0.0005$ and 34 (16) and 116 (41)%, $P < 0.005$, at 0.03 and 0.05 mg/kg respectively, for up to 12 h. vWF : Ag, FVIII, vWF multimers and cleaving protease activity were unchanged. Template bleeding time (BT) was normal at all AJW200 doses, but the PFA-100 showed prolonged closure times 1 h post-infusion, with both CADP: 146 (22) and 82 (10) and 202 (78) and 80 (19) s, $P < 0.01$; and CEPI cartridges: 182 (28) and 108 (16) and 233 (56) and 107 (19) s, $P < 0.01$, at 0.03 and 0.05 mg/kg AJW200, respectively. This effect lasted for 3–6 h with the lower and up to 12 h for the higher dose. The RiCof and PFA-100 responses showed dose dependent inhibition and the magnitude of the effect appeared to be related to the baseline vWF level. AJW200 showed inhibition of vWF : RiCof activity and PFA-100 responses, without prolonging the skin bleeding time, and merits further clinical studies for thrombotic disorders. This is the first study demonstrating the effect of a monoclonal antibody that blocks GPIb/vWF in humans.

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Phase I study of the adoptive transfer of tetramer selected cytomegalovirus-specific cytotoxic T cells after allogeneic stem cell transplantation

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Cytomegalovirus (CMV) remains an important cause of morbidity and mortality following stem cell transplantation (SCT). Adoptive transfer of donor CMV-specific CD8+ T cell clones has been shown to protect against CMV reactivation in previous studies. However, this procedure has not been widely used due to the need for extensive *ex vivo* culture of T cells prior to infusion. We have combined HLA-peptide tetramer staining with the use of magnetic beads against phycoerythrin to facilitate the rapid selection of CMV-specific CTL from donor peripheral blood using a CliniMACS machine. In this study we infused tetramer selected donor CMV-CTL to six allogeneic stem cell transplant (SCT) recipients following CMV reactivation. An additional patient had undergone transplantation from a matched unrelated donor and CTL were infused for treatment of CMV reactivation refractory to Foscarnet, Ganciclovir and Cidofovir. In the 6 sibling allografts between 10^5 and 1.6×10^6 (mean 6×10^5) CMV-specific CD8+ T cells were infused from 250 mL of donor peripheral blood. The unrelated donor transplant patient received 2×10^6 CMV-CTL isolated from a cryopreserved lymphopheresis product. No infusion reactions were noted. One patient developed grade II GvHD which responded to treatment. CMV-CTL were not detectable prior to infusion in three of six patients but were detected in all patients following infusion. CMV-CTL expanded to represent up to 22.1% of the CD8 compartment. This expansion occurred in the first 14 days followed by a contraction to a basal level. Four patients did not require antiviral therapy. In the unrelated transplant recipient CMV-specific CTL reached a peak of 0.6% of the CD8 compartment and CMV antigenaemia became negative 6 days following CTL infusion. This novel approach to the adoptive transfer of antigen-specific CTL is rapid and relatively straightforward. It has considerable potential in the development of antigen-specific T cell therapy after allogeneic transplantation.

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Bone marrow mesenchymal stromal cells protect chronic lymphocytic leukaemia (CLL) from spontaneous and drug-induced apoptosis

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CLL cells are able to survive for prolonged periods *in vitro* and yet in most instances undergo rapid spontaneous apoptosis when cultured *in vitro*. Sustained viability *in vitro* is probably maintained by contact with 'nurse-like' cells in the peripheral blood (PB) and the bone marrow (BM) microenvironment. An *in vitro* model to mimic *in vitro* conditions was developed using adherent BM mesenchymal stromal cells (MSCs) from the iliac crest of normal donors. This model was used to test protection of CLL PB mononuclear cells both from apoptosis and from the effects of CD20 (Rituximab) and CD52 (CAMPATH-1H) therapeutic monoclonal antibodies, the proteasomal inhibitor MG132 and Bis9, an inhibitor of protein kinase C. Levels of apoptosis were determined by flow cytometry, using AnnexinV-FITC and propidium iodide. BM MSCs abrogated spontaneous apoptosis of CLL cells over a 5-day period of culture (80–90% viability, $n = 9$). Protection required CLL cells to be in close contact with the MSCs; separation by a microporous membrane induced apoptosis, comparable to that seen in control samples cultured without MSCs (30–40% viability). Rituximab treatment did not have any obvious apoptotic effect on CLL cells *in vitro* but CAMPATH-1H was able to induce apoptosis in a subpopulation of cells even in the presence of MSCs. Treatment with Bis9 and MG132 caused substantial and rapid apoptosis of CLL cells (0–15% viability $n = 3$). At lower doses of both drugs (200 nM), this apoptosis was prevented by MSC co-culture (70–90% viability). MSCs can therefore maintain CLL viability *in vitro* and can influence drug-induced apoptosis. The critical cell–cell interactions remain unknown, but are likely to include the beta-integrin, TNFSF13B and the CXCR4/CXCRL12 pathways. The expression of these molecules and their possible interactions in maintaining CLL viability will be investigated to assess if any might be targeted therapeutically.

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Targeting FGFR3 in multiple myeloma: the use of SU5402 and PD173074 to inhibit t(4;14) positive cells

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The t(4;14)(p16.3;q32), associated with 10–20% of cases of multiple myeloma (MM), deregulates the expression of two genes, MMSET and FGFR3. Most t(4;14) positive myeloma patients express FGFR3 and constitutive activation of this receptor is seen in some cases. To assess the potential of FGFR3 as a drug target we evaluated the effects of three FGFR inhibitors (SU6668, SU5402 and PD173074) on control and MM cell lines. Activity was assessed initially against Ba/F3 cells transformed to IL-3 independence by ZNF198-FGFR1, a constitutively active fusion gene associated with the 8p11 myeloproliferative syndrome (EMS), and compared to activity against control BaF3 cells grown with IL-3. Three methods were employed: MTS to assess proliferation/metabolic activity, trypan blue staining to determine cell viability and TUNEL to assess apoptosis. SU6668 inhibited the growth of both transformed and control cells lines at concentrations of >20 μM and therefore was not pursued further. SU5402 and PD173074 specifically inhibited the growth of ZNF198-FGFR1 transformed cells in a dose-dependent fashion with an IC50 of 5 μM and 5 nM, respectively. Both compounds also caused decreased cell viability and increased apoptosis. These compounds were then tested against two t(4;14) positive MM lines, KMS-11 and OPM-2, which express FGFR3 and harbour activating mutations of this gene. Dose-dependent growth inhibition was observed in both lines with IC50s similar to those in ZNF198-FGFR1 transformed cells. Importantly, inhibition was still observed in the presence of IL-6. Both compounds induced a dose-dependent reduction in cell viability and an increase in apoptosis, accompanied by a decrease in FGFR3 and ERK phosphorylation. In contrast, no inhibition was seen with either compound against the t(4;14) negative lines U266 and HS-Sultan, or the t(4;14) positive, FGFR3 negative cell line KMS-18. FGFR3 is thus a plausible candidate for targeted therapy in a subset of MM patients.

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Myeloid Malignancy

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Decreased expression of a novel growth regulatory protein, Nov, in chronic myeloid leukaemia

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Chronic myeloid leukaemia (CML) is characterized by the constitutively active Bcr-Abl tyrosine kinase. We have used a murine multipotent haematopoietic stem cell line, FDCP-MIX, expressing a temperature-sensitive mutant Bcr-Abl (ts Bcr-Abl FDCP-MIX), to investigate changes in gene expression using DNA microarray analysis. RNA was prepared from cells expressing the Bcr-Abl tyrosine kinase and from control cells, labelled with biotin and hybridized to an Affymetrix Mu6500 array (Affymetrix, USA). The Bcr-Abl kinase activity resulted in differential expression of 300 genes, the most significant being a 25-fold decrease in expression of a novel gene called nephroblastoma overexpressed (Nov). Northern blot analysis detected a 4-fold reduction in Nov expression in the Bcr-Abl positive cells in comparison with the control cells. Western blot analysis of immunoaffinity-purified Nov detected a two-fold reduction in expression as a result of the Bcr-Abl kinase activity. Confocal microscopy revealed strong vesicular cytoplasmic staining for Nov in FDCP-MIX control cells whilst fluorescence was barely detectable in cells expressing Bcr-Abl. A similar pattern of Nov expression was observed in primary human cells (five CML, five normal controls). Normal CD34+ progenitor cells uniformly expressed an intense fluorescence signal for Nov expression. In contrast, the majority of CD34+ cells within the CML patient samples were negative ($P < 0.001$). Nov expression has previously been associated with cell differentiation, quiescence and has been shown to promote cell adhesion. Loss of these properties is consistent with the CML phenotype.

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Reduced hTR expression in CML CD34+ cells at diagnosis: a novel mechanism for telomere loss in leukaemia

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CML is characterized by massive myeloid expansion, and significant telomere loss has been documented at diagnosis and during disease progression. Critical telomere shortening results in chromosomal instability, which can lead to disease progression. The regulatory mechanisms and expression levels of the key telomerase components (hTERT and hTR, the reverse transcriptase and RNA template respectively) in the CML stem cell compartment have not been described; however studies in solid tumours and cell lines generally show increased expression of both components. We selected CD34+ cells from 16 CML diagnostic leucapheresis specimens and 12 non-CML mobilized PBSC (control population) (median purity 94.1, 96.3% respectively; BCR-ABL positive in CML group). High-resolution cell cycle analysis demonstrated increased cycling in the CML CD34+ fraction. Q-RT-PCR for full-length (functional) hTERT showed an approximately two-fold rise in CML expression compared with control cells. RT-PCR with primers designed to detect the full-length hTERT transcript plus the three non-functional splice variants (with quantitative analysis) demonstrated a shift away from splicing in the CML samples. By combining these two complementary techniques we determined that expression of all (i.e. full length plus splice variants) transcripts are not different between CML and control cells: the increased levels of functional hTERT mRNA appear to be accounted for mainly by a reduction in the splicing out of the active sites. Surprisingly, Q-RT-PCR for hTR demonstrated a five-fold reduction in hTR levels in CML compared with control cells. Even more striking was the difference in hTERT/hTR ratio: 1 : 180 in CML cells versus 1 : 1800 in non-CML cells. We therefore hypothesize that despite increased hTERT levels there is a disassociated reduction in hTR, which in conjunction with increased cell cycling and replication

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induced telomere loss, could alter telomere homeostasis in CML. This could contribute to disease evolution via chromosomal instability.

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Reduced-intensity allogeneic haematopoietic stem cell transplantation (HSCT) using fludarabine, busulphan and alemtuzumab conditioning in 63 patients with myelodysplastic syndromes (MDS)

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We report the largest series of patients with MDS who have received a reduced-intensity HSCT using Fludarabine 30 mg/m^2 i.v. (day −9 to −5); busulphan 4 mg/kg/day orally (day −3 to −2) and alemtuzumab 20 mg i.v. (day −5 to −1). Immunosuppression comprised ciclosporin from day −1 titrated to plasma troughs of 150–200 ng/L. 63 patients with MDS (RA = 16, RAEB-I = 9, RAEB-II = 11, CMML = 5, TLD-AML = 21, aCML = 1) have received transplants from matched sibling (MSD) (24) or volunteer unrelated donors (VUD) (39). Except for RA, all had received at least one course of chemotherapy. Twenty-two of 24 MSD utilized PBSCs compared with 19 of 39 VUD HSCTs, with a median CD34+ cells of 4.6×10^6/kg (1.83–17.5) and 3.9×10^6/kg (0.44–16.7) respectively. There was no difference in engraftment latency, with the median time to neutrophil engraftment of 15 days (4–57) and platelets 18 days (7–105). 85% achieved full donor chimerism with a 6% incidence of grade III/IV aGvHD (43% grade I–IV) and 3% extensive cGvHD. At a median follow-up of 343 days (50–1213) for MSD, and 248 days (37–1316) for VUD recipients, 63 and 62% were alive respectively. The median age of sibling allografts recipients was 54 years (41–70). At day +200, the TRM was 5% and DFS 68%. The median age for VUD recipients was 49 years (25–58). The TRM at day +200 was 21% with DFS 65%. At 1 year, the estimated OS was 66% for VUD and 77% for MSD, with a DFS of 57 and 53% respectively. Twenty-one received DLI at a median of day +273 (126–1290); 11 for relapse and 10 for declining donor chimerism. A graft versus leukaemia effect was demonstrable. Although longer follow-up is required, RIC allogeneic HSCT appears to be safe and feasible in this high-risk group of patients.

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Combined clofibric acid and medroxyprogesterone acetate exert multiple anti-leukaemic actions against acute myeloid leukaemia cells and cell lines

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Poor survival rates in acute myeloid leukaemia (AML) relate to both the failure of cytotoxic chemotherapy and inability of older patients to tolerate that therapy. Thus non-toxic drugs that specifically target the differentiation and apoptotic pathways of AML cells are urgently required. We and others have demonstrated that fibrates such as clofibric acid (CA) and inhibitors of the aldo-keto reductase AKR1C3, including medroxyprogesterone acetate (MPA), potentiate all trans-retinoic acid (ATRA) induced HL-60 cell differentiation. Fibrates are pharmacological PPARalpha ligands commonly used in the treatment of hyperlipidaemia whereas MPA is used to treat breast carcinoma. We recently demonstrated that, potentiation of HL-60 differentiation by AKR1C3 inhibitors occurs via the consequent activation of PPARgamma. Thus combined CA and MPA (CA/MPA) provide the means to simultaneously target PPARalpha and gamma using established drugs with low systemic toxicities. Here we demonstrate that CA/MPA killed cultured primary AML blasts in the presence or absence of IL3 and SCF ($P = 0.0001$) but not normal CD34+ve myeloid blasts. CA/MPA also sensitized AML cell killing by suboptimal doses of daunorubicin and Ara-C ($P < 0.003$) highlighting the drugs' potential as chemosensitizing agents. Although ATRA did not enhance AML cell killing by CA/MPA, all three drugs together resulted in the differentiation of the surviving cells from three of seven non-acute promyelocytic AML samples. Finally, high serum Nm23-H1 protein concentrations have been associated with the poorest prognosis in AML. Here we provide the rationale for this by demonstrating that Nm23-H1 acts as an autocrine survival factor. Importantly we also demonstrate that CA/MPA rapidly down-regulated Nm23-H1 expression in those AMLs that had highest initial expression ($P = 0.001$). We conclude that CA/MPA exert multiple antileukemic actions against AML cells and should be considered as adjuncts in the treatment of elderly and relapsed AML.

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The utility of *in vitro* testing for imatinib sensitivity in BCR-ABL negative myeloproliferative disorders

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Imatinib mesylate (STI571, Glivec) inhibits the tyrosine kinase activity of the protein tyrosine kinases ABL, ARG, PDGFRA, PDGFRB and KIT, and has proven clinical efficacy in the management of patients with BCR-ABL positive CML and MPDs with PDGFRB rearrangements. However, it is not known to what extent patients with BCR-ABL negative MPDs may harbour activating rearrangements or point mutations of imatinib-responsive genes. To test for imatinib responsiveness we have employed two *in vitro* assays. First, blood or marrow mononuclear cells (MNC) were grown in methylcellulose supplemented with growth factors and treated with 0, 1 or 5 μM imatinib. Colonies were counted on days 7 and 14. Secondly, blood or marrow MNCs were placed in liquid culture with 0, 1 and 5 μM imatinib and cell numbers were enumerated over 2–3 weeks. Imatinib-treated cells from CML patients showed a reduction in colony and cell numbers over time compared with untreated cells and treated cells from normal individuals. We have obtained results using one or both methods in 38 BCR-ABL, TEL-PDGFRB negative patients who had been diagnosed with atypical CML, BCR-ABL negative MPD, CMML, mastocytosis, myelofibrosis or hypereosinophilic syndrome (HES). Eleven patients showed a positive response, four of whom had a PDGFRB rearrangement as determined by FISH and one had TEL-ABL. The remaining six patients had HES ($n = 2$), secondary MDS with eosinophilia following treatment for AML ($n = 1$), aCML ($n = 1$), MF ($n = 1$) or mastocytosis ($n = 1$). Six

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patients (five with PDGFRB or ABL rearranged MPD and one with HES) were treated and all showed a cytogenetic and/or haematological response. We conclude that *in vitro* cultures are useful for predicting patients who might respond clinically to imatinib. Evidence to date suggests that most BCR-ABL negative patients who are imatinib-responsive have elevated eosinophil counts.

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Identification of a novel gene, GEMS, that is fused to FGFR1 in the 8p11 myeloproliferative syndrome

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The 8p11 myeloproliferative syndrome (EMS) is an atypical stem-cell myeloproliferative disorder which is caused by activation of the transmembrane receptor tyrosine kinase FGFR1. This typically occurs as a result of fusion of the tyrosine kinase domain of FGFR1 to an unrelated partner gene, which promotes dimerisation and consequent activation of the kinase in the absence of an appropriate ligand. To date, five FGFR1 partner genes have been identified: ZNF198 at 13q12, FOP at 6q27, CEP110 at 9q33, BCR at 22q11 and HERV-K at 19q13. In this study, we describe a patient with a clinical picture typical of EMS, who was found to have an acquired ins(12;8)(p11;?p11p22) by cytogenetics. Southern blotting and FISH were used to confirm that FGFR1 was disrupted and RT-PCR analysis indicated that the patient did not harbour any of the known FGFR1 fusion mRNAs or BCR-ABL. To identify the partner gene in this patient we employed 5′-RACE PCR, exploiting the fact that all known FGFR1 translocations generate mRNA fusions in which the partner gene is spliced to FGFR1 exon 9. A 629-bp product was obtained which was found to consist of sequence derived from chromosome band 12p11 fused to exon 9 of FGFR1. Analysis of ESTs and other clones indicated that the sequence was derived from a widely expressed novel gene that we have called GEMS (gene disrupted in eight p11 myeloproliferative syndrome). GEMS is predicted to be translated into a 29-kDa protein that contains an N-terminal coiled-coil domain but no other recognisable motifs. RT-PCR confirmed the presence of the GEMS-FGFR1 fusion but the reciprocal chimaeric mRNA was not detected. Experiments to determine the transforming ability of this novel fusion gene are underway.

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Haemostasis and Thrombosis 2

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Venous thromboembolism (VTE) associated with the management of acute thrombotic thrombocytopenic purpura (TTP)

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VTE is reported to occur in 3% of TTP/HUS cases following plasma exchange (PEX) therapy. From May 1997 to May 2002, 68 patients with acute TTP were treated. Eight documented VTE episodes (six DVTs and three PEs) were identified in seven patients (all female, mean age 31 years) during therapy for acute TTP (five primary presentation and three relapses). All six DVTs were associated with central lines at the site of thrombosis. Other precipitating factors included pregnancy (one), immobility (eight), obesity (three) and FV Leiden heterozygosity (one). VTE occurred at a mean of 53 days following the first PEX. Solvent detergent plasma (Octaplas, Octapharma, Austria) was the last plasma to be used in PEX prior to the VTE in seven of eight events. Octaplas was used as the sole plasma for three TTP episodes and in the other episodes was substituted for cryosupernatant or FFP owing to poor response (four) or allergy (one). The mean volume of Octaplas replacement prior to the VTE was 100 L (range 22–206). The mean platelet count at VTE was 161 (range 12–232) $\times 10^9$/L. The platelet count had been in the normal range for a mean of 18 days prior to the VTE in those with a normal platelet count (six of eight). One patient received prophylactic LMW heparin from initial TTP diagnosis and seven of eight received aspirin. In conclusion, we identified VTE in 12% of TTP cases and 88% of these were associated with Octaplas replacement in PEX. VTE is a multifactorial disease and several known precipitating factors are present in TTP patients. The use of large volumes of Octaplas in PEX may be an additional risk factor, possibly associated with protein S (PS) deficiency, particularly as the median functional PS in Octaplas is 58 IU/dL compared with 91 IU/dL and 102 IU/dL in FFP and cryosupernatant respectively.

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Results of D-dimer in the UK NEQAS (Blood Coagulation) proficiency testing programme: urgent need for improvements in standardisation of testing and reporting

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We report the proficiency testing of D-dimer in diagnostic departments. Two lyophilised samples were tested for D-dimer by over 240 centres using different methods. Around 30% used semi-quantitative tile-based latex agglutination assays and 70% used automated techniques including 20% who reported in fibrinogen equivalent units (FEU). Six automated methods were used in 10 or more centres and the median and range of results obtained with these methods are shown below in ng/mL. For sample 1, 70% of centres reported a result above the cut-off for DVT. For one method the median test result was within the normal range, whereas for other methods the median was 40–80% above the normal range depending on the technique. It was a major cause for concern that 24% reported a normal result for this sample. There appeared to be a poor understanding of the difference between the upper limit of normal and the cut-off for DVT diagnosis. For the 103 centres who reported using a cut-off for DVT diagnosis the value in use ranged from 80 to 1000 ng/mL. For sample 2, a pooled normal plasma, six centres reported their result as positive, abnormal or consistent with DVT. As reconstituted lyophilised plasma is unsuitable for the Nycocard system a different test sample was tested by 67 users of the Nycocard system who obtained results in the range of 80 to 1300 ng/mL for

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a liquid control sample having a median result of 500 ng/mL. We conclude that standardization of D-dimer testing and reporting is currently poor and that there is an urgent need for international reference preparations and recommendations.

		Sample 1		Sample 2		
	n	Median	Range	Median	Range	Median upper limit normal
Biomerieux VIDAS*	10	872	798–983	169	149–250	500
Dade-Behring DD Plus	10	170	120–219	100	60–170	190
Instrumentation Lab	68	352	240–848	103	10–280	260
Organon (FEU)*	18	915	770–4320	200	80–830	500
Organon (other units)	10	433	400–850	80	30–100	300
Stago*	15	890	800–1360	220	130–640	500

* FEU, fibrinogen equivalent units.

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Progression of liver disease in hepatitis C infected haemophiliacs assessed by serial liver biopsy

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More than 90% of haemophiliacs treated with plasma-derived factor concentrates prior to 1984 are infected with the hepatitis C virus. Although the timescale for progression to cirrhosis is many years, most patients will develop chronic liver disease. It has been suggested that liver biopsy in haemophiliacs is particularly hazardous and should be avoided. However, current treatment guidelines use liver biopsy to determine those who require therapy with ribavirin/interferon. We have previously reported our experience of liver biopsies in 35 patients with haemophilia/von Willebrand's disease and hepatitis C. We now report follow-up data on repeat biopsies on the same patient group 3–5 years following initial biopsy to assess progression of liver disease in this group. Patients who were HCV-PCR negative and those with inhibitors were excluded. To date, 25 patients have undergone repeat biopsy. Biopsies were graded according to fibrosis and inflammatory scores. Ten patients have progressed by one fibrosis grade. Three patients have progressed from grade 2 or less to grade 3 thereby qualifying for combination therapy. One patient has progressed from grade 0 to grade 3 and another progressed from grade 0 to grade 5. Both these patients had a history of excessive alcohol consumption. Thirteen patients showed no evidence of progression. Over a time course of at least 18 years only four patients have developed cirrhosis. There have been no bleeding complications related to the biopsies. We have performed 70 biopsies in patients with haemophilia/severe VWD and shown it to be a safe procedure. We have demonstrated the slow progression of liver disease in this group. The majority of our patients do not qualify for combination therapy according to national guidelines.

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UK surveillance of acquired haemophilia A: May 2001–April 2002

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Reports on acquired haemophilia (AH) are exclusively from referral centres which may have lead to publication bias. This study aims to establish the incidence and clinical characteristics of AH in all UK patients. In the first 12 months 256 of 257 UK Trusts circularised reported 98 cases (incidence 1.7/million/year). Clinical details are available on 68 patients. Median age is 80 (range 32–97). 39% had died at 6 months: 10% infection (pneumonia/sepsis), 4% bleeding, 7% malignancy, 7% cardiac and 11% others. Clinical associations are malignancy 29%, inflammation 20%, pregnancy 3%, pemphigoid 3% and none 59%. Median (range) factor VIII and anti-human inhibitor levels were 3 IU/dL (<1–25) and 13 BU/mL (<0.4–717). Bleeding manifestations are subcutaneous 78%, muscular 37%, gastrointestinal 22%, retroperitoneal 9%, haemarthrosis 7%, haematuria 7% and intracranial 3%. Subcutaneous bleeding was the only manifestation in 28%. Bleeding manifestations did not vary between CCC, haemophilia centres (HC) and other centres. Haemostatic treatment: none 38%, FEIBA 34%, rFVIIa 24%, human FVIII 22% and porcine FVIII 4%. No haemostatic treatment was required in 26% of patients at CCC, 55% at HC and 45% at other centres. Immunosuppression induced remission in 68% but only 44% of the cohort was alive and inhibitor-free at 6 months. There was no difference in remission rate between steroids alone 73% or cytotoxics and steroids 69% (IVIG made no significant difference); however, these results must be interpreted with caution as they are retrospective and not randomized. The remission rate at CCC, HC and other centres was 63, 65 and 91% respectively. These interim data suggest that overall survival is worse than previously reported, more patients died of infection than bleeding and many patients required no haemostatic treatment. Patients treated at non-haemophilia centres require less haemostatic treatment and are more likely to achieve remission suggesting that previously published data may be biased to a more severe phenotype.

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Procoagulant changes in human macrophages induced by environmental microparticles [PM10]: a possible pathogenetic mechanism in cardiovascular disease

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In epidemiological studies a strong relationship has been confirmed between increasing concentrations of environmental particles [PM10 and PM25] and acute cardiovascular events including myocardial infarction and stroke. No plausible pathogenetic mechanism

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has been demonstrated to explain this association. Recently it has been demonstrated that inhaled particles pass into the systemic circulation in humans (1). Therefore we hypothesized that vascular events may be triggered by coagulation activation on cell surfaces induced by contact with environmental particles. Human peripheral blood monocyte-derived macrophages and a human bronchial epithelial cell line were exposed to PM10 and supernatants examined for procoagulant activity in a plasma recalcification time assay. Control experiments employed culture medium only or hydrogen peroxide. Both PM10 and hydrogen peroxide induced an increase of procoagulant activity in culture supernatants from both cell types [shortening of clotting time versus control with PM10 was 31% with macrophages and 19% with epithelial cells; mean of three experiments, $P < 0.001$ and < 0.01 respectively]. In order to characterise the procoagulant activity from macrophages induced by PM10 we went on to determine the effects of these particles on surface expression of negatively charged phospholipid by annexin V binding, and tissue factor mRNA expression by RT-PCR. Finally we measured macrophage interleukin 8 (IL8) mRNA expression and release in response to PM10. Environmental particles induced macrophage tissue factor mRNA expression and significantly increased bound annexin V. Furthermore IL8 message and release were significantly enhanced also. We have demonstrated, for the first time, that environmental particles associated with increased cardiovascular risk can activate macrophages *in vitro* with transformation to a procoagulant phenotype which may be mediated through apoptosis/negatively charged phospholipid exposure and tissue factor expression. This may represent a novel prothrombotic mechanism triggered when environmental particles reach the systemic circulation.

Reference

1 Nemmar *et al. Circulation* 2002; **105**: 411–4.

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Is early activation of the clotting cascade involved in the pathogenesis of verotoxin-producing *Escerichia coli*-associated haemolytic uraemic syndrome?

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In most cases, haemolytic uraemic syndrome (HUS) is a thrombotic complication of infection with *Escherichia coli* O157 : H7. It usually follows bloody diarrhoea caused by verotoxin secreted by the bacteria, and comprises acute renal failure, non-immune, microangiopathic haemolytic anaemia and thrombocytopaenia. HUS is primarily a disorder of the vascular endothelium, with platelet aggregation at sites of endothelial cell damage in the renal microvasculature. Several *in vitro* studies demonstrate changes in endothelial cell anticoagulant properties when exposed to cytokines and under apoptotic conditions. Of interest are changes in tissue factor (TF) expression and activity, TF being crucial in initiating the coagulation cascade. Additionally, the expression of antithrombotic membrane components, notably thrombomodulin, has been shown to be downgraded. We examined the changes in TF and thrombomodulin on endothelial cells exposed to the *E. coli* products, verotoxin and lipopolysaccharide (LPS), and the inflammatory cytokines, interferon-gamma (IFNgamma) and tumour necrosis factor-alpha (TNFalpha), and their relationship to apoptosis. Macrovascular [primary culture of human umbilical vein endothelial cells (HUVEC)] and microvascular [human microvascular endothelial cells (HMEC)] endothelial cells were cultured with LPS and verotoxin in the presence and absence of cytokines. Surface TF and thrombomodulin expression and apoptosis were assessed using flow cytometry. Increases in TF expression were observed in activated HUVECs; changes were less apparent in HMECs which constitutively express TF. Thrombomodulin was consistently reduced on HMECs under similar conditions. It appeared not to be the apoptotic population that changed their expression of membrane proteins. HUVECs cultured with LPS and TNFalpha demonstrated the greatest increase in TF. Decreases in thrombomodulin were greatest on HMECs exposed to verotoxin. We demonstrate an up-regulation of TF and a down-regulation of thrombomodulin on endothelial cells stimulated with LPS, verotoxin and inflammatory cytokines. These cells are rendered more thrombogenic, mechanisms that may be involved in the pathogenesis of HUS.

Oral Communication

Paediatric and General Haematology

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Revealed: D-dimers are being inappropriately requested. Results of audit to influence subsequent requesting

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D-dimers, cross-linked fibrin degradation products, are extremely sensitive, but non-specific markers for coagulation activation; a process that complicates many conditions. They have been shown to be useful in negatively predicting for deep vein thrombosis (DVT), particularly in combination with a clinical assessment score for DVT. Junior medical staff and GPs are increasingly using the D-dimer test to screen for the presence of DVT. Radiology departments use a positive D-dimer to 'gate-keep' their Doppler service. Haematology departments have therefore identified a dramatic increase in D-dimer, requesting and associated cost (£26,000 in 2002 at Derriford Hospital). Over-investigation may lead to unnecessary, and potentially dangerous, further investigation and anticoagulation. This audit was conducted to address these concerns. The case-notes were reviewed from 50 consecutive patients presenting to/within Derriford Hospital with suspected DVT (34%) or pulmonary embolism (66%) in 2002. Not surprisingly, junior-most of the doctors requested the bulk of these investigations, 11% by registrars and none by the consultants. The reasons for requesting were documented in all but seven patients. The Biomericux MDA D-dimer assay kit was previously evaluated locally, giving a positive cut-off level of 0.5 mg/mL for DVT with >95% sensitivity. In the current group of patients, 28% fell below the cut-off value. Of the positive results, only 61% were investigated further. The management was not affected by the D-dimer result in 45% of cases. Of the original cohort, only four DVTs and two PEs were confirmed. One PE was diagnosed in spite of negative D-dimer. These data support the theory regarding over-requesting of D-dimers. The low specificity is highlighted by only six confirmed DVT/PEs of 72% patients having a raised D-dimer. Locally, the co-importance of validated clinical assessment score is emphasized and the futility/danger of performing D-dimers on

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in-patients and those with suspected PE until a specific cut-off value has been established.

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Anti-D immunoglobulin in the treatment of immune thrombocytopenic purpura in adults in the United Kingdom: an evaluation of clinical outcomes and resource utilization

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Immune thrombocytopenic purpura [ITP] is an autoimmune disorder distinguished by a low platelet count and mucocutaneous bleeding. Estimated incidence is 10 per 100,000 persons per year. ITP is classified as primary, or secondary to an underlying disorder, and acute (of 6 months or less in duration), or chronic. In 70% of children, resolution occurs in 6 months. By contrast, ITP in adults has an insidious onset, and is chronic in nature. Given comparable efficacy of anti-D immunoglobulin and IVIg in the treatment of ITP, this inquiry was designed to contrast health resource utilization patterns stemming from the use of each agent in the treatment of ITP in adults. Stemming from the schematic developed by Cines and Blanchette (1), and information contained in the report of the British Committee for Standards in Haematology, General Haematology Task Force (Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children, and in pregnancy) we employed Monte Carlo simulation to evaluate the utility (cost implications) of various treatment schema (intent-to-treat format; steroids; IVIg; anti-D immunoglobulin) for the treatment of ITP. Utilizing 100,000 replicate simulations per year of age examined [15 through 80 years (5-year intervals); quadratic regression equations] we discerned a significant ($P < 0.05$) efficacy/health service utilization-based saving (percent reduction) for the 'steroids to anti-D immunoglobulin' arm, relative to the 'steroids to IVIg' arm [34.0% (SE = 0.1%) for males; 33.4% (SE = 0.1%) for females]. In the United Kingdom, use of anti-D immunoglobulin in the management of ITP is estimated to result in reduced health service expenditures relative to IVIg. Specifically, use of anti-D immunoglobulin reduces the time required (cost) for medication administration, patient monitoring, and occupancy of health care facilities. The effect on patient's quality-of-life warrants further research.

Reference
1 Cines and Blanchett. *NEJM* 2002; 346: 995–1008.

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Reduced numbers and antigen unresponsiveness of natural killer T-cells in idiopathic (autoimmune) thrombocytopenia (ITP)

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Natural killer T cells (NKT cells) are a rare cell subset implicated in the control of autoimmunity. Here, untreated ITP patients and healthy controls were compared for the number of circulating NKT cells and their proliferative response to the NKT cell antigen alpha-galactosylceramide. The cells were identified as Valpha24 Vbeta11 double-positive (CD4 positive or CD4 CD8 double-negative) lymphocytes using flow cytometry. In controls, NKT cell frequency ranged from 0.008 to 0.351% of total lymphocytes (mean 0.13%, $n = 9$). In the ITP group, a low NKT cell frequency was much more common, resulting in a mean of 0.04% (range 0.002–0.336%, $n = 14$). The capacity for alpha-galactosylceramide-driven NKT cell expansion was variable in the ITP group, which could be divided into responders (55% of tested donors, $n = 13$) and non-responders (45%). In comparison, 92% of controls ($n = 13$) responded. Interestingly, repeated testing of a limited number of ITP patients showed that proliferative response to the antigen or unresponsiveness was not constant over time, thus one and the same patient may respond at one time but not another. Furthermore, while IL-2 increased antigen-induced NKT cell proliferation in the majority of controls, this was not observed in the ITP group. In conclusion, the number of circulating NKT cells is reduced in ITP and a proportion of ITP patients have both antigen and IL-2 unresponsive NKT cells. The lack of proliferative response after antigen exposure and the IL-2 unresponsiveness may be due to adaptive tolerance, or *in vivo* anergy. This is believed to be caused by a prior incomplete antigen activation *in vivo*. These observations therefore suggest that NKT cells from ITP patients are anergic and this may contribute to the autoimmune process. Future research is planned to assess NKT cell function and involvement in autoimmune thrombocytopenia.

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Outcome heterogeneity in childhood high hyperdiploid acute lymphoblastic leukaemia

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High hyperdiploid (HeH) karyotypes (51–65 chromosomes) are found in approximately one-third of children with acute lymphoblastic leukaemia (ALL). Generally, these patients respond well to standard therapy and enjoy a superior outcome compared with other children. It has been suggested that specific cytogenetic features might further refine this prognosis and provide a method for identifying those with a poorer outlook, however no consensual picture has yet emerged. We analysed outcome data from 700 HeH ALL children enrolled on two MRC trials (UKALLXI and ALL97) to examine the effect of sex, age, white blood cell (WBC) count, individual trisomics, modal number and structural abnormalities on survival. Univariate analysis showed that age (1–9 years), sex (female), trisomies of chromosomes 4, 10 and 18, and a modal number of 54–65 chromosomes were all associated with improved survival, whereas the presence or absence of structural abnormalities and WBC had no effect on outcome. Multivariate analysis revealed that only age, sex, trisomy 4 (+4) and trisomy 18 (+18) were independent indicators of outcome. These results enabled the construction of two hazard scores for predicting relapse and mortality. Three risk groups were found using age, sex and +18, with event-free survival (EFS) rates of 86, 75 and 50% at 5 years ($P < 0.0001$). The group with the highest risk of relapse comprised:

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boys aged >9 years, boys aged 1–9 years without +18 and girls >9 years without +18. While girls aged 1–9 years with +18 made up the group with the best EFS. In terms of mortality, those aged <10 years with both +4 and +18 had a significantly improved survival (96 versus 84% at 5 years, $P < 0.0001$). These findings confirm that the outcome of children with HeH ALL is heterogeneous and information regarding specific trisomies can help identify those with a greater risk of treatment failure.

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Correction of the anaemia of chronic disease (ACD) with small doses of recombinant erythropoietin

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The anaemia associated with chronic disease such as infectious, inflammatory or neoplastic conditions, contributes significantly to morbidity. It has been recognized recently that a similar anaemia is also seen in congestive cardiac failure (CCF) and diabetes without significant renal failure. Pharmacological doses of recombinant erythropoietin (EPO) correct ACD associated with rheumatoid arthritis, inflammatory bowel disease, and AIDS. Good responses to EPO have also been seen in cardiac failure, and type 1 diabetes. Large doses of EPO are used in some centres to treat anaemia associated with cancer and its treatment. We corrected ACD with small doses of EPO in 10 patients: six males and four females, age range 58–88 years. Three patients had inflammatory disease, three were diabetic, and four had CCF. Six had either normal or reactive bone marrow histology, normal cytogenetics, and normal or increased iron stores. Six were transfusion dependent prior to EPO therapy. The dose of subcutaneous recombinant human erythropoietin (Eprex) was titrated according to the haemoglobin. Nine patients were evaluated, one having died from his underlying disease. The mean dose required was 9000 units/week (range 3000–14,000). The average maximum increase in haemoglobin was 4.1 g/dL (range 1.3–6.8). Few side effects were encountered. One patient suffered transient cerebro-vascular ischaemia, possibly related to a rapidly rising haemoglobin. All patients became transfusion independent. All reported improved general health without the peak and trough effect associated with blood transfusion. Avoidance of red cell transfusion is desirable in view of the possible infective and non-infective complications of transfusion and concern over the availability and cost of blood products. Although EPO is expensive, the doses required were comparable with those used in chronic renal failure, and much smaller than usually required to treat anaemia associated with cancer and its treatment. The cost therefore, compared favourably with blood transfusion.

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Epidemiological evidence for an infectious origin for childhood leukaemia

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The aim of the study was to interpret the results of several analyses of childhood leukaemia data from the Manchester Children's Tumour Registry (MCTR), in view of current hypotheses regarding an infectious origin. The MCTR is population-based and has been collecting case data from a geographically defined region of North West England since 1954. Trend tests, space-time clustering tests (Knox and *K*-function), ecological regression and geographical mapping were used to study the data on the leukaemias, broken down by sub-type (ALL, AML). For the most recent period (since 1980), ALL was further examined by immunophenotypic sub-type. There was an increasing incidence of ALL, due mainly to an increase of the precursor B-cell sub-type in the childhood peak. Space-time clustering was evident for cases of ALL, aged 0–4 years, for the time period 1954–1985, mainly based on date of diagnosis and place of birth. For the time period 1980–2001, space-time clustering was confined to cases of precursor B-cell ALL, aged 18–54 months, based on date and place of birth. The incidence of ALL was greater in more densely populated wards, largely because of cases of non-precursor B-cell ALL. The studies present a complex picture, but are supportive of an infectious aetiology, particularly for precursor B-cell ALL. It is concluded that there may be at least two infectious mechanisms operating, with a different mechanism predominating in the earlier years from that in the later years.

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Eculizumab

Prop Inn; USAN

[redacted]

Humanized Anti-C5 Monoclonal Antibody

h5G1.1
5G1.1

Immunoglobulin, anti-(human complement C5 α-chain) (human-mouse monoclonal 5G1.1 heavy chain), disulfide with human-mouse monoclonal 5G1.1 light chain, dimer

CAS: 219685-50-4

EN: 239304

Abstract

Eculizumab (5G1.1) is a recombinant, fully humanized monoclonal antibody that inhibits the activation of terminal complement components. Eculizumab binds specifically to the terminal human complement protein C5, preventing the release of the inflammatory mediator C5a and the formation of C5b-9 by inhibiting the cleavage of human complement C5 to C5a and C5b. Preclinical studies have demonstrated that the antibody has high binding affinity for C5, blocks the formation of C5a and C5b-9, and protects mammalian cells from C5b-9-mediated damage. Favorable clinical results have been reported in patients with membranous nephritis, rheumatoid arthritis and paroxysmal nocturnal hemoglobinuria (PNH) and the antibody is in phase II clinical development for these disorders; phase I trials have also been conducted in dermatomyositis.

Introduction

There is increasing evidence that activated components of the complement system contribute significantly to the pathogenesis of a large number of acute and chronic inflammatory diseases including rheumatoid arthritis, atherosclerosis, acute myocardial infarction and vascular injury after cardiopulmonary bypass (1-5). Furthermore, complement plays a significant role in the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH), characterized by intravascular hemolysis and venous thrombosis, and associated with aplastic anemia. Patients with PNH have a deficiency in CD59 and CD55 proteins on the surface of erythrocytes, platelets and other blood cells, which normally protect cells from complement-mediated injury (6-8). Patients with PNH experience fatigue, dysphagia, pain and hemoglobinuria, and the disease is associated with about 50% mortality (9).

Strategies that inhibit the damaging effects of complement on disease pathogenesis have therefore become a major focus in pharmacological research. In particular, inhibition of the complement cascade at C5, the cleavage of which results in the generation of the potent inflammatory mediators C5a and C5b-9 (10), while allowing the upstream generation of C3b, essential for combatting infections and preventing the development of autoimmune diseases (11), would appear to represent a promising approach.

Eculizumab is a recombinant, fully humanized monoclonal antibody that inhibits the activation of terminal complement components. Eculizumab binds specifically to the terminal complement protein C5 and prevents the release of the inflammatory mediator C5a and the formation of C5b-9 by inhibiting the cleavage of human complement C5 to C5a and C5b (12). The antibody is being developed for chronic indications including rheumatoid arthritis, membranous nephritis, dermatomyositis, lupus nephritis and paroxysmal nocturnal hemoglobinuria (13).

Pharmacological Actions

Eculizumab, as well as Fab and scFv fragments thereof, was evaluated for binding to complement and inhibition of complement activation *in vitro*. Eculizumab had similar binding affinity for human C5 compared to the murine antibody (IC_{50} ~ 2 nM), as determined in an ELISA, and it inhibited human serum complement-induced lysis of porcine aortic endothelial cells (PAEC) at the same concentration as the murine antibody (12).

Pharmacokinetics

A double-blind, randomized, placebo-controlled study was conducted with eculizumab (8 mg/kg i.v. once or

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twice monthly) to evaluate the pharmacokinetics/pharmacodynamics in 117 patients with idiopathic membranous nephropathy (mean creatinine clearance = 111 ml/min; 24-h proteinuria = 9 g; protein/creatinine ratio = 5; serum cholesterol = 270 mg/dl; hemoglobin = 12.0 g/dl; serum albumin = 2.8 g/dl). Preliminary results were reported for 11 patients. Mean steady-state trough serum eculizumab levels were 18 μg/ml in the twice-monthly treatment group and 8 μg/ml in the monthly treatment group. Mean steady-state mid-dose interval levels (1 week postdose on day 119 of the study) were 47 μg/ml for patients treated twice monthly and 35 μg/ml for those treated once monthly. Urine levels of eculizumab at midtrial and at the end of the trial were below the level of quantification in 8 patients, and 3 patients excreted 65-400 μg/24 h of eculizumab. All eculizumab-treated patients demonstrated < 10% of the hemolytic activity of placebo-treated patients at 1 h postinfusion. Serum eculizumab levels > 38 μg/ml inhibited hemolysis in all patients. Treatment with eculizumab was found to be safe and well tolerated, with no hypersensitivity reactions noted (14).

Pharmacokinetics and pharmacodynamics were also examined in an open-label pilot study in 11 transfusion-dependent patients with PNH who received treatment with eculizumab (600 mg weekly for 4 weeks, then 900 mg every 2 weeks for 2 months). Peak and trough serum levels of eculizumab were measured 1 h after the initial dose and then at weeks 3, 4, 6, 10 and 12, and in 10 patients eculizumab levels were > 35 μg/ml from 1 h after the first dose to the end of the study period. In 1 patient, eculizumab levels were > 35 μg/ml for the initial 10 weeks but < 35 μg/ml at week 12. In 10 patients, serum hemolytic activity was 20% or less for the entire treatment period, representing complete blockade, with only 1 patient showing increased hemolytic activity at week 12, which corresponded with a decline in eculizumab levels to < 35 μg/ml (15).

Clinical Studies

The safety and biological activity of a single i.v. dose of eculizumab (0.1-8 mg/kg) were investigated in 40 patients with mild to moderate rheumatoid arthritis. Mean C-reactive protein (CRP) levels were significantly reduced at day 7 compared to baseline after the highest dose and returned to baseline by day 14. Patients treated at this dose showed a trend for reductions in the signs and symptoms of rheumatoid arthritis. Doses of 2 mg/kg and above resulted in > 80% inhibition of human complement C5 for at least 10 h, and a single dose of 8 mg/kg provided > 80% inhibition for 10 days, with a return to baseline levels after 17 days. Eculizumab was safe and well tolerated across patient groups (16).

Another study examined the safety, efficacy and pharmacokinetics of eculizumab following multiple doses in 209 patients with rheumatoid arthritis. Patients were randomized to receive placebo, eculizumab 8 mg/kg every 2 weeks, eculizumab 8 mg/kg every week x 5 weeks, then every 2 weeks, or eculizumab 8 mg/kg every week x 5 weeks, then monthly, and evaluated both after 3 months of treatment and 3 months after terminating treatment. After 3 months of treatment, the induction/monthly group met the primary endpoint of improvement in ACR20 score (44% *vs.* 18% on placebo), and both induction groups met the secondary endpoint of decrease in CRP. Baseline C5b-9 levels were correlated with increased CRP levels and therefore systemic inflammation. Baseline C5b-9 levels > 200 mg/ml were associated with clinical response to eculizumab. In cases of elevated C5b-9, an ACR20 response was obtained in 9% of patients in the placebo group, 33% of patients in the twice-monthly eculizumab treatment group, 57% of patients in the induction/monthly treatment group and 50% of patients in the induction/twice-monthly treatment group. The induction regimens completely suppressed complement-mediated serum hemolytic activity up to week 4. The half-life of eculizumab was 10-14 days (17-20).

A randomized, double-blind, placebo-controlled phase IIb clinical trial evaluated the efficacy and safety of eculizumab in 368 adult patients with mild to moderate active rheumatoid arthritis undergoing treatment with methotrexate and/or leflunomide. The patients were enrolled at 69 sites in the U.S. and Canada and were randomized to receive placebo, twice-monthly eculizumab (600 mg i.v. weekly for 5 weeks, then 600 mg once every 2 weeks) or monthly eculizumab (600 mg i.v. weekly for 5 weeks, then 600 mg monthly). The primary endpoint was improvement in ACR20 score after 6 months of treatment. Monthly administration of eculizumab following an induction period was associated with a statistically significant, moderate improvement in the ACR20 score compared to placebo at 6 months. The percentage of patients who achieved an ACR20 response was 34%, 24% and 22%, respectively, on monthly eculizumab, twice-monthly eculizumab and placebo. Monthly therapy was also associated with evidence of a long-term antiinflammatory effect, with a significant reduction in erythrocyte sedimentation rate at 6 months. The incidence of adverse events was slightly higher among patients given eculizumab every 2 weeks, although the incidence of injection-site reactions remained low (21).

A double-blind, placebo-controlled phase I pilot study was performed to determine the safety and tolerability of eculizumab (8 mg/kg weekly for 5 weeks, then once every 2 weeks) in 10 patients with dermatomyositis compared with placebo (n=3). After 2 months of treatment, results showed moderate improvements in skin score, physician global score and manual muscle test in the eculizumab group compared with controls. The placebo group showed deterioration of physician global score, but improvements in skin score and manual muscle test compared to baseline. Disease intensity measured by magnetic resonance imaging (MRI) and various parameters on skin biopsy were also improved in the treatment group compared with placebo. Eculizumab was safe and well

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tolerated, with no significant differences in adverse events between treatment groups (22).

Alexion has sponsored phase II trials in patients with membranous nephritis, with results showing good tolerance. One trial did not reach the primary clinical efficacy endpoint of reduction in proteinuria after 4 months, while in the other trial an increase in remission rate and significant improvements in proteinuria, hyperlipidemia, hypertriglyceridemia and hypoalbuminemia were obtained after 12 months. The company also began a phase II study in lupus nephritis several years ago following the observation of positive effects on proteinuria in a study in patients with systemic lupus erythematosus (13, 23).

Further results regarding efficacy and safety were reported from the open-label pilot study in 11 transfusion-dependent patients with PNH treated with eculizumab (600 mg weekly for 4 weeks, then 900 mg every 2 weeks for 2 months). After 3 months of initial treatment, all patients were enrolled into an open-label extension study and went on to receive eculizumab 900 mg every 2 weeks for over a year. In the 12 months prior to entrance into the study, patients had received over 4 red blood cell transfusions, had detectable glycosylphosphatidylinositol (GPI)-deficient hematopoietic clone, and had a negative throat culture for *Neisseria meningitidis* and *Neisseria gonorrhoeae*. After the first 3 months of treatment, serum hemolytic activity was completely inhibited in the 10 patients who showed serum trough levels of eculizumab above 35 μg/ml; quality-of-life markers were also significantly improved, transfusion rates decreased and serum levels of lactate dehydrogenase (LDH), an indicator of hemolysis, were significantly reduced. Increased levels of PNH type III erythrocytes were thought to be due to prolongation of PNH red cell survival and confirmed that treatment with eculizumab inhibits complement-mediated hemolysis. The proportion of PNH neutrophils, monocytes and platelets did not change. The reduction in transfusions was greatest for patients (n=7) without thrombocytopenia. Biochemical markers of hemolysis showed dramatic improvement 1 week after beginning treatment with eculizumab. The most common adverse events were headache and upper respiratory tract infections, followed by influenza-like symptoms, rigors, dizziness, nausea, nasal congestion and joint aches. No episodes of thrombosis were reported. No adverse events were considered to be drug-related and although 2 patients had serious adverse events, all patients completed the 12-week study (15, 24-26).

Reports of the cumulative results after 6 and 12 months of eculizumab treatment showed that patients with purely hemolytic disease (macroscopic hemoglobinuria with a normal platelet count; n=6) had the best response to treatment, with type III erythrocytes increased from 39% to 67%, median transfusions reduced from 2.0 units/patient/month to 0 units/patient/month, and suppression of hemolysis. Four patients who did not become transfusion-independent were treated with additional subcutaneous erythropoietin with some improvement. Two patients reported breakthroughs in complement activity, demonstrated by increased lactate dehydrogenase levels, hemoglobinuria and symptoms. These symptoms resolved immediately after the next dose of eculizumab and patients were managed by reducing the dosing interval. The most commonly reported adverse events after 12 months of eculizumab treatment were flu-like symptoms, sore throat, headache and upper respiratory infection. Four serious adverse events were reported, but there were no study discontinuations as a result of adverse events and all patients elected to continue eculizumab treatment. It was concluded that eculizumab was well tolerated, decreased hemolysis and transfusion requirements, and improved symptoms of PNH for over 12 months (27, 28).

Conclusions

Eculizumab is a promising new monoclonal antibody that binds to human complement C5 and inhibits its cleavage by C5 convertase. Eculizumab has been granted orphan drug status for the treatment of PNH in both the U.S. and Europe. A pilot program has been completed in patients with PNH and plans are being finalized with the FDA for the next stage of development. Pilot programs have also been completed in patients with dermatomyositis and eculizumab is in phase II trials for rheumatoid arthritis and membranous nephritis.

Source

Alexion Pharmaceuticals, Inc. (US).

References

1. Morgan, B.P. *Clinical complementology: Recent progress and future trends.* Eur J Clin Invest 1994, 24: 219-28.

2. Morgan, B.P. *Complement and rheumatic disease.* In: Complement, Clinical Aspects and Relevance to Disease. B.P. Morgan (Ed.), Academic Press, New York, 1990, 130.

3. Torzewski, M., Klouche, M., Hock, J., Messner, M., Dorweiler, B., Torzewski, J., Gabbert, H.E., Bhakdi, S. *Immunohistochemical demonstration of enzymatically modified human LDL and its colocalization with the terminal complement complex in the early atherosclerotic lesion.* Arterioscler Thromb Vasc Biol 1998, 18: 369-78.

4. Rus, H.G., Niculescu, F., Vlaicu, R. *The relationship between macrophages and C5b-9 complement complexes in human atherosclerosis.* Clin Immunol Immunopathol 1988, 48: 307-16.

5. Kilgore, K.S., Friedrichs, G.S., Homeister, J.W., Lucchesi, B.R. *The complement system in myocardial ischaemia/reperfusion injury.* Cardiovasc Res 1994, 28: 437-44.

6. Holguin, M.H., Fredrick, L.R., Bernshaw, N.J., Wilcox, L.A., Parker, C.J. *Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of*

Samsung Bioepis Exhibit No. 1002
Page 6452

the erythrocytes of paroxysmal nocturnal hemoglobinuria. J Clin Invest 1989, 84: 7-17.

7. Rollins, S.A., Sims, P.J. *The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9.* J Immunol 1990, 144: 3478-83.

8. Morgan, B.P. *Isolation and characterization of the complement-inhibiting protein CD59 antigen from platelet membranes.* Biochem J 1992, 282: 409-13.

9. Hillmen, P., Lewis, S.M., Bessler, M., Luzzatto, L., Dacie, J.V. *Natural history of paroxysmal nocturnal hemoglobinuria.* New Engl J Med 1995, 333: 1253-8.

10. Matis, L.A., Rollins, S.A. *Complement-specific antibodies: Designing novel anti-inflammatories.* Nat Med 1995, 1: 839-42.

11 Liszewski, M.K., Atkinson, J.P. *The complement system.* In: Fundamental Immunology, W.E. Paul (Ed.), Raven Press, New York, 1993, 917-39.

12. Thomas, T.C., Rollins, S.A., Rother, R.P., Giannoni, M.A., Hartman, S.L., Elliott, E.A., Nye, S.H., Matis, L.A., Squinto, S.P., Evans, M.J. *Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv.* Mol Immunol 1996, 33: 1389-401.

13. *Product profiles: Autoimmune.* Alexion Pharmaceuticals Web Site July 13, 2004.

14. Appel, G., Nachman, P., Hogan, S. et al. *Eculizumab (C5 complement inhibitor) in the treatment of idiopathic membranous nephropathy (IMN): Preliminary baseline and pharmacokinetic (PK)/pharmcodynamic (PD) data.* J Am Soc Nephrol 2002, 13: Abst SU-P0970.

15. Hillmen, P., Hall, C., Marsh, J.C.W. et al. *Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria.* New Engl J Med 2004, 350: 552-9.

16. Jain, R.I., Moreland, L.W., Caldwell, J.R., Rollins, S.A., Mojcik, C.F. *A single dose, placebo controlled, double-blind, phase I study of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* 63rd Annu Meet Am Coll Rheumatol (Nov 13-17, Boston) 1999, Abst 42.

17. Kivitz, A., Burch, F., Birbara, C., Rollins, S., Tesser, J. *Pharmacokinetics (PK) and pharmacodynamics (PD) of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S214.

18. Burch, F., Tesser, J., Bell, L. *Baseline C5b-9 level correlates with CRP and ACR 20 response to the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S214.

19. Tesser, J., Kivitz, A., Fleischmann, R., Mojcik, C.F., Bombara, M., Burch, F. *Safety and efficacy of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S274.

20. *Phase II clinical results for 5G1.1 anticomplement antibody support further testing in RA.* DailyDrugNews.com (Daily Essentials) Nov 19, 2001.

21. Mojcik, C.F., Kremer, J., Bingham, C., Burch, F., Vitiello, S., McCroskery, E., Bookbinder, S. *Results of a phase 2B study of the humanized anti-C5 antibody eculizumab in patients with rheumatoid arthritis.* Ann Rheum Dis 2004, 63(Suppl. 1): Abst FRI0170.

22. Takada, K., Bookbinder, S., Furie, R., Oddis, C., Mojcik, C., Bombara, M., Plotz, P., Kissel, J. *A pilot study of eculizumab in patients with dermatomyositis.* 66th Annu Meet Am Coll Rheumatol (Oct 25-29, New Orleans) 2002, Abst 1300.

23. *Alexion reports presentation of membranous nephritis clinical trials.* Alexion Pharmaceuticals Press Release November 4, 2002.

24. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Cullen, M., Richards, S.J., Rother, R.P. *Eculizumab, a C5 complement blocking antibody, is the first therapy to reduce transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria (PNH).* Br J Haematol 2003, 121(Suppl. 1): Abst 257.

25. Rother, R.P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Richards, S., Cullen, M., Hillmen, P. *Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and reduces transfusion dependency in patients with paroxysmal nocturnal hemoglobinuria (PNH).* Mol Immunol 2003, 40(2-4): Abst 54.

26. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Richards, S., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and renders hemolytic patients with paroxysmal nocturnal hemoglobinuria (PNH) transfusion independent.* Blood 2002, 100(11, Part 1): Abst 154.

27. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Richards, S., Rollins, S.A., Mojcik, C.F., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, controls hemolysis in paroxysmal nocturnal hemoglobinuria (PNH) with responses maintained over a prolonged period of therapy.* Blood 2003, 102(11, Part 1): Abst 1858.

28. Hillmen, P., Hall, C., Marsh, J.C.W., Elebute, M., Richards, S.J., Rollins, S.A., Mojcik, C.F., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, controls haemolysis in paroxysmal nocturnal haemoglobinuria (PNH) with responses maintained over a prolonged period of therapy.* Br J Haematol 2004, 125(Suppl. 1): Abst 5.

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ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

Section Editor: Craig M. Kessler, MD

Eculizumab for the Treatment of Paroxysmal Nocturnal Hemoglobinuria

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H&O Can you give a short background on the standard of care for paroxysmal nocturnal hemoglobinuria before the advent of eculizumab?

AH Until recently, the treatment for paroxysmal nocturnal hemoglobinuria (PNH) was predominantly supportive. Red blood cell (RBC) transfusions were administered when appropriate, and warfarin or other anticoagulation therapy was given following a thrombosis. In the UK we practice primary prophylaxis with anticoagulant therapy depending on the neutrophil PNH clone size. Other therapies, such as corticosteroids or androgen therapy, have also been used, but less commonly and with efficacy in only a few patients; the mechanism of action behind these treatments is unclear. Stem cell transplantation has also been used in the past, but of course this approach is associated with serious complications. It has been difficult to develop an evidence base for treatment because of the heterogeneous nature and rarity of this disease. Before eculizumab (Alexion), treatment for PNH was limited to alleviating symptoms and improving patient quality of life. Eculizumab is a monoclonal antibody that blocks the complement system at protein C5.

H&O What is the rationale for the development of eculizumab for the treatment of PNH?

AH We know the hemolysis in PNH is complement-mediated, thus this drug should be effective in preventing hemolysis. The pathogenesis of PNH is known to be due to complement sensitivity of RBCs against complement attack. RBCs in PNH have lost their protective proteins (eg, CD55 and CD59) that would normally prevent complement-mediated lysis. It was hypothesized that if the complement attack on the RBCs could be blocked, the hemolysis in PNH and its associated symptoms could be prevented. Based on patients who have inherited complement deficiencies, it appears that patients with deficiencies in complement proteins C5–C9 suffer few clinical consequences compared to those with deficiencies in the complement proteins before C5. Therefore, C5 seemed to be a logical target for blocking the complement cascade. Eculizumab was developed for the purpose of blocking the complement system safely and without severe complications.

H&O Eculizumab has been evaluated for the treatment of rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune conditions. Is there a similarity between these conditions and PNH that spurred investigators to evaluate eculizumab in PNH?

AH The research strategy has been to develop therapeutic approaches that block complement activity, which contributes to a variety of disorders. There is some evidence that some components of autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus are complement-mediated. In PNH, however, almost all of the clinical symptoms are due to the uncontrolled activity of complement. Therefore, whereas there are many possible disorders in which complement blockade might have some efficacy, PNH is the disorder in which we had little doubt that eculizumab would be effective.

H&O What were some of the initial findings with eculizumab in PNH?

AH In May 2002, we began the pilot study of eculizumab in PNH. Patients enrolled had been having regular transfusions, and most exhibited dark urine (a sign of intravascular hemolysis) and severe symptoms of PNH such as difficulty swallowing (dysphagia) and abdominal pain. Men complained of erectile dysfunction. Additionally, they had biochemical parameters indicative of active, ongoing intravascular hemolysis. We found that just 1 infusion of the drug caused a resolution of the

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dark urine by the next morning. Other symptoms, such as abdominal pain and difficulty in swallowing, resolved quickly. Of the 6 male patients who had experienced erectile dysfunction, 5 experienced complete resolution of this symptom. The patients felt better quickly and, in terms of biochemical parameters, we found quick improvements in lactate dehydrogenase levels as well.

H&O What were the next steps after this initial study?

AH The pilot study was initially planned to be 12 weeks in duration, but at the end of the 12 weeks, all patients wanted to continue with eculizumab therapy. The extension phase of the trial commenced and is still continuing with 10 of the 11 patients. Patients have experienced a dramatic improvement in their quality of life and generally prefer 2 weekly infusions of eculizumab rather than their previous treatment, which might have been, for example, 4 weekly blood transfusions. Some patients have become transfusion-independent.

H&O What side effects are associated with eculizumab?

AH The side effects associated with eculizumab have been very minimal. One patient in the pilot study had a reaction to the initial infusion, consisting of shivering and fever. She required observation only and has tolerated every infusion after that extremely well with no further reactions; she is still on the drug more than 3 years later. No other patients experienced any infusion-related reactions. The only minor side effect has been headache after the first or second infusions in some of the patients, but otherwise toxicity has not been a problem. There is a theoretical risk of meningitis, but this has not been a major issue to date.

H&O Is there any sense about whether eculizumab treats all patients equally or are there variations between patients that might change the efficacy of the agent?

AH We found that patients who have the classic purely hemolytic PNH respond better to the drug than those who have a hypoplastic bone marrow. The reason for this difference is simply that marrow failure can sometimes contribute more significantly than hemolysis to anemia, in which case, although the hemolysis will be blocked by eculizumab, marrow failure will most likely still lead to anemia and therefore continuing transfusions will be required. Certainly any patient with classical PNH who is hemolyzing frequently is a good candidate for eculizumab therapy. This agent would also be appropriate for patients with biochemical and clinical evidence of hemolysis because their quality of life can be improved.

H&O What is the clinical trial status of eculizumab?

AH Two phase III trials opened worldwide in the last year: TRIUMPH and SHEPHERD. The first opened about a year ago and the second opened earlier this year. The trials are now closed to recruitment, and we are eagerly awaiting the results. If the results of this trial are positive, I would expect that applications for product licenses would be made relatively quickly.

H&O Does eculizumab appear to have any effect on thrombosis, the most significant morbidity associated with PNH?

AH Although at the moment we have no evidence that eculizumab will prevent thrombosis, it is reasonable to hypothesize that thrombosis may be prevented by this terminal complement blockade through its mechanism of action. Theories for the causes of thrombosis in PNH include activation of PNH platelets by complement attack and nitric oxide depletion through free hemoglobin scavenging. Eculizumab should prevent both consequences. This possibility will be very interesting to examine in the future. In the pilot study there was no evidence of thromboses in any of the patients.

Suggested Reading

Hill A, Hillmen P, Richards SJ, et al. Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. *Blood*. 2005;106:2559-2565.

Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2004;350:552-559.

Parker C, Omine M, Richards S, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. *Blood*. 2005; [Epub ahead of print].

Poggi A, Negrini S, Zocchi MR, et al. Patients with paroxysmal nocturnal hemoglobinuria have a high frequency of peripheral-blood T cells expressing activating isoforms of inhibiting superfamily receptors. *Blood*. 2005;106:2399-2408.

Young NS. Paroxysmal nocturnal hemoglobinuria: current issues in pathophysiology and treatment. *Curr Hematol Rep*. 2005;4:103-109.

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(54) **STRATIFICATION OF PATIENT POPULATIONS HAVING OR SUSPECTED OF HAVING RHEUMATOID ARTHRITIS**

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(57) **ABSTRACT**

The present disclosure is directed to screening, diagnosing and treating patients having, or suspected of having, rheumatoid arthritis. Levels of glucose-6-phophate isomerase or antibodies to glucose-6-phophate isomerase are assayed in test subjects or populations to determine susceptibility to, or existence of, an antibody mediated form of rheumatoid arthritis in such test subjects or populations. The results of the assays provide guidelines for therapeutic intervention with complement inhibiting agents.

[redacted]

STRATIFICATION OF PATIENT POPULATIONS HAVING OR SUSPECTED OF HAVING RHEUMATOID ARTHRITIS

BACKGROUND

[0001] 1. Technical Field

[0002] The present disclosure relates to stratifying patients having, or suspected of having rheumatoid arthritis, and selection of therapeutic agents useful in the treatment thereof.

[0003] 2. Background of Related Art

[0004] Rheumatoid arthritis (RA) is a chronic disease which can exhibit a variety of systemic manifestations. This disease has an unknown etiology and characteristically exhibits a persistent inflammatory synovitis which usually involves peripheral joints in a symmetric distribution. Despite the destructive potential of RA, its course can be quite variable. In many patients, a chronic polyarthritis begins insidiously with fatigue, anorexia, generalized weakness and vague musculoskeletal symptoms until the appearance of synovitis becomes apparent. In about 10% of patients, the onset can be more acute with rapid development of polyarthritis. In about one-third of patients, symptoms may initially be confined to one or a few joints. Overall, most patients will experience an intermediate course of the disease. The potential of inflammation to cause cartilage destruction, bone erosions and, ultimately, joint deformities is the most important feature of this disease.

[0005] Many drugs have been used to treat rheumatoid arthritis without complete relief of the symptoms. Conventional drugs include non-steroidal anti-inflammatory drugs (NSAIDs, aspirin, ibuprofen), gold salt, penicillamine, TNFα inhibitors, IL-1 receptor antagonists, and steroidal hormones. The steroidal hormones, which are considered to be the most potent and effective, have side effects when taken for long periods. NSAIDs also have side effects such as stomach irritation and can be ulcerigenic. Recently, a compound which blocks the C5 component of the complement cascade (h5G1.1-mAb) has been shown to be an effective therapy in a murine model of RA. This compound is now in human clinical trials for the treatment of RA.

[0006] Although the etiologic stimulus for RA has not been identified, established rheumatoid synovitis is characterized by persistent immunologic activity. The presence of rheumatoid factor, which is an autoantibody reactive with IgG, suggests that there may be an underlying immune dysfunction in association with RA. This factor was initially described by Waaler in 1940 and later described by Rose and co-workers in 1948. Rheumatoid factors consist of IgA, IgG and/or IgM immunoglobulin classes. The idiotypic nature of rheumatoid factor autoantibodies has been described and partially characterized. This suggests that there may be a perturbation in the idiotype/anti-idiotype humoral immune network of patients with RA. In addition, the presence of rheumatoid factor can be of prognostic significance because patients with high titers tend to have more severe and progressive disease with extraarticular manifestations. Rheumatoid factor can also be employed to confirm the diagnosis of RA in patients with a clinical presentation of RA and, if at high titer, to designate patients at risk for severe systemic disease.

[0007] The examination of established rheumatoid synovitis reveals a characteristic constellation of features which include hyperplasia and hypertrophy of the synovial lining cells, focal or segmental vascular changes and infiltration with mononuclear cells often collected into aggregates or follicles around small blood vessels. The predominant infiltrating cells are T lymphocytes with T4 (helper-inducer) cells predominating over T8 (suppressor-cytotoxic) cells. Evidence of B cell activation can also be found in the inflamed synovium and plasma cells producing immunoglobulin and rheumatoid factor are characteristic features of rheumatoid synovitis. The resultant production of immunoglobulin and rheumatoid factor can lead to the formation of immune complexes with subsequent complement activation and exacerbation of the inflammatory process through the production of anaphylatoxins and chemotactic factors. Indeed, levels of total hemolytic complement, C3 and C4 are markedly diminished in synovial fluid relative to total protein concentration as a result of activation of the classic complement pathway by locally produced immune complexes. All these findings suggest that progression of RA is an immunologically mediated event.

[0008] The most widely used immunodiagnostic assay of RA is the so-called Waaler-Rose assay discussed above. This assay is based upon the antibody (rheumatoid factor) to the Fc region of IgG. Rheumatoid factor (RF) is present in about 60% to 70% of those individuals afflicted with RA. However, the test may not always be accurate because it has been found to give false positives or negatives, and it does not assess the response to therapy or predict activation or reactivation of the disease process.

[0009] Since there is no unambiguous test distinguishing RA from other acute or chronic inflammatory diseases, differentiating RA from other arthritides, such as systemic lupus erythematosus (SLE), ankylosing spondylitis (AS), polyarticular gout (PAG), or psoriatic arthritis (PsA) is often difficult. Diagnosis of RA is usually made according to American Rheumatism Association (ARA) criteria, i.e.:

[0010] (1) morning stiffness;

[0011] (2) joint tenderness or pain on motion;

[0012] (3) soft-tissue swelling of the joint;

[0013] (4) soft-tissue swelling of a second joint (within three months);

[0014] (5) soft-tissue swelling of symmetrical joints (excludes distal interphalangeal joint);

[0015] (6) subcutaneous nodules;

[0016] (7) X-ray changes;

[0017] (8) serum positive for rheumatoid factors;

[0018] wherein diagnosis of 3 or 4 of these factors is considered representative of probable RA and diagnosis of 5 or more of the factors is considered representative of definite RA.

[0019] Recently, a transgenic mouse model (K/BxNT cell receptor) was found to exhibit spontaneous inflammatory arthritis resembling many of the features of human RA. See, Matsumoto et al., Science, 286, 1732-1735 (1999) and Ji et al. J. Exp. Med., 194 (3) 321-330 (2001). The inflammatory arthritis was traced to the presence of glucose-6-phosphate

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isomerase as an antigen targeted by T-cells and IgG immunoglobulins. Glucose-6-phosphate isomerase ("GPI") is a dimeric enzyme that catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate. The protein functions in different capacities inside and outside the cell. In the cytoplasm, the gene product is involved in glycolysis and gluconeogenesis, while outside the cell it functions as a neurotrophic factor for spinal and sensory neurons. The enzyme has also been known as glucosephosphate isomerase, phosphohexose isomerase (PHI), phosphoglucose isomerase (PGI), autocrine motility factor (AMF) and neuroleukin (NLK). In addition, it has recently been shown that a majority of humans with RA have elevated levels of anti-GPI IgG immunoglobulin in sera and elevated levels of soluble GPI in the sera and synovial fluid. See, e.g., Schaller et al., Nature Immunology, Vol. 2, No. 6, 746-753 (2001).

[0020] In light of the ambiguity and relative subjectivity of existing tests for RA there is a need for diagnostic procedures that allow non-subjective determination of the existence of RA and which provide a concrete indication of a course of therapeutics.

SUMMARY

[0021] In one aspect, a method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent is provided which includes conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of a patient to determine the level of GPI in the patient's serum or synovial fluid, and comparing the level of GPI in the serum or synovial of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The baseline serum GPI levels may encompass a mean concentration of 0.069+/−0.048 U/ml, P<0.0001. The baseline synovial fluid GPI levels may encompass a mean concentration of 0.060+/−0.052 U/ml P<0001. The patient may be a mammal such as a human.

[0022] In another aspect, a method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent is provided which includes conducting an assay for glucose-6-phosphate isomerase antibody (anti-GPI) in the serum or synovial fluid of a patient to determine the level of anti-GPI in the patient's serum or synovial fluid, and comparing the level of anti-GPI in the serum or synovial of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The baseline serum anti-GPI levels may encompass a mean concentration of (A_{405}) 0.059+/−0.037, P<0.0001. The baseline synovial fluid anti-GPI levels may encompass a mean concentration of (A_{405}) 0.645+/−0.209 P<0001. The patient may be a mammal such as a human.

[0023] In another aspect, a method for screening a population of subjects for antibody mediated rheumatoid arthritis and determining treatment thereof is provided which includes conducting a glucose-6-phophate isomerase (GPI) assay on the serum or synovial fluid of a population of subjects, and identifying subjects having GPI assay results which are statistically significantly greater than (P<0.05) the respective mean GPI serum or synovial fluid assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent. The subjects so identified may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G 1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0024] In another aspect, a method for screening a population of subjects for rheumatoid arthritis and determining treatment thereof is provided which includes conducting an assay for glucose-6-phophate isomerase antibody (anti-GPI) on the serum or synovial fluid of a population of subjects, and identifying subjects having anti-GPI assay results which are statistically significantly greater than (P<0.05) the respective mean anti-GPI serum or synovial fluid assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent. The subjects so identified may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0025] In another aspect, a method for predicting susceptibility of a patient to rheumatoid arthritis and to treatment with a complement inhibiting agent is provided which includes conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid, and comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of susceptibility to rheumatoid arthritis in the patient which is further susceptible to treatment with a complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement

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inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0026] In another aspect, a method for predicting susceptibility of a patient to rheumatoid arthritis and to treatment with a complement inhibiting agent is provided which includes conducting an assay for glucose-6-phosphate isomerase antibody (anti-GPI) of the serum or synovial fluid of the patient to determine the level of anti-GPI in the serum or synovial fluid, and comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of susceptibility to rheumatoid arthritis in the patient which is further susceptible to treatment with a complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0027] The present disclosure provides convenient, reliable methods to screen and treat patients having, or suspected of having, rheumatoid arthritis (RA). Recognition that glucose-6-phosphate isomerase (GPI) is an antigen which triggers an autoimmune response in a significant portion of the population afflicted with RA provides the basis for these methods. Detection of elevated levels of GPI or antibodies to GPI (anti-GPI) in the sera or synovial fluid of such individuals is indicative of antibody mediated complement activation and provides a signpost for therapeutic intervention through complement inhibition. Thus, by checking for elevated levels of serum or synovial GPI or anti-GPI antibodies in a population of subjects, a determination can be made as to whether members of that population will respond to therapeutic agents that inhibit complement.

[0028] In one aspect, patients already diagnosed with RA are stratified with respect to susceptibility to treatment with complement inhibitors. By determining the level of GPI or anti-GPI in serum or synovial fluid, a decision can be made by medical practitioners to treat the RA with a complement inhibitor or some other therapeutic approach. For example, a patient with RA who exhibits elevated GPI or anti-GPI levels may be treated with any pharmaceutically acceptable complement inhibitor. Those RA patients who do not exhibit elevated levels of GPI or anti-GPI would be directed to treatments which are not based on complement inhibition.

[0029] It is contemplated that any technique known to those skilled in the art can be utilized to determine the level of GPI or anti-GPI in the serum or synovial fluid. While enzyme-linked immunosorbent assay (ELISA) is preferred for detection of GPI or anti-GPI levels, other assays, e.g., enzyme activity assays, fluid phase assays, radioimmunoassay, precipitation, agglutination, direct and indirect immunofluorescence and complement fixation can be used. These assays may employ any protocol such as competitive, inhibition or sandwich.

[0030] Once measured, the GPI or anti-GPI concentrations in serum and/or synovial fluid of non-rheumatoid arthritis patients can be calculated to create a baseline GPI test standard. For example, an assay involving spectrophotometric analysis of enzyme activity to determine the level of GPI in the sera of normal individuals provided a human mean concentration baseline standard of 0.069+/−0.048 U/ml. See, Schaller et al., Nature Immunology, Vol. 2, No. 6, 746-753 (2001). An example of a baseline level of GPI in synovial fluid is 0.060+/−0.052 U/ml, P<0.001. Id. An example of baseline levels of anti-GPI in sera and synovial fluid of healthy individuals was determined by ELISA to be (A_{405}) 0.059+/−0.037, P<0.001, and (A_{405}) 0.645+/−0.209 P<0001, respectively. Id. Baseline levels may otherwise be determined in accordance with established principles and various techniques known to those skilled in the art.

[0031] One can then numerically compare the GPI or anti-GPI assay results of a test subject to the respective GPI or anti-GPI assay results of a rheumatoid arthritis-free population to determine whether the GPI or anti-GPI assay results of the test subject exceed the numerical range of the test results of the rheumatoid arthritis-free population. The numerical comparison involves comparing one or more of the above referenced GPI or anti-GPI results from the test subject to determine if one or more of the results is outside of the numerical range of the test results of the rheumatoid arthritis-free population. Optionally, statistical analysis can be performed to determine if the GPI or anti-GPI test results of the test subject exceed about two standard deviations or more above the mean test results of the rheumatoid arthritis-free population. In another option, statistical analysis can be performed to determine if the GPI or anti-GPI test results of the test subject exceed about one standard deviation or more above the mean test results of the rheumatoid arthritis-free population. These findings are used to support a diagnosis in the test subject of a predisposition to a positive response to complement inhibition therapies.

[0032] It should be understood by those of ordinary skill in the art that, in one aspect, this method is useful to diagnose a predisposition for the subsequent development of RA in a test subject or population of test subjects so that an early therapeutic regimen of complement inhibiting agents may be instituted. It should also be understood that not all test subjects with elevated level test results will always develop RA (i.e., there may be false positives). The test results from normal (NL) and RA populations may have overlapping ranges, with RA patients generally having the higher numerical range. The strength of the diagnosis relates to the test results obtained as follows. In a case in which the test results of a test subject lies above, that is, exceeds the numerical range of, the test results obtained from a rheumatoid arthritis-free population, it is said that the test subject is likely to develop RA and that serious consideration of therapy involving complement inhibiting agents should be given. However, not all test subjects that will develop RA will have test results exceeding the numerical range of test results from a rheumatoid arthritis-free population. Therefore, one can perform a statistical analysis to determine if a test result of the test subject is more than two standard deviations above the mean of the test results from a rheumatoid arthritis-free population. The test result of the test subject can be greater than two standard deviations or more above the mean and also be within the absolute numerical range of the results from a rheumatoid arthritis-free popu-

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lation. In this case, it is said that the test subject is likely to develop RA and that complement inhibiting agents should be given consideration. In a final case, the test result of a test subject can be more than one standard deviation above the mean of the test results from a rheumatoid arthritis-free population and also be within the absolute numerical range of test results for RA confirmed patients. The diagnosis of a predisposition for RA and complement inhibiting agent therapy based on these results is less clear. In this case, a test subject may develop RA. However, the percentage of individuals that ultimately develop RA will be smaller than those with test results about two standard deviations or more above normal or those exceeding the numerical range of normal (i.e., an increase in the incidence of false positives).

[0033] As mentioned above, it is possible to determine the susceptibility of an entire population of subjects to RA and to determine whether complement inhibiting agents should be given prophylactically in accordance with the present disclosure. The method of screening a population of subjects for the presence or absence of a predisposition toward developing RA includes: (1) performing a GPI or anti-GPI assay on a population of subjects; (2) calculating the results of the GPI or anti-GPI assay for the population; and (3) diagnosing in the test subjects the presence or absence of a predisposition toward developing RA by statistical analysis of the GPI or anti-GPI assay results. If there is a predisposition toward developing RA, prophylactic administration of complement inhibiting compounds should be considered.

[0034] The GPI or anti-GPI assay is performed on the population to be screened essentially as described above. Of course, the larger the population of subjects screened, the greater the potential accuracy of the diagnosis. Preferably, the population includes at least about 20 subjects. The GPI or anti-GPI test results for the population of subjects are calculated as described above. The test results are then analyzed statistically to identify subjects having GPI or anti-GPI assay results that are significantly greater than the mean GPI or anti-GPI assay results of the entire population of subjects. For example, a linear regression outlier analysis can be performed on the GPI or anti-GPI assay results for the entire population. Those subjects having one or more GPI or anti-GPI assay results that are statistically significantly greater than the mean values for the population (i.e., the test results is an outlier) are diagnosed as having a predisposition toward developing RA. Although the confidence level for the statistical analysis can be as low as $p<0.001$, typically, a $p<0.05$ is used. Linear regression outlier analysis is a known statistical analysis that identifies a data point that differs significantly (i.e., at a given p value) from the mean of a pool of data points from which the data point has been selected. The difference between the data point and the mean of the pool of data points being statistically significantly greater than that which is consistent with the inherent variability of the variable which the data point represents. "p value" or "P<" refers to the probability of observing a sample at least as unlikely as the one observed if the null hypothesis is true. For example, a sample result is statistically significant at the 5% level if, and only if, it leads to a rejection of the null hypothesis when the type I error probability alpha (i.e., the significance level) is 0.05.

[0035] Once a determination is made that a subject has elevated GPI or anti-GPI levels, a decision can be made regarding therapeutic intervention. The fact that soluble GPI is present in the synovial fluid of inflamed RA joints indicates that anti-GPI IgG forms immune complexes with the soluble GPI, which precipitate on the surface of the synovial lining with subsequent activation of the complement cascade. See Schaller et al., supra. Accordingly, compounds that interfere with the complement cascade can be utilized to treat subjects having GPI associated RA.

[0036] The complement cascade has been studied in depth and is fairly well understood. The complement system is an important means by which a host defends itself against infection. The complement cascade system is a component of the immune system that helps provide a natural immunity against invading microbes and is also an effector arm of antibody mediated humoral immunity. Complement is responsible for activating cells and other molecules involved in the inflammatory process as well as being directly related to the destruction of microbial invaders. The activation of complement involves a cascade of proteolytic reactions that lead to the release of inflammatory mediators and result in the assembly of the terminal complement complex. This cascade system has been characterized as containing at least thirty serum and membrane proteins that are activated by antibody-antigen complexes or by the invasion, in a host or experimentally in culture, by a microorganism, or other antigenic molecules.

[0037] The complement cascade consists of two major branches, the classical and alternative pathways. Though these pathways are initiated differently, they converge at the step of complement protein C3 activation. The complement cascade can mediate undesirable cellular damage in inflammatory, immune or autoimmune (auto-antibody-mediated) conditions. A series of regulatory proteins are involved in the control of the complement cascade. These proteins are considered part of the complement system and act to block endogenous complement activity at various stages of the complement cascade.

[0038] The classical pathway of the complement system is initialized by complement protein C1 binding to antigen bound IgG or IgM. C4 and C2 are then activated in the formation of C3 convertase at which point an amplification takes place that generates literally thousands of C3a and C3b fragments. C3b fragments can bind to complement protein complex C4b2a to form C4b2a C3b which is called C5 convertase and generates thousands of C5a and C5b fragments. C3b can also be used to regenerate C3 convertase which causes a greater amplification of complement activation. One approach to inhibit complement mediated effects is by depleting complement.

[0039] Any compounds that bind to or otherwise block the generation and/or activity of any of the human complement components, such as, for example, antibodies specific to a human complement component are useful herein and are referred to as complement inhibitors or complement inhibiting agents. Some complement inhibiting agents include 1) antibodies or antibody fragments directed against complement components C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2 and 2) naturally occurring or soluble forms of complement inhibitory compounds such as CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, serine protease inhibitors and K76 COOH.

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[0040] Particularly suitable complement inhibiting agents for use herein are antibodies or fragments thereof that reduce, directly or indirectly, the conversion of complement component C5 into complement components C5a and C5b. One class of useful antibodies are those having at least one antibody-antigen binding site and exhibiting specific binding to human complement component C5, wherein the specific binding is targeted to the alpha chain of human complement component C5. Such an antibody 1) inhibits complement activation in a human body fluid; 2) inhibits the binding of purified human complement component C5 to either human complement component C3 or human complement component C4; and 3) does not specifically bind to the human complement activation product for C5a. Particularly useful complement inhibitors are compounds which reduce the generation of C5a and/or C5b-9 by greater than about 30%. A particularly useful anti-C5 antibody is 5G1.1-mAb. Also particularly useful is a single chain Fv version of 5G1.1-mAb referred to as 5G1.1-scFv. In a preferred embodiment, humanized versions of these antibodies or scFv referred to as h5g1.1-mAb and h5G1.1-scFv are utilized. Methods for the preparation of these antibodies including h5G1.1-mAb and h5G1.1-scFv are described in U.S. patent application Ser. No. 08/487,283 filed Jun. 7, 1995, now U.S. Pat. No. 6,355,245, and "Inhibition of Complement Activity by Humanized Anti-C5 Antibody and Single Chain Fv", Thomas et al., *Molecular Immunology*, Vol. 33, No. 17/18, pages 1389-1401, 1996, the disclosures of which are incorporated herein in their entirety by reference. It is contemplated that Fab fragments, Fab' fragments, $F(ab')_2$ fragments, diabodies, and other forms of antibody fragments directed to the C5 component are suitable as well.

[0041] It is intended that the methods of the present disclosure are applicable to various animals such as mammals, e.g., humans, dogs, cats, horses, etc., which can all be benefited by screening and treatment herein. The complement inhibitors can be utilized in pharmaceutical compositions by adding an effective amount of one or more complement inhibitors to a suitable pharmaceutically acceptable diluent or carrier. Those skilled in the art are familiar with numerous techniques and formulations utilized to compound pharmaceutical compositions. The pharmaceutical compositions herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, (pulmonary, e.g., by inhalation or insufflation of liquids, powders or aerosols, including by nebulizer; intratracheal, intranasal, enteral, epidermal and transdermal), oral, sublingual, buccal or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intramedullary or intracranial, e.g., intrathecal or intraventricular, administration. Direct injection into affected joints is contemplated as well.

[0042] Pharmaceutical compositions for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, troches or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients. Pharmaceutical compositions herein include, but are not limited to, solutions, emulsions, suspensions, foams and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids, according to conventional methods, by one of skill in the art.

[0043] The pharmaceutical formulations herein, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product. Further details on techniques for formulation and administration of numerous dosage forms may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.). The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

[0044] Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0045] Pharmaceutical compositions suitable for use herein include compositions wherein the complement inhibitors are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically

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effective dose refers to that amount of active ingredient, which ameliorates, partially or completely, the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for animal or human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0046] The exact dosage will be determined by the practitioner in light of factors related to the subject that require treatment. Dosage and administration are adjusted to provide sufficient levels of the complement inhibitors to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation. Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g per kilogram, depending upon the route of administration. For example, anti-C5 monoclonal antibodies may be administered at about 8 mg/kg or at a 600 mg dose. Anti-C5 scFv may be administered at about 2 mg/kg. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art.

[0047] Although preferred and other embodiments have been described herein, modifications of the embodiments and further embodiments may be envisioned by those skilled in the art without departing from the scope of the invention as defined by the following claims.

What is claimed is:

1. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent comprising:

conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid; and

comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis patient to treatment with the complement inhibiting agent.

2. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 1 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2

3. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 2 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

4. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 3 wherein the antibody is 5G1.1-mAb or h5G1.1-mAb.

5. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 3 wherein the functional antibody fragment is 5G1.1-scFv or hG51.1-scFv

6. A method for determining the eligibility of a patient having rheumatoid arthritis to treatment with a complement inhibiting agent according to claim 1 wherein the baseline serum GPI levels encompass a mean concentration of 0.069+/−0.048 U/ml, P<0.0001 and the baseline synovial fluid GPI levels encompass a mean concentration of 0.060+/−0.052 U/ml P<0001.

7. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 1 wherein the patient is a mammal.

8. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 7 wherein the patient is a human.

9. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent comprising:

conducting an assay for antibodies to glucose-6-phosphate isomerase (anti-GPI) in the serum or synovial fluid of the patient to determine the level of ant-GPI in the serum or synovial fluid; and

comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent.

10. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 9 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

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11. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 10 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

12. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 11 wherein the antibody is 5G1.1-mAb or h5G1.1-mAb.

13. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 11 wherein the functional antibody fragment is 5G1.1-scFv or hG51.1-scFv.

14. A method for determining the eligibility of a patient having rheumatoid arthritis to treatment with a complement inhibiting agent according to claim 9 wherein the baseline serum anti-GPI levels encompass a mean concentration of (A_{405}) 0.059+/−0.037, P<0.0001 and the baseline synovial fluid anti-GPI levels encompass a mean concentration of (A_{405}) 0.645+/−0.209 P<0001.

15. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 9 wherein the patient is a mammal.

16. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 15 wherein the patient is a human.

17. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof comprising:

conducting a glucose-6-phophate isomerase (GPI) assay on the serum or synovial fluid of a population of subjects;

identifying subjects having GPI assay results which are statistically significantly greater than (P<0.05) the mean GPI assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent.

18. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 17 further comprising treating the subjects so identified with a complement inhibiting agent.

19. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 18 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

20. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 19 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

21. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 20 wherein the antibody is 5G1.1 or h5G1.1.

22. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 20 wherein the C5 complement inhibiting antibody wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

23. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 17 wherein the subjects are mammals.

24. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis according to claim 23 wherein the subjects are humans.

25. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof comprising:

conducting an assay for antibodies to glucose-6-phophate isomerase (anti-GPI) in the serum or synovial fluid of a population of subjects;

identifying subjects having anti-GPI assay results which are statistically significantly greater than (P<0.05) the mean anti-GPI assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent.

26. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 25 further comprising treating the subjects so identified with a complement inhibiting agent.

27. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 26 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

28. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 27 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

29. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 28 wherein the antibody is 5G1.1 or h5G1.1.

30. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 28 wherein the C5 complement inhibiting antibody wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

31. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 25 wherein the subjects are mammals.

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32. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis according to claim 31 wherein the subjects are humans.

33. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent comprising: conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid; and comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of a predisposition for antibody mediated rheumatoid arthritis in the patient which is susceptible to treatment with a complement inhibiting agent.

34. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 further comprising treating the patient exceeding the numerical range with a complement inhibiting agent.

35. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

36. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 35 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

37. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 36 wherein the wherein the antibody is 5G1.1 or h5G1.1.

38. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 36 wherein the antibody fragment is 5G1.1-scFV or h5G10.1-scFv.

39. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 wherein the patient is a mammal.

40. A method for predicting susceptibility of a patient to rheumatoid arthritis according to claim 39 wherein the patient is a human.

41. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent comprising:

conducting an assay for antibodies to glucose-6-phosphate isomerase (anti-GPI) in the serum or synovial fluid of the patient to determine the level of anti-GPI in the serum or synovial fluid; and

comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of a predisposition for antibody mediated rheumatoid arthritis in the patient which is susceptible to treatment with a complement inhibiting agent.

42. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 41 further comprising treating the patient exceeding the numerical range with a complement inhibiting agent.

43. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 42 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

44. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 43 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

45. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 44 wherein the wherein the antibody is 5G1.1 or h5G1.1.

46. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 44 wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

47. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 41 wherein the patient is a mammal.

48. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 47 wherein the patient is a human.

* * * * *

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Reference	Application No./Patent No.
AMG17203OP OPPO 01	11001632.6 - 1466 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

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Eculizumab Composition / ALEXION

Opposition to: **EP 2 359 834 B1 (11001632.6)**

Proprietor: **Alexion Pharmaceuticals, Inc.**
Cheshire, CT 06410 (US)

1892125 2017

Opponent: **Hoffmann Eitle**
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This is in response to the Request for Documents pursuant Rule 83 EPC dated August 17, 2017, and the Brief Communication dated September 25, 2017.

We herewith submit the documents cited as D2, D4, D5 and D6 in our Notice of Opposition dated August 9, 2017.

Leo Polz

DR. LEO POLZ
European Patent Attorney
Hoffmann Eitle Patent-und Rechtsanwälte PartmbB
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WEDNESDAY, 9 APRIL 2003

Oral Communication

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Eculizumab, a C5 complement blocking antibody, is the first therapy to reduce transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria (PNH)

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Paroxysmal nocturnal haemoglobinuria (PNH) is characterized by intravascular haemolysis and venous thrombosis due to the unopposed effect of complement on PNH red cells and platelets, respectively. Eculizumab is a humanized monoclonal antibody that binds C5 and inhibits terminal complement activation. Eleven transfusion-dependent PNH patients have completed a 3-month pilot study receiving 600 mg eculizumab by intravenous infusion over 30 min weekly for four doses and then 900 mg every other week. Adverse events were relatively minor and self-limiting. The biochemical parameters of haemolysis dramatically improved for all patients within a week. Mean LDH decreased from 3089 ± 599 U/L before to 597 ± 32 U/L after treatment ($P = 0.002$) and AST from 77 ± 20 to 26 ± 3 U/L ($P = 0.04$). Episodes of haemoglobinuria reduced by 90% ($P < 0.001$). Quality of life (EORTC QLC30) improved significantly post-eculizumab compared to pre-eculizumab. One patient began treatment during a severe acute haemolytic episode with almost black urine and within 24 h there was a complete resolution of her haemoglobinuria and symptoms. Transfusions for all patients were significantly reduced in the 3 months of eculizumab compared with the previous 12 months from 2.2 to 0.7 units/month ($P = 0.003$). The reduction in transfusions was most dramatic in the seven patients without thrombocytopenia from 2.3 to 0.2 units/month ($P = 0.001$). There was an increase in PNH type III red cells from a mean of 35.6% pre-eculizumab to 58.7% post-eculizumab ($P = 0.005$) due to their protection from complement-mediated lysis and the reduction in transfusions. There was no change in the proportion of PNH neutrophils, monocytes or platelets. All patients have continued on eculizumab in an extension study. In summary, eculizumab is well tolerated, decreases haemolysis, improves symptoms and significantly reduces transfusions in PNH patients. Eculizumab appears to be the first effective therapy for haemolysis in PNH and merits further development. This is the first trial of an anti-complement antibody therapy in PNH. The responses were remarkable both in reduction in haemolysis and transfusions and in the improvement in quality of life. Thus it is the first effective therapy for haemolysis in PNH and would also be expected to reduce the risk of thrombosis (this study could not address this). The trial was performed at two centres in the UK only.

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The induction of tolerance to RhD in HLA DR15 transgenic mice

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The RhD protein is a clinically important red cell alloantigen that induces the production of anti-D in RhD-negative individuals. Because of the immunogenicity of RhD, all blood donations are typed and matched for this antigen, and RhD-negative women are managed for the prevention of haemolytic disease of the newborn with prophylactic anti-D immunoglobulin. Understanding the mechanisms by which helper T-cells control the antibody response to RhD would enable the design of alternative strategies such as active toleration. We have shown that CD4+ helper T cells from alloimmunized donors can proliferate and produce both IFN-gamma and IL-4 in response to synthetic peptides derived from the RhD sequence, indicating a Th0 balanced cytokine response. The responses are HLA class II restricted, with HLA-DR15 being over-represented in good responders. Certain peptides are immunodominant, and induce proliferative responses in most alloimmunized donors. These peptides are candidates for the induction of tolerance to the RhD protein by the mucosal route. On the other hand, some peptides from the RhD protein preferentially induce IL-10 production by putative Tr1 regulatory cells *in vitro*, and these peptides inhibit T-cell proliferation in response to stimulation with purified RhD protein *in vitro*. These peptides may be used to induce tolerance by the parenteral route. We have used a HLA-DR15 transgenic mouse model to show that T-cells from mice immunized with the RhD protein proliferate in response to purified RhD protein and to RhD peptides, and serum antibodies specific for human erythrocytes are also produced. Mucosal administration of immunodominant RhD peptides, or parenteral administration of IL-10 inducing peptides prior to immunization with RhD protein, inhibits both T-cell proliferation and antibody production. These results suggest that synthetic RhD peptides can induce tolerance to the RhD protein and may be of value in the prevention of immunization in RhD-negative individuals.

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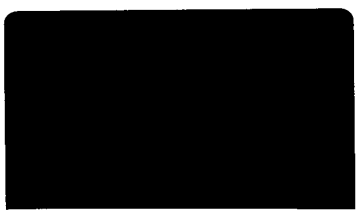
A humanised monoclonal antibody against vWF A1 domain inhibits vWF:RiCof activity and platelet adhesion in human volunteers

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AJW200 (Ajinomoto), a humanized IgG4 monoclonal antibody directed against the A1 domain of von Willebrand factor (vWF), specifically inhibits vWF : platelet GPIb receptor interactions. At

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high shear, it inhibits platelet adhesion, aggregation and activation, and in a dog model, inhibits arterial thrombus formation, without prolonging the bleeding time. In a randomized, double blind study, placebo, 0.01, 0.03, or 0.05 mg/kg AJW200, were infused i.v., into 24 healthy men. No clinically significant adverse events were recorded and there was no evidence of immunogenicity. Mean PK results were: C_{max} 204.8, 586.2, 833.2 ng/ml; T_{max} 1.0, 0.92, 0.57 h, T/2 23.5, 24.3, 27.2 h, for ascending doses. The maximum vWF occupancy by AJW200 was 19.4, 51.0 and 62.4%, showing correlation with plasma AJW200 concentration. Ristocetin cofactor (RiCof) was reduced 1 h post-infusion compared to baseline: mean (SD) 58 (22) and 110 (25)%, $P < 0.0005$ and 34 (16) and 116 (41)%, $P < 0.005$, at 0.03 and 0.05 mg/kg respectively, for up to 12 h. vWF : Ag, FVIII, vWF multimers and cleaving protease activity were unchanged. Template bleeding time (BT) was normal at all AJW200 doses, but the PFA-100 showed prolonged closure times 1 h post-infusion, with both CADP: 146 (22) and 82 (10) and 202 (78) and 80 (19) s, $P < 0.01$; and CEPI cartridges: 182 (28) and 108 (16) and 233 (56) and 107 (19) s, $P < 0.01$, at 0.03 and 0.05 mg/kg AJW200, respectively. This effect lasted for 3–6 h with the lower and up to 12 h for the higher dose. The RiCof and PFA-100 responses showed dose dependent inhibition and the magnitude of the effect appeared to be related to the baseline vWF level. AJW200 showed inhibition of vWF : RiCof activity and PFA-100 responses, without prolonging the skin bleeding time, and merits further clinical studies for thrombotic disorders. This is the first study demonstrating the effect of a monoclonal antibody that blocks GPIb/vWF in humans.

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Phase I study of the adoptive transfer of tetramer selected cytomegalovirus-specific cytotoxic T cells after allogeneic stem cell transplantation

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Cytomegalovirus (CMV) remains an important cause of morbidity and mortality following stem cell transplantation (SCT). Adoptive transfer of donor CMV-specific CD8+ T cell clones has been shown to protect against CMV reactivation in previous studies. However, this procedure has not been widely used due to the need for extensive *ex vivo* culture of T cells prior to infusion. We have combined HLA-peptide tetramer staining with the use of magnetic beads against phycoerythrin to facilitate the rapid selection of CMV-specific CTL from donor peripheral blood using a CliniMACS machine. In this study we infused tetramer selected donor CMV-CTL to six allogeneic stem cell transplant (SCT) recipients following CMV reactivation. An additional patient had undergone transplantation from a matched unrelated donor and CTL were infused for treatment of CMV reactivation refractory to Foscarnet, Ganciclovir and Cidofovir. In the 6 sibling allografts between 10^5 and 1.6×10^6 (mean 6×10^5) CMV-specific CD8+ T cells were infused from 250 mL of donor peripheral blood. The unrelated donor transplant patient received 2×10^6 CMV-CTL isolated from a cryopreserved lymphopheresis product. No infusion reactions were noted. One patient developed grade II GvHD which responded to treatment. CMV-CTL were not detectable prior to infusion in three of six patients but were detected in all patients following infusion. CMV-CTL expanded to represent up to 22.1% of the CD8 compartment. This expansion occurred in the first 14 days followed by a contraction to a basal level. Four patients did not require antiviral therapy. In the unrelated transplant recipient CMV-specific CTL reached a peak of 0.6% of the CD8 compartment and CMV antigenaemia became negative 6 days following CTL infusion. This novel approach to the adoptive transfer of antigen-specific CTL is rapid and relatively straightforward. It has considerable potential in the development of antigen-specific T cell therapy after allogeneic transplantation.

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Bone marrow mesenchymal stromal cells protect chronic lymphocytic leukaemia (CLL) from spontaneous and drug-induced apoptosis

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CLL cells are able to survive for prolonged periods *in vitro* and yet in most instances undergo rapid spontaneous apoptosis when cultured *in vitro*. Sustained viability *in vitro* is probably maintained by contact with 'nurse-like' cells in the peripheral blood (PB) and the bone marrow (BM) microenvironment. An *in vitro* model to mimic *in vitro* conditions was developed using adherent BM mesenchymal stromal cells (MSCs) from the iliac crest of normal donors. This model was used to test protection of CLL PB mononuclear cells both from apoptosis and from the effects of CD20 (Rituximab) and CD52 (CAMPATH-1H) therapeutic monoclonal antibodies, the proteasomal inhibitor MG132 and Bis9, an inhibitor of protein kinase C. Levels of apoptosis were determined by flow cytometry, using AnnexinV-FITC and propidium iodide. BM MSCs abrogated spontaneous apoptosis of CLL cells over a 5-day period of culture (80–90% viability, $n = 9$). Protection required CLL cells to be in close contact with the MSCs; separation by a microporous membrane induced apoptosis, comparable to that seen in control samples cultured without MSCs (30–40% viability). Rituximab treatment did not have any obvious apoptotic effect on CLL cells *in vitro* but CAMPATH-1H was able to induce apoptosis in a subpopulation of cells even in the presence of MSCs. Treatment with Bis9 and MG132 caused substantial and rapid apoptosis of CLL cells (0–15% viability $n = 3$). At lower doses of both drugs (200 nM), this apoptosis was prevented by MSC co-culture (70–90% viability). MSCs can therefore maintain CLL viability *in vitro* and can influence drug-induced apoptosis. The critical cell–cell interactions remain unknown, but are likely to include the beta-integrin, TNFSF13B and the CXCR4/CXCRL12 pathways. The expression of these molecules and their possible interactions in maintaining CLL viability will be investigated to assess if any might be targeted therapeutically.

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Targeting FGFR3 in multiple myeloma: the use of SU5402 and PD173074 to inhibit t(4;14) positive cells

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The t(4;14)(p16.3;q32), associated with 10–20% of cases of multiple myeloma (MM), deregulates the expression of two genes, MMSET and FGFR3. Most t(4;14) positive myeloma patients express FGFR3 and constitutive activation of this receptor is seen in some cases. To assess the potential of FGFR3 as a drug target we evaluated the effects of three FGFR inhibitors (SU6668, SU5402 and PD173074) on control and MM cell lines. Activity was assessed initially against Ba/F3 cells transformed to IL-3 independence by ZNF198-FGFR1, a constitutively active fusion gene associated with the 8p11 myeloproliferative syndrome (EMS), and compared to activity against control BaF3 cells grown with IL-3. Three methods were employed: MTS to assess proliferation/metabolic activity, trypan blue staining to determine cell viability and TUNEL to assess apoptosis. SU6668 inhibited the growth of both transformed and control cells lines at concentrations of >20 μM and therefore was not pursued further. SU5402 and PD173074 specifically inhibited the growth of ZNF198-FGFR1 transformed cells in a dose-dependent fashion with an IC50 of 5 μM and 5 nM, respectively. Both compounds also caused decreased cell viability and increased apoptosis. These compounds were then tested against two t(4;14) positive MM lines, KMS-11 and OPM-2, which express FGFR3 and harbour activating mutations of this gene. Dose-dependent growth inhibition was observed in both lines with IC50s similar to those in ZNF198-FGFR1 transformed cells. Importantly, inhibition was still observed in the presence of IL-6. Both compounds induced a dose-dependent reduction in cell viability and an increase in apoptosis, accompanied by a decrease in FGFR3 and ERK phosphorylation. In contrast, no inhibition was seen with either compound against the t(4;14) negative lines U266 and HS-Sultan, or the t(4;14) positive, FGFR3 negative cell line KMS-18. FGFR3 is thus a plausible candidate for targeted therapy in a subset of MM patients.

Oral Communication

Myeloid Malignancy

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Decreased expression of a novel growth regulatory protein, Nov, in chronic myeloid leukaemia

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Chronic myeloid leukaemia (CML) is characterized by the constitutively active Bcr-Abl tyrosine kinase. We have used a murine multipotent haematopoietic stem cell line, FDCP-MIX, expressing a temperature-sensitive mutant Bcr-Abl (ts Bcr-Abl FDCP-MIX), to investigate changes in gene expression using DNA microarray analysis. RNA was prepared from cells expressing the Bcr-Abl tyrosine kinase and from control cells, labelled with biotin and hybridized to an Affymetrix Mu6500 array (Affymetrix, USA). The Bcr-Abl kinase activity resulted in differential expression of 300 genes, the most significant being a 25-fold decrease in expression of a novel gene called nephroblastoma overexpressed (Nov). Northern blot analysis detected a 4-fold reduction in Nov expression in the Bcr-Abl positive cells in comparison with the control cells. Western blot analysis of immunoaffinity-purified Nov detected a two-fold reduction in expression as a result of the Bcr-Abl kinase activity. Confocal microscopy revealed strong vesicular cytoplasmic staining for Nov in FDCP-MIX control cells whilst fluorescence was barely detectable in cells expressing Bcr-Abl. A similar pattern of Nov expression was observed in primary human cells (five CML, five normal controls). Normal CD34+ progenitor cells uniformly expressed an intense fluorescence signal for Nov expression. In contrast, the majority of CD34+ cells within the CML patient samples were negative ($P < 0.001$). Nov expression has previously been associated with cell differentiation, quiescence and has been shown to promote cell adhesion. Loss of these properties is consistent with the CML phenotype.

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Reduced hTR expression in CML CD34+ cells at diagnosis: a novel mechanism for telomere loss in leukaemia

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CML is characterized by massive myeloid expansion, and significant telomere loss has been documented at diagnosis and during disease progression. Critical telomere shortening results in chromosomal instability, which can lead to disease progression. The regulatory mechanisms and expression levels of the key telomerase components (hTERT and hTR, the reverse transcriptase and RNA template respectively) in the CML stem cell compartment have not been described; however studies in solid tumours and cell lines generally show increased expression of both components. We selected CD34+ cells from 16 CML diagnostic leucapheresis specimens and 12 non-CML mobilized PBSC (control population) (median purity 94.1, 96.3% respectively; BCR-ABL positive in CML group). High-resolution cell cycle analysis demonstrated increased cycling in the CML CD34+ fraction. Q-RT-PCR for full-length (functional) hTERT showed an approximately two-fold rise in CML expression compared with control cells. RT-PCR with primers designed to detect the full-length hTERT transcript plus the three non-functional splice variants (with quantitative analysis) demonstrated a shift away from splicing in the CML samples. By combining these two complementary techniques we determined that expression of all (i.e. full length plus splice variants) transcripts are not different between CML and control cells: the increased levels of functional hTERT mRNA appear to be accounted for mainly by a reduction in the splicing out of the active sites. Surprisingly, Q-RT-PCR for hTR demonstrated a five-fold reduction in hTR levels in CML compared with control cells. Even more striking was the difference in hTERT/hTR ratio: 1 : 180 in CML cells versus 1 : 1800 in non-CML cells. We therefore hypothesize that despite increased hTERT levels there is a disassociated reduction in hTR, which in conjunction with increased cell cycling and replication

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induced telomere loss, could alter telomere homeostasis in CML. This could contribute to disease evolution via chromosomal instability.

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Reduced-intensity allogeneic haematopoietic stem cell transplantation (HSCT) using fludarabine, busulphan and alemtuzumab conditioning in 63 patients with myelodysplastic syndromes (MDS)

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We report the largest series of patients with MDS who have received a reduced-intensity HSCT using Fludarabine 30 mg/m^2 i.v. (day −9 to −5); busulphan 4 mg/kg/day orally (day −3 to −2) and alemtuzumab 20 mg i.v. (day −5 to −1). Immunosuppression comprised ciclosporin from day −1 titrated to plasma troughs of 150–200 ng/L. 63 patients with MDS (RA = 16, RAEB-I = 9, RAEB-II = 11, CMML = 5, TLD-AML = 21, aCML = 1) have received transplants from matched sibling (MSD) (24) or volunteer unrelated donors (VUD) (39). Except for RA, all had received at least one course of chemotherapy. Twenty-two of 24 MSD utilized PBSCs compared with 19 of 39 VUD HSCTs, with a median CD34+ cells of 4.6×10^6/kg (1.83–17.5) and 3.9×10^6/kg (0.44–16.7) respectively. There was no difference in engraftment latency, with the median time to neutrophil engraftment of 15 days (4–57) and platelets 18 days (7–105). 85% achieved full donor chimerism with a 6% incidence of grade III/IV aGvHD (43% grade I–IV) and 3% extensive cGvHD. At a median follow-up of 343 days (50–1213) for MSD, and 248 days (37–1316) for VUD recipients, 63 and 62% were alive respectively. The median age of sibling allografts recipients was 54 years (41–70). At day +200, the TRM was 5% and DFS 68%. The median age for VUD recipients was 49 years (25–58). The TRM at day +200 was 21% with DFS 65%. At 1 year, the estimated OS was 66% for VUD and 77% for MSD, with a DFS of 57 and 53% respectively. Twenty-one received DLI at a median of day +273 (126–1290); 11 for relapse and 10 for declining donor chimerism. A graft versus leukaemia effect was demonstrable. Although longer follow-up is required, RIC allogeneic HSCT appears to be safe and feasible in this high-risk group of patients.

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Combined clofibric acid and medroxyprogesterone acetate exert multiple anti-leukaemic actions against acute myeloid leukaemia cells and cell lines

Q. T. Luong[1], S. L. Fenton[2], M. T. Drayson[3], J. C. Mountford[4], R. E. Hayden[1], T. A. Sant[3], L. A. Copsey[2], J. A. Murray[5] and C. M. Bunce[1]

[1]*School of Biosciences,* [2]*Division of Medical Sciences,* [3]*Division of Immunity and Infection, University of Birmingham, Birmingham, UK;* [4]*ATMU, Glasgow Royal Infirmary, Glasgow, UK;* [5]*Department of Haematology, University Hospitals NHS Trust, Birmingham, UK*

Poor survival rates in acute myeloid leukaemia (AML) relate to both the failure of cytotoxic chemotherapy and inability of older patients to tolerate that therapy. Thus non-toxic drugs that specifically target the differentiation and apoptotic pathways of AML cells are urgently required. We and others have demonstrated that fibrates such as clofibric acid (CA) and inhibitors of the aldo-keto reductase AKR1C3, including medroxyprogesterone acetate (MPA), potentiate all trans-retinoic acid (ATRA) induced HL-60 cell differentiation. Fibrates are pharmacological PPARalpha ligands commonly used in the treatment of hyperlipidaemia whereas MPA is used to treat breast carcinoma. We recently demonstrated that, potentiation of HL-60 differentiation by AKR1C3 inhibitors occurs via the consequent activation of PPARgamma. Thus combined CA and MPA (CA/MPA) provide the means to simultaneously target PPARalpha and gamma using established drugs with low systemic toxicities. Here we demonstrate that CA/MPA killed cultured primary AML blasts in the presence or absence of IL3 and SCF ($P = 0.0001$) but not normal CD34+ve myeloid blasts. CA/MPA also sensitized AML cell killing by suboptimal doses of daunorubicin and Ara-C ($P < 0.003$) highlighting the drugs' potential as chemosensitizing agents. Although ATRA did not enhance AML cell killing by CA/MPA, all three drugs together resulted in the differentiation of the surviving cells from three of seven non-acute promyelocytic AML samples. Finally, high serum Nm23-H1 protein concentrations have been associated with the poorest prognosis in AML. Here we provide the rationale for this by demonstrating that Nm23-H1 acts as an autocrine survival factor. Importantly we also demonstrate that CA/MPA rapidly down-regulated Nm23-H1 expression in those AMLs that had highest initial expression ($P = 0.001$). We conclude that CA/MPA exert multiple antileukemic actions against AML cells and should be considered as adjuncts in the treatment of elderly and relapsed AML.

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The utility of *in vitro* testing for imatinib sensitivity in BCR-ABL negative myeloproliferative disorders

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Imatinib mesylate (STI571, Glivec) inhibits the tyrosine kinase activity of the protein tyrosine kinases ABL, ARG, PDGFRA, PDGFRB and KIT, and has proven clinical efficacy in the management of patients with BCR-ABL positive CML and MPDs with PDGFRB rearrangements. However, it is not known to what extent patients with BCR-ABL negative MPDs may harbour activating rearrangements or point mutations of imatinib-responsive genes. To test for imatinib responsiveness we have employed two *in vitro* assays. First, blood or marrow mononuclear cells (MNC) were grown in methylcellulose supplemented with growth factors and treated with 0, 1 or 5 μM imatinib. Colonies were counted on days 7 and 14. Secondly, blood or marrow MNCs were placed in liquid culture with 0, 1 and 5 μM imatinib and cell numbers were enumerated over 2–3 weeks. Imatinib-treated cells from CML patients showed a reduction in colony and cell numbers over time compared with untreated cells and treated cells from normal individuals. We have obtained results using one or both methods in 38 BCR-ABL, TEL-PDGFRB negative patients who had been diagnosed with atypical CML, BCR-ABL negative MPD, CMML, mastocytosis, myelofibrosis or hypereosinophilic syndrome (HES). Eleven patients showed a positive response, four of whom had a PDGFRB rearrangement as determined by FISH and one had TEL-ABL. The remaining six patients had HES ($n = 2$), secondary MDS with eosinophilia following treatment for AML ($n = 1$), aCML ($n = 1$), MF ($n = 1$) or mastocytosis ($n = 1$). Six

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patients (five with PDGFRB or ABL rearranged MPD and one with HES) were treated and all showed a cytogenetic and/or haematological response. We conclude that *in vitro* cultures are useful for predicting patients who might respond clinically to imatinib. Evidence to date suggests that most BCR-ABL negative patients who are imatinib-responsive have elevated eosinophil counts.

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Identification of a novel gene, GEMS, that is fused to FGFR1 in the 8p11 myeloproliferative syndrome

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The 8p11 myeloproliferative syndrome (EMS) is an atypical stem-cell myeloproliferative disorder which is caused by activation of the transmembrane receptor tyrosine kinase FGFR1. This typically occurs as a result of fusion of the tyrosine kinase domain of FGFR1 to an unrelated partner gene, which promotes dimerisation and consequent activation of the kinase in the absence of an appropriate ligand. To date, five FGFR1 partner genes have been identified: ZNF198 at 13q12, FOP at 6q27, CEP110 at 9q33, BCR at 22q11 and HERV-K at 19q13. In this study, we describe a patient with a clinical picture typical of EMS, who was found to have an acquired ins(12;8)(p11;?p11p22) by cytogenetics. Southern blotting and FISH were used to confirm that FGFR1 was disrupted and RT-PCR analysis indicated that the patient did not harbour any of the known FGFR1 fusion mRNAs or BCR-ABL. To identify the partner gene in this patient we employed 5′-RACE PCR, exploiting the fact that all known FGFR1 translocations generate mRNA fusions in which the partner gene is spliced to FGFR1 exon 9. A 629-bp product was obtained which was found to consist of sequence derived from chromosome band 12p11 fused to exon 9 of FGFR1. Analysis of ESTs and other clones indicated that the sequence was derived from a widely expressed novel gene that we have called GEMS (gene disrupted in eight p11 myeloproliferative syndrome). GEMS is predicted to be translated into a 29-kDa protein that contains an N-terminal coiled-coil domain but no other recognisable motifs. RT-PCR confirmed the presence of the GEMS-FGFR1 fusion but the reciprocal chimaeric mRNA was not detected. Experiments to determine the transforming ability of this novel fusion gene are underway.

Oral Communication

Haemostasis and Thrombosis 2

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Venous thromboembolism (VTE) associated with the management of acute thrombotic thrombocytopenic purpura (TTP)

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VTE is reported to occur in 3% of TTP/HUS cases following plasma exchange (PEX) therapy. From May 1997 to May 2002, 68 patients with acute TTP were treated. Eight documented VTE episodes (six DVTs and three PEs) were identified in seven patients (all female, mean age 31 years) during therapy for acute TTP (five primary presentation and three relapses). All six DVTs were associated with central lines at the site of thrombosis. Other precipitating factors included pregnancy (one), immobility (eight), obesity (three) and FV Leiden heterozygosity (one). VTE occurred at a mean of 53 days following the first PEX. Solvent detergent plasma (Octaplas, Octapharma, Austria) was the last plasma to be used in PEX prior to the VTE in seven of eight events. Octaplas was used as the sole plasma for three TTP episodes and in the other episodes was substituted for cryosupernatant or FFP owing to poor response (four) or allergy (one). The mean volume of Octaplas replacement prior to the VTE was 100 L (range 22–206). The mean platelet count at VTE was 161 (range 12–232) $\times 10^9$/L. The platelet count had been in the normal range for a mean of 18 days prior to the VTE in those with a normal platelet count (six of eight). One patient received prophylactic LMW heparin from initial TTP diagnosis and seven of eight received aspirin. In conclusion, we identified VTE in 12% of TTP cases and 88% of these were associated with Octaplas replacement in PEX. VTE is a multifactorial disease and several known precipitating factors are present in TTP patients. The use of large volumes of Octaplas in PEX may be an additional risk factor, possibly associated with protein S (PS) deficiency, particularly as the median functional PS in Octaplas is 58 IU/dL compared with 91 IU/dL and 102 IU/dL in FFP and cryosupernatant respectively.

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Results of D-dimer in the UK NEQAS (Blood Coagulation) proficiency testing programme: urgent need for improvements in standardisation of testing and reporting

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We report the proficiency testing of D-dimer in diagnostic departments. Two lyophilised samples were tested for D-dimer by over 240 centres using different methods. Around 30% used semi-quantitative tile-based latex agglutination assays and 70% used automated techniques including 20% who reported in fibrinogen equivalent units (FEU). Six automated methods were used in 10 or more centres and the median and range of results obtained with these methods are shown below in ng/mL. For sample 1, 70% of centres reported a result above the cut-off for DVT. For one method the median test result was within the normal range, whereas for other methods the median was 40–80% above the normal range depending on the technique. It was a major cause for concern that 24% reported a normal result for this sample. There appeared to be a poor understanding of the difference between the upper limit of normal and the cut-off for DVT diagnosis. For the 103 centres who reported using a cut-off for DVT diagnosis the value in use ranged from 80 to 1000 ng/mL. For sample 2, a pooled normal plasma, six centres reported their result as positive, abnormal or consistent with DVT. As reconstituted lyophilised plasma is unsuitable for the Nycocard system a different test sample was tested by 67 users of the Nycocard system who obtained results in the range of 80 to 1300 ng/mL for

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a liquid control sample having a median result of 500 ng/mL. We conclude that standardization of D-dimer testing and reporting is currently poor and that there is an urgent need for international reference preparations and recommendations.

		Sample 1		Sample 2		
	n	Median	Range	Median	Range	Median upper limit normal
Biomerieux VIDAS*	10	872	798–983	169	149–250	500
Dade-Behring DD Plus	10	170	120–219	100	60–170	190
Instrumentation Lab	68	352	240–848	103	10–280	260
Organon (FEU)*	18	915	770–4320	200	80–830	500
Organon (other units)	10	433	400–850	80	30–100	300
Stago*	15	890	800–1360	220	130–640	500

* FEU, fibrinogen equivalent units.

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Progression of liver disease in hepatitis C infected haemophiliacs assessed by serial liver biopsy

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More than 90% of haemophiliacs treated with plasma-derived factor concentrates prior to 1984 are infected with the hepatitis C virus. Although the timescale for progression to cirrhosis is many years, most patients will develop chronic liver disease. It has been suggested that liver biopsy in haemophiliacs is particularly hazardous and should be avoided. However, current treatment guidelines use liver biopsy to determine those who require therapy with ribavirin/interferon. We have previously reported our experience of liver biopsies in 35 patients with haemophilia/von Willebrand's disease and hepatitis C. We now report follow-up data on repeat biopsies on the same patient group 3–5 years following initial biopsy to assess progression of liver disease in this group. Patients who were HCV-PCR negative and those with inhibitors were excluded. To date, 25 patients have undergone repeat biopsy. Biopsies were graded according to fibrosis and inflammatory scores. Ten patients have progressed by one fibrosis grade. Three patients have progressed from grade 2 or less to grade 3 thereby qualifying for combination therapy. One patient has progressed from grade 0 to grade 3 and another progressed from grade 0 to grade 5. Both these patients had a history of excessive alcohol consumption. Thirteen patients showed no evidence of progression. Over a time course of at least 18 years only four patients have developed cirrhosis. There have been no bleeding complications related to the biopsies. We have performed 70 biopsies in patients with haemophilia/severe VWD and shown it to be a safe procedure. We have demonstrated the slow progression of liver disease in this group. The majority of our patients do not qualify for combination therapy according to national guidelines.

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UK surveillance of acquired haemophilia A: May 2001–April 2002

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Reports on acquired haemophilia (AH) are exclusively from referral centres which may have lead to publication bias. This study aims to establish the incidence and clinical characteristics of AH in all UK patients. In the first 12 months 256 of 257 UK Trusts circularised reported 98 cases (incidence 1.7/million/year). Clinical details are available on 68 patients. Median age is 80 (range 32–97). 39% had died at 6 months: 10% infection (pneumonia/sepsis), 4% bleeding, 7% malignancy, 7% cardiac and 11% others. Clinical associations are malignancy 29%, inflammation 20%, pregnancy 3%, pemphigoid 3% and none 59%. Median (range) factor VIII and anti-human inhibitor levels were 3 IU/dL (<1–25) and 13 BU/mL (<0.4–717). Bleeding manifestations are subcutaneous 78%, muscular 37%, gastrointestinal 22%, retroperitoneal 9%, haemarthrosis 7%, haematuria 7% and intracranial 3%. Subcutaneous bleeding was the only manifestation in 28%. Bleeding manifestations did not vary between CCC, haemophilia centres (HC) and other centres. Haemostatic treatment: none 38%, FEIBA 34%, rFVIIa 24%, human FVIII 22% and porcine FVIII 4%. No haemostatic treatment was required in 26% of patients at CCC, 55% at HC and 45% at other centres. Immunosuppression induced remission in 68% but only 44% of the cohort was alive and inhibitor-free at 6 months. There was no difference in remission rate between steroids alone 73% or cytotoxics and steroids 69% (IVIG made no significant difference); however, these results must be interpreted with caution as they are retrospective and not randomized. The remission rate at CCC, HC and other centres was 63, 65 and 91% respectively. These interim data suggest that overall survival is worse than previously reported, more patients died of infection than bleeding and many patients required no haemostatic treatment. Patients treated at non-haemophilia centres require less haemostatic treatment and are more likely to achieve remission suggesting that previously published data may be biased to a more severe phenotype.

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Procoagulant changes in human macrophages induced by environmental microparticles [PM10]: a possible pathogenetic mechanism in cardiovascular disease

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In epidemiological studies a strong relationship has been confirmed between increasing concentrations of environmental particles [PM10 and PM25] and acute cardiovascular events including myocardial infarction and stroke. No plausible pathogenetic mechanism

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has been demonstrated to explain this association. Recently it has been demonstrated that inhaled particles pass into the systemic circulation in humans (1). Therefore we hypothesized that vascular events may be triggered by coagulation activation on cell surfaces induced by contact with environmental particles. Human peripheral blood monocyte-derived macrophages and a human bronchial epithelial cell line were exposed to PM10 and supernatants examined for procoagulant activity in a plasma recalcification time assay. Control experiments employed culture medium only or hydrogen peroxide. Both PM10 and hydrogen peroxide induced an increase of procoagulant activity in culture supernatants from both cell types [shortening of clotting time versus control with PM10 was 31% with macrophages and 19% with epithelial cells; mean of three experiments, $P < 0.001$ and < 0.01 respectively]. In order to characterise the procoagulant activity from macrophages induced by PM10 we went on to determine the effects of these particles on surface expression of negatively charged phospholipid by annexin V binding, and tissue factor mRNA expression by RT-PCR. Finally we measured macrophage interleukin 8 (IL8) mRNA expression and release in response to PM10. Environmental particles induced macrophage tissue factor mRNA expression and significantly increased bound annexin V. Furthermore IL8 message and release were significantly enhanced also. We have demonstrated, for the first time, that environmental particles associated with increased cardiovascular risk can activate macrophages *in vitro* with transformation to a procoagulant phenotype which may be mediated through apoptosis/negatively charged phospholipid exposure and tissue factor expression. This may represent a novel prothrombotic mechanism triggered when environmental particles reach the systemic circulation.

Reference

1 Nemmar *et al. Circulation* 2002; **105**: 411–4.

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Is early activation of the clotting cascade involved in the pathogenesis of verotoxin-producing *Escerichia coli*-associated haemolytic uraemic syndrome?

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In most cases, haemolytic uraemic syndrome (HUS) is a thrombotic complication of infection with *Escherichia coli* O157 : H7. It usually follows bloody diarrhoea caused by verotoxin secreted by the bacteria, and comprises acute renal failure, non-immune, microangiopathic haemolytic anaemia and thrombocytopaenia. HUS is primarily a disorder of the vascular endothelium, with platelet aggregation at sites of endothelial cell damage in the renal microvasculature. Several *in vitro* studies demonstrate changes in endothelial cell anticoagulant properties when exposed to cytokines and under apoptotic conditions. Of interest are changes in tissue factor (TF) expression and activity, TF being crucial in initiating the coagulation cascade. Additionally, the expression of antithrombotic membrane components, notably thrombomodulin, has been shown to be downgraded. We examined the changes in TF and thrombomodulin on endothelial cells exposed to the *E. coli* products, verotoxin and lipopolysaccharide (LPS), and the inflammatory cytokines, interferon-gamma (IFNgamma) and tumour necrosis factor-alpha (TNFalpha), and their relationship to apoptosis. Macrovascular [primary culture of human umbilical vein endothelial cells (HUVEC)] and microvascular [human microvascular endothelial cells (HMEC)] endothelial cells were cultured with LPS and verotoxin in the presence and absence of cytokines. Surface TF and thrombomodulin expression and apoptosis were assessed using flow cytometry. Increases in TF expression were observed in activated HUVECs; changes were less apparent in HMECs which constitutively express TF. Thrombomodulin was consistently reduced on HMECs under similar conditions. It appeared not to be the apoptotic population that changed their expression of membrane proteins. HUVECs cultured with LPS and TNFalpha demonstrated the greatest increase in TF. Decreases in thrombomodulin were greatest on HMECs exposed to verotoxin. We demonstrate an up-regulation of TF and a down-regulation of thrombomodulin on endothelial cells stimulated with LPS, verotoxin and inflammatory cytokines. These cells are rendered more thrombogenic, mechanisms that may be involved in the pathogenesis of HUS.

Oral Communication

Paediatric and General Haematology

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Revealed: D-dimers are being inappropriately requested. Results of audit to influence subsequent requesting

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D-dimers, cross-linked fibrin degradation products, are extremely sensitive, but non-specific markers for coagulation activation; a process that complicates many conditions. They have been shown to be useful in negatively predicting for deep vein thrombosis (DVT), particularly in combination with a clinical assessment score for DVT. Junior medical staff and GPs are increasingly using the D-dimer test to screen for the presence of DVT. Radiology departments use a positive D-dimer to 'gate-keep' their Doppler service. Haematology departments have therefore identified a dramatic increase in D-dimer, requesting and associated cost (£26,000 in 2002 at Derriford Hospital). Over-investigation may lead to unnecessary, and potentially dangerous, further investigation and anticoagulation. This audit was conducted to address these concerns. The case-notes were reviewed from 50 consecutive patients presenting to/within Derriford Hospital with suspected DVT (34%) or pulmonary embolism (66%) in 2002. Not surprisingly, junior-most of the doctors requested the bulk of these investigations, 11% by registrars and none by the consultants. The reasons for requesting were documented in all but seven patients. The Biomericux MDA D-dimer assay kit was previously evaluated locally, giving a positive cut-off level of 0.5 mg/mL for DVT with >95% sensitivity. In the current group of patients, 28% fell below the cut-off value. Of the positive results, only 61% were investigated further. The management was not affected by the D-dimer result in 45% of cases. Of the original cohort, only four DVTs and two PEs were confirmed. One PE was diagnosed in spite of negative D-dimer. These data support the theory regarding over-requesting of D-dimers. The low specificity is highlighted by only six confirmed DVT/PEs of 72% patients having a raised D-dimer. Locally, the co-importance of validated clinical assessment score is emphasized and the futility/danger of performing D-dimers on

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in-patients and those with suspected PE until a specific cut-off value has been established.

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Anti-D immunoglobulin in the treatment of immune thrombocytopenic purpura in adults in the United Kingdom: an evaluation of clinical outcomes and resource utilization

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Immune thrombocytopenic purpura [ITP] is an autoimmune disorder distinguished by a low platelet count and mucocutaneous bleeding. Estimated incidence is 10 per 100,000 persons per year. ITP is classified as primary, or secondary to an underlying disorder, and acute (of 6 months or less in duration), or chronic. In 70% of children, resolution occurs in 6 months. By contrast, ITP in adults has an insidious onset, and is chronic in nature. Given comparable efficacy of anti-D immunoglobulin and IVIg in the treatment of ITP, this inquiry was designed to contrast health resource utilization patterns stemming from the use of each agent in the treatment of ITP in adults. Stemming from the schematic developed by Cines and Blanchette (1), and information contained in the report of the British Committee for Standards in Haematology, General Haematology Task Force (Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children, and in pregnancy) we employed Monte Carlo simulation to evaluate the utility (cost implications) of various treatment schema (intent-to-treat format; steroids; IVIg; anti-D immunoglobulin) for the treatment of ITP. Utilizing 100,000 replicate simulations per year of age examined [15 through 80 years (5-year intervals); quadratic regression equations] we discerned a significant ($P < 0.05$) efficacy/health service utilization-based saving (percent reduction) for the 'steroids to anti-D immunoglobulin' arm, relative to the 'steroids to IVIg' arm [34.0% (SE = 0.1%) for males; 33.4% (SE = 0.1%) for females]. In the United Kingdom, use of anti-D immunoglobulin in the management of ITP is estimated to result in reduced health service expenditures relative to IVIg. Specifically, use of anti-D immunoglobulin reduces the time required (cost) for medication administration, patient monitoring, and occupancy of health care facilities. The effect on patient's quality-of-life warrants further research.

Reference
1 Cines and Blanchett. *NEJM* 2002; 346: 995–1008.

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Reduced numbers and antigen unresponsiveness of natural killer T-cells in idiopathic (autoimmune) thrombocytopenia (ITP)

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Natural killer T cells (NKT cells) are a rare cell subset implicated in the control of autoimmunity. Here, untreated ITP patients and healthy controls were compared for the number of circulating NKT cells and their proliferative response to the NKT cell antigen alpha-galactosylceramide. The cells were identified as Valpha24 Vbeta11 double-positive (CD4 positive or CD4 CD8 double-negative) lymphocytes using flow cytometry. In controls, NKT cell frequency ranged from 0.008 to 0.351% of total lymphocytes (mean 0.13%, $n = 9$). In the ITP group, a low NKT cell frequency was much more common, resulting in a mean of 0.04% (range 0.002–0.336%, $n = 14$). The capacity for alpha-galactosylceramide-driven NKT cell expansion was variable in the ITP group, which could be divided into responders (55% of tested donors, $n = 13$) and non-responders (45%). In comparison, 92% of controls ($n = 13$) responded. Interestingly, repeated testing of a limited number of ITP patients showed that proliferative response to the antigen or unresponsiveness was not constant over time, thus one and the same patient may respond at one time but not another. Furthermore, while IL-2 increased antigen-induced NKT cell proliferation in the majority of controls, this was not observed in the ITP group. In conclusion, the number of circulating NKT cells is reduced in ITP and a proportion of ITP patients have both antigen and IL-2 unresponsive NKT cells. The lack of proliferative response after antigen exposure and the IL-2 unresponsiveness may be due to adaptive tolerance, or *in vivo* anergy. This is believed to be caused by a prior incomplete antigen activation *in vivo*. These observations therefore suggest that NKT cells from ITP patients are anergic and this may contribute to the autoimmune process. Future research is planned to assess NKT cell function and involvement in autoimmune thrombocytopenia.

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Outcome heterogeneity in childhood high hyperdiploid acute lymphoblastic leukaemia

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High hyperdiploid (HeH) karyotypes (51–65 chromosomes) are found in approximately one-third of children with acute lymphoblastic leukaemia (ALL). Generally, these patients respond well to standard therapy and enjoy a superior outcome compared with other children. It has been suggested that specific cytogenetic features might further refine this prognosis and provide a method for identifying those with a poorer outlook, however no consensual picture has yet emerged. We analysed outcome data from 700 HeH ALL children enrolled on two MRC trials (UKALLXI and ALL97) to examine the effect of sex, age, white blood cell (WBC) count, individual trisomics, modal number and structural abnormalities on survival. Univariate analysis showed that age (1–9 years), sex (female), trisomies of chromosomes 4, 10 and 18, and a modal number of 54–65 chromosomes were all associated with improved survival, whereas the presence or absence of structural abnormalities and WBC had no effect on outcome. Multivariate analysis revealed that only age, sex, trisomy 4 (+4) and trisomy 18 (+18) were independent indicators of outcome. These results enabled the construction of two hazard scores for predicting relapse and mortality. Three risk groups were found using age, sex and +18, with event-free survival (EFS) rates of 86, 75 and 50% at 5 years ($P < 0.0001$). The group with the highest risk of relapse comprised:

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boys aged >9 years, boys aged 1–9 years without +18 and girls >9 years without +18. While girls aged 1–9 years with +18 made up the group with the best EFS. In terms of mortality, those aged <10 years with both +4 and +18 had a significantly improved survival (96 versus 84% at 5 years, $P < 0.0001$). These findings confirm that the outcome of children with HeH ALL is heterogeneous and information regarding specific trisomies can help identify those with a greater risk of treatment failure.

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Correction of the anaemia of chronic disease (ACD) with small doses of recombinant erythropoietin

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The anaemia associated with chronic disease such as infectious, inflammatory or neoplastic conditions, contributes significantly to morbidity. It has been recognized recently that a similar anaemia is also seen in congestive cardiac failure (CCF) and diabetes without significant renal failure. Pharmacological doses of recombinant erythropoietin (EPO) correct ACD associated with rheumatoid arthritis, inflammatory bowel disease, and AIDS. Good responses to EPO have also been seen in cardiac failure, and type 1 diabetes. Large doses of EPO are used in some centres to treat anaemia associated with cancer and its treatment. We corrected ACD with small doses of EPO in 10 patients: six males and four females, age range 58–88 years. Three patients had inflammatory disease, three were diabetic, and four had CCF. Six had either normal or reactive bone marrow histology, normal cytogenetics, and normal or increased iron stores. Six were transfusion dependent prior to EPO therapy. The dose of subcutaneous recombinant human erythropoietin (Eprex) was titrated according to the haemoglobin. Nine patients were evaluated, one having died from his underlying disease. The mean dose required was 9000 units/week (range 3000–14,000). The average maximum increase in haemoglobin was 4.1 g/dL (range 1.3–6.8). Few side effects were encountered. One patient suffered transient cerebro-vascular ischaemia, possibly related to a rapidly rising haemoglobin. All patients became transfusion independent. All reported improved general health without the peak and trough effect associated with blood transfusion. Avoidance of red cell transfusion is desirable in view of the possible infective and non-infective complications of transfusion and concern over the availability and cost of blood products. Although EPO is expensive, the doses required were comparable with those used in chronic renal failure, and much smaller than usually required to treat anaemia associated with cancer and its treatment. The cost therefore, compared favourably with blood transfusion.

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Epidemiological evidence for an infectious origin for childhood leukaemia

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The aim of the study was to interpret the results of several analyses of childhood leukaemia data from the Manchester Children's Tumour Registry (MCTR), in view of current hypotheses regarding an infectious origin. The MCTR is population-based and has been collecting case data from a geographically defined region of North West England since 1954. Trend tests, space-time clustering tests (Knox and *K*-function), ecological regression and geographical mapping were used to study the data on the leukaemias, broken down by sub-type (ALL, AML). For the most recent period (since 1980), ALL was further examined by immunophenotypic sub-type. There was an increasing incidence of ALL, due mainly to an increase of the precursor B-cell sub-type in the childhood peak. Space-time clustering was evident for cases of ALL, aged 0–4 years, for the time period 1954–1985, mainly based on date of diagnosis and place of birth. For the time period 1980–2001, space-time clustering was confined to cases of precursor B-cell ALL, aged 18–54 months, based on date and place of birth. The incidence of ALL was greater in more densely populated wards, largely because of cases of non-precursor B-cell ALL. The studies present a complex picture, but are supportive of an infectious aetiology, particularly for precursor B-cell ALL. It is concluded that there may be at least two infectious mechanisms operating, with a different mechanism predominating in the earlier years from that in the later years.

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Eculizumab

Prop Inn; USAN

[redacted]

Humanized Anti-C5 Monoclonal Antibody

h5G1.1
5G1.1

Immunoglobulin, anti-(human complement C5 α-chain) (human-mouse monoclonal 5G1.1 heavy chain), disulfide with human-mouse monoclonal 5G1.1 light chain, dimer

CAS: 219685-50-4

EN: 239304

Abstract

Eculizumab (5G1.1) is a recombinant, fully humanized monoclonal antibody that inhibits the activation of terminal complement components. Eculizumab binds specifically to the terminal human complement protein C5, preventing the release of the inflammatory mediator C5a and the formation of C5b-9 by inhibiting the cleavage of human complement C5 to C5a and C5b. Preclinical studies have demonstrated that the antibody has high binding affinity for C5, blocks the formation of C5a and C5b-9, and protects mammalian cells from C5b-9-mediated damage. Favorable clinical results have been reported in patients with membranous nephritis, rheumatoid arthritis and paroxysmal nocturnal hemoglobinuria (PNH) and the antibody is in phase II clinical development for these disorders; phase I trials have also been conducted in dermatomyositis.

Introduction

There is increasing evidence that activated components of the complement system contribute significantly to the pathogenesis of a large number of acute and chronic inflammatory diseases including rheumatoid arthritis, atherosclerosis, acute myocardial infarction and vascular injury after cardiopulmonary bypass (1-5). Furthermore, complement plays a significant role in the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH), characterized by intravascular hemolysis and venous thrombosis, and associated with aplastic anemia. Patients with PNH have a deficiency in CD59 and CD55 proteins on the surface of erythrocytes, platelets and other blood cells, which normally protect cells from complement-mediated injury (6-8). Patients with PNH experience fatigue, dysphagia, pain and hemoglobinuria, and the disease is associated with about 50% mortality (9).

Strategies that inhibit the damaging effects of complement on disease pathogenesis have therefore become a major focus in pharmacological research. In particular, inhibition of the complement cascade at C5, the cleavage of which results in the generation of the potent inflammatory mediators C5a and C5b-9 (10), while allowing the upstream generation of C3b, essential for combatting infections and preventing the development of autoimmune diseases (11), would appear to represent a promising approach.

Eculizumab is a recombinant, fully humanized monoclonal antibody that inhibits the activation of terminal complement components. Eculizumab binds specifically to the terminal complement protein C5 and prevents the release of the inflammatory mediator C5a and the formation of C5b-9 by inhibiting the cleavage of human complement C5 to C5a and C5b (12). The antibody is being developed for chronic indications including rheumatoid arthritis, membranous nephritis, dermatomyositis, lupus nephritis and paroxysmal nocturnal hemoglobinuria (13).

Pharmacological Actions

Eculizumab, as well as Fab and scFv fragments thereof, was evaluated for binding to complement and inhibition of complement activation *in vitro*. Eculizumab had similar binding affinity for human C5 compared to the murine antibody (IC_{50} ~ 2 nM), as determined in an ELISA, and it inhibited human serum complement-induced lysis of porcine aortic endothelial cells (PAEC) at the same concentration as the murine antibody (12).

Pharmacokinetics

A double-blind, randomized, placebo-controlled study was conducted with eculizumab (8 mg/kg i.v. once or

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twice monthly) to evaluate the pharmacokinetics/pharmacodynamics in 117 patients with idiopathic membranous nephropathy (mean creatinine clearance = 111 ml/min; 24-h proteinuria = 9 g; protein/creatinine ratio = 5; serum cholesterol = 270 mg/dl; hemoglobin = 12.0 g/dl; serum albumin = 2.8 g/dl). Preliminary results were reported for 11 patients. Mean steady-state trough serum eculizumab levels were 18 μg/ml in the twice-monthly treatment group and 8 μg/ml in the monthly treatment group. Mean steady-state mid-dose interval levels (1 week postdose on day 119 of the study) were 47 μg/ml for patients treated twice monthly and 35 μg/ml for those treated once monthly. Urine levels of eculizumab at midtrial and at the end of the trial were below the level of quantification in 8 patients, and 3 patients excreted 65-400 μg/24 h of eculizumab. All eculizumab-treated patients demonstrated < 10% of the hemolytic activity of placebo-treated patients at 1 h postinfusion. Serum eculizumab levels > 38 μg/ml inhibited hemolysis in all patients. Treatment with eculizumab was found to be safe and well tolerated, with no hypersensitivity reactions noted (14).

Pharmacokinetics and pharmacodynamics were also examined in an open-label pilot study in 11 transfusion-dependent patients with PNH who received treatment with eculizumab (600 mg weekly for 4 weeks, then 900 mg every 2 weeks for 2 months). Peak and trough serum levels of eculizumab were measured 1 h after the initial dose and then at weeks 3, 4, 6, 10 and 12, and in 10 patients eculizumab levels were > 35 μg/ml from 1 h after the first dose to the end of the study period. In 1 patient, eculizumab levels were > 35 μg/ml for the initial 10 weeks but < 35 μg/ml at week 12. In 10 patients, serum hemolytic activity was 20% or less for the entire treatment period, representing complete blockade, with only 1 patient showing increased hemolytic activity at week 12, which corresponded with a decline in eculizumab levels to < 35 μg/ml (15).

Clinical Studies

The safety and biological activity of a single i.v. dose of eculizumab (0.1-8 mg/kg) were investigated in 40 patients with mild to moderate rheumatoid arthritis. Mean C-reactive protein (CRP) levels were significantly reduced at day 7 compared to baseline after the highest dose and returned to baseline by day 14. Patients treated at this dose showed a trend for reductions in the signs and symptoms of rheumatoid arthritis. Doses of 2 mg/kg and above resulted in > 80% inhibition of human complement C5 for at least 10 h, and a single dose of 8 mg/kg provided > 80% inhibition for 10 days, with a return to baseline levels after 17 days. Eculizumab was safe and well tolerated across patient groups (16).

Another study examined the safety, efficacy and pharmacokinetics of eculizumab following multiple doses in 209 patients with rheumatoid arthritis. Patients were randomized to receive placebo, eculizumab 8 mg/kg every 2 weeks, eculizumab 8 mg/kg every week x 5 weeks, then every 2 weeks, or eculizumab 8 mg/kg every week x 5 weeks, then monthly, and evaluated both after 3 months of treatment and 3 months after terminating treatment. After 3 months of treatment, the induction/monthly group met the primary endpoint of improvement in ACR20 score (44% *vs.* 18% on placebo), and both induction groups met the secondary endpoint of decrease in CRP. Baseline C5b-9 levels were correlated with increased CRP levels and therefore systemic inflammation. Baseline C5b-9 levels > 200 mg/ml were associated with clinical response to eculizumab. In cases of elevated C5b-9, an ACR20 response was obtained in 9% of patients in the placebo group, 33% of patients in the twice-monthly eculizumab treatment group, 57% of patients in the induction/monthly treatment group and 50% of patients in the induction/twice-monthly treatment group. The induction regimens completely suppressed complement-mediated serum hemolytic activity up to week 4. The half-life of eculizumab was 10-14 days (17-20).

A randomized, double-blind, placebo-controlled phase IIb clinical trial evaluated the efficacy and safety of eculizumab in 368 adult patients with mild to moderate active rheumatoid arthritis undergoing treatment with methotrexate and/or leflunomide. The patients were enrolled at 69 sites in the U.S. and Canada and were randomized to receive placebo, twice-monthly eculizumab (600 mg i.v. weekly for 5 weeks, then 600 mg once every 2 weeks) or monthly eculizumab (600 mg i.v. weekly for 5 weeks, then 600 mg monthly). The primary endpoint was improvement in ACR20 score after 6 months of treatment. Monthly administration of eculizumab following an induction period was associated with a statistically significant, moderate improvement in the ACR20 score compared to placebo at 6 months. The percentage of patients who achieved an ACR20 response was 34%, 24% and 22%, respectively, on monthly eculizumab, twice-monthly eculizumab and placebo. Monthly therapy was also associated with evidence of a long-term antiinflammatory effect, with a significant reduction in erythrocyte sedimentation rate at 6 months. The incidence of adverse events was slightly higher among patients given eculizumab every 2 weeks, although the incidence of injection-site reactions remained low (21).

A double-blind, placebo-controlled phase I pilot study was performed to determine the safety and tolerability of eculizumab (8 mg/kg weekly for 5 weeks, then once every 2 weeks) in 10 patients with dermatomyositis compared with placebo (n=3). After 2 months of treatment, results showed moderate improvements in skin score, physician global score and manual muscle test in the eculizumab group compared with controls. The placebo group showed deterioration of physician global score, but improvements in skin score and manual muscle test compared to baseline. Disease intensity measured by magnetic resonance imaging (MRI) and various parameters on skin biopsy were also improved in the treatment group compared with placebo. Eculizumab was safe and well

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tolerated, with no significant differences in adverse events between treatment groups (22).

Alexion has sponsored phase II trials in patients with membranous nephritis, with results showing good tolerance. One trial did not reach the primary clinical efficacy endpoint of reduction in proteinuria after 4 months, while in the other trial an increase in remission rate and significant improvements in proteinuria, hyperlipidemia, hypertriglyceridemia and hypoalbuminemia were obtained after 12 months. The company also began a phase II study in lupus nephritis several years ago following the observation of positive effects on proteinuria in a study in patients with systemic lupus erythematosus (13, 23).

Further results regarding efficacy and safety were reported from the open-label pilot study in 11 transfusion-dependent patients with PNH treated with eculizumab (600 mg weekly for 4 weeks, then 900 mg every 2 weeks for 2 months). After 3 months of initial treatment, all patients were enrolled into an open-label extension study and went on to receive eculizumab 900 mg every 2 weeks for over a year. In the 12 months prior to entrance into the study, patients had received over 4 red blood cell transfusions, had detectable glycosylphosphatidylinositol (GPI)-deficient hematopoietic clone, and had a negative throat culture for *Neisseria meningitidis* and *Neisseria gonorrhoeae*. After the first 3 months of treatment, serum hemolytic activity was completely inhibited in the 10 patients who showed serum trough levels of eculizumab above 35 μg/ml; quality-of-life markers were also significantly improved, transfusion rates decreased and serum levels of lactate dehydrogenase (LDH), an indicator of hemolysis, were significantly reduced. Increased levels of PNH type III erythrocytes were thought to be due to prolongation of PNH red cell survival and confirmed that treatment with eculizumab inhibits complement-mediated hemolysis. The proportion of PNH neutrophils, monocytes and platelets did not change. The reduction in transfusions was greatest for patients (n=7) without thrombocytopenia. Biochemical markers of hemolysis showed dramatic improvement 1 week after beginning treatment with eculizumab. The most common adverse events were headache and upper respiratory tract infections, followed by influenza-like symptoms, rigors, dizziness, nausea, nasal congestion and joint aches. No episodes of thrombosis were reported. No adverse events were considered to be drug-related and although 2 patients had serious adverse events, all patients completed the 12-week study (15, 24-26).

Reports of the cumulative results after 6 and 12 months of eculizumab treatment showed that patients with purely hemolytic disease (macroscopic hemoglobinuria with a normal platelet count; n=6) had the best response to treatment, with type III erythrocytes increased from 39% to 67%, median transfusions reduced from 2.0 units/patient/month to 0 units/patient/month, and suppression of hemolysis. Four patients who did not become transfusion-independent were treated with additional subcutaneous erythropoietin with some improvement. Two patients reported breakthroughs in complement activity, demonstrated by increased lactate dehydrogenase levels, hemoglobinuria and symptoms. These symptoms resolved immediately after the next dose of eculizumab and patients were managed by reducing the dosing interval. The most commonly reported adverse events after 12 months of eculizumab treatment were flu-like symptoms, sore throat, headache and upper respiratory infection. Four serious adverse events were reported, but there were no study discontinuations as a result of adverse events and all patients elected to continue eculizumab treatment. It was concluded that eculizumab was well tolerated, decreased hemolysis and transfusion requirements, and improved symptoms of PNH for over 12 months (27, 28).

Conclusions

Eculizumab is a promising new monoclonal antibody that binds to human complement C5 and inhibits its cleavage by C5 convertase. Eculizumab has been granted orphan drug status for the treatment of PNH in both the U.S. and Europe. A pilot program has been completed in patients with PNH and plans are being finalized with the FDA for the next stage of development. Pilot programs have also been completed in patients with dermatomyositis and eculizumab is in phase II trials for rheumatoid arthritis and membranous nephritis.

Source

Alexion Pharmaceuticals, Inc. (US).

References

1. Morgan, B.P. *Clinical complementology: Recent progress and future trends.* Eur J Clin Invest 1994, 24: 219-28.

2. Morgan, B.P. *Complement and rheumatic disease.* In: Complement, Clinical Aspects and Relevance to Disease. B.P. Morgan (Ed.), Academic Press, New York, 1990, 130.

3. Torzewski, M., Klouche, M., Hock, J., Messner, M., Dorweiler, B., Torzewski, J., Gabbert, H.E., Bhakdi, S. *Immunohistochemical demonstration of enzymatically modified human LDL and its colocalization with the terminal complement complex in the early atherosclerotic lesion.* Arterioscler Thromb Vasc Biol 1998, 18: 369-78.

4. Rus, H.G., Niculescu, F., Vlaicu, R. *The relationship between macrophages and C5b-9 complement complexes in human atherosclerosis.* Clin Immunol Immunopathol 1988, 48: 307-16.

5. Kilgore, K.S., Friedrichs, G.S., Homeister, J.W., Lucchesi, B.R. *The complement system in myocardial ischaemia/reperfusion injury.* Cardiovasc Res 1994, 28: 437-44.

6. Holguin, M.H., Fredrick, L.R., Bernshaw, N.J., Wilcox, L.A., Parker, C.J. *Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of*

Samsung Bioepis Exhibit No. 1002
Page 6478

the erythrocytes of paroxysmal nocturnal hemoglobinuria. J Clin Invest 1989, 84: 7-17.

7. Rollins, S.A., Sims, P.J. *The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9.* J Immunol 1990, 144: 3478-83.

8. Morgan, B.P. *Isolation and characterization of the complement-inhibiting protein CD59 antigen from platelet membranes.* Biochem J 1992, 282: 409-13.

9. Hillmen, P., Lewis, S.M., Bessler, M., Luzzatto, L., Dacie, J.V. *Natural history of paroxysmal nocturnal hemoglobinuria.* New Engl J Med 1995, 333: 1253-8.

10. Matis, L.A., Rollins, S.A. *Complement-specific antibodies: Designing novel anti-inflammatories.* Nat Med 1995, 1: 839-42.

11 Liszewski, M.K., Atkinson, J.P. *The complement system.* In: Fundamental Immunology, W.E. Paul (Ed.), Raven Press, New York, 1993, 917-39.

12. Thomas, T.C., Rollins, S.A., Rother, R.P., Giannoni, M.A., Hartman, S.L., Elliott, E.A., Nye, S.H., Matis, L.A., Squinto, S.P., Evans, M.J. *Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv.* Mol Immunol 1996, 33: 1389-401.

13. *Product profiles: Autoimmune.* Alexion Pharmaceuticals Web Site July 13, 2004.

14. Appel, G., Nachman, P., Hogan, S. et al. *Eculizumab (C5 complement inhibitor) in the treatment of idiopathic membranous nephropathy (IMN): Preliminary baseline and pharmacokinetic (PK)/pharmcodynamic (PD) data.* J Am Soc Nephrol 2002, 13: Abst SU-P0970.

15. Hillmen, P., Hall, C., Marsh, J.C.W. et al. *Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria.* New Engl J Med 2004, 350: 552-9.

16. Jain, R.I., Moreland, L.W., Caldwell, J.R., Rollins, S.A., Mojcik, C.F. *A single dose, placebo controlled, double-blind, phase I study of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* 63rd Annu Meet Am Coll Rheumatol (Nov 13-17, Boston) 1999, Abst 42.

17. Kivitz, A., Burch, F., Birbara, C., Rollins, S., Tesser, J. *Pharmacokinetics (PK) and pharmacodynamics (PD) of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S214.

18. Burch, F., Tesser, J., Bell, L. *Baseline C5b-9 level correlates with CRP and ACR 20 response to the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S214.

19. Tesser, J., Kivitz, A., Fleischmann, R., Mojcik, C.F., Bombara, M., Burch, F. *Safety and efficacy of the humanized anti-C5 antibody h5G1.1 in patients with rheumatoid arthritis.* Arthritis Rheum 2001, 44(9, Suppl.): S274.

20. *Phase II clinical results for 5G1.1 anticomplement antibody support further testing in RA.* DailyDrugNews.com (Daily Essentials) Nov 19, 2001.

21. Mojcik, C.F., Kremer, J., Bingham, C., Burch, F., Vitiello, S., McCroskery, E., Bookbinder, S. *Results of a phase 2B study of the humanized anti-C5 antibody eculizumab in patients with rheumatoid arthritis.* Ann Rheum Dis 2004, 63(Suppl. 1): Abst FRI0170.

22. Takada, K., Bookbinder, S., Furie, R., Oddis, C., Mojcik, C., Bombara, M., Plotz, P., Kissel, J. *A pilot study of eculizumab in patients with dermatomyositis.* 66th Annu Meet Am Coll Rheumatol (Oct 25-29, New Orleans) 2002, Abst 1300.

23. *Alexion reports presentation of membranous nephritis clinical trials.* Alexion Pharmaceuticals Press Release November 4, 2002.

24. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Cullen, M., Richards, S.J., Rother, R.P. *Eculizumab, a C5 complement blocking antibody, is the first therapy to reduce transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria (PNH).* Br J Haematol 2003, 121(Suppl. 1): Abst 257.

25. Rother, R.P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Richards, S., Cullen, M., Hillmen, P. *Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and reduces transfusion dependency in patients with paroxysmal nocturnal hemoglobinuria (PNH).* Mol Immunol 2003, 40(2-4): Abst 54.

26. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Mojcik, C.F., Rollins, S.A., Richards, S., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, abolishes hemolysis and renders hemolytic patients with paroxysmal nocturnal hemoglobinuria (PNH) transfusion independent.* Blood 2002, 100(11, Part 1): Abst 154.

27. Hillmen, P., Hall, C., Marsh, J., Elebute, D., Richards, S., Rollins, S.A., Mojcik, C.F., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, controls hemolysis in paroxysmal nocturnal hemoglobinuria (PNH) with responses maintained over a prolonged period of therapy.* Blood 2003, 102(11, Part 1): Abst 1858.

28. Hillmen, P., Hall, C., Marsh, J.C.W., Elebute, M., Richards, S.J., Rollins, S.A., Mojcik, C.F., Rother, R.P. *Eculizumab, a C5 complement-blocking antibody, controls haemolysis in paroxysmal nocturnal haemoglobinuria (PNH) with responses maintained over a prolonged period of therapy.* Br J Haematol 2004, 125(Suppl. 1): Abst 5.

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ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

Section Editor: Craig M. Kessler, MD

Eculizumab for the Treatment of Paroxysmal Nocturnal Hemoglobinuria

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H&O Can you give a short background on the standard of care for paroxysmal nocturnal hemoglobinuria before the advent of eculizumab?

AH Until recently, the treatment for paroxysmal nocturnal hemoglobinuria (PNH) was predominantly supportive. Red blood cell (RBC) transfusions were administered when appropriate, and warfarin or other anticoagulation therapy was given following a thrombosis. In the UK we practice primary prophylaxis with anticoagulant therapy depending on the neutrophil PNH clone size. Other therapies, such as corticosteroids or androgen therapy, have also been used, but less commonly and with efficacy in only a few patients; the mechanism of action behind these treatments is unclear. Stem cell transplantation has also been used in the past, but of course this approach is associated with serious complications. It has been difficult to develop an evidence base for treatment because of the heterogeneous nature and rarity of this disease. Before eculizumab (Alexion), treatment for PNH was limited to alleviating symptoms and improving patient quality of life. Eculizumab is a monoclonal antibody that blocks the complement system at protein C5.

H&O What is the rationale for the development of eculizumab for the treatment of PNH?

AH We know the hemolysis in PNH is complement-mediated, thus this drug should be effective in preventing hemolysis. The pathogenesis of PNH is known to be due to complement sensitivity of RBCs against complement attack. RBCs in PNH have lost their protective proteins (eg, CD55 and CD59) that would normally prevent complement-mediated lysis. It was hypothesized that if the complement attack on the RBCs could be blocked, the hemolysis in PNH and its associated symptoms could be prevented. Based on patients who have inherited complement deficiencies, it appears that patients with deficiencies in complement proteins C5–C9 suffer few clinical consequences compared to those with deficiencies in the complement proteins before C5. Therefore, C5 seemed to be a logical target for blocking the complement cascade. Eculizumab was developed for the purpose of blocking the complement system safely and without severe complications.

H&O Eculizumab has been evaluated for the treatment of rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune conditions. Is there a similarity between these conditions and PNH that spurred investigators to evaluate eculizumab in PNH?

AH The research strategy has been to develop therapeutic approaches that block complement activity, which contributes to a variety of disorders. There is some evidence that some components of autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus are complement-mediated. In PNH, however, almost all of the clinical symptoms are due to the uncontrolled activity of complement. Therefore, whereas there are many possible disorders in which complement blockade might have some efficacy, PNH is the disorder in which we had little doubt that eculizumab would be effective.

H&O What were some of the initial findings with eculizumab in PNH?

AH In May 2002, we began the pilot study of eculizumab in PNH. Patients enrolled had been having regular transfusions, and most exhibited dark urine (a sign of intravascular hemolysis) and severe symptoms of PNH such as difficulty swallowing (dysphagia) and abdominal pain. Men complained of erectile dysfunction. Additionally, they had biochemical parameters indicative of active, ongoing intravascular hemolysis. We found that just 1 infusion of the drug caused a resolution of the

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dark urine by the next morning. Other symptoms, such as abdominal pain and difficulty in swallowing, resolved quickly. Of the 6 male patients who had experienced erectile dysfunction, 5 experienced complete resolution of this symptom. The patients felt better quickly and, in terms of biochemical parameters, we found quick improvements in lactate dehydrogenase levels as well.

H&O What were the next steps after this initial study?

AH The pilot study was initially planned to be 12 weeks in duration, but at the end of the 12 weeks, all patients wanted to continue with eculizumab therapy. The extension phase of the trial commenced and is still continuing with 10 of the 11 patients. Patients have experienced a dramatic improvement in their quality of life and generally prefer 2 weekly infusions of eculizumab rather than their previous treatment, which might have been, for example, 4 weekly blood transfusions. Some patients have become transfusion-independent.

H&O What side effects are associated with eculizumab?

AH The side effects associated with eculizumab have been very minimal. One patient in the pilot study had a reaction to the initial infusion, consisting of shivering and fever. She required observation only and has tolerated every infusion after that extremely well with no further reactions; she is still on the drug more than 3 years later. No other patients experienced any infusion-related reactions. The only minor side effect has been headache after the first or second infusions in some of the patients, but otherwise toxicity has not been a problem. There is a theoretical risk of meningitis, but this has not been a major issue to date.

H&O Is there any sense about whether eculizumab treats all patients equally or are there variations between patients that might change the efficacy of the agent?

AH We found that patients who have the classic purely hemolytic PNH respond better to the drug than those who have a hypoplastic bone marrow. The reason for this difference is simply that marrow failure can sometimes contribute more significantly than hemolysis to anemia, in which case, although the hemolysis will be blocked by eculizumab, marrow failure will most likely still lead to anemia and therefore continuing transfusions will be required. Certainly any patient with classical PNH who is hemolyzing frequently is a good candidate for eculizumab therapy. This agent would also be appropriate for patients with biochemical and clinical evidence of hemolysis because their quality of life can be improved.

H&O What is the clinical trial status of eculizumab?

AH Two phase III trials opened worldwide in the last year: TRIUMPH and SHEPHERD. The first opened about a year ago and the second opened earlier this year. The trials are now closed to recruitment, and we are eagerly awaiting the results. If the results of this trial are positive, I would expect that applications for product licenses would be made relatively quickly.

H&O Does eculizumab appear to have any effect on thrombosis, the most significant morbidity associated with PNH?

AH Although at the moment we have no evidence that eculizumab will prevent thrombosis, it is reasonable to hypothesize that thrombosis may be prevented by this terminal complement blockade through its mechanism of action. Theories for the causes of thrombosis in PNH include activation of PNH platelets by complement attack and nitric oxide depletion through free hemoglobin scavenging. Eculizumab should prevent both consequences. This possibility will be very interesting to examine in the future. In the pilot study there was no evidence of thromboses in any of the patients.

Suggested Reading

Hill A, Hillmen P, Richards SJ, et al. Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. *Blood*. 2005;106:2559-2565.

Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2004;350:552-559.

Parker C, Omine M, Richards S, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. *Blood*. 2005; [Epub ahead of print].

Poggi A, Negrini S, Zocchi MR, et al. Patients with paroxysmal nocturnal hemoglobinuria have a high frequency of peripheral-blood T cells expressing activating isoforms of inhibiting superfamily receptors. *Blood*. 2005;106:2399-2408.

Young NS. Paroxysmal nocturnal hemoglobinuria: current issues in pathophysiology and treatment. *Curr Hematol Rep*. 2005;4:103-109.

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(54) **STRATIFICATION OF PATIENT POPULATIONS HAVING OR SUSPECTED OF HAVING RHEUMATOID ARTHRITIS**

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(57) **ABSTRACT**

The present disclosure is directed to screening, diagnosing and treating patients having, or suspected of having, rheumatoid arthritis. Levels of glucose-6-phophate isomerase or antibodies to glucose-6-phophate isomerase are assayed in test subjects or populations to determine susceptibility to, or existence of, an antibody mediated form of rheumatoid arthritis in such test subjects or populations. The results of the assays provide guidelines for therapeutic intervention with complement inhibiting agents.

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STRATIFICATION OF PATIENT POPULATIONS HAVING OR SUSPECTED OF HAVING RHEUMATOID ARTHRITIS

BACKGROUND

[0001] 1. Technical Field

[0002] The present disclosure relates to stratifying patients having, or suspected of having rheumatoid arthritis, and selection of therapeutic agents useful in the treatment thereof.

[0003] 2. Background of Related Art

[0004] Rheumatoid arthritis (RA) is a chronic disease which can exhibit a variety of systemic manifestations. This disease has an unknown etiology and characteristically exhibits a persistent inflammatory synovitis which usually involves peripheral joints in a symmetric distribution. Despite the destructive potential of RA, its course can be quite variable. In many patients, a chronic polyarthritis begins insidiously with fatigue, anorexia, generalized weakness and vague musculoskeletal symptoms until the appearance of synovitis becomes apparent. In about 10% of patients, the onset can be more acute with rapid development of polyarthritis. In about one-third of patients, symptoms may initially be confined to one or a few joints. Overall, most patients will experience an intermediate course of the disease. The potential of inflammation to cause cartilage destruction, bone erosions and, ultimately, joint deformities is the most important feature of this disease.

[0005] Many drugs have been used to treat rheumatoid arthritis without complete relief of the symptoms. Conventional drugs include non-steroidal anti-inflammatory drugs (NSAIDs, aspirin, ibuprofen), gold salt, penicillamine, TNFα inhibitors, IL-1 receptor antagonists, and steroidal hormones. The steroidal hormones, which are considered to be the most potent and effective, have side effects when taken for long periods. NSAIDs also have side effects such as stomach irritation and can be ulcerigenic. Recently, a compound which blocks the C5 component of the complement cascade (h5G1.1-mAb) has been shown to be an effective therapy in a murine model of RA. This compound is now in human clinical trials for the treatment of RA.

[0006] Although the etiologic stimulus for RA has not been identified, established rheumatoid synovitis is characterized by persistent immunologic activity. The presence of rheumatoid factor, which is an autoantibody reactive with IgG, suggests that there may be an underlying immune dysfunction in association with RA. This factor was initially described by Waaler in 1940 and later described by Rose and co-workers in 1948. Rheumatoid factors consist of IgA, IgG and/or IgM immunoglobulin classes. The idiotypic nature of rheumatoid factor autoantibodies has been described and partially characterized. This suggests that there may be a perturbation in the idiotype/anti-idiotype humoral immune network of patients with RA. In addition, the presence of rheumatoid factor can be of prognostic significance because patients with high titers tend to have more severe and progressive disease with extraarticular manifestations. Rheumatoid factor can also be employed to confirm the diagnosis of RA in patients with a clinical presentation of RA and, if at high titer, to designate patients at risk for severe systemic disease.

[0007] The examination of established rheumatoid synovitis reveals a characteristic constellation of features which include hyperplasia and hypertrophy of the synovial lining cells, focal or segmental vascular changes and infiltration with mononuclear cells often collected into aggregates or follicles around small blood vessels. The predominant infiltrating cells are T lymphocytes with T4 (helper-inducer) cells predominating over T8 (suppressor-cytotoxic) cells. Evidence of B cell activation can also be found in the inflamed synovium and plasma cells producing immunoglobulin and rheumatoid factor are characteristic features of rheumatoid synovitis. The resultant production of immunoglobulin and rheumatoid factor can lead to the formation of immune complexes with subsequent complement activation and exacerbation of the inflammatory process through the production of anaphylatoxins and chemotactic factors. Indeed, levels of total hemolytic complement, C3 and C4 are markedly diminished in synovial fluid relative to total protein concentration as a result of activation of the classic complement pathway by locally produced immune complexes. All these findings suggest that progression of RA is an immunologically mediated event.

[0008] The most widely used immunodiagnostic assay of RA is the so-called Waaler-Rose assay discussed above. This assay is based upon the antibody (rheumatoid factor) to the Fc region of IgG. Rheumatoid factor (RF) is present in about 60% to 70% of those individuals afflicted with RA. However, the test may not always be accurate because it has been found to give false positives or negatives, and it does not assess the response to therapy or predict activation or reactivation of the disease process.

[0009] Since there is no unambiguous test distinguishing RA from other acute or chronic inflammatory diseases, differentiating RA from other arthritides, such as systemic lupus erythematosus (SLE), ankylosing spondylitis (AS), polyarticular gout (PAG), or psoriatic arthritis (PsA) is often difficult. Diagnosis of RA is usually made according to American Rheumatism Association (ARA) criteria, i.e.:

[0010] (1) morning stiffness;

[0011] (2) joint tenderness or pain on motion;

[0012] (3) soft-tissue swelling of the joint;

[0013] (4) soft-tissue swelling of a second joint (within three months);

[0014] (5) soft-tissue swelling of symmetrical joints (excludes distal interphalangeal joint);

[0015] (6) subcutaneous nodules;

[0016] (7) X-ray changes;

[0017] (8) serum positive for rheumatoid factors;

[0018] wherein diagnosis of 3 or 4 of these factors is considered representative of probable RA and diagnosis of 5 or more of the factors is considered representative of definite RA.

[0019] Recently, a transgenic mouse model (K/BxNT cell receptor) was found to exhibit spontaneous inflammatory arthritis resembling many of the features of human RA. See, Matsumoto et al., Science, 286, 1732-1735 (1999) and Ji et al. J. Exp. Med., 194 (3) 321-330 (2001). The inflammatory arthritis was traced to the presence of glucose-6-phosphate

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isomerase as an antigen targeted by T-cells and IgG immunoglobulins. Glucose-6-phosphate isomerase ("GPI") is a dimeric enzyme that catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate. The protein functions in different capacities inside and outside the cell. In the cytoplasm, the gene product is involved in glycolysis and gluconeogenesis, while outside the cell it functions as a neurotrophic factor for spinal and sensory neurons. The enzyme has also been known as glucosephosphate isomerase, phosphohexose isomerase (PHI), phosphoglucose isomerase (PGI), autocrine motility factor (AMF) and neuroleukin (NLK). In addition, it has recently been shown that a majority of humans with RA have elevated levels of anti-GPI IgG immunoglobulin in sera and elevated levels of soluble GPI in the sera and synovial fluid. See, e.g., Schaller et al., Nature Immunology, Vol. 2, No. 6, 746-753 (2001).

[0020] In light of the ambiguity and relative subjectivity of existing tests for RA there is a need for diagnostic procedures that allow non-subjective determination of the existence of RA and which provide a concrete indication of a course of therapeutics.

SUMMARY

[0021] In one aspect, a method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent is provided which includes conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of a patient to determine the level of GPI in the patient's serum or synovial fluid, and comparing the level of GPI in the serum or synovial of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The baseline serum GPI levels may encompass a mean concentration of 0.069+/−0.048 U/ml, P<0.0001. The baseline synovial fluid GPI levels may encompass a mean concentration of 0.060+/−0.052 U/ml P<0001. The patient may be a mammal such as a human.

[0022] In another aspect, a method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent is provided which includes conducting an assay for glucose-6-phosphate isomerase antibody (anti-GPI) in the serum or synovial fluid of a patient to determine the level of anti-GPI in the patient's serum or synovial fluid, and comparing the level of anti-GPI in the serum or synovial of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The baseline serum anti-GPI levels may encompass a mean concentration of (A_{405}) 0.059+/−0.037, P<0.0001. The baseline synovial fluid anti-GPI levels may encompass a mean concentration of (A_{405}) 0.645+/−0.209 P<0001. The patient may be a mammal such as a human.

[0023] In another aspect, a method for screening a population of subjects for antibody mediated rheumatoid arthritis and determining treatment thereof is provided which includes conducting a glucose-6-phophate isomerase (GPI) assay on the serum or synovial fluid of a population of subjects, and identifying subjects having GPI assay results which are statistically significantly greater than (P<0.05) the respective mean GPI serum or synovial fluid assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent. The subjects so identified may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G 1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0024] In another aspect, a method for screening a population of subjects for rheumatoid arthritis and determining treatment thereof is provided which includes conducting an assay for glucose-6-phophate isomerase antibody (anti-GPI) on the serum or synovial fluid of a population of subjects, and identifying subjects having anti-GPI assay results which are statistically significantly greater than (P<0.05) the respective mean anti-GPI serum or synovial fluid assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent. The subjects so identified may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0025] In another aspect, a method for predicting susceptibility of a patient to rheumatoid arthritis and to treatment with a complement inhibiting agent is provided which includes conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid, and comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of susceptibility to rheumatoid arthritis in the patient which is further susceptible to treatment with a complement inhibiting agent. The patient who exceeds the numerical range may then be treated with the complement inhibiting agent. The complement inhibiting agent may be a C5 complement

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inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

[0026] In another aspect, a method for predicting susceptibility of a patient to rheumatoid arthritis and to treatment with a complement inhibiting agent is provided which includes conducting an assay for glucose-6-phosphate isomerase antibody (anti-GPI) of the serum or synovial fluid of the patient to determine the level of anti-GPI in the serum or synovial fluid, and comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of susceptibility to rheumatoid arthritis in the patient which is further susceptible to treatment with a complement inhibiting agent. The complement inhibiting agent may be a C5 complement inhibiting antibody or fragment thereof. The C5 complement inhibiting antibody or fragment thereof may be 5G1.1-mAb, h5G1.1-mAb, 5G1.1-scFv or h5G1.1-scFv. The patient may be a mammal such as a human.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0027] The present disclosure provides convenient, reliable methods to screen and treat patients having, or suspected of having, rheumatoid arthritis (RA). Recognition that glucose-6-phosphate isomerase (GPI) is an antigen which triggers an autoimmune response in a significant portion of the population afflicted with RA provides the basis for these methods. Detection of elevated levels of GPI or antibodies to GPI (anti-GPI) in the sera or synovial fluid of such individuals is indicative of antibody mediated complement activation and provides a signpost for therapeutic intervention through complement inhibition. Thus, by checking for elevated levels of serum or synovial GPI or anti-GPI antibodies in a population of subjects, a determination can be made as to whether members of that population will respond to therapeutic agents that inhibit complement.

[0028] In one aspect, patients already diagnosed with RA are stratified with respect to susceptibility to treatment with complement inhibitors. By determining the level of GPI or anti-GPI in serum or synovial fluid, a decision can be made by medical practitioners to treat the RA with a complement inhibitor or some other therapeutic approach. For example, a patient with RA who exhibits elevated GPI or anti-GPI levels may be treated with any pharmaceutically acceptable complement inhibitor. Those RA patients who do not exhibit elevated levels of GPI or anti-GPI would be directed to treatments which are not based on complement inhibition.

[0029] It is contemplated that any technique known to those skilled in the art can be utilized to determine the level of GPI or anti-GPI in the serum or synovial fluid. While enzyme-linked immunosorbent assay (ELISA) is preferred for detection of GPI or anti-GPI levels, other assays, e.g., enzyme activity assays, fluid phase assays, radioimmunoassay, precipitation, agglutination, direct and indirect immunofluorescence and complement fixation can be used. These assays may employ any protocol such as competitive, inhibition or sandwich.

[0030] Once measured, the GPI or anti-GPI concentrations in serum and/or synovial fluid of non-rheumatoid arthritis patients can be calculated to create a baseline GPI test standard. For example, an assay involving spectrophotometric analysis of enzyme activity to determine the level of GPI in the sera of normal individuals provided a human mean concentration baseline standard of 0.069+/−0.048 U/ml. See, Schaller et al., Nature Immunology, Vol. 2, No. 6, 746-753 (2001). An example of a baseline level of GPI in synovial fluid is 0.060+/−0.052 U/ml, P<0.001. Id. An example of baseline levels of anti-GPI in sera and synovial fluid of healthy individuals was determined by ELISA to be (A_{405}) 0.059+/−0.037, P<0.001, and (A_{405}) 0.645+/−0.209 P<0001, respectively. Id. Baseline levels may otherwise be determined in accordance with established principles and various techniques known to those skilled in the art.

[0031] One can then numerically compare the GPI or anti-GPI assay results of a test subject to the respective GPI or anti-GPI assay results of a rheumatoid arthritis-free population to determine whether the GPI or anti-GPI assay results of the test subject exceed the numerical range of the test results of the rheumatoid arthritis-free population. The numerical comparison involves comparing one or more of the above referenced GPI or anti-GPI results from the test subject to determine if one or more of the results is outside of the numerical range of the test results of the rheumatoid arthritis-free population. Optionally, statistical analysis can be performed to determine if the GPI or anti-GPI test results of the test subject exceed about two standard deviations or more above the mean test results of the rheumatoid arthritis-free population. In another option, statistical analysis can be performed to determine if the GPI or anti-GPI test results of the test subject exceed about one standard deviation or more above the mean test results of the rheumatoid arthritis-free population. These findings are used to support a diagnosis in the test subject of a predisposition to a positive response to complement inhibition therapies.

[0032] It should be understood by those of ordinary skill in the art that, in one aspect, this method is useful to diagnose a predisposition for the subsequent development of RA in a test subject or population of test subjects so that an early therapeutic regimen of complement inhibiting agents may be instituted. It should also be understood that not all test subjects with elevated level test results will always develop RA (i.e., there may be false positives). The test results from normal (NL) and RA populations may have overlapping ranges, with RA patients generally having the higher numerical range. The strength of the diagnosis relates to the test results obtained as follows. In a case in which the test results of a test subject lies above, that is, exceeds the numerical range of, the test results obtained from a rheumatoid arthritis-free population, it is said that the test subject is likely to develop RA and that serious consideration of therapy involving complement inhibiting agents should be given. However, not all test subjects that will develop RA will have test results exceeding the numerical range of test results from a rheumatoid arthritis-free population. Therefore, one can perform a statistical analysis to determine if a test result of the test subject is more than two standard deviations above the mean of the test results from a rheumatoid arthritis-free population. The test result of the test subject can be greater than two standard deviations or more above the mean and also be within the absolute numerical range of the results from a rheumatoid arthritis-free popu-

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lation. In this case, it is said that the test subject is likely to develop RA and that complement inhibiting agents should be given consideration. In a final case, the test result of a test subject can be more than one standard deviation above the mean of the test results from a rheumatoid arthritis-free population and also be within the absolute numerical range of test results for RA confirmed patients. The diagnosis of a predisposition for RA and complement inhibiting agent therapy based on these results is less clear. In this case, a test subject may develop RA. However, the percentage of individuals that ultimately develop RA will be smaller than those with test results about two standard deviations or more above normal or those exceeding the numerical range of normal (i.e., an increase in the incidence of false positives).

[0033] As mentioned above, it is possible to determine the susceptibility of an entire population of subjects to RA and to determine whether complement inhibiting agents should be given prophylactically in accordance with the present disclosure. The method of screening a population of subjects for the presence or absence of a predisposition toward developing RA includes: (1) performing a GPI or anti-GPI assay on a population of subjects; (2) calculating the results of the GPI or anti-GPI assay for the population; and (3) diagnosing in the test subjects the presence or absence of a predisposition toward developing RA by statistical analysis of the GPI or anti-GPI assay results. If there is a predisposition toward developing RA, prophylactic administration of complement inhibiting compounds should be considered.

[0034] The GPI or anti-GPI assay is performed on the population to be screened essentially as described above. Of course, the larger the population of subjects screened, the greater the potential accuracy of the diagnosis. Preferably, the population includes at least about 20 subjects. The GPI or anti-GPI test results for the population of subjects are calculated as described above. The test results are then analyzed statistically to identify subjects having GPI or anti-GPI assay results that are significantly greater than the mean GPI or anti-GPI assay results of the entire population of subjects. For example, a linear regression outlier analysis can be performed on the GPI or anti-GPI assay results for the entire population. Those subjects having one or more GPI or anti-GPI assay results that are statistically significantly greater than the mean values for the population (i.e., the test results is an outlier) are diagnosed as having a predisposition toward developing RA. Although the confidence level for the statistical analysis can be as low as $p<0.001$, typically, a $p<0.05$ is used. Linear regression outlier analysis is a known statistical analysis that identifies a data point that differs significantly (i.e., at a given p value) from the mean of a pool of data points from which the data point has been selected. The difference between the data point and the mean of the pool of data points being statistically significantly greater than that which is consistent with the inherent variability of the variable which the data point represents. "p value" or "P<" refers to the probability of observing a sample at least as unlikely as the one observed if the null hypothesis is true. For example, a sample result is statistically significant at the 5% level if, and only if, it leads to a rejection of the null hypothesis when the type I error probability alpha (i.e., the significance level) is 0.05.

[0035] Once a determination is made that a subject has elevated GPI or anti-GPI levels, a decision can be made regarding therapeutic intervention. The fact that soluble GPI is present in the synovial fluid of inflamed RA joints indicates that anti-GPI IgG forms immune complexes with the soluble GPI, which precipitate on the surface of the synovial lining with subsequent activation of the complement cascade. See Schaller et al., supra. Accordingly, compounds that interfere with the complement cascade can be utilized to treat subjects having GPI associated RA.

[0036] The complement cascade has been studied in depth and is fairly well understood. The complement system is an important means by which a host defends itself against infection. The complement cascade system is a component of the immune system that helps provide a natural immunity against invading microbes and is also an effector arm of antibody mediated humoral immunity. Complement is responsible for activating cells and other molecules involved in the inflammatory process as well as being directly related to the destruction of microbial invaders. The activation of complement involves a cascade of proteolytic reactions that lead to the release of inflammatory mediators and result in the assembly of the terminal complement complex. This cascade system has been characterized as containing at least thirty serum and membrane proteins that are activated by antibody-antigen complexes or by the invasion, in a host or experimentally in culture, by a microorganism, or other antigenic molecules.

[0037] The complement cascade consists of two major branches, the classical and alternative pathways. Though these pathways are initiated differently, they converge at the step of complement protein C3 activation. The complement cascade can mediate undesirable cellular damage in inflammatory, immune or autoimmune (auto-antibody-mediated) conditions. A series of regulatory proteins are involved in the control of the complement cascade. These proteins are considered part of the complement system and act to block endogenous complement activity at various stages of the complement cascade.

[0038] The classical pathway of the complement system is initialized by complement protein C1 binding to antigen bound IgG or IgM. C4 and C2 are then activated in the formation of C3 convertase at which point an amplification takes place that generates literally thousands of C3a and C3b fragments. C3b fragments can bind to complement protein complex C4b2a to form C4b2a C3b which is called C5 convertase and generates thousands of C5a and C5b fragments. C3b can also be used to regenerate C3 convertase which causes a greater amplification of complement activation. One approach to inhibit complement mediated effects is by depleting complement.

[0039] Any compounds that bind to or otherwise block the generation and/or activity of any of the human complement components, such as, for example, antibodies specific to a human complement component are useful herein and are referred to as complement inhibitors or complement inhibiting agents. Some complement inhibiting agents include 1) antibodies or antibody fragments directed against complement components C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2 and 2) naturally occurring or soluble forms of complement inhibitory compounds such as CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, serine protease inhibitors and K76 COOH.

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[0040] Particularly suitable complement inhibiting agents for use herein are antibodies or fragments thereof that reduce, directly or indirectly, the conversion of complement component C5 into complement components C5a and C5b. One class of useful antibodies are those having at least one antibody-antigen binding site and exhibiting specific binding to human complement component C5, wherein the specific binding is targeted to the alpha chain of human complement component C5. Such an antibody 1) inhibits complement activation in a human body fluid; 2) inhibits the binding of purified human complement component C5 to either human complement component C3 or human complement component C4; and 3) does not specifically bind to the human complement activation product for C5a. Particularly useful complement inhibitors are compounds which reduce the generation of C5a and/or C5b-9 by greater than about 30%. A particularly useful anti-C5 antibody is 5G1.1-mAb. Also particularly useful is a single chain Fv version of 5G1.1-mAb referred to as 5G1.1-scFv. In a preferred embodiment, humanized versions of these antibodies or scFv referred to as h5g1.1-mAb and h5G1.1-scFv are utilized. Methods for the preparation of these antibodies including h5G1.1-mAb and h5G1.1-scFv are described in U.S. patent application Ser. No. 08/487,283 filed Jun. 7, 1995, now U.S. Pat. No. 6,355,245, and "Inhibition of Complement Activity by Humanized Anti-C5 Antibody and Single Chain Fv", Thomas et al., *Molecular Immunology*, Vol. 33, No. 17/18, pages 1389-1401, 1996, the disclosures of which are incorporated herein in their entirety by reference. It is contemplated that Fab fragments, Fab' fragments, $F(ab')_2$ fragments, diabodies, and other forms of antibody fragments directed to the C5 component are suitable as well.

[0041] It is intended that the methods of the present disclosure are applicable to various animals such as mammals, e.g., humans, dogs, cats, horses, etc., which can all be benefited by screening and treatment herein. The complement inhibitors can be utilized in pharmaceutical compositions by adding an effective amount of one or more complement inhibitors to a suitable pharmaceutically acceptable diluent or carrier. Those skilled in the art are familiar with numerous techniques and formulations utilized to compound pharmaceutical compositions. The pharmaceutical compositions herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, (pulmonary, e.g., by inhalation or insufflation of liquids, powders or aerosols, including by nebulizer; intratracheal, intranasal, enteral, epidermal and transdermal), oral, sublingual, buccal or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intramedullary or intracranial, e.g., intrathecal or intraventricular, administration. Direct injection into affected joints is contemplated as well.

[0042] Pharmaceutical compositions for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, troches or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients. Pharmaceutical compositions herein include, but are not limited to, solutions, emulsions, suspensions, foams and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids, according to conventional methods, by one of skill in the art.

[0043] The pharmaceutical formulations herein, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product. Further details on techniques for formulation and administration of numerous dosage forms may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.). The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

[0044] Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0045] Pharmaceutical compositions suitable for use herein include compositions wherein the complement inhibitors are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically

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effective dose refers to that amount of active ingredient, which ameliorates, partially or completely, the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for animal or human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0046] The exact dosage will be determined by the practitioner in light of factors related to the subject that require treatment. Dosage and administration are adjusted to provide sufficient levels of the complement inhibitors to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation. Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g per kilogram, depending upon the route of administration. For example, anti-C5 monoclonal antibodies may be administered at about 8 mg/kg or at a 600 mg dose. Anti-C5 scFv may be administered at about 2 mg/kg. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art.

[0047] Although preferred and other embodiments have been described herein, modifications of the embodiments and further embodiments may be envisioned by those skilled in the art without departing from the scope of the invention as defined by the following claims.

What is claimed is:

1. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent comprising:

conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid; and

comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis patient to treatment with the complement inhibiting agent.

2. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 1 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2

3. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 2 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

4. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 3 wherein the antibody is 5G1.1-mAb or h5G1.1-mAb.

5. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 3 wherein the functional antibody fragment is 5G1.1-scFv or hG51.1-scFv

6. A method for determining the eligibility of a patient having rheumatoid arthritis to treatment with a complement inhibiting agent according to claim 1 wherein the baseline serum GPI levels encompass a mean concentration of 0.069+/−0.048 U/ml, P<0.0001 and the baseline synovial fluid GPI levels encompass a mean concentration of 0.060+/−0.052 U/ml P<0001.

7. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 1 wherein the patient is a mammal.

8. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 7 wherein the patient is a human.

9. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent comprising:

conducting an assay for antibodies to glucose-6-phosphate isomerase (anti-GPI) in the serum or synovial fluid of the patient to determine the level of ant-GPI in the serum or synovial fluid; and

comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population indicate susceptibility of the rheumatoid arthritis to treatment with the complement inhibiting agent.

10. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 9 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

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11. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 10 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

12. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 11 wherein the antibody is 5G1.1-mAb or h5G1.1-mAb.

13. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 11 wherein the functional antibody fragment is 5G1.1-scFv or hG51.1-scFv.

14. A method for determining the eligibility of a patient having rheumatoid arthritis to treatment with a complement inhibiting agent according to claim 9 wherein the baseline serum anti-GPI levels encompass a mean concentration of (A_{405}) 0.059+/−0.037, P<0.0001 and the baseline synovial fluid anti-GPI levels encompass a mean concentration of (A_{405}) 0.645+/−0.209 P<0001.

15. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 9 wherein the patient is a mammal.

16. A method for determining the eligibility of a patient having rheumatoid arthritis for treatment with a complement inhibiting agent according to claim 15 wherein the patient is a human.

17. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof comprising:

conducting a glucose-6-phophate isomerase (GPI) assay on the serum or synovial fluid of a population of subjects;

identifying subjects having GPI assay results which are statistically significantly greater than (P<0.05) the mean GPI assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent.

18. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 17 further comprising treating the subjects so identified with a complement inhibiting agent.

19. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 18 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

20. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 19 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

21. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 20 wherein the antibody is 5G1.1 or h5G1.1.

22. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 20 wherein the C5 complement inhibiting antibody wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

23. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 17 wherein the subjects are mammals.

24. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis according to claim 23 wherein the subjects are humans.

25. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof comprising:

conducting an assay for antibodies to glucose-6-phophate isomerase (anti-GPI) in the serum or synovial fluid of a population of subjects;

identifying subjects having anti-GPI assay results which are statistically significantly greater than (P<0.05) the mean anti-GPI assay results of the population by linear regression analysis, wherein the subjects so identified are diagnosed as having rheumatoid arthritis which is susceptible to treatment with a complement inhibiting agent.

26. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 25 further comprising treating the subjects so identified with a complement inhibiting agent.

27. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 26 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

28. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 27 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

29. A method for screening a population of subjects for the presence or absence of antibody mediated rheumatoid arthritis and determining treatment thereof according to claim 28 wherein the antibody is 5G1.1 or h5G1.1.

30. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 28 wherein the C5 complement inhibiting antibody wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

31. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis and determining treatment thereof according to claim 25 wherein the subjects are mammals.

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32. A method for screening a population of subjects for the presence or absence of rheumatoid arthritis according to claim 31 wherein the subjects are humans.

33. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent comprising: conducting a glucose-6-phosphate isomerase (GPI) assay of the serum or synovial fluid of the patient to determine the level of GPI in the serum or synovial fluid; and comparing the level of GPI in the serum or synovial fluid of the patient to respective baseline GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of a predisposition for antibody mediated rheumatoid arthritis in the patient which is susceptible to treatment with a complement inhibiting agent.

34. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 further comprising treating the patient exceeding the numerical range with a complement inhibiting agent.

35. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

36. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 35 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

37. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 36 wherein the wherein the antibody is 5G1.1 or h5G1.1.

38. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 36 wherein the antibody fragment is 5G1.1-scFV or h5G10.1-scFv.

39. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 33 wherein the patient is a mammal.

40. A method for predicting susceptibility of a patient to rheumatoid arthritis according to claim 39 wherein the patient is a human.

41. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent comprising:

conducting an assay for antibodies to glucose-6-phosphate isomerase (anti-GPI) in the serum or synovial fluid of the patient to determine the level of anti-GPI in the serum or synovial fluid; and

comparing the level of anti-GPI in the serum or synovial fluid of the patient to respective baseline anti-GPI serum or synovial fluid levels established by the test results of a rheumatoid arthritis-free population, wherein the anti-GPI serum or synovial fluid assay results of the patient exceeding the numerical range of the rheumatoid arthritis-free population are diagnostic of a predisposition for antibody mediated rheumatoid arthritis in the patient which is susceptible to treatment with a complement inhibiting agent.

42. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 41 further comprising treating the patient exceeding the numerical range with a complement inhibiting agent.

43. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 42 wherein the complement inhibiting agent is selected from the group consisting of CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, y bind protein, complestatin, K76 COOH, serine protease inhibitor and antibodies and functional antibody fragments directed against complement components C1, C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2.

44. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 43 wherein the complement inhibiting agent is a C5 complement inhibiting antibody or functional antibody fragment thereof.

45. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 44 wherein the wherein the antibody is 5G1.1 or h5G1.1.

46. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 44 wherein the antibody fragment is 5G1.1-scFV or h5G1.1-scFv.

47. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 41 wherein the patient is a mammal.

48. A method for predicting susceptibility of a patient to antibody mediated rheumatoid arthritis and treatment with a complement inhibiting agent according to claim 47 wherein the patient is a human.

* * * * *

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27. Okt. 2017

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European Patent Office

80298 M U N I C H

Opposition against EP Patent 2359834
(EP 11 00 1632.6)
Patentee: Alexion Pharmaceuticals, Inc.
Opposition by: Amgen Inc.; Hoffmann - Eitle
Vossius Ref.: P2913 OPP(EP/1)

München, October 27, 2017
WA/FLG/MUA

We herewith resign from representation of the above-identified opposition matter.

Please direct all future correspondence to

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European Patent Attorneys
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Ms. Amanda Simons
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GB

It is respectfully requested that this change is registered in the official Register.

Dr. Joachim Wachenfeld
European Patent Attorney

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J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

30 October 2017

Dear Sirs

European Patent No. 2359834
ALEXION PHARMACEUTICALS, INC.
Our Ref: C411599EP PXT

As per the letter of 27 October 2017, J A Kemp have taken over as representatives in respect of this Opposition. Please record the new representative as J A Kemp, Association No. 811. Our reference will be C411599EP.

I request an extension of two months to the term set by the Communication dated 13 September 2017 requesting a response to the two Notices of Opposition.

It is believed that an exceptional two month extension is appropriate in the circumstances of the present case, for the following reasons.

1 Multiple Oppositions have been filed against the present patent. Over 30 documents have been referred to by the Opponents, between them. One of the Opponents has prepared new experimental data. The Grounds of Opposition include many different issues, *inter alia* allegations of added subject matter, lack of sufficiency, lack of priority and lack of inventive step. The Patentee does not consider that these Grounds are well founded. However, the Opposition Division will appreciate that the response needs to ensure that all of the issues raised, by all of the Opponents, are addressed fully.

2 As evidenced by the enclosed press release, the Patentee, Alexion, is currently undergoing a significant restructuring program. Alexion is a global biopharmaceutical company and a restructuring program on this scale is a highly unusual event. The exceptional circumstance of this major restructuring and its effect on the preparation of a full response, as discussed below, is considered to justify an extension in the present case.

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A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

The restructuring was announced on 12 September 2017, a day before issuance of the Notice inviting response to the Oppositions. The restructuring is expected to continue for a number of months and will not be complete before mid 2018. The restructuring will therefore be taking place throughout the entire period set for response to the Communication of Notices of Opposition.

As the Opposition Division will see from the enclosed press release, the restructuring is a significant change to the entire company and involves a reduction in the global work force of 20%. This reduction in the work force has impacted the IP team at Alexion, who has responsibility for managing the present Opposition, and has directly affected the scientists who have been involved in the development of Eculizumab, the drug which is the subject of the present patent.

In addition to the reduction of the work force, a significant portion of the remaining Alexion staff has been asked to relocate to a new headquarters in Boston. These employees' decisions to either relocate to Boston or to leave the company will have a large impact on the day-to-day activities of Alexion's remaining employees during the entire period set for response to the Notices of Opposition.

Significant input from both the IP team and the relevant scientists will be needed in formulating a full response to the Notices of Opposition. The restructuring and consequent relocation and/or departure of numerous staff will make this task significantly more difficult and is expected to lead to delays in gathering relevant data, including periods where the relevant personnel will be unavailable for input. It is clearly important that full scientific consideration is given to all aspects of the case, in particular the new data filed by one of the Opponents. Given the exceptional circumstance of a major restructuring and relocation of a global biopharmaceutical company, it is considered appropriate to request additional time to prepare the Patentee's response.

3 In view of the filing of new experimental data by one of the Opponents, it is important that the Patentee be afforded sufficient time, under the current circumstances, to assess whether to file data in reply and to prepare any necessary data. The Patentee presently proposes to file new data in relation to eculizumab, the antibody which is the subject of the patent, and/or other antibodies. Clearly these activities will require additional time and resources, especially in light of the significant restructuring that is currently taking place at Alexion.

4 The patent relates to the treatment of PNH, a rare and life-threating disease. PNH is a chronic condition stemming from complement dysregulation which was largely untreatable until eculizumab was approved. If untreated, PNH can lead to a significant reduction in both quality of life and life expectancy of patients. Treatments available in the past have included transfusion, and on occasion treatment with steroids to reduce the severity of hemolytic events, but the effectiveness of steroid treatment was problematic. Eculizumab, the PNH treatment

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which is the subject of the present patent, was the first and only treatment approved for PNH and provides patients with significant improvements in quality of life and life expectancy.

It is important for the Patentee to file a fully considered response to the Oppositions against the patent, which is of clear medical and commercial importance. The unfortunate coincidental timing of the filing of the Oppositions and the internal restructuring program should not be allowed to damage the Patentee's position in defending this important patent.

In view of all of the above considerations, it is requested, exceptionally, that a two month extension be allowed for responding to the Notices of Opposition. As an Auxiliary Request, I request an extension of one month to the response term.

Merely as a precaution, the patent Proprietor intends to defend the patent and requests that the patent is maintained as granted. Oral proceedings are requested if the Opposition Division contemplates any other outcome.

Yours faithfully

Electronically Signed
AMANDA SIMONS
ASSOCIATION NO. 811

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Europäisches Patentamt
European Patent Office
Office européen des brevets

Submission in opposition proceedings
made following summons to attend oral proceedings

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- representing the proprietor(s):	Alexion Pharmaceuticals, Inc.
Proprietor/representative's reference	C411599EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Requests:

- An extension of time limit is hereby requested

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - Press Release	Alexion Announces Restructuring.PDF	OTHER-1.PDF
2	Substantiation of the request for time extension	Extension Request C411599EP ALS.PDF	TIMEEXTREQ.PDF

Signatures

Place:	
Date:	**30 October 2017**
Signed by:	**Amanda Simons 23469**
Association:	**J A Kemp**
Representative name:	**/Amanda Simons/**
Capacity:	**(Representative)**

Alexion Announces Restructuring to Advance Corporate Strategy

Release Date:
Tuesday, September 12, 2017 8:00 am EDT

Terms:
Financial News Company News

- *Expect to Deliver Approximately $270 Million in GAAP and Approximately $250 Million in Non-GAAP Pre-tax Savings Annually by 2019*
- *Global Workforce Reduction of Approximately 20%*
- *Expect to Reinvest Approximately $100 Million Annually to Build Pipeline Through Disciplined Business Development and Additional Complement Indications*
- *Maximize Leadership in Complement and Grow Rare Disease Business by Focusing Investments in Priority Growth Areas*
- *Research Center of Excellence to be Based in New Haven, CT*
- *New Headquarters in Boston, MA to Support Innovation and Future Growth*
- *Management to Host Conference Call at 8:30 AM EDT to Discuss Restructuring*

NEW HAVEN, Conn.--(BUSINESS WIRE)--Alexion Pharmaceuticals, Inc. (NASDAQ:ALXN) today announced an operational plan to re-align the global organization with its refocused corporate strategy. The plan is expected to deliver approximately $270 million in GAAP and approximately $250 million in non-GAAP pre-tax savings annually by 2019. The restructuring will reduce the Company's global workforce by approximately 20%. The resulting savings will allow Alexion to prioritize investments to advance growth opportunities, optimize capabilities across the organization, and position Alexion to deliver on its financial ambitions, including growing GAAP operating margin to 37% and non-GAAP operating margin to 50% in 2019. Alexion expects that the increased financial flexibility will allow the Company to reinvest approximately $100 million a year into research and development through disciplined business development and additional complement proof-of-concept studies starting in 2018. Alexion anticipates the total pre-tax restructuring and related expenses associated with today's announcement to be in the range of $340 million to $440 million.

"By streamlining our operations we will create a leaner organization with greater financial flexibility that is highly focused on delivering for patients, growing our rare disease business, and both leveraging our leadership in complement and pursuing disciplined business development to expand the pipeline," said Ludwig Hantson, Chief Executive Officer of Alexion. "These types of changes are difficult and we recognize that they have a personal impact on people who have been dedicated to the mission of Alexion. We thank our employees for their contributions to the achievements of Alexion. While difficult, these changes were necessary to enable the Company to deliver sustainable long-term performance to support our ability to continue to develop and deliver life-changing therapies for patients."

Alexion's Strategic Focus

Alexion previously announced the Company's strategy to drive its next phase of growth by:

- Growing and maximizing our rare disease business
- Focusing research on complement and development on core areas of hematology, nephrology, neurology and metabolic disorders to enhance productivity
- Pursuing disciplined business development to expand the pipeline
- Optimizing infrastructure to deliver margin expansion, EPS growth and increased cash flow

Restructuring Details

The restructuring will achieve savings by focusing the development portfolio, simplifying business structures and processes across the Company's global operations, and consolidating manufacturing capabilities. This includes:

- Research & Development: Eliminating spend and headcount associated with previously announced de-prioritized pipeline programs as well as optimizing additional R&D spend. As previously disclosed, these de-prioritized programs include ALXN1101 (cPMP replacement therapy) and ALXN6000 (samalizumab) as well as partnerships with Moderna Therapeutics, Blueprint Medicines and Arbutus Biopharma.
- Manufacturing and Site Consolidation: The new facilities strategy will result in the closing of multiple Alexion sites, including the Alexion Rhode Island manufacturing facility and certain regional and country-based offices. Alexion has had a positive working relationship with the state of Rhode Island, however, the Company is aligning its manufacturing facilities with its ongoing multi-product network manufacturing strategy, which utilizes Alexion's manufacturing operations in the U.S. and Ireland, and manufacturing capacity through its manufacturing partners.
- Commercial: Aligning investments with its core, high growth markets, optimizing the global operating structure to leverage synergies across regions and countries, reducing operating redundancies and focusing on continuity of the Company's field teams.
- General & Administrative: In parallel with the Research & Development and Commercial organizational changes, the Company is right-sizing certain G&A functions, such as Human Resources, Finance and Information Technology (IT), including out-sourcing certain non-core Finance and IT roles to support the realigned business. Alexion will continue to invest in other G&A functions, such as Legal and Compliance, to support its global business.

Implementing these changes is expected to deliver approximately $270 million in GAAP pre-tax savings annually, inclusive of $20 million of share-based compensation savings, and approximately $250 million in non-GAAP pre-tax savings annually by 2019. These changes will position Alexion to deliver on its financial ambitions, including growing GAAP operating margin to 37% and non-GAAP operating margin to 50% in 2019.

Establishing New Headquarters in Boston, MA; Research Center of Excellence to be Based in New Haven, CT

As part of these initiatives, Alexion plans to relocate its headquarters to Boston, MA by mid-2018. The Company plans to have approximately 400 positions in Boston. As the Company reorganizes to deliver on its re-focused strategy, Boston will provide access to a larger biopharmaceutical talent pool and a variety of life-sciences partners to further support future growth initiatives.

"Alexion's 25 year history began in New Haven, and Connecticut remains a critical part of our future. We value our relationship with the state of Connecticut, and our New Haven-based research team is critical to growing and strengthening Alexion's leadership in complement, which will allow us to fulfill our mission of serving patients and families with rare and ultra-rare diseases," said Hantson.

New Haven, CT will be the Company's Center of Excellence for its world-class complement research and process development teams, which are dedicated to advancing Alexion's innovation engine. Approximately 450 positions will be based in New Haven, including employees working in the research and process development laboratories, the clinical supply and quality teams, nurse case management and a number of important enterprise business services.

Financial Update

The Company expects to record cumulative pre-tax restructuring and related expenses ranging between approximately $340 million and $440 million. Included in these amounts are: (i) employee separation costs of approximately $80 million to $90 million; (ii) asset-related charges of approximately $170 million to $210 million primarily related to asset impairments and accelerated depreciation of facilities to be closed; (iii) costs associated with relocating employees of approximately $40 million to $60 million and (iv) contract termination and other costs of approximately $50 million to $80 million.

Approximately 50% of the restructuring and related expenses will result in cash outlays. Pre-tax restructuring and related expenses of approximately $240 million to $300 million are expected to be recorded in 2017.

The Company's 2017 revenue guidance remains unchanged. GAAP EPS guidance will be impacted by restructuring and related expenses and 2017 non-GAAP EPS guidance is unchanged.

Conference Call/Webcast Information:

Alexion will host a conference call/audio webcast to discuss the operational plan at 8:30 a.m. Eastern Time. To participate in the call, dial 800-406-5345 (USA) or 719-325-4744 (International), passcode 9877152 shortly before 8:30 a.m. Eastern Time. A replay of the call will be available for a limited period following the call. The replay number is 888-203-1112 (USA) or 719-457-0820 (International), passcode 9877152. The audio webcast can be accessed on the Investor page of Alexion's website at: http://ir.alexion.com .

About Alexion

Alexion is a global biopharmaceutical company focused on developing and delivering life-transforming therapies for patients with devastating and rare disorders. Alexion is the global leader in complement inhibition and has developed and commercializes the first and only approved complement inhibitor to treat patients with paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and refractory generalized myasthenia gravis (gMG). In addition, Alexion has two highly innovative enzyme replacement therapies for patients with life-threatening and ultra-rare metabolic disorders, hypophosphatasia (HPP) and lysosomal acid lipase deficiency (LAL-D). As the leader in complement biology for over 20 years, Alexion focuses its research efforts on novel molecules and targets in the complement cascade, and its development efforts on the core therapeutic areas of hematology, nephrology, neurology, and metabolic disorders. This press release and further information about Alexion can be found at: www.alexion.com.

[ALXN-G]

Forward-Looking Statements

This press release contains forward-looking statements, including among others, statements related to anticipated savings, costs and expenses resulting from the restructuring, the size and nature of future investments by the Company, anticipated changes to the Company's global workforce, development portfolio, global operations, and manufacturing capabilities, and

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Page 6498

the relocation of the Company’s headquarters. Forward-looking statements are subject to factors that may cause the Company's results and plans to differ from those expected, including for example, risks related to whether the Company’s expenses are as predicted, potential disruptions to our business as a result of the restructuring, including the anticipated reduction in the global workforce and consolidation of facilities, and a variety of other risks set forth from time to time in Alexion's filings with the U.S. Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Quarterly Report on Form 10-Q for the period ended June 30, 2017 and in our other filings with the U.S. Securities and Exchange Commission. Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof, except when a duty arises under law.

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ALEXION PHARMACEUTICALS, INC.

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Operating margin (% total revenues)	
GAAP operating margin	37%
Share-based compensation	6%
Amortization of purchased intangible assets	7%
Change in fair value of contingent consideration	0%
Non-GAAP operating margin	50%

Contact:

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Kim Diamond, 475-230-3775
Executive Director, Corporate Communications
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Vice President, Investor Relations
or
Catherine Hu, 475-230-3599
Director, Investor Relations

Source URL: http://news.alexion.com/press-release/financial-news/alexion-announces-restructuring-advance-corporate-strategy

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following submission by the proprietor:

Submission number	5745442
Application number	EP11001632.6
Patent number	EP2359834
Date of receipt	30 October 2017
Your reference	C411599EP
Proprietor	Alexion Pharmaceuticals, Inc.
Title	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Documents submitted	package-data.xml ep-oppo.pdf (2 p.) OTHER-1.PDF\Alexion Announces Restructuring.PDF (3 p.) ep-opposition-data.xml TIMEEXTREQ.PDF\Extension Request C411599EP ALS.PDF (3 p.)
Submitted by	CN=Amanda Simons 23469
Method of submission	Online
Date and time receipt generated	30 October 2017, 17:15 (CET)
Message Digest	84:CE:E1:A5:5C:7A:C3:33:B4:6C:B5:F9:FF:7A:A8:E5:77:03:EE:D9

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82,

OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

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European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
08.11.17

new reference C411599EP PXT

Reference	Application No./Patent No.
P2913 EP/1	11001632.6 - 1466 / 2359834

Applicant/Proprietor
Alexion Pharmaceuticals, Inc.

Communication of amended entries concerning the representative (R. 143(1)(h) EPC)

As requested, for the above-mentioned European patent application / European patent the entries concerning the representative have been amended as follows:

J A Kemp
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London WC1R 5JJ
GB

The amendment will be recorded in the Register of European Patents.

For the Opposition Division

Samsung Bioepis Exhibit No. 1002
Page 6502

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ROYAUME UNI

Date
09.11.2017

Reference	Application No./Patent No.
C411599EP PXT	11001632.6 - 1466 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

Extension of time limit pursuant to Rule 132 EPC

Opposition procedure

With reference to your request, the time limit for replying to the communication dated 13.09.2017 has been extended

by 2 months

to a total of 6 months

from the date of notification of the above-mentioned communication.

Please note: To the extent that your request exceeded the above extension, your request has been refused.

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For the Opposition Division

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Page 6503

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European Patent Office
Office européen des brevets

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80298 MUNICH
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Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Dörries, Hans Ulrich
df-mp
Fünf Höfe
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80333 München
ALLEMAGNE

Date
09.11.2017

Reference	Application No./Patent No.
AMG17203OP OPPO 01	11001632.6 - 1466 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

BRIEF COMMUNICATION

Subject:
- ☐ Your letter of
- ☐ Our telephone conversation of
- ☐ Communication of
- ☐

Enclosure(s):
- ☒ Letter from the proprietor of the patent of 30.10.2017
- ☐ Letter from the opponent of
- ☒ Copy (copies) EPO Form 2548 / 2944C

Communication:

☐ A letter from the opponent /proprietor was received on ..... . The documents specified as patent documents in this letter are available via the European Patent Register at **www.epo.org/register.** Should you wish to receive these documents in paper format, they will be supplied to you free of charge if specifically requested on receipt of this communication (OJ EPO 2009, 434).

☐

For the Opposition Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

☐ **Registered letter**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Submission in opposition proceedings
made following summons to attend oral proceedings

Representative:

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United Kingdom

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Tel. +31(0)70 340-2040 | Fax -3016

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Tel. +49(0)30 25901-0 | Fax -840

- representing the proprietor(s):	Alexion Pharmaceuticals, Inc.
Proprietor/representative's reference	C411599EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Requests:

- An extension of time limit is hereby requested

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - Press Release	Alexion Announces Restructuring.PDF	OTHER-1.PDF
2	Substantiation of the request for time extension	Extension Request C411599EP ALS.PDF	TIMEEXTREQ.PDF

Signatures

Place:	
Date:	**30 October 2017**
Signed by:	**Amanda Simons 23469**
Association:	**J A Kemp**
Representative name:	**/Amanda Simons/**
Capacity:	**(Representative)**

J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

30 October 2017

Dear Sirs

European Patent No. 2359834
ALEXION PHARMACEUTICALS, INC.
Our Ref: C411599EP PXT

As per the letter of 27 October 2017, J A Kemp have taken over as representatives in respect of this Opposition. Please record the new representative as J A Kemp, Association No. 811. Our reference will be C411599EP.

I request an extension of two months to the term set by the Communication dated 13 September 2017 requesting a response to the two Notices of Opposition.

It is believed that an exceptional two month extension is appropriate in the circumstances of the present case, for the following reasons.

1 Multiple Oppositions have been filed against the present patent. Over 30 documents have been referred to by the Opponents, between them. One of the Opponents has prepared new experimental data. The Grounds of Opposition include many different issues, *inter alia* allegations of added subject matter, lack of sufficiency, lack of priority and lack of inventive step. The Patentee does not consider that these Grounds are well founded. However, the Opposition Division will appreciate that the response needs to ensure that all of the issues raised, by all of the Opponents, are addressed fully.

2 As evidenced by the enclosed press release, the Patentee, Alexion, is currently undergoing a significant restructuring program. Alexion is a global biopharmaceutical company and a restructuring program on this scale is a highly unusual event. The exceptional circumstance of this major restructuring and its effect on the preparation of a full response, as discussed below, is considered to justify an extension in the present case.

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • MUNICH

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mail@jakemp.com **www.jakemp.com**

A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

The restructuring was announced on 12 September 2017, a day before issuance of the Notice inviting response to the Oppositions. The restructuring is expected to continue for a number of months and will not be complete before mid 2018. The restructuring will therefore be taking place throughout the entire period set for response to the Communication of Notices of Opposition.

As the Opposition Division will see from the enclosed press release, the restructuring is a significant change to the entire company and involves a reduction in the global work force of 20%. This reduction in the work force has impacted the IP team at Alexion, who has responsibility for managing the present Opposition, and has directly affected the scientists who have been involved in the development of Eculizumab, the drug which is the subject of the present patent.

In addition to the reduction of the work force, a significant portion of the remaining Alexion staff has been asked to relocate to a new headquarters in Boston. These employees' decisions to either relocate to Boston or to leave the company will have a large impact on the day-to-day activities of Alexion's remaining employees during the entire period set for response to the Notices of Opposition.

Significant input from both the IP team and the relevant scientists will be needed in formulating a full response to the Notices of Opposition. The restructuring and consequent relocation and/or departure of numerous staff will make this task significantly more difficult and is expected to lead to delays in gathering relevant data, including periods where the relevant personnel will be unavailable for input. It is clearly important that full scientific consideration is given to all aspects of the case, in particular the new data filed by one of the Opponents. Given the exceptional circumstance of a major restructuring and relocation of a global biopharmaceutical company, it is considered appropriate to request additional time to prepare the Patentee's response.

3 In view of the filing of new experimental data by one of the Opponents, it is important that the Patentee be afforded sufficient time, under the current circumstances, to assess whether to file data in reply and to prepare any necessary data. The Patentee presently proposes to file new data in relation to eculizumab, the antibody which is the subject of the patent, and/or other antibodies. Clearly these activities will require additional time and resources, especially in light of the significant restructuring that is currently taking place at Alexion.

4 The patent relates to the treatment of PNH, a rare and life-threating disease. PNH is a chronic condition stemming from complement dysregulation which was largely untreatable until eculizumab was approved. If untreated, PNH can lead to a significant reduction in both quality of life and life expectancy of patients. Treatments available in the past have included transfusion, and on occasion treatment with steroids to reduce the severity of hemolytic events, but the effectiveness of steroid treatment was problematic. Eculizumab, the PNH treatment

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which is the subject of the present patent, was the first and only treatment approved for PNH and provides patients with significant improvements in quality of life and life expectancy.

It is important for the Patentee to file a fully considered response to the Oppositions against the patent, which is of clear medical and commercial importance. The unfortunate coincidental timing of the filing of the Oppositions and the internal restructuring program should not be allowed to damage the Patentee's position in defending this important patent.

In view of all of the above considerations, it is requested, exceptionally, that a two month extension be allowed for responding to the Notices of Opposition. As an Auxiliary Request, I request an extension of one month to the response term.

Merely as a precaution, the patent Proprietor intends to defend the patent and requests that the patent is maintained as granted. Oral proceedings are requested if the Opposition Division contemplates any other outcome.

Yours faithfully

Electronically Signed
AMANDA SIMONS
ASSOCIATION NO. 811

Samsung Bioepis Exhibit No. 1002
Page 6509

Alexion Announces Restructuring to Advance Corporate Strategy

Release Date:
Tuesday, September 12, 2017 8:00 am EDT

Terms:
Financial News Company News

- *Expect to Deliver Approximately $270 Million in GAAP and Approximately $250 Million in Non-GAAP Pre-tax Savings Annually by 2019*
- *Global Workforce Reduction of Approximately 20%*
- *Expect to Reinvest Approximately $100 Million Annually to Build Pipeline Through Disciplined Business Development and Additional Complement Indications*
- *Maximize Leadership in Complement and Grow Rare Disease Business by Focusing Investments in Priority Growth Areas*
- *Research Center of Excellence to be Based in New Haven, CT*
- *New Headquarters in Boston, MA to Support Innovation and Future Growth*
- *Management to Host Conference Call at 8:30 AM EDT to Discuss Restructuring*

NEW HAVEN, Conn.--(BUSINESS WIRE)--Alexion Pharmaceuticals, Inc. (NASDAQ:ALXN) today announced an operational plan to re-align the global organization with its refocused corporate strategy. The plan is expected to deliver approximately $270 million in GAAP and approximately $250 million in non-GAAP pre-tax savings annually by 2019. The restructuring will reduce the Company's global workforce by approximately 20%. The resulting savings will allow Alexion to prioritize investments to advance growth opportunities, optimize capabilities across the organization, and position Alexion to deliver on its financial ambitions, including growing GAAP operating margin to 37% and non-GAAP operating margin to 50% in 2019. Alexion expects that the increased financial flexibility will allow the Company to reinvest approximately $100 million a year into research and development through disciplined business development and additional complement proof-of-concept studies starting in 2018. Alexion anticipates the total pre-tax restructuring and related expenses associated with today's announcement to be in the range of $340 million to $440 million.

"By streamlining our operations we will create a leaner organization with greater financial flexibility that is highly focused on delivering for patients, growing our rare disease business, and both leveraging our leadership in complement and pursuing disciplined business development to expand the pipeline," said Ludwig Hantson, Chief Executive Officer of Alexion. "These types of changes are difficult and we recognize that they have a personal impact on people who have been dedicated to the mission of Alexion. We thank our employees for their contributions to the achievements of Alexion. While difficult, these changes were necessary to enable the Company to deliver sustainable long-term performance to support our ability to continue to develop and deliver life-changing therapies for patients."

Alexion's Strategic Focus

Alexion previously announced the Company's strategy to drive its next phase of growth by:

- Growing and maximizing our rare disease business
- Focusing research on complement and development on core areas of hematology, nephrology, neurology and metabolic disorders to enhance productivity
- Pursuing disciplined business development to expand the pipeline
- Optimizing infrastructure to deliver margin expansion, EPS growth and increased cash flow

Restructuring Details

The restructuring will achieve savings by focusing the development portfolio, simplifying business structures and processes across the Company's global operations, and consolidating manufacturing capabilities. This includes:

- Research & Development: Eliminating spend and headcount associated with previously announced de-prioritized pipeline programs as well as optimizing additional R&D spend. As previously disclosed, these de-prioritized programs include ALXN1101 (cPMP replacement therapy) and ALXN6000 (samalizumab) as well as partnerships with Moderna Therapeutics, Blueprint Medicines and Arbutus Biopharma.
- Manufacturing and Site Consolidation: The new facilities strategy will result in the closing of multiple Alexion sites, including the Alexion Rhode Island manufacturing facility and certain regional and country-based offices. Alexion has had a positive working relationship with the state of Rhode Island, however, the Company is aligning its manufacturing facilities with its ongoing multi-product network manufacturing strategy, which utilizes Alexion's manufacturing operations in the U.S. and Ireland, and manufacturing capacity through its manufacturing partners.
- Commercial: Aligning investments with its core, high growth markets, optimizing the global operating structure to leverage synergies across regions and countries, reducing operating redundancies and focusing on continuity of the Company's field teams.
- General & Administrative: In parallel with the Research & Development and Commercial organizational changes, the Company is right-sizing certain G&A functions, such as Human Resources, Finance and Information Technology (IT), including out-sourcing certain non-core Finance and IT roles to support the realigned business. Alexion will continue to invest in other G&A functions, such as Legal and Compliance, to support its global business.

Implementing these changes is expected to deliver approximately $270 million in GAAP pre-tax savings annually, inclusive of $20 million of share-based compensation savings, and approximately $250 million in non-GAAP pre-tax savings annually by 2019. These changes will position Alexion to deliver on its financial ambitions, including growing GAAP operating margin to 37% and non-GAAP operating margin to 50% in 2019.

Establishing New Headquarters in Boston, MA; Research Center of Excellence to be Based in New Haven, CT

As part of these initiatives, Alexion plans to relocate its headquarters to Boston, MA by mid-2018. The Company plans to have approximately 400 positions in Boston. As the Company reorganizes to deliver on its re-focused strategy, Boston will provide access to a larger biopharmaceutical talent pool and a variety of life-sciences partners to further support future growth initiatives.

"Alexion's 25 year history began in New Haven, and Connecticut remains a critical part of our future. We value our relationship with the state of Connecticut, and our New Haven-based research team is critical to growing and strengthening Alexion's leadership in complement, which will allow us to fulfill our mission of serving patients and families with rare and ultra-rare diseases," said Hantson.

New Haven, CT will be the Company's Center of Excellence for its world-class complement research and process development teams, which are dedicated to advancing Alexion's innovation engine. Approximately 450 positions will be based in New Haven, including employees working in the research and process development laboratories, the clinical supply and quality teams, nurse case management and a number of important enterprise business services.

Financial Update

The Company expects to record cumulative pre-tax restructuring and related expenses ranging between approximately $340 million and $440 million. Included in these amounts are: (i) employee separation costs of approximately $80 million to $90 million; (ii) asset-related charges of approximately $170 million to $210 million primarily related to asset impairments and accelerated depreciation of facilities to be closed; (iii) costs associated with relocating employees of approximately $40 million to $60 million and (iv) contract termination and other costs of approximately $50 million to $80 million.

Approximately 50% of the restructuring and related expenses will result in cash outlays. Pre-tax restructuring and related expenses of approximately $240 million to $300 million are expected to be recorded in 2017.

The Company's 2017 revenue guidance remains unchanged. GAAP EPS guidance will be impacted by restructuring and related expenses and 2017 non-GAAP EPS guidance is unchanged.

Conference Call/Webcast Information:

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Page 6511

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Executive Director, Corporate Communications
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Investors
Elena Ridloff, CFA, 475-230-3601
Vice President, Investor Relations
or
Catherine Hu, 475-230-3599
Director, Investor Relations

Source URL: http://news.alexion.com/press-release/financial-news/alexion-announces-restructuring-advance-corporate-strategy

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Questions about this communication ?
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J A Kemp
14 South Square
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ROYAUME UNI

Date
08.11.17

new reference C411599EP PXT

Reference	Application No./Patent No.
P2913 EP/1	11001632.6 - 1466 / 2359834

Applicant/Proprietor
Alexion Pharmaceuticals, Inc.

Communication of amended entries concerning the representative (R. 143(1)(h) EPC)

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GB

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For the Opposition Division

Samsung Bioepis Exhibit No. 1002
Page 6513

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ROYAUME UNI

Date
09.11.2017

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Alexion Pharmaceuticals, Inc.	

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For the Opposition Division

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Questions about this communication ?
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ALLEMAGNE

Date: 09.11.2017

Reference	Application No./Patent No.
198 441 u6/ne OPPO 02	11001632.6 - 1466 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

BRIEF COMMUNICATION

Subject:
- ☐ Your letter of
- ☐ Our telephone conversation of
- ☐ Communication of
- ☐

Enclosure(s):
- ☒ Letter from the proprietor of the patent of 30.10.2017
- ☐ Letter from the opponent of
- ☒ Copy (copies) EPO Form 2548 / 2944C

Communication:

☐ A letter from the opponent /proprietor was received on ..... . The documents specified as patent documents in this letter are available via the European Patent Register at **www.epo.org/register.** Should you wish to receive these documents in paper format, they will be supplied to you free of charge if specifically requested on receipt of this communication (OJ EPO 2009, 434).

☐

For the Opposition Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

☐ **Registered letter**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Submission in opposition proceedings
made following summons to attend oral proceedings

Representative:

J A Kemp
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Tel. +49(0)30 25901-0 | Fax -840

- representing the proprietor(s):	Alexion Pharmaceuticals, Inc.
Proprietor/representative's reference	C411599EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Requests:

- An extension of time limit is hereby requested

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - Press Release	Alexion Announces Restructuring.PDF	OTHER-1.PDF
2	Substantiation of the request for time extension	Extension Request C411599EP ALS.PDF	TIMEEXTREQ.PDF

Signatures

Place:	
Date:	**30 October 2017**
Signed by:	**Amanda Simons 23469**
Association:	**J A Kemp**
Representative name:	**/Amanda Simons/**
Capacity:	**(Representative)**

J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

30 October 2017

Dear Sirs

European Patent No. 2359834
ALEXION PHARMACEUTICALS, INC.
Our Ref: C411599EP PXT

As per the letter of 27 October 2017, J A Kemp have taken over as representatives in respect of this Opposition. Please record the new representative as J A Kemp, Association No. 811. Our reference will be C411599EP.

I request an extension of two months to the term set by the Communication dated 13 September 2017 requesting a response to the two Notices of Opposition.

It is believed that an exceptional two month extension is appropriate in the circumstances of the present case, for the following reasons.

1 Multiple Oppositions have been filed against the present patent. Over 30 documents have been referred to by the Opponents, between them. One of the Opponents has prepared new experimental data. The Grounds of Opposition include many different issues, *inter alia* allegations of added subject matter, lack of sufficiency, lack of priority and lack of inventive step. The Patentee does not consider that these Grounds are well founded. However, the Opposition Division will appreciate that the response needs to ensure that all of the issues raised, by all of the Opponents, are addressed fully.

2 As evidenced by the enclosed press release, the Patentee, Alexion, is currently undergoing a significant restructuring program. Alexion is a global biopharmaceutical company and a restructuring program on this scale is a highly unusual event. The exceptional circumstance of this major restructuring and its effect on the preparation of a full response, as discussed below, is considered to justify an extension in the present case.

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
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A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

The restructuring was announced on 12 September 2017, a day before issuance of the Notice inviting response to the Oppositions. The restructuring is expected to continue for a number of months and will not be complete before mid 2018. The restructuring will therefore be taking place throughout the entire period set for response to the Communication of Notices of Opposition.

As the Opposition Division will see from the enclosed press release, the restructuring is a significant change to the entire company and involves a reduction in the global work force of 20%. This reduction in the work force has impacted the IP team at Alexion, who has responsibility for managing the present Opposition, and has directly affected the scientists who have been involved in the development of Eculizumab, the drug which is the subject of the present patent.

In addition to the reduction of the work force, a significant portion of the remaining Alexion staff has been asked to relocate to a new headquarters in Boston. These employees' decisions to either relocate to Boston or to leave the company will have a large impact on the day-to-day activities of Alexion's remaining employees during the entire period set for response to the Notices of Opposition.

Significant input from both the IP team and the relevant scientists will be needed in formulating a full response to the Notices of Opposition. The restructuring and consequent relocation and/or departure of numerous staff will make this task significantly more difficult and is expected to lead to delays in gathering relevant data, including periods where the relevant personnel will be unavailable for input. It is clearly important that full scientific consideration is given to all aspects of the case, in particular the new data filed by one of the Opponents. Given the exceptional circumstance of a major restructuring and relocation of a global biopharmaceutical company, it is considered appropriate to request additional time to prepare the Patentee's response.

3 In view of the filing of new experimental data by one of the Opponents, it is important that the Patentee be afforded sufficient time, under the current circumstances, to assess whether to file data in reply and to prepare any necessary data. The Patentee presently proposes to file new data in relation to eculizumab, the antibody which is the subject of the patent, and/or other antibodies. Clearly these activities will require additional time and resources, especially in light of the significant restructuring that is currently taking place at Alexion.

4 The patent relates to the treatment of PNH, a rare and life-threating disease. PNH is a chronic condition stemming from complement dysregulation which was largely untreatable until eculizumab was approved. If untreated, PNH can lead to a significant reduction in both quality of life and life expectancy of patients. Treatments available in the past have included transfusion, and on occasion treatment with steroids to reduce the severity of hemolytic events, but the effectiveness of steroid treatment was problematic. Eculizumab, the PNH treatment

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which is the subject of the present patent, was the first and only treatment approved for PNH and provides patients with significant improvements in quality of life and life expectancy.

It is important for the Patentee to file a fully considered response to the Oppositions against the patent, which is of clear medical and commercial importance. The unfortunate coincidental timing of the filing of the Oppositions and the internal restructuring program should not be allowed to damage the Patentee's position in defending this important patent.

In view of all of the above considerations, it is requested, exceptionally, that a two month extension be allowed for responding to the Notices of Opposition. As an Auxiliary Request, I request an extension of one month to the response term.

Merely as a precaution, the patent Proprietor intends to defend the patent and requests that the patent is maintained as granted. Oral proceedings are requested if the Opposition Division contemplates any other outcome.

Yours faithfully

Electronically Signed
AMANDA SIMONS
ASSOCIATION NO. 811

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Page 6520

Alexion Announces Restructuring to Advance Corporate Strategy

Release Date:
Tuesday, September 12, 2017 8:00 am EDT

Terms:
Financial News Company News

- *Expect to Deliver Approximately $270 Million in GAAP and Approximately $250 Million in Non-GAAP Pre-tax Savings Annually by 2019*
- *Global Workforce Reduction of Approximately 20%*
- *Expect to Reinvest Approximately $100 Million Annually to Build Pipeline Through Disciplined Business Development and Additional Complement Indications*
- *Maximize Leadership in Complement and Grow Rare Disease Business by Focusing Investments in Priority Growth Areas*
- *Research Center of Excellence to be Based in New Haven, CT*
- *New Headquarters in Boston, MA to Support Innovation and Future Growth*
- *Management to Host Conference Call at 8:30 AM EDT to Discuss Restructuring*

NEW HAVEN, Conn.--(BUSINESS WIRE)--Alexion Pharmaceuticals, Inc. (NASDAQ:ALXN) today announced an operational plan to re-align the global organization with its refocused corporate strategy. The plan is expected to deliver approximately $270 million in GAAP and approximately $250 million in non-GAAP pre-tax savings annually by 2019. The restructuring will reduce the Company's global workforce by approximately 20%. The resulting savings will allow Alexion to prioritize investments to advance growth opportunities, optimize capabilities across the organization, and position Alexion to deliver on its financial ambitions, including growing GAAP operating margin to 37% and non-GAAP operating margin to 50% in 2019. Alexion expects that the increased financial flexibility will allow the Company to reinvest approximately $100 million a year into research and development through disciplined business development and additional complement proof-of-concept studies starting in 2018. Alexion anticipates the total pre-tax restructuring and related expenses associated with today's announcement to be in the range of $340 million to $440 million.

"By streamlining our operations we will create a leaner organization with greater financial flexibility that is highly focused on delivering for patients, growing our rare disease business, and both leveraging our leadership in complement and pursuing disciplined business development to expand the pipeline," said Ludwig Hantson, Chief Executive Officer of Alexion. "These types of changes are difficult and we recognize that they have a personal impact on people who have been dedicated to the mission of Alexion. We thank our employees for their contributions to the achievements of Alexion. While difficult, these changes were necessary to enable the Company to deliver sustainable long-term performance to support our ability to continue to develop and deliver life-changing therapies for patients."

Alexion's Strategic Focus

Alexion previously announced the Company's strategy to drive its next phase of growth by:

- Growing and maximizing our rare disease business
- Focusing research on complement and development on core areas of hematology, nephrology, neurology and metabolic disorders to enhance productivity
- Pursuing disciplined business development to expand the pipeline
- Optimizing infrastructure to deliver margin expansion, EPS growth and increased cash flow

Restructuring Details

The restructuring will achieve savings by focusing the development portfolio, simplifying business structures and processes across the Company's global operations, and consolidating manufacturing capabilities. This includes:

- Research & Development: Eliminating spend and headcount associated with previously announced de-prioritized pipeline programs as well as optimizing additional R&D spend. As previously disclosed, these de-prioritized programs include ALXN1101 (cPMP replacement therapy) and ALXN6000 (samalizumab) as well as partnerships with Moderna Therapeutics, Blueprint Medicines and Arbutus Biopharma.
- Manufacturing and Site Consolidation: The new facilities strategy will result in the closing of multiple Alexion sites, including the Alexion Rhode Island manufacturing facility and certain regional and country-based offices. Alexion has had a positive working relationship with the state of Rhode Island, however, the Company is aligning its manufacturing facilities with its ongoing multi-product network manufacturing strategy, which utilizes Alexion's manufacturing operations in the U.S. and Ireland, and manufacturing capacity through its manufacturing partners.
- Commercial: Aligning investments with its core, high growth markets, optimizing the global operating structure to leverage synergies across regions and countries, reducing operating redundancies and focusing on continuity of the Company's field teams.
- General & Administrative: In parallel with the Research & Development and Commercial organizational changes, the Company is right-sizing certain G&A functions, such as Human Resources, Finance and Information Technology (IT), including out-sourcing certain non-core Finance and IT roles to support the realigned business. Alexion will continue to invest in other G&A functions, such as Legal and Compliance, to support its global business.

Implementing these changes is expected to deliver approximately $270 million in GAAP pre-tax savings annually, inclusive of $20 million of share-based compensation savings, and approximately $250 million in non-GAAP pre-tax savings annually by 2019. These changes will position Alexion to deliver on its financial ambitions, including growing GAAP operating margin to 37% and non-GAAP operating margin to 50% in 2019.

Establishing New Headquarters in Boston, MA; Research Center of Excellence to be Based in New Haven, CT

As part of these initiatives, Alexion plans to relocate its headquarters to Boston, MA by mid-2018. The Company plans to have approximately 400 positions in Boston. As the Company reorganizes to deliver on its re-focused strategy, Boston will provide access to a larger biopharmaceutical talent pool and a variety of life-sciences partners to further support future growth initiatives.

"Alexion's 25 year history began in New Haven, and Connecticut remains a critical part of our future. We value our relationship with the state of Connecticut, and our New Haven-based research team is critical to growing and strengthening Alexion's leadership in complement, which will allow us to fulfill our mission of serving patients and families with rare and ultra-rare diseases," said Hantson.

New Haven, CT will be the Company's Center of Excellence for its world-class complement research and process development teams, which are dedicated to advancing Alexion's innovation engine. Approximately 450 positions will be based in New Haven, including employees working in the research and process development laboratories, the clinical supply and quality teams, nurse case management and a number of important enterprise business services.

Financial Update

The Company expects to record cumulative pre-tax restructuring and related expenses ranging between approximately $340 million and $440 million. Included in these amounts are: (i) employee separation costs of approximately $80 million to $90 million; (ii) asset-related charges of approximately $170 million to $210 million primarily related to asset impairments and accelerated depreciation of facilities to be closed; (iii) costs associated with relocating employees of approximately $40 million to $60 million and (iv) contract termination and other costs of approximately $50 million to $80 million.

Approximately 50% of the restructuring and related expenses will result in cash outlays. Pre-tax restructuring and related expenses of approximately $240 million to $300 million are expected to be recorded in 2017.

The Company's 2017 revenue guidance remains unchanged. GAAP EPS guidance will be impacted by restructuring and related expenses and 2017 non-GAAP EPS guidance is unchanged.

Conference Call/Webcast Information:

Alexion will host a conference call/audio webcast to discuss the operational plan at 8:30 a.m. Eastern Time. To participate in the call, dial 800-406-5345 (USA) or 719-325-4744 (International), passcode 9877152 shortly before 8:30 a.m. Eastern Time. A replay of the call will be available for a limited period following the call. The replay number is 888-203-1112 (USA) or 719-457-0820 (International), passcode 9877152. The audio webcast can be accessed on the Investor page of Alexion's website at: http://ir.alexion.com .

About Alexion

Alexion is a global biopharmaceutical company focused on developing and delivering life-transforming therapies for patients with devastating and rare disorders. Alexion is the global leader in complement inhibition and has developed and commercializes the first and only approved complement inhibitor to treat patients with paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and refractory generalized myasthenia gravis (gMG). In addition, Alexion has two highly innovative enzyme replacement therapies for patients with life-threatening and ultra-rare metabolic disorders, hypophosphatasia (HPP) and lysosomal acid lipase deficiency (LAL-D). As the leader in complement biology for over 20 years, Alexion focuses its research efforts on novel molecules and targets in the complement cascade, and its development efforts on the core therapeutic areas of hematology, nephrology, neurology, and metabolic disorders. This press release and further information about Alexion can be found at: www.alexion.com.

[ALXN-G]

Forward-Looking Statements

This press release contains forward-looking statements, including among others, statements related to anticipated savings, costs and expenses resulting from the restructuring, the size and nature of future investments by the Company, anticipated changes to the Company's global workforce, development portfolio, global operations, and manufacturing capabilities, and

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the relocation of the Company’s headquarters. Forward-looking statements are subject to factors that may cause the Company's results and plans to differ from those expected, including for example, risks related to whether the Company’s expenses are as predicted, potential disruptions to our business as a result of the restructuring, including the anticipated reduction in the global workforce and consolidation of facilities, and a variety of other risks set forth from time to time in Alexion's filings with the U.S. Securities and Exchange Commission, including but not limited to the risks discussed in Alexion's Quarterly Report on Form 10-Q for the period ended June 30, 2017 and in our other filings with the U.S. Securities and Exchange Commission. Alexion does not intend to update any of these forward-looking statements to reflect events or circumstances after the date hereof, except when a duty arises under law.

In addition to financial information prepared in accordance with GAAP, this press release also contains non-GAAP financial measures that Alexion believes, when considered together with the GAAP information, provide investors and management with supplemental information relating to performance, trends and prospects that promote a more complete understanding of our operating results and financial position during different periods. The non-GAAP results exclude the impact of the following GAAP items: share-based compensation expense, fair value adjustment of inventory acquired, amortization of purchased intangible assets, changes in fair value of contingent consideration, acquisition-related costs, restructuring and related expenses, upfront and milestone payments related to licenses and collaborations, impairment of intangible assets and adjustments to income tax expense. These non-GAAP financial measures are not intended to be considered in isolation or as a substitute for, or superior to, the financial measures prepared and presented in accordance with GAAP and should be reviewed in conjunction with the relevant GAAP financial measures. Please refer to the attached table for the GAAP to non-GAAP 2019 Operating Margin reconciliation.

ALEXION PHARMACEUTICALS, INC.

RECONCILIATION GAAP TO NON-GAAP 2019 OPERATING MARGIN

	Twelve months ended December 31, 2019
Operating margin (% total revenues)	
GAAP operating margin	37%
Share-based compensation	6%
Amortization of purchased intangible assets	7%
Change in fair value of contingent consideration	0%
Non-GAAP operating margin	50%

Contact:

Alexion
Media
Kim Diamond, 475-230-3775
Executive Director, Corporate Communications
or
Investors
Elena Ridloff, CFA, 475-230-3601
Vice President, Investor Relations
or
Catherine Hu, 475-230-3599
Director, Investor Relations

Source URL: http://news.alexion.com/press-release/financial-news/alexion-announces-restructuring-advance-corporate-strategy

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Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
08.11.17

new reference C411599EP PXT

Reference	Application No./Patent No.
P2913 EP/1	11001632.6 - 1466 / 2359834

Applicant/Proprietor
Alexion Pharmaceuticals, Inc.

Communication of amended entries concerning the representative (R. 143(1)(h) EPC)

As requested, for the above-mentioned European patent application / European patent the entries concerning the representative have been amended as follows:

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
GB

The amendment will be recorded in the Register of European Patents.

For the Opposition Division

Samsung Bioepis Exhibit No. 1002
Page 6524

Europäisches Patentamt
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European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date: 09.11.2017

Reference	Application No./Patent No.
C411599EP PXT	11001632.6 - 1466 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

Extension of time limit pursuant to Rule 132 EPC

Opposition procedure

With reference to your request, the time limit for replying to the communication dated 13.09.2017 has been extended

by 2 months

to a total of 6 months

from the date of notification of the above-mentioned communication.

Please note: To the extent that your request exceeded the above extension, your request has been refused.

Note:
The granting of extensions to time limits is governed by the Implementing Regulations to the EPC and the Guidelines for Examination in the EPO, E-VII, 1.6. A request for extension, which would result in the total period set exceeding six months, must be reasoned and supported by evidence.

If no reply to the communication is received in due time, the procedure will be continued. Attention is drawn to Article 114(2) EPC.

For the Opposition Division

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Samsung Bioepis Exhibit No. 1002
Page 6525

dfmp
Dörries Frank-Molnia & Pohlman
Patentanwälte Rechtsanwälte PartG mbB

Patents – Trademarks – Designs

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European Patent Office
Bob-van-Benthem-Platz 1
80469 Munich

November 10, 2017

Opposition against European patent 2 359 834 (Application No. 11 001 632.6)

Title:	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement - EP 2 359 834
Proprietor:	Alexion Pharmaceuticals, Inc.
Opponent:	Amgen Inc.
Our Ref.:	AMG17203OP

In response to the Proprietor's request for an extension of term for filing a response to the Notice of Opposition

The OD has set a term for filing the Proprietor's observations on the notices of opposition within four months from notification of said communication, dated September 13, 2017. Accordingly, said term ends on January 23, 2018.

The Proprietor has requested an extraordinary extension of said term for two months on October 30, 2017.

We respectfully request that said request for extension of term is **refused**.

The SPC on the base eculizumab patent expires in May 2020. The term of the opposed patent, if maintained, only ends in March 2027.

As set forth in our notice of opposition, the opposed patent is invalid for several reasons. Any delay in final resolution of this opposition may unfairly extend Alexion's period of exclusivity and delay PNH patients' access to a lower cost alternative to Soliris® (eculizumab), one of the most expensive drugs in the world.

H. Ulrich Dörries [1234]
Dr. sc. nat., Dipl.-Biol.

David Molnia [12347]
M.S. Elec. Eng., B.S. Physics

Sandra Pohlman [568]
B.S. Biology

Michael Eder [1234]
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Oliver Schulz [1234]
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Dr. rer. nat., Mag. pharm.

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LL.M., Dipl.-Phys.

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Dr. rer. nat., MEng (Cornell)

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René Okoampah [1234]
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Julia Mössinger [1234]
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Judith Hesse [8]
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Helena Bertelsmeier [8]
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Barbara Englert [1234]
Dr. rer. nat., Dipl.-Phys.

1 European Patent Attorney
2 European Trademark Attorney
3 European Design Attorney
4 Patentanwalt
5 Solicitor (England & Wales)
6 U.S. Patent Attorney
7 U.S. Patent Agent
8 Rechtsanwalt

Partnerschaftsregister des AG München
PR-Nr. 1278

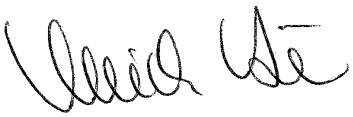
Although we can appreciate Alexion is going through a reorganization, this is a business decision of the Proprietor. Opponent 1, Amgen Inc., is spending significant time and resources to get approval of their biosimilar, and should not be subjected to unnecessary uncertainty and risk due to Alexion's business decisions.

It would thus be contrary to both public interest and the interest of Opponent 1 to delay proceedings.

Furthermore, an opposition with two opponents and "more than 30 documents" filed by the Opponents does not present extraordinary circumstances which would support a request for an extension of term.

Proprietor argues that it needs time to prepare and file new data in response to the filing of experimental data by Opponent 1. Surely during development of Soliris® ample data were produced regarding the stability of eculizumab, calling into question the need for the production of "new" data. Indeed, according to the minutes of the oral proceedings of March 10, 2016, Proprietor's then representative argued that the difference of the claim from the prior art resulted in an increased and/or unexpected stability. Unless those statements, which appear key to the decision to grant the patent, were mere conjecture, the patentee should already have data regarding the stability of eculizumab in support of these statements.

In summary, refusal of the request for extension of term is justified.



Dr. H. Ulrich Dörries
European Patent Attorney

UD/Mo/CB

Europäisches Patentamt
European Patent Office
Office européen des brevets

Submission in opposition proceedings

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- representing the opponent(s):	Amgen, Inc.
Opponent/representative's reference	AMG17203OP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - Response to Request for Extension of time	EPO submission 10Nov2017.pdf	OTHER-1.pdf

Signatures

Place:	**Munich, Germany**
Date:	**10 November 2017**
Signed by:	**Hans Doerries 13689**
Representative name:	**H. Ulrich Dörries**
Capacity:	**(Representative)**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following submission by the opponent:

Submission number	5774785	
Application number	EP11001632.6	
Patent number	EP2359834	
Date of receipt	10 November 2017	
Your reference	AMG17203OP	
Opponent	Amgen, Inc.	
Title	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement	
Documents submitted	package-data.xml ep-oppo.pdf (1 p.)	ep-opposition-data.xml OTHER-1.pdf\EPO submission 10Nov2017.pdf (2 p.)
Submitted by	CN=Hans Doerries 13689	
Method of submission	Online	
Date and time receipt generated	10 November 2017, 10:43 (CET)	
Message Digest	33:D1:8D:D4:8A:4C:39:0C:8A:C0:8D:D8:21:F2:7A:98:61:26:AF:39	

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

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J A Kemp
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ROYAUME UNI

Date: 15.11.2017

Reference	Application No./Patent No.
C411599EP PXT	11001632.6 - 1466 / 2359834

Applicant/Proprietor: Alexion Pharmaceuticals, Inc.

BRIEF COMMUNICATION

Subject:
- ☐ Your letter of
- ☐ Our telephone conversation of
- ☐ Communication of
- ☐

Enclosure(s):
- ☐ Letter from the proprietor of the patent of
- ☒ Letter from the opponent O1 of 10.11.2017
- ☐ Copy (copies)

Communication:

☐ A letter from the opponent /proprietor was received on ..... . The documents specified as patent documents in this letter are available via the European Patent Register at **www.epo.org/register.** Should you wish to receive these documents in paper format, they will be supplied to you free of charge if specifically requested on receipt of this communication (OJ EPO 2009, 434).

☐

For the Opposition Division

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☐ **Registered letter**

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European Patent Office
Office européen des brevets

Submission in opposition proceedings

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- representing the opponent(s):	Amgen, Inc.
Opponent/representative's reference	AMG17203OP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - Response to Request for Extension of time	EPO submission 10Nov2017.pdf	OTHER-1.pdf

Signatures

Place:	**Munich, Germany**
Date:	**10 November 2017**
Signed by:	**Hans Doerries 13689**
Representative name:	**H. Ulrich Dörries**
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November 10, 2017

Opposition against European patent 2 359 834 (Application No. 11 001 632.6)

Title:	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement - EP 2 359 834
Proprietor:	Alexion Pharmaceuticals, Inc.
Opponent:	Amgen Inc.
Our Ref.:	AMG17203OP

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We respectfully request that said request for extension of term is **refused**.

The SPC on the base eculizumab patent expires in May 2020. The term of the opposed patent, if maintained, only ends in March 2027.

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Partnerschaftsregister des AG München
PR-Nr. 1278

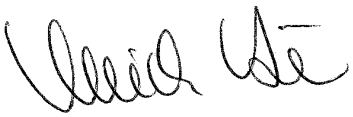
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In summary, refusal of the request for extension of term is justified.



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European Patent Attorney

UD/Mo/CB

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European Patent Office
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Questions about this communication ?
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ALLEMAGNE

Date: 15.11.2017

Reference	Application No./Patent No.
198 441 u6/ne OPPO 02	11001632.6 - 1466 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

BRIEF COMMUNICATION

Subject:
- ☐ Your letter of
- ☐ Our telephone conversation of
- ☐ Communication of
- ☐

Enclosure(s):
- ☐ Letter from the proprietor of the patent of
- ☒ Letter from the opponent O1 of 10.11.2017
- ☐ Copy (copies)

Communication:

☐ A letter from the opponent /proprietor was received on ..... . The documents specified as patent documents in this letter are available via the European Patent Register at **www.epo.org/register.** Should you wish to receive these documents in paper format, they will be supplied to you free of charge if specifically requested on receipt of this communication (OJ EPO 2009, 434).

☐

For the Opposition Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

☐ **Registered letter**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Submission in opposition proceedings

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November 10, 2017

Opposition against European patent 2 359 834 (Application No. 11 001 632.6)

Title:	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement - EP 2 359 834
Proprietor:	Alexion Pharmaceuticals, Inc.
Opponent:	Amgen Inc.
Our Ref.:	AMG17203OP

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7 U.S. Patent Agent
8 Rechtsanwalt

Partnerschaftsregister des AG München
PR-Nr. 1278

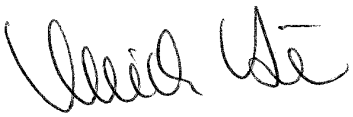
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In summary, refusal of the request for extension of term is justified.



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BY ONLINE FILING

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22 March 2018

Dear Sirs

Opposition to European Patent No. 2359834
(Application No. 11001632.6)
ALEXION PHARMACEUTICALS, INC.
Our Ref: C411599EP PXT/ALS

In response to the Communication dated 13 September 2017, I attach the Patentee's response to the two Oppositions. Attached are:

- the Response;
- documents D33 to D38 as listed in section 2 of the response; and
- a consolidated list of documents D1 to D38.

The Patentee's requests are set out in section 1 of the response. Specifically, it is requested that the Opposition Division maintains the patent as granted. If the Opposition Division is minded to refuse this request, I request that oral proceedings be appointed. If oral proceedings are appointed, I shall speak in English and shall require interpretation into English should any other party speak in a language other than English.

Yours faithfully

Electronically Signed
DR PAMELA M TUXWORTH
ASSOCIATION NO. 811

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22 March 2018

Our Ref: C411599EP

RESPONSE TO NOTICE OF OPPOSITION

Patent No:	2359834
Application No:	11001632.6
Patentee:	Alexion Pharmaceuticals, Inc.
Opponents:	(1) Amgen Inc. (2) Hoffmann Eitle

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CONTENTS

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Page 6540

1 REQUESTS

1.1 **Main Request**

1.1.1 The Patent Proprietor requests that the Opposition Division maintain the Patent as granted.

1.1.2 If the Opposition Division contemplates any decision other than the maintenance of the Patent as granted, then Oral Proceedings are requested. If Oral Proceedings are appointed, I shall speak in English. I shall require interpretation into English should any other party speak in a language other than English.

1.2 **Auxiliary Requests**

1.2.1 The Patent Proprietor requests the opportunity to file further claim requests if it is appropriate to do so during these Opposition Proceedings.

2 DOCUMENTS

2.1 The following documents are cited:

D33 Declaration of Dr Miano

D34 Rouet *et al*; FEBS Letters 588 (2014) 269-277

D35 Summary of antibodies listed in D20/D21 Tables

D36 Graphical representation of D20 Table 2

D37 Summary of antibody products approved by EMA since the filing date

D38 Extracts from Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991)

2.2 We also enclose a consolidated list of all of the documents cited by both Opponents, and including the additional documents mentioned above.

3 No Added Subject Matter

3.1 Opponent 1 (O1) argues that claim 1 includes added subject matter compared with the application as filed. O1's reasoning is that claim 1 of the patent as granted does not include the term "single unit dosage form" which is found both in the passage on page 5, lines 3 to 9 and that on page 22, lines 7 to 14 of the application as filed. O1 argues that this is presented as an essential element of the pharmaceutical composition and

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therefore its omission from claim 1 leads to inadmissible subject matter. Notably, Opponent 2 (O2) has not raised the same objection and makes no argument that the claim as granted includes added subject matter.

3.2 The Patentee disagrees with O1's position. Basis for the wording used in claim 1 of the patent as granted is provided at the following passages of the application as filed:

- Page 5, lines 9 to 10: *"In certain embodiments, the 300mg single-use dosage form comprises 30ml of a 10mg/ml sterile, preservative free solution."*
- Page 6, lines 21 to 23: *"In certain embodiments, the pharmaceutical composition comprises a 300mg single-use formulation of 30ml of a 10mg/ml sterile, preservative free solution."*
- Page 22, lines 11 to 13: *"In certain embodiments, the pharmaceutical composition comprises a 300mg single-use formulation of 30ml of a 10mg/ml sterile, preservative free solution."*

3.3 As is apparent from the above extracts from the application as filed, in none of these passages is the term "single-unit dosage form" presented as an essential feature. Accordingly, these passages are sufficient to provide basis for the wording used in claim 1 of the present patent. There is no addition of subject matter in contravention of Article 123 (2) EPC.

3.4 For completeness, it is noted that the Patentee disagrees that D10 supports O1's argument. D10 does not define either the terms "single-use" or "single-unit dosage form". For at least this reason, D10 is therefore not relevant to the interpretation of these terms.

4 Inventive Step: State of the Art

4.1 The inventive step of the claimed invention will be discussed with reference to the problem and solution approach in section 5 below. It is useful, however, firstly to provide a discussion of the situation in the art at the time the invention was made, in particular the general knowledge of the skilled person in the art in relation to eculizumab and formulating antibodies in general.

4.2 *Eculizumab and treatment of PNH*

4.2.1 Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematologic disease which results in PNH blood cells which have a partial or complete deficiency of GPI-anchored proteins. Anemia is a significant problem for PNH patients leading to an impaired quality of life which is disproportionate to the degree of anemia. Many PNH patients depend on blood transfusions to maintain adequate haemoglobin levels.

4.2.2 The nature of PNH is such that patients, once identified, need to start on treatment very quickly. Thus, the required drug, in the present case

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eculizumab, must be available and ready for use as soon as possible. It is therefore stored on site or at a location nearby (within a certain radius) such that it is essentially ready for use when a patient is identified.

4.2.3 PNH is, however, an ultra-rare disease, so the frequency with which patients are diagnosed is low. The person of skill in the art of PNH treatment would therefore be aware of the need for a long shelf-life product, in order that it can be stored where it is needed for a long period of time without loss of drug activity.

4.2.4 Eculizumab has been identified as a useful treatment for PNH and is the first antibody of its kind to be brought to market. Prior to eculizumab, the vast majority of antibodies brought to market were of IgG1 type, or occasionally IgG2 or IgG4. Eculizumab is none of these IgG types. Instead, appended to eculizumab's variable region is a unique, non-naturally occurring CH1-hinge-CH2-CH3 sequence. No analogously-engineered antibodies were even in development at the filing date of the patent and certainly none had been approved for clinical use. Even today there are no antibodies on the market with the same uniquely-engineered CH1-hinge-CH2-CH3 sequence as eculizumab.

4.3 *Formulation of Antibodies is a Complex Challenge*

4.3.1 A number of antibody drugs were in development at the time the present invention was made, and a small number had already reached the market. However, the issue of the formulation of antibody drugs was at the time regarded as a highly complex and unpredictable issue. As with other protein drugs, it is very important to maintain the integrity of the antibody and to minimize any possible degradation pathways. Therefore, in order to maintain activity of the antibody, both in storage and for administration, a balance is required between maintaining functionality of the antibody and minimizing the inevitable processes of protein degradation that occur in solution. Providing formulations in which antibodies maintain good stability, however, had proved to be a challenging problem.

4.3.2 These issues are discussed in D20 and D21, review articles published in 2007 and 2006 respectively. As Wang *et al* put it in the abstract of D20:

> *"Although antibodies share certain structural similarities, development of commercially viable antibody pharmaceuticals has not been straightforward because of their unique and somewhat unpredictable solution behaviour."*

4.3.3 The various different degradation pathways to which antibodies may be susceptible are described in the section beginning at page 8 onwards of D20. These include denaturation, aggregation, surface adsorption, disulphide bond formation/exchange, non-reducible cross-linking, deamidation, isomerisation, oxidation, formation of acidic or basic species, C-terminal clipping, fragmentation and Maillard reaction. The skilled person looking to formulate an antibody therefore has a multitude of

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different problems to consider, each of which will apply in different ways to each individual antibody.

4.3.4 Of course, it is not the case that only one degradation pathway will necessarily dominate for any one antibody. In the right-hand column, top paragraph of D20, Wang *et al* states that:

> *"In many cases, multiple degradation pathways can occur at the same time and the degradation mechanism may change depending on the stress conditions."*

4.3.5 Moreover, the strategies available to minimise the various degradation pathways may conflict with one another. For instance, deamidation, a major degradation pathway in antibodies, has been shown to have a strong pH-dependence, with less deamidation occurring at pH 4 compared to pH 7 (D20, page 12, right-hand column penultimate paragraph). In contrast, antibody precipitation can be a significant problem at low pH values. D20 reports (page 11, left-hand column first paragraph) that incubation of two mouse monoclonal antibodies (IgG2a and IgG1) at 37°C for 32 days caused precipitation at pH 3 and pH 4 solutions for both antibodies. It is therefore difficult to create a stable antibody formulation due to the numerous degradation pathways possible, and the potentially conflicting strategies available to the formulation chemist for dealing with each of them in the context of each individual antibody.

4.3.6 This issue is also highlighted in D21. In the passage set out below, the authors comment not only on this issue but also on the critical need to devise a formulation that remains compatible with delivery:

> *"In general,* ***successful antibody formulation designs are simply the best compromise of minimizing several competing degradation pathways****. One approach is to find a pH where the minimal amount of degradation events such as deamidation and oxidation will occur. Another is to identify a buffer system that can stabilize the formulation at that pH without damaging the antibody. One common tactic taken to reduce the rate of many of these chemical degradation events is to prepare lyophilized or freeze-dried antibody preparation but even these can be compromised by aggregation issues due to thermodynamic stress that occurs during freezing, drying and reconstitution. A number of protective excipients, mostly carbohydrates, can reduce the propensity of antibodies to aggregate during these stressful events.* ***Another issue is to produce a formulation that is compatible with delivery — neither hypotonic — nor hypertonic solutions are well-tolerated upon injection.****"* (D21, page 701 right-hand column, first full paragraph emphasis added)

4.4 *Each Antibody is Unique*

4.4.1 Despite the various structural similarities between some of the different antibody drugs available in 2007, different antibodies have significantly

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different behaviours and each requires completely separate study in terms of its formulation. Daugherty et al put it like this in D21:

> " *A priori, one might assume that by finding a stable formulation for one of these antibody drugs, that such a formulation would be good for most if not all, similar antibodies. If this were borne out by experience, there would be no need for a review such as this.* ***Instead, each antibody seems to have a unique personality relating to its requirements for stability****; a phenomenon derived from the fact that the small differences between these antibodies are focused on surface-exposed amino acid differences that stipulate antigen specificity. Thus, the interfacial surface of each antibody drug is unique and thus requires specific formulation components to provide maximal stability and retention of activity."* (D21, page 690, right-hand column, first paragraph, emphasis added)

4.4.2 This need to treat even very similar antibodies individually in terms of their stability characteristics is illustrated at page 693, left-hand column of D21. Here the authors describe a recombinant monoclonal antibody that recognises vascular endothelial growth factor (rhMab-VEGF) which was studied for its kinetic and thermodynamic aspects of antibody solution dimerization. The results are reported by Daugherty et al as follows:

> *"It was found that for this IgG1 class antibody, aggregation rates were greater in slightly alkaline (pH 7.5-8.5) compared to slightly acidic (pH 6.5-7.5) conditions. A high salt environment (1 M NaCl) also enhanced dimerization. …. One of the most striking aspects of these dimerization studies on rhMab-VEGF is that trastuzumab, another genetically engineered antibody with the same IgG1 framework and a 92% sequence homology with rhMab- VEGF, fails to demonstrate aggregation events under the same conditions."* (D21, page 693, left-hand column)

4.4.3 This example therefore illustrates that antibodies having very close structural similarity can behave in very different ways in terms of their degradation pathways. Strategies to provide stable antibody formulations therefore must be considered on a case by case basis for each individual antibody.

4.4.4 It is also notable that the vast majority of the discussion of both D20 and D21 relates to IgG1 antibodies. There is a brief discussion in the first paragraph of page 689 of D21 of engineered antibody molecules. D21 notes that such structurally-different antibodies could have very different properties. D21 goes on to state that:

> "*as these new approaches gain prominence, more information will be acquired about their unique requirements for successful formulation and delivery. At present, however, there is little information on formulation approaches for antibodies produced using these production processes.*"

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4.4.5 Therefore, the authors of D21 acknowledge that experience gained in relation to IgG1 antibodies may well not be applicable at all to structurally different antibodies, for example the non-natural, uniquely-engineered structure of eculizumab.

4.5 *No Single Technique Provides Stability*

4.5.1 It can be seen from the discussion above that the formulation of antibody therapeutics was a highly complex field, multiple degradation pathways needed to be taken into account and potentially conflicting strategies were available to the skilled person for dealing with these degradation pathways. Further, each antibody must be individually studied, since small differences in antibody structure were known to lead to significant differences in antibody stability and degradation behaviour. It follows, therefore, that no single technique, no single group of excipients, and no single formulation strategy was available to the skilled person to provide a stable antibody formulation. This is evident from the variety of strategies discussed in the art. Not only do D20 and D21 themselves describe a wide variety of different strategies for effecting antibody stability, but this is also apparent from other prior art documents cited by the Opponents. For example:

- D28 describes a technique for providing stable lyophilised protein formulations using a lyoprotectant such as sucrose or trehalose (see paragraph 8). This is exemplified only in two specific antibodies, an anti-HER2 antibody and an anti-IGE antibody.

- In D29 a very different strategy is used in which the antibodies are not subject to lyophilisation but particular formulation pH and excipients are considered. This strategy was applied to two specific antibodies, an anti-CD18 antibody and an anti-CD20 antibody. Notably, D29 provides an entirely separate study of the suitable pH, buffer and excipients for each of these two separate antibodies.

4.6 *Antibody Concentrations Used in Earlier Formulations*

4.6.1 The Opponents have argued that Table 1 of D20 and Table 1 of D21 demonstrate that antibodies were commonly formulated at 10mg/ml in the art and that therefore this would be an obvious choice of formulation concentration for eculizumab. That 10mg/ml is not the obvious choice for eculizumab is explained in detail below with reference to the problem and solution approach. However, it is useful here to consider what, if any, information the skilled person could derive from the Tables of D20 and D21 when considering suitable formulations for any future antibody drug.

4.6.2 The enclosed table D35 lists each antibody of Table 1 of D20 and Table 1 of D21. The type of antibody is listed, as well as the antibody concentration (in the form in which the product is stored), together with details of the formulation. D35 therefore summarises the information provided in Table 1 of D20 and Table 1 of D21, whilst additionally providing the antibody type. IgG2 and IgG4 antibodies are highlighted in bold.

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4.6.3 A number of observations can be made based on D35 regarding the antibody products on the market before the filing date of the patent:

- The concentrations of products provided in liquid form range from 1 to 50mg/ml with a wide variety of different concentrations being used for different products.
- Those products that can be stored in solution form include five products having a concentration from 1 to 2 mg/ml; four products having a concentration of 10 mg/ml; and three products having a concentration of 20 mg/ml or above.
- Eight products are provided in lyophilised form.
- Half of the antibodies provided in solution form were formulated at concentrations of 5 mg/ml or less.
- The vast majority of antibody drugs which had reached the market before the filing date are IgG1 antibodies.

4.6.4 It is difficult, if not impossible, to derive any pattern in terms of antibody formulation based on the wide variety of formulations used for marketed drugs. The only possible conclusion is that since each antibody is unique for its binding specificity to an antigen, a unique formulation must be developed individually for each antibody. It certainly cannot be concluded that 10 mg/ml is a "typical" concentration for an antibody formulation. Some commercial antibodies were formulated at 10 mg/ml, but the majority were not and it is not possible to determine from the information in Table 1 of D20 and Table 1 of D22 why each formulation was selected. There is no indication as to whether other future antibodies might or might not be suitable for formulation at 10 mg/ml.

4.6.5 D35 also shows that the largest group of antibodies are lyophilised. Again, there is no indication in the Tables of D20 and D21 as to why these particular products are lyophilised for storage. However, the first paragraph of the left-hand column of page 18 of D20 hints that this could be due to instabilities:

> "*Like most proteins, some antibodies are not stable enough in a liquid form and lyophilized dosage forms will have to be considered*".

4.6.6 The wide variety of different formulation types, concentrations and the varying use of lyophilisation which is apparent from D35 demonstrates the unpredictability of antibody formulation. Each antibody needs separate consideration, and the behaviour of other antibodies clearly has not in the past led to any consistency in the way that these products are formulated. The same is true for any future antibody product: the evidence of Table 1 of D20 and Table 1 of D21 is no guide to a suitable formulation for eculizumab.

4.6.7 This is particularly the case given that eculizumab is a non-natural, uniquely-engineered antibody, whilst the vast majority of those antibodies which had reached the market by the filing date were IgG1 type

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antibodies. There are only five examples of non-IgG1 antibodies on the market before the filing date. These were formulated at 1 mg/ml, 1.1 mg/ml, 14 mg/ml, 20 mg/ml and in lyophilised form. The skilled person simply cannot predict from this information a suitable formulation for any future non-IgG1 product. It is certainly not possible to predict from this information a suitable formulation type for a product such as eculizumab. No antibody of this type had reached the market before the filing date.

4.6.8 As well as providing information on the formulation of marketed antibodies, D20 also provides information on antibody stability, including aggregation, in Table 2. Table 2 lists five antibodies where the percent aggregates following storage of a liquid formulation is provided. In D36, the Patentee has plotted the concentration of the formulation against the percent aggregates for these five examples. It is notable that there is no clear trend evident from this graph. Aggregate formation does not vary linearly as antibody concentration changes. There is no direct relationship between these two parameters and thus there are other factors at play in determining the degree of aggregation. In particular, the tendency towards aggregation is very different for different antibodies.

4.6.9 In summary, formulation of antibody drugs remains to this day a challenging topic. There is no single technique or set of excipients and buffer/pH conditions, which formulation experts have at their disposal, which is guaranteed to lead to stability of any particular antibody product. Rather, each antibody has a unique biochemical behaviour. The process of developing a suitable formulation for an antibody, which maintains its biological integrity and activity over time, is one which needs to be carried out individually for each new antibody product. This is particularly the case for an antibody having a novel type of structure, such as the uniquely-engineered structure of eculizumab.

5 Inventive Step: Problem and Solution Approach

5.1 Closest Prior Art

5.1.1 Both Opponents have suggested that D1 is a suitable prior art document. The problem and solution analysis below is accordingly based on D1 as the closest prior art.

5.2 Difference from D1

5.2.1 The claims of the patent differ from the disclosure of D1 in that:

- The formulation is a single use 300mg dosage form
- The concentration is 10 mg/ml
- The volume is 30ml
- The solution is preservative free

5.3 *Technical Problem to be solved*

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5.3.1 The patent aims to provide treatments for PNH. Any such treatment must, to ensure efficacy, be provided in a form which maintains its biological integrity and activity during any period of storage. This is clearly important so that the drug remains an effective treatment for PNH. The treatment must also be in a suitable form for a clinician to administer to a patient.

5.3.2 The specific pharmaceutical composition of eculizumab set out in claim 1 of the patent provides the ideal balance of these properties. The composition is easy for the clinician to use: it is a liquid formulation so there is no need for reconstitution which brings inherent inconvenience for clinical staff and risks of incomplete dissolution. Further, the composition provides a balance of (a) excellent long-term storage stability without loss of biological integrity, (b) minimising the bulk of the stored product, and (c) a suitable concentration for use to provide convenient administration in an acceptable infusion time. All of these factors make the product an excellent treatment for PNH, which has led to the approval of the claimed composition as a treatment for PNH by the regulatory bodies.

5.3.3 It is, of course, critical that the antibody maintains its biological integrity and activity in the period between manufacture and use. Aggregation is a common indicator of antibody stability, and accordingly, any formulation for the treatment of PNH needs to have a low incidence of aggregation on long-term storage. The enclosed declaration by Doctor Miano (D33) provides evidence of the long-term stability of eculizumab 10 mg/ml formulations, and the improved stability of these formulations compared with 30 mg/ml eculizumab formulations.

5.3.4 In paragraph 2 and Exhibits 2 and 3 of D33, Dr Miano provides evidence demonstrating that the eculizumab formulation at 10 mg/ml (i.e. the commercial formulation, Soliris®) is stable for a total period of 48 months. The product is stable for 18 months when stored as the drug substance, i.e. in bulk, and for a further 30 months when stored as the drug product in individual vials. Even over this long storage period, the product meets the commercial requirement that at least 98% monomer antibody remains.

5.3.5 Dr Miano also provides data demonstrating the improved stability of the 10 mg/ml formulation compared with a formulation which is identical in all respects, except that the eculizumab concentration is 30 mg/ml. The studies were carried out at 2 to 8°C and also at 23 to 27°C. As Dr Miano explains at paragraph 5 of D33, these higher temperature stability studies provide a good indication of effects which might be seen at 2 to 8°C over a longer period of time.

5.3.6 As is apparent from Figure 1 of D33, after only six months storage at 23 to 27°C the 30 mg/ml concentration led to an unacceptable level of aggregate formation, with the % monomer dropping to below the threshold of 98% which is required for release. In contrast, the 10 mg/ml concentration maintained over 99% monomer under the same storage conditions.

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5.3.7 Similar trends are seen in the studies at 2 to 8°C, where the % monomer retained after storage is significantly higher for the 10 mg/ml concentration compared with the 30 mg/ml concentration. Based on this data, Dr Miano concludes that the 30 mg/ml concentration would not meet the release standards after 36 months of storage.

5.3.8 Dr Miano's evidence therefore demonstrates that the 10 mg/ml composition of eculizumab provides extremely good long-term stability such that the antibody maintains its integrity and biological activity. It also demonstrates that the 10 mg/ml concentration of eculizumab provides improved stability compared with other concentrations.

5.3.9 The 10 mg/ml concentration combines this enhanced stability with ease of use for the clinician due to the low-volume liquid formulation. Firstly, because it is a liquid formulation, there is no need for reconstitution. Secondly, simple dilution of product required by the label conveniently provides the required 600 or 900 mg administration dosages. Thirdly, the 600 or 900 mg doses required by the patient can be administered via IV infusion of only 120 or 180 ml of solution (following dilution for administration). This relatively low volume enables the drug to be administered by IV infusion over relatively short periods of time and without the need for high infusion volumes which are undesirable for the patient. Finally, the low volume of product in the storage vials (30 mL per vial) minimises the volume which must be stored on site at each clinic.

5.3.10 The technical problem which is solved by the invention is therefore the provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion times and which is convenient to store.

5.3.11 The solution to this problem is the 300 mg single-use dosage form comprising 30 mL of a 10mg/ml preservative-free solution.

5.4 *Response to Opponents' Comments on Technical Solution*

5.4.1 The Opponents argue that the 10 mg/ml concentration of the formulation is an arbitrary selection. This is not the case, for the reasons set out above: the 10 mg/ml formulation shows enhanced maintenance of biological integrity following long-term storage, combined with low volumes for convenient dilution, administration and storage. The formulation as claimed is therefore not only extremely effective but is improved over alternative formulations of eculizumab.

5.4.2 Opponent 1 has filed a declaration by Dr Treuheit (D23) which is said to show the equivalence of differing concentrations of eculizumab. The evidence provided by Dr Treuheit does not show the equivalence of the different concentrations for the following reasons:

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- Dr Treuheit tested stability only over a 13 week period. This is significantly less than the 48-month stability demonstrated for the 10 mg/ml eculizumab product in D33. As the Opposition Division will clearly appreciate, where stability tests are carried out for a very short period of time, differences between the samples tested may not be detected, or may be very small.

- Even at the short period of time over which Dr Treuheit's test was conducted, an increase in formation of aggregates can be seen at elevated temperature for both the 30 mg/ml and 50 mg/ml formulations. These trends support the 30 mg/mL data presented in Dr. Miano's declaration on behalf of the Patentee.

5.4.3 As Dr Treuheit explains in paragraph 5 of his declaration, aggregation of the antibody was studied by size-exclusion ultra high-performance liquid chromatography (SE-UHPLC). The results are shown in Appendix A to his declaration. The lower panel of Appendix A shows that the 30 mg/ml and 50 mg/ml formulations resulted in a reduced percentage of intact antibody after 13 weeks. In both cases, the percent antibody remaining after this period of time was lower than at base line. However, the 10 mg/ml formulation did not show any reduction in percent antibody after 13 weeks even after storage at 25°C.

5.4.4 This result is in accordance with the longer 6 month study carried out by Dr Miano where the 30 mg/ml concentration had a reduced proportion of intact antibody as storage progressed compared with the 10 mg/ml concentration.

5.4.5 The SEC data in Appendix A of Dr Treuheit's declaration shows that the 30 mg/ml and 50 mg/ml formulations had close to 98% antibody remaining after 13 weeks of storage at 25°C. This antibody content falls below the release parameter for commercial lots of eculizumab, indicating that this drug would not be sufficiently stable over this period of time for clinical use.

5.4.6 Opponent 1 also comments on D25 which discloses formulations of anti-C5 antibodies, including eculizumab, of various concentrations. Opponent 1 highlights formulations of eculizumab at various concentrations which were found to be stable. However, the evidence of D25 does not provide any indication of the relative stability of these high concentration formulations compared to the claimed 10 mg/ml formulation. As is clear from the formulation flow chart depicted in the abstract of D25, the formulation is changed on moving from the 10 mg/ml phosphate-based solution to the higher concentration solutions. Thus, it is not possible to directly compare the 10 mg/ml solutions with the higher concentration solutions of D25. Indeed, the skilled person might assume that the higher concentration solutions of eculizumab needed particular treatment, with variation of buffers and excipients, since simply increasing the concentration of the 10 mg/ml solution did not lead to sufficient stability.

5.4.7 In summary, in light of D1,

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- The claimed subject matter provides a specific formulation for eculizumab which is a 300 mg single use dosage form comprising 30 mL of a 10 mg/ml preservative free solution.
- This formulation has extremely good storage stability (48 months at 2-8°C) which means that clinics can store the product for long periods of time and still rely on the biological activity of the product being suitable for treatment of PNH.
- The 10 mg/ml concentration has an *improved* storage stability compared with 30 mg/ml as demonstrated by Dr Miano (D33).
- The volume of the formulation is only 30 ml, such that the volume of product which needs to be stored and administered is low. This has benefits in terms of acceptable infusion volumes and times for the treatment of PNH as well as providing the ability to store eculizumab at various sites to ensure that PNH patients are able to quickly receive this critical therapy.
- The product can be stored in liquid form, avoiding risks of incomplete/incorrect reconstitution by a clinician and providing convenience of use in the treatment of patients having PNH.

5.4.8 The claimed subject matter therefore solves the technical problem of providing a composition of eculizumab which demonstrates the ideal balance of enhanced maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low volume liquid formulation.

5.5 *The Solution is Not Obvious From the Art: Overview*

5.5.1 As is set out in the Guidelines for Examination, the consideration of whether or not a claimed invention would have been obvious involves asking:

> *"whether there is any teaching in the prior art as a whole that **would** (not simply could, but would) have prompted the skilled person, faced with the objective technical problem, to modify or adapt the closest prior art while taking account of that teaching, thereby arriving at something falling within the terms of the claims, and thus achieving what the invention achieves (see **G-VII, 4**).*
>
> *In other words, the point is not whether the skilled person could have arrived at the invention by adapting or modifying the closest prior art, but whether he **would have done** so because the prior art incited him to do so in the hope of solving the objective technical problem or in expectation of some improvement or advantage (see **T 2/83**)."*

5.5.2 It is therefore necessary in the present case to consider whether the skilled person *would* have arrived at the claimed 300 mg preservative-free dosage form containing 30ml of a 10 mg/ml eculizumab solution, with a reasonable expectation that such a composition would provide the ideal balance of maintaining biological integrity of the antibody following storage in a low-

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volume liquid formulation (not merely whether he/she *could* have done so).

5.5.3 The Boards of Appeal have further held that it is necessary to consider whether a skilled person would have followed a particular course of action with a reasonable expectation of successfully solving the technical problem. A reasonable expectation of success has been distinguished from a "hope to succeed" or from a situation where it is "obvious to try" (T296/93). Rather, what is required is that the skilled person can predict, before starting a project, whether or not there is a realistic expectation of a successful conclusion to a project (i.e. of solving the technical problem.)

5.5.4 The Boards of Appeal have, in a few occasions, denied an inventive step on the basis that the skilled person is in a "try and see" situation, as mentioned by Opponent 1 (T333/97). However, a "try and see" situation is considered by the Boards to be limited to particular situations, i.e. where the prior art clearly envisages a specific feature, and the skilled person merely has to determine by routine tests whether such feature does indeed provide a desired effect. In T333/97, for instance, the skilled person is faced with the problem of providing a method for blocking in plants a given pathogenic target RNA strand activity. A specific, suitable method was known in E coli and all parties agreed that it would be obvious to try this specific method in plants. However, it was not known whether or not the method would succeed. A lack of inventive step was found since a single course of action, agreed to be the route obvious to the skilled person, merely had to be tested to see if it worked.

5.5.5 In the present case, as will be discussed further below, none of the documents cited by the Opponents gives the skilled person a reasonable expectation of achieving, in a formulation of eculizumab, good stability and low liquid volume via *any* type of formulation. There is certainly no suggestion that a liquid formulation of 30 ml at 10 mg/ml would provide the ideal balance of properties. It is also clear that the present situation is not a "try and see" situation. The prior art does not provide an indication of a single suitable formulation which could be tested to see if it is effective. Rather, a wide variety of different formulations are used for different antibodies. To determine a suitable composition for the uniquely- engineered eculizumab antibody, the skilled person would have to conduct a significant research program to fully investigate the stability and biological integrity of the antibody, as well as the various factors (and their interplay) affecting each.

5.5.6 Indeed, the Opponents largely seek to rely on references which relate to formulations of other antibodies. As discussed in detail above in Section 4, each antibody has individual properties in terms of its stability, even where structure is very similar. In the present case, eculizumab is a non-naturally occurring, non-IgG1 isotype and is therefore *not* structurally similar to the largely IgG1 antibodies referred to by the Opponents. No conclusions can therefore be drawn from prior art which refers to suitable formulations of different antibodies.

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5.6 *Claimed Subject Matter is Not Obvious over D1 combined with D19-D21*

5.6.1 In light of D1, the technical problem to be solved is to provide a composition for eculizumab which has the right balance of properties providing biological integrity following storage and a low volume liquid formulation for ease of storage and administration. The question in relation to inventive step is: would the skilled person have chosen a 30ml single use formulation of 10 mg/ml eculizumab in a preservative free solution, based on the teaching in D19 to D21, with a reasonable expectation of solving this problem?

5.6.2 In Section 4 above we discussed the knowledge in the art in relation to antibody formulations in D20 and D21. This can be summarised as follows:

- Formulation of antibodies was a complex area with a wide array of different stability considerations to be taken into account for each individual antibody.
- Each antibody is unique and needed separate consideration in terms of determining a suitable composition, even where antibodies were structurally similar.
- There was no single "common" formulation, or antibody concentration, which the skilled person would turn to first.
- The knowledge in the art was largely in relation to IgG1 antibodies. Non-IgG1 antibodies with non-naturally occurring, engineered constant regions were known to act differently, but little work had been done on formulation of such antibodies.
- A wide array of different formulations had been used for various antibodies on the market, giving no clear guidance as to preferable or even suitable formulations for future therapeutic molecules.

5.6.3 It is clear from the above, therefore, that the skilled person would not have known which concentration to use in a pharmaceutical composition of eculizumab. The skilled person would not have had any awareness as to how eculizumab would be affected by the wide variety of potential stability issues to consider. The skilled person would not have had any awareness of which of the many tools available to the formulation scientist (pH, excipients, antibody concentration, lyophilisation) would be effective in addressing the as yet unknown stability issues. The skilled person would certainly not have any indication that 30ml of a 10 mg/ml liquid composition of eculizumab would provide the combined benefits of improved biological integrity of the antibody on storage at up to 48 months and low volume liquid formulation for improved administration. Rather, a full research project would be needed to arrive at such a suitable and ideal composition.

5.6.4 Importantly, the skilled person would be aware that the knowledge about formulation in D20 and D21 largely relates to IgG1 isotype antibodies. However, as set out in D21, little information was available about engineered antibodies:

> *"A number of engineered antibody-like molecules are now being examined for therapeutic applications. Some engineered fragments,*

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such as diabodies and minibodies, are much smaller than intact, full length IgG1 and have very different properties of systemic distribution and clearance. Additionally, methods to prepare other antibody types such as dimeric secretory IgA and methods to prepare antibodies using non-mammalian cell systems, such as plants, from the eggs of chimeric chickens and from transgenic animals, are now being explored by academic and industrial groups. As these new approaches gain prominence, more information will be acquired about their unique requirements for successful formulation and delivery. At present, however, there is little information on formulation approaches for antibodies produced using these production processes." (D21, p689, LH column)

5.6.5 Eculizumab is an example of an engineered antibody having a unique, non-naturally-occurring CH1-hinge-CH2-CH3 structure. Given the unpredictability of behaviour even between IgG1-type antibodies, the skilled person would not have been able to use the information about IgG1 antibodies in the art to predict the behaviour of eculizumab. Indeed, the skilled person would not have been able to predict whether a liquid formulation would have been possible at all for this new type of antibody. There is certainly no reasonable expectation of successfully providing a formulation which maintains the biological integrity of the antibody, in a low volume liquid formulation, over a period of 48 months. The claimed subject matter is therefore not obvious from D1 in combination with D20 and D21.

5.6.6 D19 is also relied on by O1 to show that it is desirable to exclude preservatives for regulatory reasons. However, there is no suggestion in D19 that a 30 ml eculizumab formulation at a concentration of 10 mg/ml would have beneficial properties. D19 is not therefore relevant to solving the objective technical problem.

5.7 *Antibody Concentrations After The Filing Date*

5.7.1 The Opponents have indicated that 10 mg/ml was a typical antibody concentration and it would have been obvious for a skilled person developing a new antibody to use this concentration based on the common knowledge. If the Opponents are right in their assertion, it could be expected that antibodies developed after the filing date would generally have a concentration of 10 mg/ml. However, this is not the case.

5.7.2 D37 summarises data available from the European Medicines Association (EMA) website (http://www.ema.europa.eu). D37 provides a list of active antibodies (excluding biosimilars) which have been given EMA authorisation after the filing date of the patent. The nature of the product is also indicated, including the product concentration, where the product is provided as a solution.

5.7.3 As is evident from D37, only six out of 44 antibody products were approved having a formulation of a 10 mg/ml solution. It is quite clear, therefore, that other factors are considered by developers when selecting an

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appropriate concentration for a formulation of any new drug. 10 mg/ml is not an automatic choice. Indeed, as for antibodies formulated before the filing date, the largest group of products were provided in lyophilised form.

5.7.4 A recent review article on antibody formulation by Rouet *et al* (D34) confirms that, even well after the filing date of the present patent, the issue of providing a stable formulation for an antibody product remains a challenging problem. Even in 2014, Rouet considered that:

> *"Despite recent advances, the identification of suitable formulation conditions for a specific monoclonal antibody remains challenging and cannot be determined from its amino acid sequence. Indeed, a considerable proportion of human monoclonal antibody candidates fail formulation studies, often at relatively late pre-clinical stage, placing heavy burdens onto drug development pipelines."* (Page 271, right-hand column, second paragraph).

5.7.5 In the first paragraph of the right-hand column of page 271, D34 gives the example of therapeutic monoclonal antibody cetuximab (Erbitux). Even the initial suggested formulation conditions of 2 mg/ml in phosphate buffer at pH 6.0 led to visible aggregates under mechanical stress and had to be reformulated. Notably, the new formulation was at a higher concentration – 5 mg/mL – but at a different pH and using different excipients.

5.8 *Claimed Subject Matter is Not Obvious over D1 Combined with D22*

5.8.1 O1 relies on D22 which relates to a nebulisable formulation. A critical factor in considering a suitable formulation of any drug is the manner in which it is administered. Clearly, a topically administered drug will be formulated very differently from an orally or parenterally administered drug. Similarly, the skilled person looking for a suitable formulation for the D1 treatments, which are administered by IV, would not consider nebulized formulations. Thus, the skilled person would not consider D22 when seeking suitable formulations in light of the D1 disclosure.

5.8.2 Moreover, even if the skilled person had considered D22, this would not have led him to the claimed solution. As O1 points out, D22 exemplifies at para [170] to [172] formulations described as eculizumab formulations at 30 mg/ml. However, this example would, if anything, lead the skilled person *away* from the claimed 10 mg/ml concentration. There is no hint or suggestion in D22 that 10 mg/ml would provide improved biological integrity of eculizumab following storage compared with 30 mg/ml. Indeed, there is no information whatsoever regarding stability in D22.

5.8.3 Moreover, a skilled person seeking a dosage form of eculizumab having a *low volume* would not consider reducing the concentration of the 30 mg/ml solution as this would inherently *increase* the volume of solution needed to provide the same drug content. This document would not, therefore, have led the skilled person to the claimed invention starting from D1.

5.9 *Claimed Subject Matter is Not Obvious over D1 combined with D29*

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5.9.1 Starting from D1, the skilled person is seeking a suitable formulation for a unique antibody having a non-naturally-occurring, engineered heavy chain. D29 would not be of assistance to the skilled person since it relates to very different antibodies, which the skilled person would understand to likely have very different stability properties.

5.9.2 D29 is primarily related to formulations for anti-CD18 and anti-CD20 antibodies. This is clear, for example, from column 2, lines 19 to 22 which state that: *"There is a need in the art for a stable aqueous pharmaceutical formulation comprising an antibody, such as an anti-CD18 or anti-CD20 antibody, which is suitable for therapeutic use"*. The only examples of specific formulations given in D29 are for anti-CD18 and anti-CD20 antibodies. Specifically, Example 1 relates to rhuMAb CD18 which is a $F(ab')_2$ molecule (column 24, line 42) and Example 2 relates to rhuMAb CD20.

5.9.3 Eculizumab, on the other hand, is not at all similar to the antibodies exemplified in D29. Eculizumab has a uniquely engineered CH1-hinge-CH2-CH3 sequence and is structurally remote from the antibodies described in D29. The skilled person would have no expectation that the formulation behaviour and stability of the anti-CD18 and anti-CD20 antibodies described in D29 would shed any light at all on a composition for eculizumab. The skilled person would not therefore consider combining D29 with D1.

5.9.4 Further, even if the skilled person had considered D29, the focus of the document is on the excipients used in the formulation. As set out in the summary of the invention at column 2, beginning at line 25, the invention of D29 suggests that a buffer providing a pH in the range 4.5 to 6.0, a surfactant and a polyol are useful in providing a formulation which is "*preferably* […] stable at a temperature of 2-8°C for at least one year, and/or is stable at a temperature of about 30°C for at least one month" (emphasis added). Had the skilled person followed the suggestions of D29, they would have considered primarily the excipients in the formulation to achieve stability. The concentration is not mentioned in the summary of the invention at all. Further, they would not have expected to be able to achieve stability (of a very different antibody) for as long as 48 months, given D29's suggestion of 12 months.

5.9.5 D29 does make some reference to antibody concentration at column 22, as pointed out by O2. However, even D29 itself makes clear that in the actual selection of antibody concentration to use, other factors need to be taken into account. At column 22, line 10 onwards, D29 explains, for example, that the amount of antibody is determined by taking into account the desired dose volumes and mode(s) of administration. The actual concentrations used in the Examples of D1 are very different for the two antibodies: Example 1 uses 1 mg/ml, whereas Example 2 uses 40 mg/ml. Notably, neither is within the narrowest range of 2 to 10 mg/ml which O2 suggests would be the obvious choice of concentration for *any* antibody formulation.

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5.9.6 Based on this information, there is no hint that 10 mg/ml is an obvious choice for a skilled person. Indeed, a skilled person could just as easily consider 1 mg/ml, 40 mg/ml or any other concentration in the range of 1 to 50 mg/ml. There is certainly no suggestion that the specific concentration of 10 mg/ml, when used for an eculizumab formulation, would provide an ideal balance of maintaining biological integrity on storage and low volume liquid formulation.

5.9.7 In summary, D29 gives the skilled person no additional information than they would already be aware of from his common general knowledge with regard to antibody concentration. The skilled person knows that many antibodies (where liquid formulation is possible) may be formulated in the range of 1 mg/ml to 50 mg/ml, and that some of those are formulated in the range of 2 mg/ml to 10 mg/ml. But, the skilled person also knows that various considerations must be taken into account when selecting a suitable concentration for any particular antibody. The skilled person also knows that each antibody requires its own individual study into suitable formulations: concentrations of antibody in a given formulation that lead to stability for that one antibody may not do so for another antibody. This is borne out by the contrast between the concentrations used in Examples 1 and 2 of D29. D29 therefore does not lead the skilled person any closer to the present invention.

5.9.8 Finally, it is noted that O2 draws comparison between the commercial formulation of rituximab, an anti-CD20 antibody, and D29. This does not have any bearing on the scope of D29 itself, which suggests a different concentration of 40 mg/ml in Example 2. Moreover, given the significant structural differences between rituximab and eculizumab, the skilled person would have had no expectation that a formulation for rituximab would prove useful for eculizumab, let alone that it would provide a storage stable composition for at least 48 months.

5.10 *Definition of the technical problem*

5.10.1 The discussion above does, of course, make reference to stability data for eculizumab. The Guidelines for Examination in the European Patent Office confirm that any technical effect derivable from the application as filed can be used as a basis to reformulate the objective technical problem. The relevant passage in the Guidelines reads as follows (G-VII-5.2).

> *"As a matter of principle any effect provided by the invention may be used as a basis for the reformulation of the technical problem, as long as said effect is derivable from the application as filed (see T386/89).* ***It is also possible to rely on new effects submitted subsequently during the proceedings by the applicant, provided that the skilled person would recognise these effects as implied by or related to the technical problem initially suggested*** *(see G-VII, 11 and T184/82)".* (Emphasis added.)

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5.10.2 The technical effects at issue in the present case are in the maintenance of the biological integrity of the antibody following storage, and suitable volumes for storage and administration to a patient. These properties are clearly intimately connected with the underlying problem of providing a useful treatment for PNH. Unless the biological integrity of the antibody is maintained, the treatment cannot be expected to be efficacious. Similarly, the ability of the clinician to safely and effectively administer the drug to a patient is clearly connected to the ability to treat PNH.

5.10.3 As noted in the passage of the Guidelines set out above, effects which are related to the technical problem set out in the application as filed may be relied upon in the formulation of the technical problem. The relevant passage is set out at G-VII-11:

> *"Subsequently, the applicant submits new evidence which shows that the claimed composition exhibits an unexpected advantage in terms of low toxicity. In this case, it is allowable to reformulate the technical problem by including the aspect of toxicity,* ***since pharmaceutical activity and toxicity are related in the sense that the skilled person would always contemplate the two aspects together****".* (Emphasis added.)

5.10.4 It is therefore clearly acceptable to rely on the evidence set out above in the formulation of the objective technical problem for the purposes of a problem/solution analysis for inventive step.

5.11 *Inventive Step: Summary*

5.11.1 In summary, starting from D1, the technical problem which is solved by the present invention is the provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced stability and thus maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion times and which is convenient to store.

5.11.2 Based on D20 and D21, the skilled person would have been aware that formulation of antibodies is a complex area and that each antibody needs to be treated as its own unique formulation project. The skilled person would also have treated with scepticism any information in the prior art that was based on IgG1 antibodies given the non-IgG1, non-naturally occurring, engineered structure of eculizumab: the skilled person would not have any expectation that formulations which had been successful with previous antibodies would be useful in the case of eculizumab.

5.11.3 Moreover, based on D20 and D21, the skilled person would see that there is no clear starting place for considering antibody formulation and that a separate research program would be needed to find the ideal formulation for eculizumab. The result of that research program, carried out by the inventors, was the finding that 30 ml of a 10 mg/ml liquid formulation of eculizumab is surprisingly stable over a period of 48 months, having

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superior maintenance of biological integrity compared with other concentrations of eculizumab, whilst also providing a low volume of liquid formulation. The skilled person could not have had any reasonable expectation of this result in advance of carrying out the research.

5.11.4 Moreover, neither D22 nor D29 cited by the Opponents provides any assistance. D22 relates to a different type of formulation from D1 so would not be considered relevant. In any case, it directs the skilled person to inferior 30 mg/ml formulations. D29 relates primarily to the excipients used in antibody formulation, in particular anti-CD18 and anti-CD20 formulations. Again, it would give the skilled person no guidance for suitable compositions for eculizumab.

5.11.5 The claimed subject matter is therefore inventive over the art.

6 Sufficiency

6.1 The skilled person would be able to carry out the invention without undue burden.

6.2 There is no confusion as to what the heavy and light chain amino acid sequences of eculizumab are. The patent discloses the variable heavy and variable light chain regions of eculizumab (SEQ ID NO: 1 and SEQ ID NO: 3) and the full heavy chain and light chain sequences of eculizumab (SEQ ID NO: 2 and SEQ ID NO: 4).

6.3 The skilled person would immediately recognise, based on his common general knowledge, that SEQ ID NO: 4 (and also the light chain variable region sequence of SEQ ID NO: 3) includes the N-terminal leader sequence which is not present in the mature antibody.

6.4 It was well known in the art at the filing date of the application that a signal peptide is present at the N-terminus of immature heavy and light chain polypeptides. Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991) (D38), which is a database of biological sequences containing sequence alignments and is evidence of the skilled person's common general knowledge, shows the signal peptides of various human light chains.

6.5 The attached page 1 from Kabat *et al.* shows the 22-residue signal peptides of human kappa light chains. The first three residues are predominantly Met Asp Met and the last two residues are predominantly Arg Cys. In SEQ ID NO: 4 of the patent, the first three residues are Met Asp Met and residues 21 and 22 are Arg Cys. Therefore, the skilled person would recognise that SEQ ID NO: 4 is the amino acid sequence of the immature light chain that contains a signal peptide that is not present in the mature eculizumab antibody.

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6.6 Furthermore, the skilled person would know that the framework of human kappa light chains commonly starts with the residues Asp Ile Gln Met as shown in the Table at pages 103 to 108 of Kabat *et al*. Residues 23 to 26 of SEQ ID NO: 4 are Asp Ile Gln Met, which the skilled person would recognise as being the start of the mature light chain of eculizumab.

6.7 The existence of post-published D25 that includes the mature sequence of SEQ ID NO: 4 has no bearing on whether the disclosure of the patent is sufficient. The date for determining sufficiency of disclosure is the filing date of the patent. At any rate, D25 merely confirms what the skilled person would know from reading the patent in light of his common general knowledge, i.e. that SEQ ID NO: 4 contains the light chain signal peptide as well as the light chain sequence present in the mature antibody.

6.8 It should also be pointed out that the skilled person would produce eculizumab using cell-based recombinant methods, and not by artificially synthesising the polypeptide chains. The cells used to make eculizumab would process the light chain polypeptide sequence to remove the signal sequence during antibody production in the cells. Therefore, contrary to the assertion of O1, the skilled person would not use an antibody that is not eculizumab to treat PNH because he would not be able make an antibody that contains the whole sequence of SEQ ID NO:4.

6.9 Thus, the skilled person seeking to reproduce the claimed invention based on the disclosure in the patent of SEQ ID NOs: 2 and SEQ ID NO: 4 would be able to make and formulate eculizumab as required by claim 1 using routine methods known in the art.

6.10 The claimed subject matter is therefore sufficiently disclosed in the patent.

7 Summary

7.1 For the reasons discussed above, the patent does not contain added matter, is inventive over the art and is sufficient. The oppositions should therefore be dismissed and the patent maintained as granted.

Signed

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Review

Stability engineering of the human antibody repertoire

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ABSTRACT

Human monoclonal antibodies often display limited thermodynamic and colloidal stabilities. This behavior hinders their production, and places limitations on the development of novel formulation conditions and therapeutic applications. Antibodies are highly diverse molecules, with much of the sequence variation observed within variable domain families and, in particular, their complementarity determining regions. This has complicated the development of comprehensive strategies for the stability engineering of the human antibody repertoire. Here we provide an overview of the field, and discuss recent advances in the development of robust and aggregation resistant antibody therapeutics.

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1. Introduction

The number of human monoclonal antibody candidates has increased rapidly in recent years, and now represent the largest single class of molecules entering clinical studies [1]. Despite the rapid growth of the antibody therapeutics market over the past twenty to thirty years, hurdles remain that limit their manufacture. Key factors relate to the variable and often limited stability of human antibodies, which negatively impact on many production processes including expression [2,3], purification [4] and formulation [5].

Antibodies are complex multidomain proteins and mechanisms governing their thermodynamic [6] and, in particular, colloidal [7] stability are not fully understood. Human antibodies of the commonly used immunoglobulin G (IgG) type consist of a total of twelve domains, which can be further divided into two chains (heavy and light) and variable (V_H, V_L) and constant (C_H1, C_H2, C_H3, C_L) domains (Fig. 1). Only limited diversity is observed among constant domains, with a total of four isotype (IgG1–4) and two light chain ([κ] kappa and [λ] lambda) classes expressed in humans. Consequently, isotype differences can explain only a limited proportion of the observed stability variation in the human antibody repertoire [8]. A higher proportion of sequence diversity is observed among antibody variable domains, which are assembled through genetic recombination of variable, diverse (V_H only) and joining segments (VDJ-recombination). Further diversity is then introduced into framework, and in particular complementarity determining regions (CDR), through somatic hypermutation, followed by clonal selection of the repertoire. As a result of these processes, the overwhelming majority of sequence variation within the human antibody repertoire is observed within the CDR regions of a limited range of human variable domain families. In this review, we summarize the influence of the observed antibody diversity on colloidal and thermodynamic stabilities and discuss recent advances to improve these properties through engineering approaches.

2. Protein aggregation

Aggregation is a complex process by which proteins can form alternative colloidal states, which are different from the native state, but otherwise energetically favorable [9,10]. It is generally believed that such aggregate species are predominantly formed via unfolded or partially unfolded states [11]. Protein aggregation is increasingly recognized as a problem affecting the manufacturability of human therapeutic antibodies, shelf life and efficacy [12]. Importantly, the presence of aggregates has also been linked to increased immunogenicity, with effects ranging from mild skin irritation to anaphylaxis [13].

Although it can be assumed that stabilization of the native state over alternative aggregate states has occurred during evolution [14], it is important to note that the production of monoclonal antibodies exposes these molecules to a wide range of non-physiological

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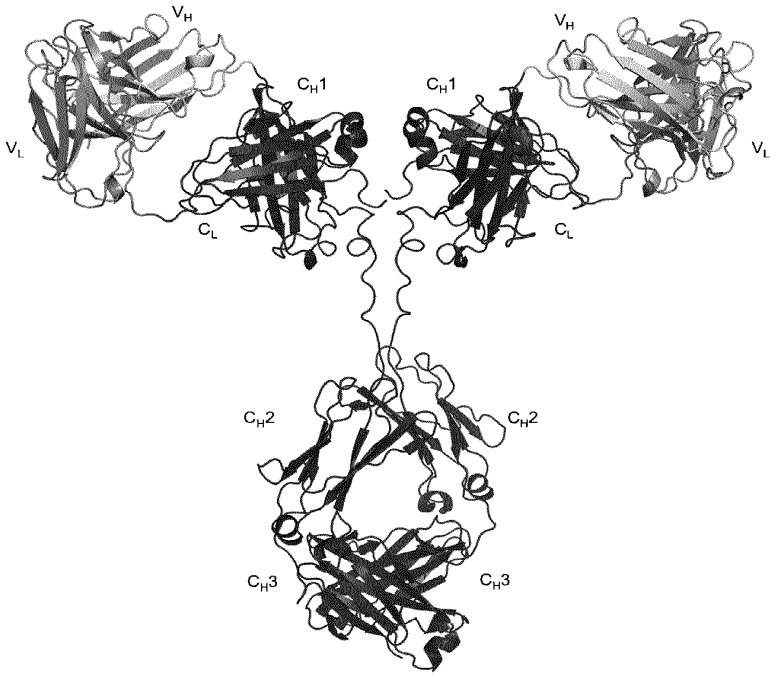


Fig. 1. Structure of the human IgG molecule. The molecule is formed by two heavy chains (consisting of V_H, C_H1, C_H2 and C_H3 domains) and two light chains (consisting of V_L and C_L). Figure was generated using PYMOL.

processes and conditions. These include recombinant expression, purification, concentration, viral inactivation, filtration, formulation, freeze/drying, transport and long-term storage. Throughout these steps the antibody molecule may encounter several stress factors that can dramatically increase its propensity to aggregate (including variations of temperature, pH, protein concentrations, ionic strength, exposure to air–water interfaces and mechanical stress). A further driver of protein aggregation has been a growing trend towards formulations that allow sub-cutaneous administration routes. This requires formulation at high protein concentrations (at around 100 mg/ml) in syringes for self-injection, which places increased demands on colloidal stability [15].

3. Stability of human antibody isotypes and constant domains

The four human IgG isotypes differ in their stabilities and biological functions, namely their potential to induce cellular killing through antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). While the human IgG1 isotype induces powerful ADCC and CDC responses, this is not the case for human IgG2, which is particularly suitable for applications where cellular killing is not required (such as neutralization of soluble ligands) [16]. Unlike the other human isotypes, IgG4 also has a naturally occurring potential to form a bispecific molecule [17] (it has been recently shown that this dual specificity can be grafted onto other isotypes [18]). Human IgG3 is not commonly used due to its longer hinge region, which renders it susceptible to proteolysis [19]. The vast majority of human antibody therapeutics currently in clinical practice and development are of the IgG1 isotype [16].

Conflicting evidence exists in respect to the colloidal stability of the various human IgG isotypes. A recent study of eleven different IgG1 and IgG2 antibodies concluded that, after high temperature storage, the IgG2 isotype is more prone to aggregation [20]. Similar findings were also obtained when subjecting these isotypes to high salt conditons [21] and from isotype switching studies [22]. The latter study confirmed that the human IgG1 isotype is less prone to aggregation when compared to IgG2 or IgG4. However, the authors also found that this isotype is more prone to fragmentation, especially at low pH, due to a non-enzymatic site in the upper hinge region. The hinge regions have also been implicated in the observed differences in aggregation propensity as the IgG2 hinge contains two additional cysteines in each heavy chain compared to IgG1, and is prone to the display of free cysteine residues [23,24]. Differences in the thermal stability of human constant domains have also been reported, with the C_H3 domains of human IgG1 and IgG2 displaying similar melting temperatures, much higher than what was observed for IgG4 [8]. In general, C_H3 domains exhibit more favorable biophysical properties than C_H2 domains [25]. This is reflected by melting temperatures of 8–10 °C higher than those observed for C_H2 domains [8,26].

Although human IgG1 therefore appears less aggregation prone than other isotypes, this is not universally the case. For example, a recent study found that the IgG1 isotype variant of an anti-LINGO-1 antibody exhibited a much higher propensity to aggregate than an IgG2 variant containing identical variable regions [27]. Studies such as these demonstrate that predicting the aggregation propensities of IgG molecules based on isotype can be difficult at the best of times. Moreover, evidence suggests that the aggregation propensity of human IgG molecules is greatly influenced by their variable domains [8,27,28]. This has the effect that antibodies with identical constant domains, but different variable domains, can vary widely in their stability profiles [5].

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4. Stability of human antibody variable domains

Human antibody variable domains can be grouped into several homologous families, which differ in their biophysical properties. A seminal study of consensus domains of human V_H and V_L families has revealed considerable differences of expression yields and thermodynamic stability [6]. In the case of human V_H domains, stabilities ranging from ΔG_{N-U} 14–53 kJ/mol were observed, with soluble expression yields ranging from 1–2.4 mg/l (Table 1). Intriguingly, Ewert et al. also reported that members of the (odd numbered) V_H 1, 3 and 5 families generally displayed superior biophysical properties in comparison with the (even numbered) 2, 4 and 6 families. These differences were observed for both thermodynamic stabilities and expression yield. In particular, the human V_H3 family was identified as uniquely stable and well expressed. This family is also among the most abundant in the human repertoire [29]. The overall stability trends were confirmed in a more recent study, which analyzed the effect of pairing of different human variable domain families on expression, thermal and colloidal stabilities of Fab fragments and IgG monoclonals [30].

More limited differences in biophysical properties were observed among human V_L families, with thermodynamic stabilities ranging from ΔG_{N-U} 15–24 kJ/mol for lambda and ΔG_{N-U} 29–35 kJ/mol for kappa [6]. Similarly, higher expression yields were observed for kappa variable light domains (5–17 mg/l) than for lambda domains (0.3–2 mg/l). The observed differences between antibody variable light domains were much less pronounced when pairing these with human V_H in an scFv format, while considerable differences between variable heavy domain families remained. Overall, the authors found the V_H/V_L combinations H3κ3, H1bκ3, H5κ3 and H3κ1 to be superior in respect to expression yield and thermodynamic stability [6]. However, despite the clear influence of variable domain family (and framework regions), the study also observed a strong influence of CDR sequence on biophysical properties. Indeed, initial attempts of the authors to express human V_H domain in isolation failed to yield protein (with the exception of V_H3). Only the grafting of a solubilizing CDR3 region allowed the soluble expression of human V_H1, and of V_H2, V_H4, V_H5 and V_H6 through refolding from inclusion bodies.

Although several of the human variable domain families are thermodynamically stable, they nevertheless often display poor colloidal stability and readily aggregate when heated above their melting temperatures. This behavior is exemplified by the V_H3 model domain DP47 [31] and by studies of synthetic antibody repertoires on phage, which have demonstrated that as few as one in a thousand VH domains resists heat induced aggregation [32].

Table 1
Biophysical properties of human antibody variable domains. Adapted from Ref. [6].

	Human family	Solubilizing CDR3 required	Expression yield	ΔG_{N-U}
V_H	1a	Y	+	+
	1b	Y	+	++
	2	Y	Refold	nd
	3	N	+	+++
	4	Y	Refold	nd
	5	Y	Refold	+
	6	Y	Refold	nd
V_L	κ1	N	+	++
	κ2	N	+++	++
	κ3	N	+++	+++
	κ4	N	++	-
	λ1	N	+	++
	λ2	N	+	+
	λ3	N	+	+

5. Increasing antibody stability through formulation

The most common method for controlling the biophysical properties of monoclonal antibodies and other biologics is through changes of buffer conditions and excipients. Formulation allows for the control of ionic strength, pH and the addition of excipient agents such as sugars or surfactants, which have been shown to stabilize monoclonal antibody preparations [33]. Although formulation can shield proteins from some chemical and physical challenges (including deamidation, oxidation, hydrolysis/fragmentation and isomerization), aggregation remains difficult to control [12]. Consequently, finding appropriate formulation conditions for an individual product is often an arduous process. This is exemplified by the therapeutic monoclonal antibody cetuximab (Erbitux). Its initial formulation conditions (2 mg/ml in phosphate buffer pH 6.0) generated visible aggregates under mechanical stress [34]. To address this issue, conditions had to be empirically optimized and were changed to 5 mg/ml in citrate buffer pH 5.5 with the addition of glycine and polysorbate 80 as stabilizers.

In recent years, more high-throughput techniques for formulation optimization have been developed [35], and stabilizers and excipients with more general applicability have been identified. Among these, arginine is commonly used to improve refolding yields [36,37], and more recently has been used in formulation to reduce heat-induced aggregation [38]. Another commonly used class of excipient molecules are polysorbates, which accumulate at the air–solvent interface and offer protection against shaking and other mechanical stresses [39]. Despite recent advances, the identification of suitable formulation conditions for a specific monoclonal antibody remains challenging and cannot be determined from its amino acid sequence. Indeed, a considerable proportion of human monoclonal antibody candidates fail formulation studies, often at relatively late pre-clinical stage, placing heavy burdens onto drug development pipelines [5].

6. Stability engineering of human antibody constant domains

Many efforts have been made to increase the stability of the different human antibody isotypes. Early work in this field focused on sequences analyses to predict stabilizing mutations. This approach allowed the identification of a set of substitutions (371K, 376G and 392L), which considerably increased thermal stability (from 76 °C to 86 °C) of the C_H3 domain [40]. The study was based on frequency analyses within 19 mammalian species to generate a consensus sequence. Structural approaches have also been utilized to increase stability of the C_H1/C_L interface [41]. More recently, it was shown that the introduction of additional intra-domain disulfide bridges in the human C_H3 domain resulted in the thermal stabilization of an isolated IgG1 Fc fragment [42] (Fig. 2). In particular, the introduction of a link between the N-terminal A strand (position 343) and the F strand (position 431) caused a 8 °C increase in the melting temperature, while a linkage between positions 375 and 396 resulted in a 4 °C increase (combined 13 °C). These findings were successfully applied to a HER2 specific Fcab molecule (Fc fragment with engineered binding loops) resulting in an increased in the thermal stability of 19 °C. A similar finding was observed for a human IgG1 C_H3 single domain, which could be stabilized by introduction of an intra-domain disulfide bridge [43]. The authors used a two-step approach, by initially introducing mutations that rendered the C_H3 domain monomeric, resulting in a overall low thermal stability (41 °C compared to 82 °C for the native dimeric form). Using structure-based design, two additional cysteines (at positions 343 and 431) were then introduced to generate a disulfide bridge, which increased the thermal stability to 76 °C (a

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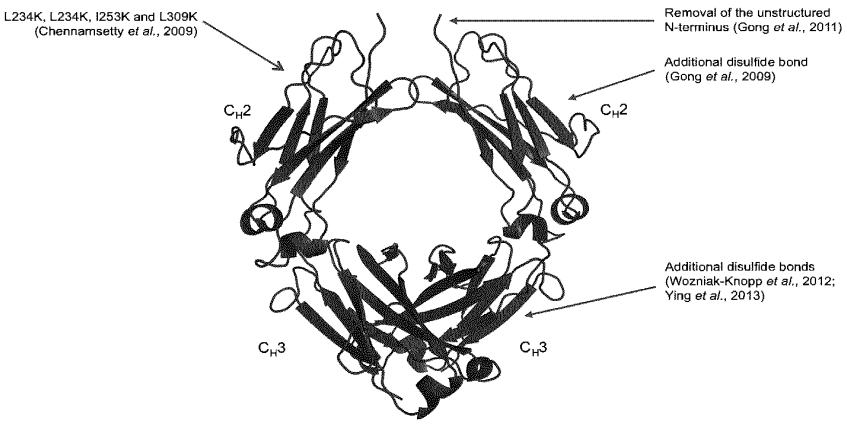


Fig. 2. Stability engineering of human constant domains.

35 °C increase) [43]. Similar strategies have been also reported for C_H2 domains [26]. Here the authors reported the engineering of an isolated human C_H2 domain, resulting in an increase in thermal stability of 20 °C (from 54 °C to 74 °C) [26]. More recently, the same group reported the stabilization of a C_H2 domain through removal of an unstructured region at the N-terminus. Combined with the intra-domain disulfide bond, this resulted in a further increase of melting temperature of 10 °C [44]. For all of the above studies, stability of constant domains was evaluated in the context of fragments or isolated domains.

Other studies have investigated the effects of such mutations on the stability of full-length human IgG. In the case of human IgG4, alteration of the disulfide bond network in the Fab region was shown to lead to an increase of thermal stability of between 3 and 7 °C [45]. This effect was achieved through removal of a cysteine at position 127 in the C_H1 domain, while an additional inter-domain disulfide bond between position 229 and C_L was introduced (similar to what is observed in human IgG1). Importantly, the authors reported that the introduced mutations did not affect antigen binding [45]. More recently, molecular dynamic simulations have been used to predict the spatial aggregation propensity (SAP) of human IgG molecules [46]. The authors later showed that by mutating identified surface-exposed hydrophobic patches within the hinge and Fc regions of an IgG, aggregation resistance and, to a more limited extent, thermodynamic stability could be improved [47].

7. Increased stability through charged fusion tags

In addition to the modification of constant domains, it was recently shown that the addition of charged fusion tags to the N-terminus of human variable domains could considerably improve aggregation propensity [48]. The authors noted that batches of the same IgG monoclonal expressed in yeast were less prone to aggregation than those expressed in mammalian cells. Mass spectrometry and sequencing analyses revealed the presence of a non-processed "EAEA" leader sequence within both heavy and light chains. In contrast to colloidal stability, the authors did not observe improved thermodynamic stability, and the effect seemed to be particularly pronounced for antibodies with a low global net charge. In addition to terminal fusion tags, a different study explored the internal use of a "DED" motif [49]. It was shown that the insertion of the tag into the CDR3 region of aggregation-prone V_H domain antibodies reduced their aggregation propensity, while reducing retention on gel filtration matrices.

8. Stability engineering of human antibody variable domains

8.1. Engineering of human V_H domains through mutation of the light chain interface

The poor biophysical properties of isolated human antibody variable domains are in marked contrast to those of the variable domains of camels and llamas [50]. Although these immunoglobulin domains (V_{HH}) display a relatively high level of sequence identity with human V_H domains, they are generally well expressed and soluble [51] and reversibly unfold after incubation at temperatures as high as 90 °C [52]. In addition to the observed differences in biophysical nature, camelid domains also display an important difference in chain structure, as they are naturally devoid of light chain partners [50]. Their single domain nature results in unique structural features, compared to human antibody V_H domains. These include CDR length and conformation, as well as framework residues. A particular feature of V_{HH} domains is their long flexible CDR3 loop capable of folding back on itself, thereby protecting an area forming the V_H/V_L interface in human antibodies. In addition, these naturally occurring single domains display a marked reduction in hydrophobicity of the former light chain interface, due to a limited number of changes in their respective germlines (Fig. 3). The most common and significant changes compared to humans, coined the "V_{HH} tetrad", are located at positions: G44Q, L45R/C, W47G/I and V37F/Y (human/camelid). The latter residue is nucleating a small core involving Y91, W103, R45 and other hydrophobic residues within the CDR3 loop [53]. Structural investigation of V_{HH} has also shown the CDR3 loop covering residue 37, thereby shielding hydrophobicity in an area corresponding to the light chain interface in human V_H [54]. In addition, camelid V_{HH} often contain an additional disulfide bridge between CDR1 and CDR3, which increases their thermal stability [55,56].

In order to improve the biophysical properties of human V_H domains, elements of the V_{HH} tetrad have been incorporated into human frameworks ("camelization") [57]. Although the introduced framework changes improved the solubility of human V_H, they also reduced expression levels, induced conformational changes, decreased thermodynamic stability and did not considerably improve the colloidal stability of the domains [58,59]. In contrast, combining the approach with the introduction of an additional disulfide bond between CDR1 and CDR3, yielded domains with high colloidal stability, although at the expense of antigen binding affinity [60].

As an alternative to camelid domains, shark single domain antibodies (V_{NAR}) could also potentially serve as a blueprint for improving

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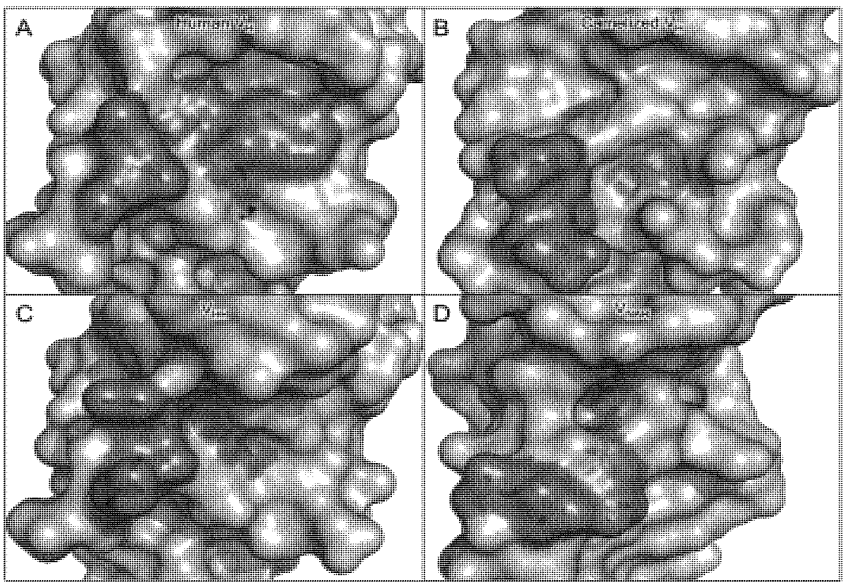


Fig. 3. Engineering of human VH domains through mutation of the light chain interface. Shown are surface representations of the light chain interface in human V_H (PDB 2VXS) and of the equivalent surfaces in camelized V_H (PDB 1VHP), camelid V_{HH} (PDB 1ZVH) and shark V_{NAR} domains (PDB 1T6V). Residues forming the camelid 'tetrad' at positions 37, 44, 45 and 47 are highlighted in green. Camelized V_H, V_{HH} and V_{NAR} domains display a considerable reduction in surface hydrophobicity in comparison to human V_H domains. Figure was generated using PYMOL.

the stability of human antibody V_H domains (although they are more distantly related to human domains [61]). As observed for camelid domains, shark V_{NAR} domains are characterized by a long protruding CDR3 [62]. The CDR loop is constrained by additional disulfide bonds, thereby folding over an area equivalent to the former V_H/V_L interface of human antibodies [61]. A recent study revealed that V_{NAR} domains are resistant to thermal denaturation, acidic denaturation, and lyophilisation denaturation and are capable of returning to their native state after thermal unfolding [63].

Rather than focusing on direct grafting of camelid residues, more recent approaches for the generation of autonomous single domains have focused on the identification of novel mutations. This has been possible due to the use of phage display based selection strategies and the use of protein A to select for folded domains (pA superantigen binds to folded human V_H3 domains, but not to unfolded or aggregated domains). Using this approach, an extensive mutagenesis study has identified hydrophilic substitutions within the V_H3/V_L interface [53]. The authors were able to demonstrate that their mutations (including H35G, Q39R, L45E, R50S) improved solubility, thermal refolding and melting temperature. Importantly, some of the mutations improved protein A binding (and presumably stability), independently of CDR3 sequence diversity. As CDR3 of V_H provides the majority of binding energy in antibody–antigen interactions, this indicated broad compatibility with antigen binding and suggested that the engineered frameworks could be used for the construction of stable repertoires of autonomous V_H domains.

8.2. Engineering of human V_H domains through CDR grafting and framework mutations

In addition to their use in the engineering of autonomous antibody single domains, homology-based approaches have also been used for the identification of mutations outside the interface regions, thereby retaining the ability of human V_H to interact with variable light domains. One of the earliest examples of such strategies relates to the use of CDR grafting for the improvement of biophysical properties. Developed by Jones and Winter in the mid-1980s, CDR grafting was initially used to reduce the immunogenicity of mouse monoclonals, by transplantation of murine CDR regions onto a human framework [64]. However, it was later shown that by grafting onto a stable and well-behaved framework (such as the Herceptin 4D5 [V_H3] framework) considerably improved expression levels and thermodynamic stability, while maintaining binding affinity of the parental antibody fragment [65]. A second approach is based on the use of consensus sequences to improve stability [66]. This approach has been applied to a range of protein scaffolds [67], and utilizes amino acid observed with high frequency within the repertoire [68]. This approach was also used in the construction of the HuCAL human antibody library, for which the authors have reported good expression yields [69]. More recent studies from the same group have identified additional framework mutations that transfer biophysical properties between human V_H3 and V_H6 families [70,71].

As an alternative to homology-based approaches, other studies have focused on the computational prediction of aggregation hotspots in antibody sequences. Early studies on peptides and proteins involved in neurodegenerative diseases have highlighted physicochemical properties that correlate with an increase in aggregate formation, namely hydrophobicity, β-sheet propensity and reduced net charge [72]. Algorithms have been developed to detect such aggregation-prone regions [73–76]. Two of these algorithms, TANGO and PAGE, have been used to identify potential aggregation-prone regions in commercial therapeutic antibodies, which are predominately located in variable domains, and particularly within CDR and adjacent framework residues [28].

8.3. Engineering of human variable domains through CDR mutations

The role of CDR residues in determining thermodynamic and in particular colloidal stability of human antibody variable domains is further highlighted by studies on the biophysical properties of the

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HEL4 model domain [31]. HEL4 was originally isolated through phage display selection from a synthetic CDR-only repertoire based on the human V_H3-23 DP47 germline [77]. As phage is remarkably resistant to chemical, proteolytic and thermal denaturation [78–80], the authors were able to heat the phage displayed antibody fragments at high temperature (80 °C), followed by cooling and binding to antigen (hen egg white lysozyme). The method thereby selected for domains that unfolded reversibly, and could be captured by binding to antigen or superantigen. Unlike other human V_H3 domains, HEL4 displayed highly favorable biophysical properties, including heat-refoldability, high expression levels in bacteria, and the absence of 'stickiness' on gel-filtration [31]. Indeed, it was later shown that aggregation resistance on phage is a general indicator of the solution behavior of human V_H domains [81]. In addition to HEL4, several other V_H domains, that were isolated using this method, showed a high level of resistance to aggregation. Intriguingly, despite their high level of colloidal stability, all of the domains actually had lower thermodynamic stabilities than the DP47 domain from which they had been derived from (ΔG_{N-U} = 15–20 kJ/mol vs 35 kJ/mol). In contrast, DP47 readily aggregated upon heating, further highlighting differences between thermodynamic and colloidal stability. As an alternative to heat-based selection on phage, a more recent study explored the use of pH stress (pH 3.2) for the selection of aggregation-resistant V_H domains [82]. In contrast to domains isolated after thermal denaturation, the selected V_H domains displayed increased thermodynamic stabilities and increased colloidal stability at low pH, reflecting the different denaturation conditions experienced during the selection process.

Although the denaturation method on phage was capable of selecting human V_H domains with CDR sequences that promoted a high level of aggregation resistance, it was also evident that such domains were rare within the repertoire. To increase the frequency of such clones, a human V_H library was constructed through combinatorial assembly of CDR regions that had been pre-selected for aggregation resistance in phage. Binders were successfully selected from this repertoire, which exhibited good antigen-binding properties together with favorable biophysical properties [32].

Despite clear improvements over other human V_H domains, detailed determinants of the observed aggregation resistance initially had remained unclear. As HEL4 and other clones had been selected from synthetic CDR-only repertoires, it was apparent that the

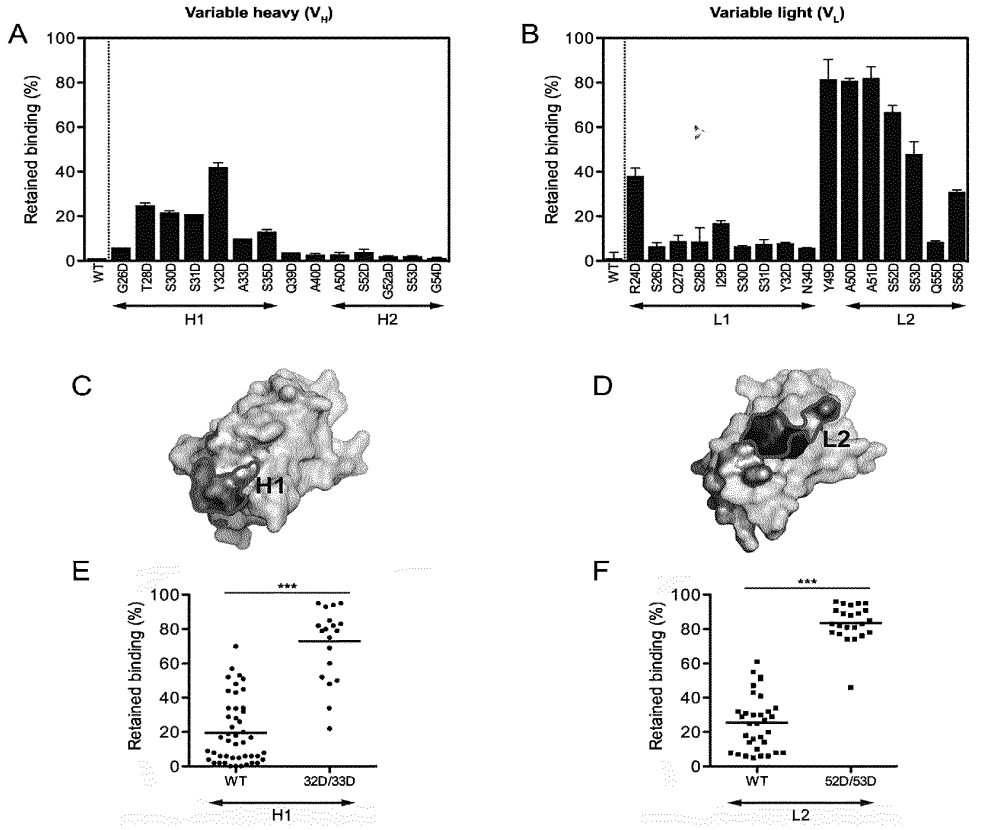


Fig. 4. Effect of mutations in human antibody V_H and V_L domains on aggregation resistance. Surface residues in variable heavy and light domains were targeted for substitution with aspartate. Aggregation resistance of the domains was determined by measuring binding to protein A and protein L superantigen after heating to 80 °C on phage. Complementarily determining regions are indicated (A) Mutations in human V_H. (B) Mutations in human V_L. Introduction of aspartate into CDR H1 and CDR L2 increases aggregation resistance of human variable domains up to 40-fold for V_H and up to 80-fold for V_L. (C) Mutations mapped onto the human V_H surface. (D) Mutations mapped onto the human V_L surface (blue: 100% retained superantigen binding, white: 0%). (E) Stability engineering of human V_H repertoire. (F) Stability engineering of human V_L repertoire. The mutant repertoires were generated through introduction of aspartate mutations in CDR H1 and CDR L2 (at positions 32/33 and 52/53, respectively). Introduction of the mutations significantly increases the aggregation resistance of human antibody repertoires, independent of sequence diversity at other CDR positions. Adapted from Ref. [7].

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exhibited biophysical properties were a result of changes in the complementarity determining regions, without any further influence of framework residues [31]. Crystal structure of the HEL4 model domain had indicated a potential effect of a serine to glycine mutation at position 35 [77]. Indeed, introduction of this mutation into DP47, resulted in increased solubility and reduced "stickiness" on gel-filtration. However, the mutant domain continued to readily aggregate when heated above its melting temperature. The authors also reported that many of the selected domains had a low isoelectric point (pI). Similar findings were also obtained in a second study, which reported that a low pI, together with the non-canonical inter-CDR disulfide linkages, contribute to improved biophysical properties human V_H domains [83]. Using transient heating to pre-select for proteins reversible thermal unfolding properties, the authors found that many of the isolated domains had pI values of less than six, although they also observed domains with values higher than eight. The authors concluded that the improved biophysical characteristics of these V_H domains were caused by a combination of global pI effects, together with an increase of stability through inter-CDR disulfide linkage (other studies by the same group demonstrated that, although the additional disulfide bridge significantly increased thermal stability and protease resistance [84], it also induced structural changes in human V_H as indicated by a marked reduction of protein A superantigen binding [85]). A global increase in charge was also observed in a different study, analyzing larger sets of V_H domain sequences after heat selection on phage, together with an overall reduction of hydrophobicity and a decrease in beta-sheet propensity [86].

More recently, an extensive mutagenesis study on phage has provided detailed insights into mutations that increase the colloidal stability of human V_H and V_L antibody domains [7]. Using superantigen binding after heat denaturation on phage as a mean to select for reversible unfolding, the investigators analyzed the effect of charged mutations at all CDR positions (excluding CDR3 to limit effects on antigen binding). This revealed that the introduction of negative charge (and in particular aspartate) at CDR-H1 positions (28, 30–33, 35) considerably increased the aggregation resistance of human V_H domains (up to 40-fold) (Fig. 4A). This is in excellent agreement with the observations for the model HEL4 domain, which contains negatively charged amino acids at a subset of the identified positions (as highlighted by CDR grafting studies [87–89]).

Intriguingly, this was not the case for human V_L domains, for which the introduction of mutations at equivalent positions had little effect. In contrast to what was observed for human V_H, increased aggregation resistance (up to 80-fold) was instead endowed by the introduction of asparate at CDR-L2 positions (49, 50–53, 56) (Fig. 4B). As observed in other studies [81], the improved properties on phage correlated with increased aggregation resistance in solution, improved expression yields and reduced retention on gel-filtration. Importantly, the observed increases in aggregation resistance were not only observed in model domains, but also when introducing CDR-H1 and CDR-L2 mutations into diverse antibody libraries [7] (Fig. 4E/F).

In addition, and unlike what had been observed for camelized and other engineered variable domains [53,59,85], crystal structures of mutant V_H and V_L domains showed no detectable conformational changes in either CDR conformations or the V_H/V_L interface. Taken together, these results indicated broad compatibility with high affinity antigen binding, which was confirmed by the introduction of CDR-H1 and CDR-L2 mutations into the antibody therapeutic Herceptin [7]. Further evidence for the influence of the identified mutations on colloidal stability has recently been reported in a study of an aggregation-prone human IgG1 monoclonal [90]. The authors found that the aspartic acid at position 49 of the V_L domain considerably reduced aggregation rate of the antibody during long-term stability studies (from 35% to 0.2% per month).

9. Conclusion – towards stable human antibody therapeutics

The increasing number of human monoclonals in development and clinical practice, together with a trend towards high concentration formulations for subcutaneous delivery places increasing demands on the stability of human antibody therapeutics. While the thermodynamic stability of human antibodies is relatively well studied and can be further increased through homology, disulfide and computational approaches [71,83,47], determinants of their colloidal stability are just beginning to emerge due to a combination of rational and high-throughput approaches on phage [7,31,46,91]. In particular, the recent availability of human V_L domains with high levels of aggregation resistance [7] will greatly assist in the generation of paired antibody fragments and IgG. Further advances in the generation of human antibody therapeutics with high thermodynamic and colloidal stability will not only improve the production and formulation of human antibody therapeutics, but may also open up new administration routes and therapeutic applications.

References

[1] Nelson, A.L., Dhimolea, E. and Reichert, J.M. (2010) Development trends for human monoclonal antibody therapeutics. Nat. Rev. Drug Discov. 9, 767–774.
[2] Finke, J.M., Roy, M., Zimm, B.H. and Jennings, P.A. (2000) Aggregation events occur prior to stable intermediate formation during refolding of interleukin 1beta. Biochemistry 39, 575–583.
[3] Baneyx, F. and Mujacic, M. (2004) Recombinant protein folding and misfolding in *Escherichia coli*. Nat. Biotechnol. 22, 1399–1408.
[4] Bondos, S.E. and Bicknell, A. (2003) Detection and prevention of protein aggregation before, during, and after purification. Anal. Biochem. 316, 223–231.
[5] Lowe, D., Dudgeon, K., Rouet, R., Schofield, P., Jermutus, L. and Christ, D. (2011) Aggregation, stability, and formulation of human antibody therapeutics. Adv. Protein Chem. Struct. Biol. 84, 41–61.
[6] Ewert, S., Huber, T., Honegger, A. and Pluckthun, A. (2003) Biophysical properties of human antibody variable domains. J. Mol. Biol. 325, 531–553.
[7] Dudgeon, K., Rouet, R., Kokmeijer, I., Schofield, P., Stolp, J., Langley, D., Stock, D. and Christ, D. (2012) General strategy for the generation of human antibody variable domains with increased aggregation resistance. Proc. Natl. Acad. Sci. USA 109, 10879–10884.
[8] Garber, E. and Demarest, S.J. (2007) A broad range of Fab stabilities within a host of therapeutic IgGs. Biochem. Biophys. Res. Commun. 355, 751–757.
[9] Wolynes, P.G., Onuchic, J.N. and Thirumalai, D. (1995) Navigating the folding routes. Science 267, 1619–1620.
[10] Sambashivan, S., Liu, Y., Sawaya, M.R., Gingery, M. and Eisenberg, D. (2005) Amyloid-like fibrils of ribonuclease A with three-dimensional domain-swapped and native-like structure. Nature 437, 266–269.
[11] Dobson, C.M. (2003) Protein folding and misfolding. Nature 426, 884–890.
[12] Wang, W. (2005) Protein aggregation and its inhibition in biopharmaceutics. Int. J. Pharm. 289, 1–30.
[13] Rosenberg, A.S. (2006) Effects of protein aggregates: an immunologic perspective. AAPS J. 8, E501–E507.
[14] Wright, C.F., Teichmann, S.A., Clarke, J. and Dobson, C.M. (2005) The importance of sequence diversity in the aggregation and evolution of proteins. Nature 438, 878–881.
[15] Shire, S.J. (2009) Formulation and manufacturability of biologics. Curr. Opin. Biotechnol. 20, 708–714.
[16] Salfeld, J.G. (2007) Isotype selection in antibody engineering. Nat. Biotechnol. 25, 1369–1372.
[17] Schuurman, J., Van Ree, R., Perdok, G.J., Van Doorn, H.R., Tan, K.Y. and Aalberse, R.C. (1999) Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Immunology 97, 693–698.
[18] Labrijn, A.F., Meesters, J.I., de Goeij, B.E., van den Bremer, E.T., Neijssen, J., van Kampen, M.D., Strumane, K., Verploegen, S., Kundu, A., Gramer, M.J., van Berkel, P.H., van de Winkel, J.G., Schuurman, J. and Parren, P.W. (2013) Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange. Proc. Natl. Acad. Sci. USA 110, 5145–5150.
[19] Carter, P.J. (2006) Potent antibody therapeutics by design. Nat. Rev. Immunol. 6, 343–357.
[20] Franey, H., Brych, S.R., Kolvenbach, C.G. and Rajan, R.S. (2010) Increased aggregation propensity of IgG2 subclass over IgG1: role of conformational changes and covalent character in isolated aggregates. Protein Sci. 19, 1601–1615.

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[21] Hari, S.B., Lau, H., Razinkov, V.I., Chen, S. and Latypov, R.F. (2010) Acid-induced aggregation of human monoclonal IgG1 and IgG2: molecular mechanism and the effect of solution composition. Biochemistry 49, 9328–9338.
[22] Ito, T. and Tsumoto, K. (2013) Effects of subclass change on the structural stability of chimeric, humanized, and human antibodies under thermal stress. Protein Sci. 22 (11), 1542–1551.
[23] Wypych, J., Li, M., Guo, A., Zhang, Z., Martinez, T., Allen, M.J., Fodor, S., Kelner, D.N., Flynn, G.C., Liu, Y.D., Bondarenko, P.V., Ricci, M.S., Dillon, T.M. and Balland, A. (2008) Human IgG2 antibodies display disulfide-mediated structural isoforms. J. Biol. Chem. 283, 16194–16205.
[24] Lacy, E.R., Baker, M. and Brigham-Burke, M. (2008) Free sulfhydryl measurement as an indicator of antibody stability. Anal. Biochem. 382, 66–68.
[25] Demarest, S.J. and Glaser, S.M. (2008) Antibody therapeutics, antibody engineering, and the merits of protein stability. Curr. Opin. Drug Discov. Devel. 11, 675–687.
[26] Gong, R., Vu, B.K., Feng, Y., Prieto, D.A., Dyba, M.A., Walsh, J.D., Prabakaran, P., Veenstra, T.D., Tarasov, S.G., Ishima, R. and Dimitrov, D.S. (2009) Engineered human antibody constant domains with increased stability. J. Biol. Chem. 284, 14203–14210.
[27] Pepinsky, R.B., Silvian, L., Berkowitz, S.A., Farrington, G., Lugovskoy, A., Walus, L., Eldredge, J., Capili, A., Mi, S., Graff, C. and Garber, E. (2010) Improving the solubility of anti-LINGO-1 monoclonal antibody Li33 by isotype switching and targeted mutagenesis. Protein Sci. 19, 954–966.
[28] Wang, X., Das, T.K., Singh, S.K. and Kumar, S. (2009) Potential aggregation prone regions in biotherapeutics: a survey of commercial monoclonal antibodies. MAbs 1, 254–267.
[29] Tomlinson, I.M., Walter, G., Marks, J.D., Llewelyn, M.B. and Winter, G. (1992) The repertoire of human germline V_H sequences reveals about fifty groups of V_H segments with different hypervariable loops. J. Mol. Biol. 227, 776–798.
[30] Tiller, T., Schuster, I., Deppe, D., Siegers, K., Strohner, R., Herrmann, T., Berenguer, M., Poujol, D., Stehle, J., Stark, Y., Heßling, M., Daubert, D., Felderer, K., Kaden, S., Kölln, J., Enzelberger, M. and Urlinger, S. (2013) A fully synthetic human Fab antibody library based on fixed V_H/V_L framework pairings with favorable biophysical properties. MAbs 5, 445–470.
[31] Jespers, L., Schon, O., Famm, K. and Winter, G. (2004) Aggregation-resistant domain antibodies selected on phage by heat denaturation. Nat. Biotechnol. 22, 1161–1165.
[32] Christ, D., Famm, K. and Winter, G. (2007) Repertoires of aggregation-resistant human antibody domains. Protein Eng. Des. Sel. 20, 413–416.
[33] Wang, W., Singh, S., Zeng, D.L., King, K. and Nema, S. (2007) Antibody structure, instability, and formulation. J. Pharm. Sci. 96, 1–26.
[34] Lahlou, A., Blanchet, B., Carvalho, M., Paul, M. and Astier, A. (2009) Mechanically-induced aggregation of the monoclonal antibody cetuximab. Ann. Pharm. Fr. 67, 340–352.
[35] Daugherty, A.L. and Mrsny, R.J. (2006) Formulation and delivery issues for monoclonal antibody therapeutics. Adv. Drug Deliv. Rev. 58, 686–706.
[36] Buchner, J. and Rudolph, R. (1991) Renaturation, purification and characterization of recombinant Fab-fragments produced in *Escherichia coli*. Biotechnology (NY) 9, 157–162.
[37] Lee, C.M., McGuire, H., Basten, A., King, C. and Christ, D. (2010) Expression, purification and characterization of recombinant interleukin-21. J. Immunol. Methods 362, 185–189.
[38] Arakawa, T., Tsumoto, K., Kita, Y., Chang, B. and Ejima, D. (2007) Biotechnology applications of amino acids in protein purification and formulations. Amino Acids 33, 587–605.
[39] Mahler, H.C., Senner, F., Maeder, K. and Mueller, R. (2009) Surface activity of a monoclonal antibody. J. Pharm. Sci. 98, 4525–4533.
[40] Demarest, S.J., Rogers, J. and Hansen, G. (2004) Optimization of the antibody C(H)3 domain by residue frequency analysis of IgG sequences. J. Mol. Biol. 335, 41–48.
[41] Teerinen, T., Valjakka, J., Rouvinen, J. and Takkinen, K. (2006) Structure-based stability engineering of the mouse IgG1 Fab fragment by modifying constant domains. J. Mol. Biol. 361, 687–697.
[42] Wozniak-Knopp, G., Stadlmann, J. and Ruker, F. (2012) Stabilisation of the Fc fragment of human IgG1 by engineered intradomain disulfide bonds. PLoS One 7, e30083.
[43] Ying, T., Chen, W., Feng, Y., Wang, Y., Gong, R. and Dimitrov, D.S. (2013) Engineered soluble monomeric IgG1 C_H3 domain: generation, mechanisms of function, and implications for design of biological therapeutics. J. Biol. Chem. 288, 25154–25164.
[44] Gong, R., Wang, Y., Feng, Y., Zhao, Q. and Dimitrov, D.S. (2011) Shortened engineered human antibody C_H2 domains: increased stability and binding to the human neonatal Fc receptor. J. Biol. Chem. 286, 27288–27293.
[45] Peters, S.J., Smales, C.M., Henry, A.J., Stephens, P.E., West, S. and Humphreys, D.P. (2012) Engineering an improved IgG4 molecule with reduced disulfide bond heterogeneity and increased Fab domain thermal stability. J. Biol. Chem. 287, 24525–24533.
[46] Chennamsetty, N., Helk, B., Voynov, V., Kayser, V. and Trout, B.L. (2009) Aggregation-prone motifs in human immunoglobulin G. J. Mol. Biol. 391, 404–413.
[47] Chennamsetty, N., Voynov, V., Kayser, V., Helk, B. and Trout, B.L. (2009) Design of therapeutic proteins with enhanced stability. Proc. Natl. Acad. Sci. USA 106, 11937–11942.
[48] Schaefer, J.V. and Pluckthun, A. (2012) Engineering aggregation resistance in IgG by two independent mechanisms: lessons from comparison of *Pichia pastoris* and mammalian cell expression. J. Mol. Biol. 417, 309–335.
[49] Perchiacca, J.M., Ladiwala, A.R., Bhattacharya, M. and Tessier, P.M. (2012) Aggregation-resistant domain antibodies engineered with charged mutations near the edges of the complementarity-determining regions. Protein Eng. Des. Sel. 25, 591–601.
[50] Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E.B., Bendahman, N. and Hamers, R. (1993) Naturally occurring antibodies devoid of light chains. Nature 363, 446–448.
[51] Arbabi Ghahroudi, M., Desmyter, A., Wyns, L., Hamers, R. and Muyldermans, S. (1997) Selection and identification of single domain antibody fragments from camel heavy-chain antibodies. FEBS Lett. 414, 521–526.
[52] van der Linden, R.H., Frenken, L.G., de Geus, B., Harmsen, M.M., Ruuls, R.C., Stok, W., de Ron, L., Wilson, S., Davis, P. and Verrips, C.T. (1999) Comparison of physical chemical properties of llama V_{HH} antibody fragments and mouse monoclonal antibodies. Biochim. Biophys. Acta 1431, 37–46.
[53] Barthelemy, P.A., Raab, H., Appleton, B.A., Bond, C.J., Wu, P., Wiesmann, C. and Sidhu, S.S. (2008) Comprehensive analysis of the factors contributing to the stability and solubility of autonomous human V_H domains. J. Biol. Chem. 283, 3639–3654.
[54] Vranken, W., Tolkatchev, D., Xu, P., Tanha, J., Chen, Z., Narang, S. and Ni, F. (2002) Solution structure of a llama single-domain antibody with hydrophobic residues typical of the V_H/V_L interface. Biochemistry 41, 8570–8579.
[55] Muyldermans, S., Atarhouch, T., Saldanha, J., Barbosa, J.A. and Hamers, R. (1994) Sequence and structure of V_H domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. Protein Eng. 7, 1129–1135.
[56] Ewert, S., Cambillau, C., Conrath, K. and Pluckthun, A. (2002) Biophysical properties of camelid V(HH) domains compared to those of human V(H)3 domains. Biochemistry 41, 3628–3636.
[57] Davies, J. and Riechmann, L. (1994) 'Camelising' human antibody fragments: NMR studies on V_H domains. FEBS Lett. 339, 285–290.
[58] Davies, J. and Riechmann, L. (1996) Single antibody domains as small recognition units: design and in vitro antigen selection of camelized, human VH domains with improved protein stability. Protein Eng. 9, 531–537.
[59] Riechmann, L. (1996) Rearrangement of the former V_L interface in the solution structure of a camelised, single antibody V_H domain. J. Mol. Biol. 259, 957–969.
[60] Tanha, J., Xu, P., Chen, Z., Ni, F., Kaplan, H., Narang, S.A. and MacKenzie, C.R. (2001) Optimal design features of camelized human single-domain antibody libraries. J. Biol. Chem. 276, 24774–24780.
[61] Roux, K.H., Greenberg, A.S., Greene, L., Strelets, L., Avila, D., McKinney, E.C. and Flajnik, M.F. (1998) Structural analysis of the nurse shark (new) antigen receptor (NAR): molecular convergence of NAR and unusual mammalian immunoglobulins. Proc. Natl. Acad. Sci. USA 95, 11804–11809.
[62] Greenberg, A.S., Avila, D., Hughes, M., Hughes, A., McKinney, E.C. and Flajnik, M.F. (1995) A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature 374, 168–173.
[63] Griffiths, K., Dolezal, O., Parisi, K., Angerosa, J., Dogovski, C., Barraclough, M., Sanalla, A., Casey, J., González, I., Perugini, M., Nuttall, S. and Foley, M. (2013) Shark variable new antigen receptor (VNAR) single domain antibody fragments: stability and diagnostic applications. Antibodies 2, 66–81.
[64] Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. and Winter, G. (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 321, 522–525.
[65] Jung, S. and Pluckthun, A. (1997) Improving in vivo folding and stability of a single-chain Fv antibody fragment by loop grafting. Protein Eng. 10, 959–966.
[66] Steipe, B., Schiller, B., Pluckthun, A. and Steinbacher, S. (1994) Sequence statistics reliably predict stabilizing mutations in a protein domain. J. Mol. Biol. 240, 188–192.
[67] Kohl, A., Binz, H.K., Forrer, P., Stumpp, M.T., Pluckthun, A. and Grutter, M.G. (2003) Designed to be stable: crystal structure of a consensus ankyrin repeat protein. Proc. Natl. Acad. Sci. USA 100, 1700–1705.
[68] Worn, A. and Pluckthun, A. (2001) Stability engineering of antibody single-chain Fv fragments. J. Mol. Biol. 305, 989–1010.
[69] Knappik, A., Ge, L., Honegger, A., Pack, P., Fischer, M., Wellnhofer, G., Hoess, A., Wolle, J., Pluckthun, A. and Virnekas, B. (2000) Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. J. Mol. Biol. 296, 57–86.
[70] Ewert, S., Honegger, A. and Pluckthun, A. (2003) Structure-based improvement of the biophysical properties of immunoglobulin VH domains with a generalizable approach. Biochemistry 42, 1517–1528.
[71] Schaefer, J.V. and Pluckthun, A. (2012) Transfer of engineered biophysical properties between different antibody formats and expression systems. Protein Eng. Des. Sel. 25, 485–506.
[72] Murphy, R.M. (2002) Peptide aggregation in neurodegenerative disease. Annu. Rev. Biomed. Eng. 4, 155–174.
[73] Kuhlman, B. and Baker, D. (2000) Native protein sequences are close to optimal for their structures. Proc. Natl. Acad. Sci. USA 97, 10383–10388.
[74] Fernandez-Escamilla, A.M., Rousseau, F., Schymkowitz, J. and Serrano, L. (2004) Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nat. Biotechnol. 22, 1302–1306.
[75] Pawar, A.P., Dubay, K.F., Zurdo, J., Chiti, F., Vendruscolo, M. and Dobson, C.M. (2005) Prediction of "aggregation-prone" and "aggregation-susceptible" regions in proteins associated with neurodegenerative diseases. J. Mol. Biol. 350, 379–392.

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[76] Tartaglia, G.G., Cavalli, A., Pellarin, R. and Caflisch, A. (2005) Prediction of aggregation rate and aggregation-prone segments in polypeptide sequences. Protein Sci. 14, 2723–2734.
[77] Jespers, L., Schon, O., James, L.C., Veprintsev, D. and Winter, G. (2004) Crystal structure of HEL4, a soluble, refoldable human V(H) single domain with a germ-line scaffold. J. Mol. Biol. 337, 893–903.
[78] Kristensen, P. and Winter, G. (1998) Proteolytic selection for protein folding using filamentous bacteriophages. Fold Des. 3, 321–328.
[79] Holliger, P., Riechmann, L. and Williams, R.L. (1999) Crystal structure of the two N-terminal domains of g3p from filamentous phage fd at 1.9 Å: evidence for conformational lability. J. Mol. Biol. 288, 649–657.
[80] Jermutus, L., Honegger, A., Schwesinger, F., Hanes, J. and Pluckthun, A. (2001) Tailoring in vitro evolution for protein affinity or stability. Proc. Natl. Acad. Sci. USA 98, 75–80.
[81] Dudgeon, K., Rouet, R. and Christ, D. (2013) Rapid prediction of expression and refolding yields using phage display. Protein Eng. Des. Sel. 26 (10), 671–674.
[82] Famm, K., Hansen, L., Christ, D. and Winter, G. (2008) Thermodynamically stable aggregation-resistant antibody domains through directed evolution. J. Mol. Biol. 376, 926–931.
[83] Arbabi-Ghahroudi, M., To, R., Gaudette, N., Hirama, T., Ding, W., MacKenzie, R. and Tanha, J. (2009) Aggregation-resistant VHs selected by in vitro evolution tend to have disulfide-bonded loops and acidic isoelectric points. Protein Eng. Des. Sel. 22, 59–66.
[84] Hussack, G., Hirama, T., Ding, W., Mackenzie, R. and Tanha, J. (2011) Engineered single-domain antibodies with high protease resistance and thermal stability. PLoS One 6, e28218.
[85] Kim, D.Y., Kandalaft, H., Ding, W., Ryan, S., van Faassen, H., Hirama, T., Foote, S.J., MacKenzie, R. and Tanha, J. (2012) Disulfide linkage engineering for improving biophysical properties of human V_H domains. Protein Eng. Des. Sel. 25, 581–589.
[86] Dudgeon, K., Famm, K. and Christ, D. (2009) Sequence determinants of protein aggregation in human V_H domains. Protein Eng. Des. Sel. 22, 217–220.
[87] Perchiacca, J.M., Bhattacharya, M. and Tessier, P.M. (2011) Mutational analysis of domain antibodies reveals aggregation hotspots within and near the complementarity determining regions. Proteins 79, 2637–2647.
[88] Lee, C.C., Perchiacca, J.M. and Tessier, P.M. (2013) Toward aggregation-resistant antibodies by design. Trends Biotechnol. 31 (11), 612–620.
[89] Christ, D. and Dudgeon, K. (2011) Modified variable domain molecules and methods for producing and using same. WO/2011/047442.
[90] Buchanan, A., Clementel, V., Woods, R., Harn, N., Bowen, M.A., Mo, W., Popovic, B., Bishop, S.M., Dall'Acqua, W., Minter, R., Jermutus, L. and Bedian, V. (2013) Engineering a therapeutic IgG molecule to address cysteinylation, aggregation and enhance thermal stability and expression. MAbs 5, 255–262.
[91] Lee, C.M., Iorno, N., Sierro, F. and Christ, D. (2007) Selection of human antibody fragments by phage display. Nat. Protoc. 2 (11), 3001–3008.

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Proprietor/representative's reference	C411599EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Reply of the patent proprietor to the notice(s) of opposition	Response to Notice of Opposition 22 Mar 18 C411599EP PXT_ALS.PDF	OBSO3.PDF
2	Any annexes (other than citation) to an opposition letter - Consolidated list of documents	Consolidated List of Documents.PDF	OTHER-1.PDF
3	Any annexes (other than citation) to an opposition letter - Cover letter	Enclose Response to Oppositions C411599EP PXT ALS.PDF	OTHER-2.PDF

Evidence filed subsequently:

D33	Other evidence	Declaration of Dr Miano C411599EP

		original file name: D33 Declaration of Dr Miano C411599EP.pdf attached as: Other-evidence-1.pdf
D34	Other evidence	Rouet et al Stability Engineering of the Human Antibody Repertoire original file name: D34 Rouet et al.pdf attached as: Other-evidence-5.pdf
D35	Other evidence	Summary of Antibodies listed in D20 and D21 Tables original file name: D35 Summary of Antibodies listed in D20 and D21 Tables.pdf attached as: Other-evidence-3.pdf
D36	Other evidence	Graphical Representation of D20 Table 2 original file name: D36 Graphical Representation of D20 Table 2.pdf attached as: Other-evidence-4.pdf
D37	Other evidence	EMA Approved Antibodies C411599EP original file name: D37 EMA Approved Antibodies C411599EP.pdf attached as: Other-evidence-2.pdf
D38	Other evidence	Extracts from Kabat et al., Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991) original file name: D38 Kabat Exhibit for Response C411599EP.pdf attached as: Other-evidence-6.pdf

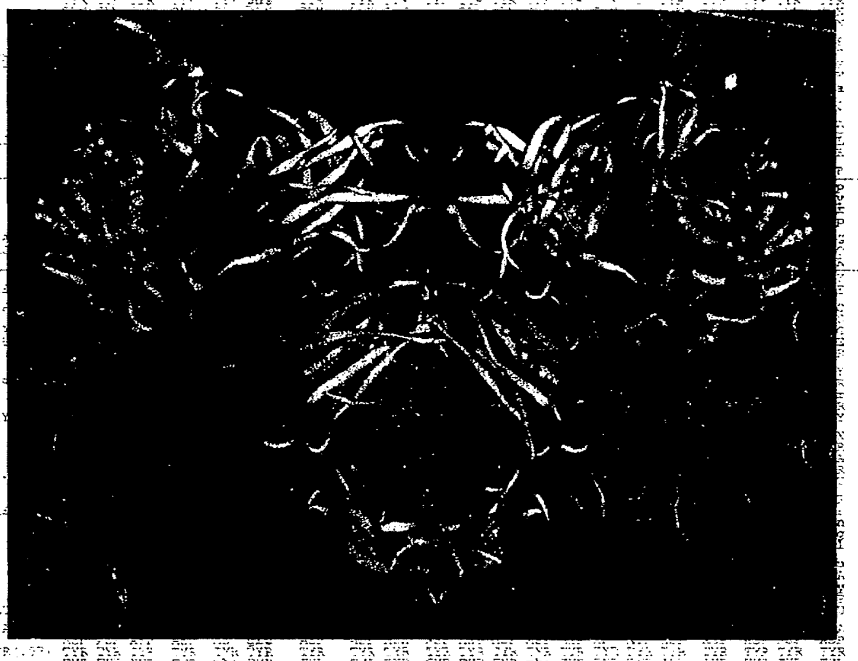
Signatures

Place:	
Date:	**22 March 2018**
Signed by:	**Amanda Simons 23469**
Association:	**J A Kemp**
Representative name:	**/Amanda Simons/**
Capacity:	**(Representative)**

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

VOL. I

FIFTH EDITION



1991

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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Page 6573

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

FIFTH EDITION

Tabulation and Analysis of
Amino Acid and Nucleic Acid Sequences of Precursors,
V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen,
T-Cell Surface Antigens, β_2-Microglobulins,
Major Histocompatibility Antigens, Thy-1, Complement,
C-Reactive Protein, Thymopoietin, Integrins, Post-gamma Globulin,
α_2-Macroglobulins, and Other Related Proteins

1991

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Public Health Service
National Institutes of Health

NIH Publication No. 91-3242

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SIGNAL PEPTIDES OF HUMAN KAPPA LIGHT CHAINS

	INVARIANT RESIDUES	1 HK101 'CL	2 HK 137 'CL	3 VKI- Chr1 'CL	4 HuVH CAMP 'CL	5 VKI-2I 'CL	6 3D6 'CL	7 V108 'CL	8 Vb 'CL	9 Vb' 'CL	10 HK102 'CL	11 2A12 'CL	12 1H1 'CL	13 2C12 'CL	14 1B11 'CL	15 Vd 'CL	16 Va' 'CL	17 Ve 'CL	18 V13 'CL	19 GM 607 'CL	20 A3 'CL	21 RPMI 6410 'CL #	22 A2 'CL	23 A23 'CL
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-20	MET(.98)	MET	MET	MET	---	VAL	MET	MET	MET	MET	MET	MET				MET	NET	MET	MET		MET	MET	MET	MET
-19		ARG	ARG	ARG	MET	ARG	ARG	ARG	ARG	MET	ARG	ARG				ARG	ARG	ARG	ARG		ARG	ARG	ARG	ARG
-18		VAL	VAL	ALA	GLY	ALA	VAL	VAL	VAL	VAL	VAL	VAL				VAL	VAL	VAL	VAL		LEU	LEU	LEU	LEU
-17		LEU	LEU	PRO	TRP	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO			PRO	PRO	PRO	PRO		PRO	PRO	PRO	LEU
-16		ALA	ALA	THR	SER	THR	ALA	ALA	ALA	ALA	ALA	ALA	ALA			ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA
-15		GLN	GLN	GLN	CYS	GLN	GLN	GLN	GLN	GLN	GLN	LEU	LEU			GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN
-14		LEU	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-13		LEU	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-12		GLY	GLY	GLY	LEU	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY			GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY
-11		LEU	LEU	LEU	PHE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-9		LEU	LEU	VAL	VAL	GLY	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		MET	MET	MET	MET
-8	LEU(.95)	LEU	LEU	LEU	ALA	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-7	TRP(.95)	CYS	CYS	TRP	THR	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP
-6		PHE	PHE	LEU	ALA	LEU	LEU	LEU	PHE	PHE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		VAL	VAL	ILE	VAL
-5		PRO	PRO	PRO	THR	PRO	PRO	ARG	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		SER	PRO	PRO	PRO
-4		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
-3		ALA	ALA	ALA	VAL	ALA	ALA	ALA	SER	SER	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	SER	SER	SER	SER	SER
-2		ARG	ARG	ARG	HIS	ARG	LYS	ARG	ARG	ARG	LYS	LYS	LYS	LYS	LYS	ARG	ARG	ARG	ARG	SER	SER	SER	SER	SER
-1		CYS	CYS	CYS	SER	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	GLY	GLY	GLY	ALA	GLY

	24 NG9 'CL (R) 'CL #	25 HIC 14.1 'CL #	26 HAH 14.2 'CL #	27 HAH 16.1 'CL #	28 HAH 'CL #	29 NOV 'CL #	30 BE10 'CL #	31 TH3 'CL #	32 Humkv 325 'CL	33 EV1-15 'CL	34 IARC/ BL41 'CL	35 Humkv 305 'CL	36 LS1 'CL	37 LS2 'CL	38 LS4 'CL	39 LS5 'CL	40 LS6 'CL	41 LS7 'CL	42 LS8 'CL	43 Vg 'CL	44 Vh 'CL	45 CLL 'CL #	46 Hum kv328 'CL #	47 Hum ka31es 'CL #
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-19	GLU	GLU	GLU	GLU	GLU				GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU		
-18	THR	THR	THR	THR	THR				THR	THR	THR	THR	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		
-17	PRO	PRO	PRO	PRO	PRO				PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		
-16	ALA	ALA	ALA	ALA	ALA				ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		
-15	GLN	GLN	GLN	GLN	GLN				GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		
-14	LEU	LEU	LEU	LEU	LEU				LEU	LEU	LEU	IEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-13	LEU	LEU	LEU	LEU	LEU				LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-12	PHE	PHE	PHE	PHE	PHE				PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE		
-11	LEU	LEU	LEU	LEU	LEU				LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-9	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-8	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-7	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		
-6	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LSU	LEU	LEU	LEU	LEU	LEU	LEU		
-5	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		
-4	VAL	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
-3	PRO	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
-2	SER	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
-1	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ARG	GLY	GLY	GLY

	48 GF4 /1.1 'CL	49 K- EV15 'CL	50 VKAPPA IV GERMLINE 'CL	51 PB17IV 'CL	52 FK-001 'CL #	53 WALKER 'CL	54 DAUDI 'CL	55 VJ1 'CL	56 HK100 'CL	57 Va 'CL	58 V2 'CL	59 V14 'CL	60 V52 'CL	61 V55 'CL	62 K- EVJK11 'CL	63 PSEUDO VKAPPA 24'CL	# OF SEQUENCES	# OF AMINO ACIDS
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-19	GLU	GLY	VAL	VAL	VAL	ARG	ARG	VAL	ARG	ARG	GLN	GLN	ARG		GLU	ARG	53	6
-18	ALA	SER	LEU	LEU	LEU	VAL	VAL	LEU	VAL	VAL	VAL	VAL	ALA		THR	CYS	53	7
-17	PRO	GLN	GLN	GLN	GLN	PRO	PRO	GLN	PRO	PRO	PRO	PRO	PRO		PRO	SER	54	5
-16	ALA	VAL	THR	THR	THR	ALA	ALA	THR	ALA	ALA	THR	THR	ALA		ALA	PRO	54	5
-15	HIS	HIS	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	HIS	54	4
-14	VAL	LEU	VAL	VAL	VAL	LEU	LEU	VAL	LEU	LEU	LEU	LEU	LEU		LEU	PHE	54	4
-13	LEU	LEU	PHE	PHE	PHE	LEU	LEU	PHE	LEU	LEU	LEU	LEU	LEU		LEU	LEU	54	3
-12	PHE	SER	ILE	ILE	ILE	GLY	GLY	ILE	GLY	GLY	GLY	GLY	GLY	GLY	PHE	GLU	55	6
-11	LEU	PHE	SER	SER	SER	LEU	LEU	SER	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	55	3
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	58	1
-9	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	VAL	VAL	LEU	PRO	LEU	VAL	58	5
-8	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	SER	LEU	PHE	58	4
-7	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	59	3
-6	LEU	ILE	ILE	ILE	ILE	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	PHE	LEU	ILE	59	5
-5	PRO	SER	SER	SER	SER	ARG	ARG	SER	SER	PRO	PRO	PRO	PRO	PRO	PRO	LEU	59	5
-4	GLU	ASP	GLY	GLY	GLY	GLY	ARG	GLY	GLY	GLY	GLY	GLY	ASP	GLY	ASP	GLU	63	5
-3	THR	THR	ALA	ALA	ALA	ALA	VAL	ALA	ALA	ALA	ALA	ALA	THR	THR	THR	VAL	63	5
-2	SER	ARG	TYR	TYR	TYR	ARG	ARG	TYR	ARG	ARG	ARG	ARG	ARG	ARG	THR	SER	63	6
-1	GLY	ALA	GLY	GLY	GLY	CYS	CYS	GLY	TYR	CYS	CYS	CYS	CYS	SER	GLY	GLY	63	6

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I

	INVARIANT RESIDUES	1 2C12 'CL #	2 1B11 'CL #	3 1H1 'CL #	4 2A12 'CL #	5 BR	6 DEN	7* 3D6 'CL	8 HK102 'CL #	9 EU	10 CAR	11 PA	12 HK 137 'CL #	13 RAU	14 KUE	15* HF2-1/17	16 Vb 'CL	17 Vb' 'CL	18 GAL (I)	19 CU (IOC)	20* WEA	21 WIL (=) #	22 WES	23 DAUDI 'CL	24 HK 134 'CL #
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1		D ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
2	ILE(.98)	I ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
3		Q GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
4		M MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET
5	THR(.98)	T THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
6		G GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
7	SER(.99)	S SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
8	PRO(.99)	P PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
9	SER(.97)	S SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
10		T THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	ser	THR	ser	ser	ser	ser	ser	ser	THR	ser	ser	ser
11		L LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	gln	LEU	val	val	LEU	LEU	LEU	LEU	val	LEU	LEU
12	SER(.97)	S SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	pro	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
13		A ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
14		S SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
15	VAL(.96)	V VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
16	GLY(.99)	G GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
17		D ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP
18		S SER	SER	SER	SER	thr	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg
19		I ILE	ILE	ILE	ILE	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
20	THR(.96)	T THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
21	ILE(.95)	I ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
22		T THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR	THR	THR	THR	THR	THR
23	CYS	C CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
24		R ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
25		A ALA	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	pro	ALA	ALA
26		S SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	gly	SER
27		Q GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	his	GLN
27A		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27B		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27C		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27D		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27E		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27F		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
28		S SER	SER	SER	SER	SER	SER	SER	SER	SER	asn	SER	gly	SER	SER	gly	gly	gly	gly	thr	gly	ala	asp	asn	gly
29		I ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	phe	ILE	ILE	ILE
30		G GLY	GLY	GLY	GLY	ser	ser	ser	ser	asn	ser	ser	ser	ser	asn	arg	ser	ser	arg	ser	arg	GLY	ser	thr	ser
31		N ASN	ASN	ASN	ASN	gly	arg	arg	ser	thr	ser	ser	ASN	ser	ile	ASN	ser	ser	ASN	ser	ASN	ser	his	ASN	ser
32		W TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	ser	tyr	TRP	asp	TRP	TRP	asp	tyr	asp	TRP	TRP	phe	TRP
33	LEU(.95)	L LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
34		A ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	gly	ALA	ALA	thr	asx	thr	ALA	ALA	ser	ALA
35	TRP		TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP
36			PHE	PHE	PHE	PHE	PHE	tyr	tyr	tyr	tyr	tyr	PHE	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr
37			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
38			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
39	PRO(.97)		LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS
40			PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ser	PRO	PRO
41			GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu
42			LYS	LYS	LYS	LYS	LYS	glu	LYS	LYS	LYS	LYS	glu	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	thr	LYS	LYS	LYS
43			ALA	ALA	ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
44	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
45			ASN	ASN	ASN	ASN	lys	lys	lys	lys	lys	gln	lys	lys	lys	lys	lys	lys	lys	ASX	lys	glu	lys	thr	lys
46			VAL	VAL	VAL	VAL	leu	VAL	leu	leu	leu	VAL	leu	ser	VAL	leu	arg	leu	glu	leu	arg	leu	leu	leu	ser
47	LEU(.98)		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
48	ILE(.98)		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	met	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
49			TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
50			LYS	LYS	LYS	LYS	LYS	gly	LYS	asp	LYS	LYS	glu	ala	ala	LYS	ala	ala	ala	ala	gly	arg	ser	ala	ala
51			ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
52			SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	SER	SER	SER
53			ASN	ASN	ASN	ASN	thr	ASN	ser	ser	ser	ser	ser	ser	thr	ser	ser	ser	ser	ASX	ser	ser	ser	ASN	ser
54	LEU(.97)		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
55			LYS	LYS	LYS	LYS	glu	glu	glu	glu	glu	glu	glu	glu	pro	glu	gln	gln	gln	his	gln	gln	glu	gln	gln
56			ASN	ASN	ASN	lys	ser	ser	ser	ser	ser	ser	ser	ser	thr	ser	ser	ser	ser	ser	ser	ser	ser	ASN	ser
57	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
58			ILE	ILE	ILE	ILE	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
59	PRO(.95)		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
60	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
61	ARG(.97)		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
62	PHE(.96)		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
63			SER	SER	SER	SER	SER	SER	SER	SER	SER	ile	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
64	GLY(.96)		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
65			SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
66	GLY(.95)		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
67			SER	SER	SER	SER	gha	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	ala	SER	SER	SER	SER	SER
68	GLY(.95)		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
69			THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR
70			GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp	GLU	asp	asp	gln	GLU	asp	asp	GLU	asx	GLU	GLU	GLU	asp
71			PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
72			THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
73			LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	phe	LEU	LEU	LEU	LEU	LEU
74			THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
75			VAL	VAL	VAL	VAL	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile
76			ILE	ILE	ILE	ILE	asn	ser	ser	ser	ser	ser	ser	ser	ser	asn	ser	ser	asr	ser	ser	asn	ser	ser	ser
77			ASN	ASN	ASN	ASN	ser	ser	ser	asn	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	gly	ser	ser	ser	ser
78	LEU(.98)		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
79			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
80			SER	SER	SER	SER	pro	SER	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
81			ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	glu	glu	ASP	glu	glu	glu	glu	glx	glu	ASP	glu	glu
82			ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
83			PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	val	PHE	PHE
84	ALA(.98)		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
85			THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
86	TYR(.98)		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
87	TYR(.95)		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR
88	CYS		CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
89			HIS	HIS	HIS	HIS	gln	gln	gln	gln	gln	gln	gln	gln	gln	leu	gln	gln	leu	glx	leu	gln	gln	gln	gln
90			HIS	HIS	HIS	HIS	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	gln	gln	gln
91			ASN	ASN	ASN	ASN	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	ASN	tyr	his	ala	ala	gln	ser	tyr	ala	ala	ASN	tyr
92			ASP	ASP	ASP	ASP	asn	ASP	ASP	asn	asn	asn	asn	tyr	ser	asn	asn	asn	asn	tyr	ser	asn	asn	his	tyr	asn
93			SER	SER	thr	SER	SER	SER	SER	SER	SER	thr	asn	SER	ile	arg	SER	SER	SER	SER	SER	arg	SER	asn	SER
94			PHE	PHE	PHE	ile	PHE	PHE	tyr	tyr	asp	PHE	leu	tyr	thr	tyr	tyr	PHE	PHE	tyr	ser	PHE	tyr	val	PHE	tyr
95			SER	SER	SER	SER	pro	pro	SER	SER	---	---	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	---	pro	SER	pro
95A			---	---	---	---	---	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	#
95B			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95C			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95D			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95E			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95F			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
96			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
97			THR	THR	THR	THR	TRP	TYR	---	---	LYS	PHE	TRP	---	THR	TYR	LEU	---	---	ARG	THR	TRP	---	LEU	PHE	---
98	PHE(.96)		PHE	PHE	PHE	PHE	PHE	PHE	PHE	---	PHE	PHE	PHE	---	PHE	PHE	PHE	---	---	PHE	PHE	PHE	PHE	PHE	PHE	---
99	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY	GLY	GLY	GLY	GLY	---
100			GLN	GLN	GLN	GLN	GLN	GLN	PRO	---	GLN	PRO	PRO	---	GLN	GLN	GLY	---	---	GLN	GLX	GLN	GLN	GLY	GLY	---
101	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY	GLY	GLY	GLY	GLY	---
102	THR(.98)		THR	THR	THR	THR	THR	THR	THR	---	THR	THR	THR	---	THR	THR	THR	---	---	THR	THR	THR	THR	THR	THR	---
103			LYS	LYS	LYS	LYS	LYS	LYS	LYS	---	LYS	LYS	LYS	---	ARG	LYS	LYS	---	---	LYS	ARG	LYS	LYS	THR	LYS	---
104			VAL	VAL	VAL	VAL	VAL	LEU	VAL	---	VAL	VAL	VAL	---	VAL	LEU	VAL	---	---	VAL	LEU	VAL	LEU	VAL	VAL	---
105			LEU	LEU	LEU	LEU	GLU	GLU	ASP	---	GLU	ASP	GLU	---	GLU	ASP	GLU	---	---	GLU	GLX	GLU	GLU	ASP	ASP	---
106			ILE	ILE	ILE	ILE	ILE	ILE	---	---	VAL	ILE	ILE	---	ILE	ILE	---	---	---	ILE	---	VAL	ILE	ILE	ASN	---
106A			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
107			LYS	LYS	LYS	LYS	LYS	LYS	LYS	---	LYS	LYS	LYS	---	LYS	LYS	LYS	---	---	LYS	LYS	LYS	LYS	LYS	LYS	---
108			ARG	ARG	ARG	ARG		ARG		---	GLY	ARG		---	ARG	ARG	ARG	---	---	ARG	ARG	ARG	ARG	ARG	---	---
109	THR							THR		---	THR	THR		---	THR	THR		---	---	THR	THR	THR	THR		---	---

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		25 Vd 'CL	26 WALKER 'CL	27 HK101 'CL #	28 Va' 'CL	29 VKI-Che1 'CL	30 AMYLOID BAN #	31 Ve 'CL	32 V13 'CL	33 DEE	34 WIL (-) #	35 AND #	36 V108 'CL #	37 VKI-ZI 'CL	38 REI	39 AU	40 ROY	41 BI	42* HuVH CAMP 'CL	43 AG #	44 SCH	45 MEV	46 KA	47* LAY	48 BEL	49 NI
	0	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ala	ASP	ASP	ala	val	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	arg	trp	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	4	leu	MET	MET	leu	MET	leu	MET	MET	MET	MET	MET	val	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	leu	MET
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
FR1	10	phe	ser	ser	ser	ser	ser	ser	leu	sec	THR	ser	ser	ser	ser	ser	pro	ser	ser	ser	ser	THR	sec	ser	ser
	11	LEU	LEU	LEU	LEU	LEU	phe	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val	val	ALA	ALA
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr
	15	VAL	VAL	VAL	VAL	VAL	VAL	thr	thr	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	gly	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	18	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	SER	arg	arg	arg	arg	arg	arg	arg	arg
	19	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	phe	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	leu
	22	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	leu
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	24	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	gln	gln	gln	gln	lys	gln	gln	ARG	glu	gln	glu
	25	ALA	ALA	ALA	ALA	ALA	ALA	ALA	met	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	26	SER	SER	arg	SER	SER	SER	SER	SER	gly	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	27	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN
	27A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	ser
	27B	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	val
CDR1	27C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	leu
	27D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	glu
	27E	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27F	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	28	gly	SER	gly	gly	gly	SER	gly	gly	SER	SER	SER	gly	gly	asp	asp	asp	asp	agn	asp	asp	SER	thr	asn	asx	SER
	29	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	val	val	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ser	val	val	ILE	gly
	30	ser	ser	ser	asn	ASP	tyr	ser	ser	ser	ser	GLY	ser	ser	asp	ser	ser	ser	asp	asn	asp	val	leu	asn	ser	asn
	31	asr	ASN	ser	ser	ASN	ser	ser	ser	lys	tyr	gly	ASN	ASN	lys	asp	ile	ASN	lys	his	lys	asp	ser	ala	lys	thr
	32	tyr	tyr	TRP	ala	asn	tyr	tyr	tyr	tyr	TRP	ser	gly	asn	tyr	tyr	phe	ser	tyr	tyr	his	tyr	tyr	tyr		phe
	33	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU
	34	ALA	asn	ALA	ALA	asn	ALA	ALA	ALA	asn	ALA	asn	ser	asn	asn	asn	asn	ile	asn	asn	asn	asn	asn	asn		ALA
	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP
	36	tyr	tyr	tyr	tyr	tyr	PHE	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr		tyr
	37	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	asp	GLN	GLN	GLN		GLN
	38	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN
	39	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	thr	LYS	LYS	LYS	LYS	§	LYS	LYS	LYS	LYS		LYS
	40	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO
	41	GLY	GLY	GLY	glu	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	§	GLY	GLY	GLY	GLY	GLY	lys
FR2	42	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	gln	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	leu	LYS	LYS
	43	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	pro	ALA	
	44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	
	45	lys	lys	lys	lys	lys	lys	lys	glu	lys	ASN	ser	thr	lys	lys	lys	lys	lys	lys	arg	lys	lys	glu	lys	lys	
	46	leu	leu	leu	ser	leu	ser	leu	leu	VAL	leu	leu	leu	leu	leu	leu	phe	leu	leu	leu	leu	leu	leu	leu	leu	
	47	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	
	48	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	
	49	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR	phe	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR	TYR
	50	ala	ala	ala	asp	ala	asp	ala	ala	ala	gln	gly	ala	ala	glu	asp	asp	asp	asn	asp	gly	asp	ala	gly	asp	asp
	51	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
CDR2	52	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	glu	asn	SER	SER	SER	SER	SER	SER	SER
	53	thr	ser	ser	ser	ser	thr	thr	thr	ser	ile	ser	ser	ser	ASN	ASN	lys	ASN	ASN	ASN	thr	ASN	ser	thr	thr	ASN
	54	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	arg	LEU	LEU
	55	gln	gln	gln	glu	gln	gln	gln	gln	LYS	asp	arg	gln	gln	gln	glu	glu	glu	gln	glu	glu	gln	glu	LYS	glu	glu
	56	ser	ser	ser	ser	ser	ser	ser	ser	ser	ala	ser	ser	ser	ala	ser	ala	ile	thr	thr	ser	thr	ala	thr	thr
	57	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	58	val	val	val	val	ILE	val	val	val	val	val	val	val	ILE	val	val	val	val	val	val	val	val	val	val	val	val
	59	PRO	thr	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	60	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	61	ARG	ARG	ARG	ARG	ARG	asn	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	62	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
	63	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	arg	SER	SER	SER	SER	SER	SER	SER	SER
	64	GLY	GLY	GLY	GLY	asp	GLY	GLY	GLY	GLY	GLY	GLY	GLY	asp	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	65	SER	SER	SER	SER	SER	SER	SER	SER	SER	asn	SER	SER	SER	SER	gly	thr	SER	SER	SER	gly	gln	SER	SER	SER	SER
	66	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ile	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	arg	GLY	GLY	GLY	GLY	GLY
	67	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	phe	SER	SER	SER	SER	SER	SER	SER
	68	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu	GLY	GLY
	69	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	ala	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	70	GLU	asp	ssp	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	his	asp	asp	asp	asp	asp	asp	asx	asp	his	asp
	71	PHE	PHE	PHE	PHE	tyr	PHE	PHE	PHE	PHE	PHE	PHE	PHE	tyr	tyr	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
FR3	72	THR	THR	THR	THR	ile	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR
	73	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	phe	phe	phe	LEU	LEU	LEU	LEU	LEU	phe	phe	LEU	phe
	74	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR
	75	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile
	76	ser	ser	ser	ser	arg	ser	ser	ser	ser	ser	thr	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser		ser	
	77	ser	ser	ser	ser	ser	cys	cys	gly	ser	ser	ser	ser	ser	ser	ser	gly	thr	ser	ser	gly	thr	ser	ser	ser	gly
	78	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU	LEU
	79	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	leu	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLX
	80	pro	pro	pro	pro	pro	pro	SER	SER	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
	81	glu	glu	glu	glu	glu	glu	glu	glu	glu	ASP	glu	glu	glu	glu	glu	glu	glu	glu	glu	ASP	glx	glu	ala	glx	
	82	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	asn	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASX	
	83	PHE	ser	PHE	PHE	PHE	PHE	PHE	PHE	PHE	ser	PHE	val	PHE	ile	ile	ile	PHE	ile	ile	ile	PHE	PHE	ile	PHE	PHE
	84	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	THR	ALA	gly	ALA	ALA	ALA	ALA	ALA
	85	THR	THR	THR	THR	THR	THR	THR	THR	THR	arg	ala	THR	ala	THR	THR	THR	THR	THR	ASN	THR	THR	THR	THR	THR	val
	86	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYA	TYR	TYR	TYR
	87	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
	88	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	89	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	leu	gln	gln	gln	gln	gln	leu	gln	gln	gln	gln	gln	gln	gln	
	90	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	glx	
	91	leu	ser	tyr	phe	ser	tyr	tyr	tyr	ser	tyr	ser	asp	ser	tyr	tyr	phe	tyr	his	tyr	tyr	ser	tyr	tyr	tyr	tyr
	92	ASP	tyr	asn	ASP	asn	tyr	tyr	tyr	asn	tyr	tyr	ASP	ASP	ile	ASP	ASP	tyr	ile	ASP	ASP	tyr	leu	asn	ASX	ASP
	93	SER	SER	SER	SER	SER	SER	SER	SER	thr	arg	SER	thr	SER	SER	tyr	asn	asn	SER	thr	asn	thr	asp	asn	his	thr
	94	tyr	thr	tyr	tyr	thr	tyr	tyr	PHE	thr	tyr	ser	thr	thr	leu	leu	leu	leu	arg	leu	val	asn	leu	trp	PHE	leu
CDR3	95	pro	leu	pro	pro	pro	pro	pro	pro	pro	---	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
	95A	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	GLU	---	---	---	---	---
	95B	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95E	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95F	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	96	---	ILE	---	---	---	TYR	---	---	TYR	---	TRP		---	TYR	TRP	LEU	TYR	ARG	ARG	ILE	VAL	ARG	PRO	LEU	SER
	97	---	THR	---	---	---	THR	---	---	THR	---	THR		---	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	98	---	PHE	---	---		PHE	---	---	PHE	PHE	PHE		---	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
	99	---	GLY	---	---		GLY	---	---	GLY	GLY	GLY		---	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	100	---	GLN	---	---		GLN	---	---	PRO	GLN	GLN		---	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	VAL
	101	---	GLY	---	---		GLY	---	---	GLY	GLY	GLY		---	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	102	---	THR	---	---		THR	---	---	THR	THR	THR		---	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	SER
FR4	103	---	ARG	---	---		LYS	---	---	LYS	LYS	LYS		---	LYS	LYS	LYS	LYS	LYS	LYS	ARG	THR	LYS	LYS	GLU	LYS
	104	---	LEU	---	---		#	---	---	VAL	LEU	VAL		---	LEU	VAL	VAL	LEU	VAL	LEU	VAL	VAL	VAL	VAL	VAL	VAL
	105	---	GLU	---	---		#	---	---	GLU	GLU	GLU		---	GLN	GLU	ASP	GLU	GLU	GLU	ASP	ASP	GLU	GLU	GLU	GLU
	106	---	ILE	---	---		ILE	---	---	MET	ILE	ILE		---	ILE	ILE	PHE	ILE	ILE	ILE	ASN	ILE	LEU	VAL	VAL	SER
	106A	---	---	---	---		---	---	---	---	---	---		---	---	---	---	---	---	---	---	---	---	---	---	---
	107	---	LYS	---	---		LYS	---	---	THR	LYS	ARG		---	THR	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS
	108	---	---	---	---		ARG	---	---	ARG	ARG			---	ARG	ARG	ARG	ARG	ARG	ARG	GLY	ARG	ARG	ARG		ARG
	109	---	---	---	---			---	---	THR	THR			---	THR	THR	THR	THR		THR	THR		THR			THR

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

	50 RZ	51* Ni RSV19 VK #	52 HBJ 4	53 26 #	54 BJ 19 #	55 RFZ	56 HA	57 PAU	58 PSM	59 JBL #	60 ME	61 FRA	62 BJ 48	63 HEJ- 16/6	64 KFZ- 1/13B	65 HFZ- 18/2	66 GR'	67 G1 'CL #	68 HOM	69 KER	70 ESH IGG	71 ESH IGH	72* DAV	73* FIN	74 PW #	75 SHE	76 ADA
0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN
4	MET	leu	MET	MET	leu	MET	MET	MET	MET	MET	leu	MET	MET	MET	MET	MET	MET		MET	MET	MET	MET	MET	MET	leu	val	
5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	THR	THR	THR	THR
6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER
8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	thr	SER	
10	ser	ser	THR	ser	ser	ser	ser	THR	ser	ser	ser	THR	ser	ser	ser	ser	THR		ser	ser	ser	ser	ser	ser	ser	ser	ser
11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER
13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA	thr	thr	ALA	ALA	val
14	SER	SER	SER	SER	SER	SER	SER	SER	SER	tyr	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	val	val	SER	SER	pha
15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP
18	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg		arg	arg	arg	arg	arg	arg	arg	arg	arg
19	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val		val	ILE	val	val	val	val	val	val	val
20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	THR	THR	THR	THR
21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	THR	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	val	phe	ILE
22	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	THR	arg	THR	THR
23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
24	gln	ARG	ARG	gln	gln	gln	gln	ARG	gln	ARG	glx	ARG		ARG	ARG	ARG	ARG		gln	gln	ARG	ARG	asp	asp	ARG	glx	glx
25	ALA	ser	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
26	asn	SER	SER	SER	SER	asn	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER		asp	SER	SER	SER	SER	SER	SER	SER	SER
27	GLN	GLN	GLN	GLN	GLN	GLN	---	GLN	GLN	GLN	GLX	GLX		GLN	GLN	GLN	arg		his	GLN	GLX	GLX	GLN	GLN	GLX	GLX	GLX
27A	---	thr	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
27B	---	leu	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
27C	---	val	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
27D	---	his	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
27E	---	thr	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
27F	---	---	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
28	val	asp	SER	SER	asp	val	---	SER	asp	SER	asx	asx		gly	gly	gly	SER		asp	asp	SER	SER	asx	asx	SER	asx	asx
29	ILE	gly	val	ILE	ILE	ILE	---	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
30	ser	asn	asn	asn	asn	ser	ser	arg	ser	arg	ser	asn		arg	arg	arg	ser		ser	lys	ser	ser	asx	asx	GLY	ser	
31	lys	thr	ASN	lys	thr	lys	ASN	ser	ser	thr	ASX	ASX		ASN	ASN	ASN	ser		ASN		ser	ser	ser	ser		ASX	ASX
32	ser	tyr	TRP	tyr	phe	ser	tyr	ser	tyr	phe	tyr	tyr		asp	asp	asp	TRP		tyr		tyr	tyr	TRP	TRP	tyr	tyr	tyr
33	LEU	LEU	LEU	ala	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU		LEU		LEU	LEU	LEU	LEU	ser	LEU	LEU
34	asn	glu	ALA	asn	asn	asn	asn	ALA	asn	#	asx	ALA		gly	gly	gly	ALA		asn		asx	asx	ile	ile	asx	asx	asx
35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP		TRP		TRP	TRP	TRP	TRP	TRP	TRP	TRP
36	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr		tyr	tyr	tyr	tyr		PHE		tyr	tyr	tyr	tyr		tyr	tyr
37	GLN	GLN	GLN	glu	GLN	GLN	GLN	GLX	GLN	GLN	GLX	GLX		GLN	GLN	GLN	GLN		GLN		GLX	GLX	GLN	GLN	GLN	his	GLX
38	GLN	GLN	glu	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLX	GLX		GLN	GLN	GLN	gly		GLN				GLN	GLN	GLN	GLX	tyr
39	arg	LYS	LYS	#	#	arg	LYS	LYS	LYS	LYS	LYS	LYS		LYS	LYS	LYS	arg		LYS				tyr	tyr	LYS	LYS	LYS
40	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	gln	PRO	PRO	PRO		PRO	PRO	PRO	PRO		PRO				PRO	PRO		PRO	PRO
41	GLY	GLY	GLY	#	#	GLY	GLY	GLY	GLY	GLY	GLY								GLY								
42	gln	LYS	glx	LYS	LYS	gln	arg	glx	LYS	LYS	glx																
43	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA														
44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO														
45	lys	lys	lys	lys	lys	lys	lys	lys	lys	lys	lys		lys														
46	leu	leu	leu	leu	leu	leu	leu	leu	leu	leu	leu																
47	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	met																
48	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE		ILE																
49	TYR	TYR	ser		TYR	TYR	TYR	TYR	TYR	TYR	TYR		gly														
50	asp	arg	LYS		asp	asp	asp	glx	asp				asp														
51	ALA	val	thr	asp	ALA	ALA	ALA		ALA				ALA														
52	SER	SER	SER	SER	SER	ala	phe		SER				SER														
53	ASN	ser	arg	ser	ASX	ASN							ser														
54	LEU	arg	LEU	LEU	LEU	LEU	LEU		LEU				LEU														
55	glu	phe	glu	glu	glu	glu	glu		glu				asx														
56	ser	ser	arg	thr	thr		ala						ser														
57	GLY	GLY	GLY	GLY	GLY	GLY	GLY						GLY														
58	val	val	val	asx	val	val	val						val					ILE									
59	PRO	PRO	PRO	PRO	glx	PRO	PRO						val					PRO									
60	SER	SER	SER	SER	SER		SER						SER					SER									
61	ARG	ARG	ARG	lys	ARG	ARG	ARG						ARG					ARG									
62	PHE	PHE	PHE	val	PHE	PHE							gln					PHE									
63	thr	SER	ala	SER	leu	thr							SER					SER									
64	GLY	GLY	GLY	GLY	GLY	GLY							GLY					GLY									
65	SER	SER	SER	SER	SER								SER					SER									
66	GLY	GLY	GLY	GLY	asx	GLY							GLY					GLY									
67	SER	SER		SER	phe								SER					SER									
68	GLY	GLY		glu	asx								GLY					GLY									
69	THR	THR		THR	THR								THR					THR									
70	asp	asp		asp	lys								asp					GLU									
71	PHE	PHE		val									pro					PHE									
72	phe	THR		THR									ser					THR									
73	LEU	pha		val									LEU					LEU									
74	THR	THR		asx									ala					THR									
75	ile	ile		glx									val					VAL									
76	ser	ser		ser									ser					ILE									
77	ser	ser		ser									arg					ASN									
78	LEU	LEU		LEU														LEU									
79	arg	GLN		GLN														GLN									
80	pro	pro		pro														SER									
81	glu	glu		glu														ASP									
82	ASP	ASP		ASP														ASP									
83	val	ile		ile														PHE									
84	ALA	ALA		ALA														ALA									
85	THR	THR		pro														THR									
86	TYR	TYR		lys														TYR		TYR							
87	phe	TYR																TYR		TYR							
88	CYS	CYS																CYS		CYS							
89	gln	phe		gln														HIS		gln							
90	gln	gln		gln														HIS		gln							
91	tyr	gly		arg	phe								gly					ASN		tyr							
92	his	ser		ASP	ASP								glu					ASP		ASP							
93	asn	his		asp	asp								SER					thr		asp							
94	leu	leu		leu	leu								asp					PHE		leu							
95	pro	pro		pro	pro								SER					SER		pro							
95A	---	---		---	---								---					---		---							
95B	---	---		---	---								---					---		---							
95C	---	---		---	---								---					---		---							
95D	---	---		---	---								---					---		---							
95E	---	---		---	---								---					---		---							
95F	---	---		---	---								---					---		---							
96	LEU	ARG		TYR	TYR								ARG					TRP		PRO							
97	THR	THR		THR	THR								THR					THR		THR							
98	PHE	PHE		LEU	PHE								PHE					PHE		PHE							
99	GLY	GLY		GLY	GLY								GLY					GLY		GLY							
100	GLY	GLY		GLY	PRO								GLN					GLN		PRO							
101	GLY	GLY		GLY	GLY								GLY					GLY		GLY							
102	THR	THR		THR	THR								THR					TER		THR							
103	LYS	LYS		LYS	LYS								LYS					LYS		LYS							
104	VAL	VAL		VAL	VAL								VAL					VAL		VAL							
105	GLU	GLU		GLU	GLU								GLU					LEU		ASP							
106	ILE	ILE		MET	LEU								VAL					ILE		LEU							
106A	---			---	---								---					---		---							
107	LYS			LYS	LYS								LYS					LYS		LYS							
108																		ARG									
109																											

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		77 AMYLOID VIII-B	78 PAUL	79 RI #	80 NAT	81* LOW	82 AMYLOID ES305 #	83 DCC	84 BOL	85 AMYLOID K	86 SAC	87 BJ	88 GO	89 F-. GUI #	90 S- GUI #	91 HF6- 21/28	92 DIE	93 CAR A	94 TEI	95 MON	96 ALE	97 JOH	98 POT	99 CON	100 TRA	101* H-G2b
	0	---	---	pca	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		val	ILE	ILE	val	ILE	ILE	ILE	ILE	GLX	ILE	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN		GLN	GLN	GLN	val	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN
	4	MET	MET	MET	MET	MET	leu	MET	ile	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET
	5	THR	THR	THR	THR	THR	THR	THR	met	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLX	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	ala	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ala	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	leu	SER	SER	SER	SER	SER	ala	SER	SER	SER
FR1	10	ser	THR	THR	ser	ser	ser	ser	ser	ser	ser		phe	ser	ser	ser	ser	ser	ser	THR		ser	ser	ser	ser
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU		val	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	val	SER	SER	SER		SER	SER	SER	pro	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	ALA		ALA	ALA	ALA	val	ALA	ALA	ALA	SER	ALA	ALA	ALA	ALA	ALA
	14	SER	SER	SER	SER	SER	SER	pro	SER	SER	SER		SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER
	15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASX	ASP	ASP		ASX	ASX	ASX	gly	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	18	arg	arg	gly	arg	arg		leu	arg	arg	---	arg	arg	arg	arg	pro	arg	arg	arg	arg	arg	arg	arg	arg	arg
	19	val	val	val	val	val	val	val	val	val	---	val	val	val	val	ala	val	val	val	val	leu	ile	val	val	val
	20	THR	THR	THR	THR	THR	THR	THR	THR	ile	---	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	#	ILE	ILE	ILE	---	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	leu	ILE	ILE	ILE	ILE
	22	THR	THR	ser	THR	THR	THR	THR	THR		---	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	ser	THR	THR	THR
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	---	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS	CYS		
	24	gln	ARG	ARG	glx	ala	gln	glx	ARG	glx	---	gln	ARG		ARG	ARG					ARG				
	25	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	---	ALA	ALA	ALA	ALA	ser									
	26	SER	SER	SER	SER	SER	SER	SER	SER		---	SER	SER			SER									
	27	GLN	GLN	GLX	GLN	GLN	glu	GLX	GLX	GLX	---	GLN				GLN									
	27A	---	---	---	---	---	---	---	---	---	---	---				ser									
	27B	---	---	---	---	---	---	---	---	---	---	---				leu									
CDR1	27C	---	---	---	---	---	---	---	---	---	---	---				val									
	27D	---	---	---	---	---	---	---	---	---	---	---				trp									
	27E	---	---	---	---	---	---	---	---	---	---	---				ser									
	27F	---	---	---	---	---	---	---	---	---	---	---				---									
	28	asx	SER	SER	asp	asx	ala	asx	gly	asx	---	asp				asp									
	29	ILE	ILE	ILE	ILE	ILE	#	ILE	ILE	ILE	---	ILE				gly									
	30	GLY	ser	ser	ser	ser		ser			---	asn				asx									
	31		ser		asp		ASN	ASX		pro	---	lys				ser									
	32	tyr	ser		tyr		tyr	tyr		tyr	---					tyr									
	33	LEU			val		LEU			LEU	---					LEU									
	34				asn						---					glu									
	35	TRP			TRP		TRP				---					TRP									
	36						tyr			tyr	---					tyr									
	37										---					GLN									
	38										---					GLN									
	39										---					LYS									
	40										---					PRO									
	41										---														
	42										---														
FR2	43										---														
	44										---														
	45										---														
	46										---														
	47										---														
	48										---														
	49										---														
	50										LYS														
	51										ser														
	52										---														
CDR2	53										---														
	54										---														
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FR3	73										---														
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	86										---	TYR													
	87										---	TYR													
	88										CYS	CYS													
	89										glx	gln													
	90										glx	gln													
	91										glx	tyr													
	92										ASX	glu													
	93										ala	asn													
CDR3	94										ala	leu													
	95										thr	pro													
	95A										---	---													
	95B										---	---													
	95C										---	---													
	95D										---	---													
	95E										---	---													
	95F										---	---													
	96										ILE	TYR													
	97										PRO														
	98										ILE														
	99										GLY														
	100										GLY														
	101										GLY														
	102										THR														
FR4	103										LYS														
	104										VAL														
	105										ASX														
	106										VAL														
	106A										---														
	107										LYS														
	108										ARG														
	109										THR														

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HUMAN KAPPA LIGET CHAINS SUBGROUP I (cont'd)

		102* K-G2	103 BRA	104 LUX	105* HEI	106 PAP	107* H-G2a	108 CRA	109 AMYLOID LEP	110* WAG	111 MOE	112 HBJ 1	113 AMYLOID 547	114 WEB	115 HBJ 10	116 LOD	117 BEN	118 MAA	119 GR	120 MUK	121 GM131 'CL #	122 AMYLOID 594	123* MAR	124 CL*
	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	---
	2	ILE	ILE	ILE	ILE	ILE	ILE	GLN	ILE	ILE	ILE	ILE	ILE	ILE	ILE	GLN	ILE	ILE	ILE	ILE		ILE	ILE	---
	3	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	leu	GLX	GLN	GLN		GLN	GLN	GLN	GLN		GLN	GLN	---
	4	MET	MET	leu	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET?		MET	leu	MET	MET		MET	leu	---
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	---
	6	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	---
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	---
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	---
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	---
FR1	10	ser	ser	phe	THR	ser	ser	ser	ser	ser	THR	ser	ser	ser	ser	THR	ser	THR	ser	ser		ser	ser	---
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU		LEU	LEU	LEU	LEU		LEU	LEU	---
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER				---
	13	ALA	val	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val					---
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER									---
	15	VAL	VAL	VAL	VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL		VAL								---
	16	GLY	GLY	GLY	GLY	GLY	GLY	arg	GLY	GLY		GLY												---
	17	ASP	glx	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP												---
	18	arg	arg	arg	arg	arg	arg	arg	arg															---
	19	val	val	val	val	val	val	val	val															---
	20	THR	THR	THR	THR	THR		THR	TER															---
	21	ILE	ILE	ILE		ILE	ILE	ILE																---
	22	THR	THR	THR		ala	THR	THR																---
			CYS																					
	23																							---
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	25																							---
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CDR1	27C																							---
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	41																							---
FR2	42																							---
	43																							---
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	47																							---
	48																							---
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CDR2	53																							---
	54																							---
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	69																							---
	70																							---
	71																							---
FR3	72																							---
	73																							---
	74																							---
	75																							---
	76																							---
	77																							---
	78																				LEU			---
	79																				GLN			---
	80																				pro			---
	81																				glu			---
	82																				ASP			---
	83																				PHE			---
	84																				ALA			---
	85																				THR			---
	86																				TYR			---
	87																				TYR			---
	88																				CYS			---
	89																				gln			---
	90																				gln			---
	91																				his			---
	92																				ASP			ASP
	93																				his			ile
	94																				leu			PHE
	95																				pro			pro
CDR3	95A																				---			---
	95B																				---			---
	95C																				---			
	95D																				---			---
	95E																				---			---
	95F																				---			---
	96																				LEU			GLY
	97																				THR			THR
	98																				PHE			PHE
	99																				GLY			GLY
	100																				GLY			GLN
	101																				GLY			GLY
	102																				THR			THR
FR4	103																				THR			LYS
	104																				VAL			VAL
	105																				GLU			GLU
	106																				MET			ILE
	106A																				---			---
	107																				LYS			LYS
	108																				ARG			ARG
	109																				THR			THR

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

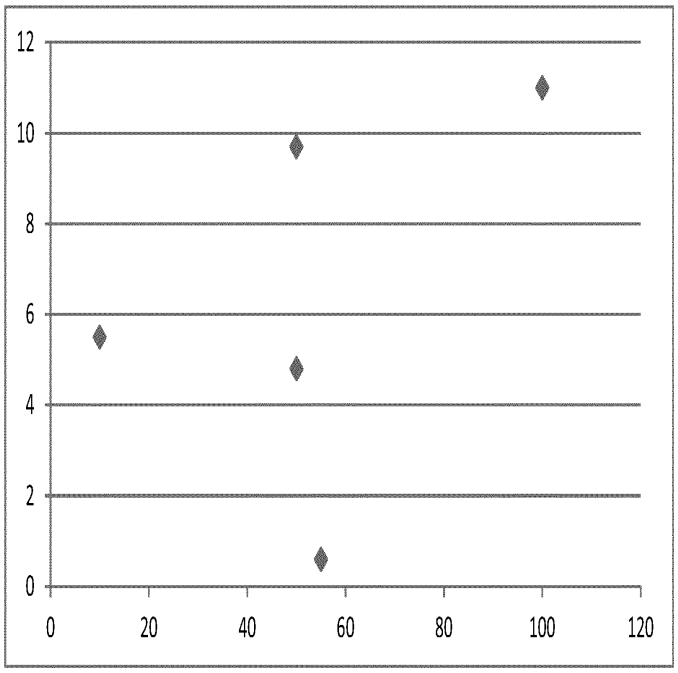
		125 AMYLOID	126 HBJ 6	127 AMYLOID MS #	128 PEN	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
	0	---	---	---	---	1	1	1(PCA)	
	1	ASP	ASP	ASP	ASP	125	3,4	122(ASP),118(ASP)	3.1,4.2
	2	ILE	ILE	#	ILE	123	2	120(ILE)	2.
	3	glu	GLN	GLN	GLN	123	7	116(GLN),112(GLN)	7.4,7.7
	4	MET	MET	MET	MET	124	4	109(MET)	4.6
	5	pro	THR	THR		124	3	122(THR)	3.
	6	GLN	GLN			123	1,2	123(GLN),116(GLN)	1.,2.1
	7	SER				121	2	120(SER)	2.
	8	PRO				121	2	120(PRO)	2.
	9	SER				121	4	117(SER)	4.1
FR1	10	ser				120	5	89(SER)	6.7
	11	LEU				119	4	111(LEU)	4.3
	12					118	3	115(SER)	3.1
	13					117	4	107(ALA)	4.4
	14					113	6	106(SER)	6.4
	15					113	3	109(VAL)	3.1
	16					109	2	108(GLY)	2.
	17					109	3,4	106(ASP),98(ASP)	3.1,4.4
	18					106	6	97(ARG)	6.6
	19					107	3	101(VAL)	3.2
	20					106	4	102(THR)	4.2
	21					104	4	99(ILE)	4.2
	22					104	6	95(THR)	6.6
	23					96	1	96(CYS)	1.
	24					88	6	57(ARG)	9.3
	25					88	6	82(ALA)	6.4
	26					85	4	79(SER)	4.3
	27					84	4	79(GLN),66(GLN)	4.3,5.1
	27A					3	2	2(SER)	
	27B					3	2	2(LEU)	
	27C					3	2	2(VAL)	
CDR1	27D					3	3	1(+)	
	27E					2	2	1(+)	
	27F								
	28					84	7	29(SER)	20.
	29					83	5	72(ILE)	5.8
	30					81	11	41(SER)	22.
	31					79	12	27(ASN),24(SER)	35.,39.
	32					79	9	34(TYR)	21.
	33					77	4	73(LEU)	4.2
	34					73	7,8	30(ALA)	17.,19.
	35					76	1	76(TRP)	1.
	36					74	2	66(TYR)	2.2
	37					73	4	68(GLN),61(GLN)	4.3,4.8
	38					71	4	68(GLN),63(GLN)	4.2,4.5
	39					68	4	62(LYS)	4.4
	40					70	3	68(PRO)	3.1
	41					58	3	54(GLY)	3.2
FR2	42					60	6	49(LYS)	7.3
	43					61	4	57(ALA)	4.3
	44					61	1	61(PRO)	1.
	45					61	7,8	46(LYS)	9.3,11.
	46					60	7	42(LEU)	10.
	47					59	2	58(LEU)	2.
	48					57	2	56(ILE)	2.
	49					59	4	55(TYR)	4.3
	50					59	9	19(ALA)	28.
	51					59	5	60(ALA)	5.9
CDR2	52					58	6	53(SER)	6.6
	53					57	6,7	26(SER)	13.,15.
	54					58	2	56(LEU)	2.1
	55					58	8,9	25(GLU)	19.,21.
	56					56	8	34(SER)	13.
	57					57	1	57(GLY)	1.
	58					58	3	50(VAL)	3.5
	59					57	4	54(PRO)	4.2
	60					57	1	57(SER)	1.
	61					58	3	56(ARG)	3.1
	62					57	3	55(PHE)	3.1
	63					57	6	50(SER)	6.8
	64					57	2	55(GLY)	2.1
	65					56	5	51(SER)	5.5
	66					57	4	54(GLY)	4.2
	67					55	3	51(SER)	3.2
	68					55	3	52(GLY)	3.2
	69					55	2	51(THR)	2.2
	70					55	5,6	33(ASP),31(ASP)	8.3,11.
	71					54	4	49(PHE)	4.4
FR3	72					54	5	50(THR)	5.4
	73					54	3	43(LEU)	3.8
	74					54	4	51(THR)	4.2
	75					54	3	47(ILE)	3.4
	76					53	5	43(SER)	6.2
	77					54	6	40(SER)	8.1
	78					54	2	53(LEU)	2.
	79					54	3,4	52(GLN),48(GLN)	3.1,4.5
	80					54	2	46(PRO)	2.3
	81					54	3,5	37(GLU),34(GLU)	4.4,7.9
	82					54	2	53(ASP),50(ASP)	2.,2.2
	83					54	4	40(PHE)	5.4
	84					54	2	53(ALA)	2.
	85					54	6	48(THR)	6.7
	86					56	2	55(TYR)	2.
	87					55	2	52(TYR)	2.1
	88					56	1	56(CYS)	1.
	89					57	4,5	46(GLN),43(GLN)	5.,6.6
	90					57	2,3	51(GLN),46(GLN)	2.2,3.7
	91					58	11,12	28(TYR)	23.,25.
	92					60	9	20(ASN),19(+)	27.,28.
	93					60	9	29(SER)	19.
	94					60	12	15(LEU)	48.
	95					56	4	44(PRO)	5.1
CDR3	95A					1	1	1(GLU)	
	95B								
	95C								
	95D								
	95E								
	95F								
	96					44	12	10(TRP)	53.
	97					43	4	39(THR)	4.4
	98					46	3	44(PHE)	3.1
	99					46	1	46(GLY)	1.
	100					46	4,5	28(GLN),27(GLN)	6.6,8.5
	101					46	1	46(GLY)	1.
	102					46	2	45(THR)	2.
FR4	103					46	4	38(LYS)	4.8
	104					45	2	36(VAL)	2.5
	105					45	4,5	29(GLU),28(GLU)	6.2,8.
	106					46	7	30(ILE)	11.
	106A								
	107					45	3	42(LYS)	3.2
	108					33	2	31(ARG)	2.1
	109					22	1	22(THR)	1.

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% Aggregation Versus Antibody Concentration

From Table 2 of D20 where concentration and % aggregates known)

Antibody	Concentration in Formulation (mg/mL)	% Aggregates
rhyMAbE25	10	5.5
Human anti-IL-8 (IgG2)	50	4.8
RhuMAb HER2	50	9.7
Human Anti-IL-8 (IgG2)	55	0.6
Anti-IL-8 (IgG2)	100	11



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IN THE MATTER OF
European Patent No. EP 2 359 834

DECLARATION OF DR. DINO MIANO

I, Dr. Dino Miano, hereby declare as follows:

1. I am Dr. Dino Miano of Alexion Pharmaceuticals, Inc., 100 College St, New Haven, Connecticut, USA. I hold a Ph.D. degree in Chemistry and Biochemistry from the University of Massachusetts, and a Bachelor of Science degree in Chemistry and Biology from the University of Hartford. I have been employed by Alexion Pharmaceuticals for nearly 20 years, and I currently hold the post of Executive Director, Global Analytical & Pharmaceutical Development. Prior to this I was employed by R.W. Johnson Pharmaceutical Research Institute in Raritan, New Jersey for approximately two years. During my employment at Alexion my responsibilities and work have focused on analytical sciences and protein characterization. I have significant expertise in formulation development and analysis, which includes designing, performing and overseeing experiments that assess the impact of protein concentration, formulation, and storage conditions on the integrity and stability of a protein over time. A copy of my resume is attached as Exhibit 1.

2. The commercial formulation of Soliris®(eculizumab) consists of a concentration of 10 mg/mL of eculizumab in a buffer of 10 mM sodium phosphate, 150 mM sodium chloride and 0.02% polysorbate 80 at pH 7.0. The commercial formulation is satisfactorily stable for 18 months when it is stored as drug substance (i.e., in bulk in the commercial formulation) and for an additional 30 months when it is stored as a drug product (i.e., in 30 mL vials as it is supplied to consumers) providing a total of 48 months of stability. (See March 16, 2007 BLA Approval Letter from U.S. FDA, attached as Exhibit 2 (indicating the drug substance is stable for 18 months and the drug product is stable for 24 months at 2-8°C), and May 8, 2009 Letter from U.S. FDA, attached as Exhibit 3 (extending the shelf-life of the drug product from 24 to 30 months at 2-8°C after supplemental stability data was obtained).)

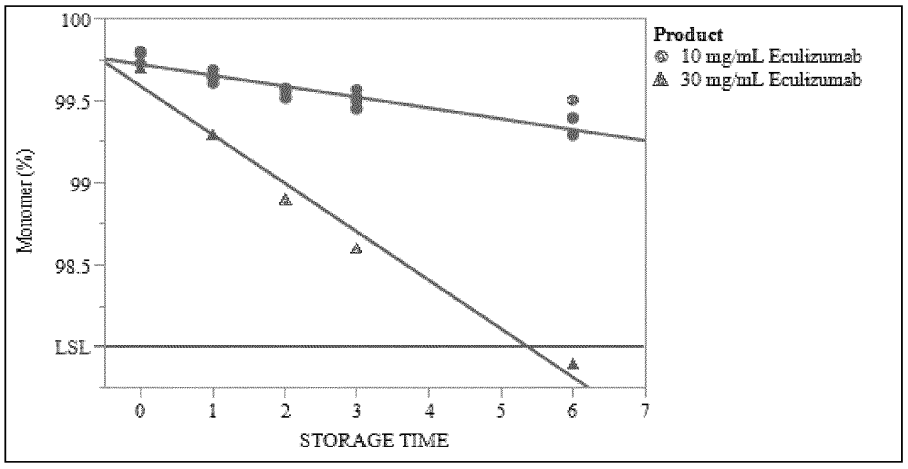
3. One of the stability parameters that the commercial formulation of eculizumab must meet in order to be deemed satisfactorily stable and thus suitable for release into the market is the percent of monomer antibody in the sample must be greater than or equal to 98.0%, which means that there must be less than or equal to 2.0% aggregates of the antibody, as determined via gel permeation high performance liquid chromatography. This release parameter for commercial lots of eculizumab ensures that the drug is satisfactorily stable for the designated storage period.

4. We generated a 30 mg/mL formulation of eculizumab (internally denoted "ALXN5100") that was identical to the commercial formulation of eculizumab, except that ALXN5100 has an eculizumab concentration of 30 mg/mL and the Soliris® commercial formulation has an eculizumab concentration of 10 mg/mL.

5. A batch of ALXN5100 was made under Good Manufacturing Practices (GMP) conditions, and stability studies on the batch were conducted by aliquotting samples into representative HyQ® CX5-14 Hyclone bags to simulate large-scale storage. The Hyclone bags were then stored horizontally at 2 to 8°C for at least 18 months and at 23 to 27°C for at least 6 months, which is how samples were stored for the stability program for the bulk drug substance for the commercial (10 mg/mL) formulation. Although the commercial formulation of eculizumab is to be stored at 2 to 8°C, when performing stability testing, researchers often store test samples at higher temperatures than commercial supplies are typically stored at, such as 23 to 27°C, because storage and stability testing at higher temperatures can provide a good indication of the stability of the formulation in a shorter test period of time. Gel Permeation High Performance Liquid Chromatography (GP-HPLC) was used to determine if there were any changes in monomer and aggregate drug at the two storage temperatures over time.

6. Figures 1 and 2 below demonstrate that the stability of eculizumab at 10 mg/mL is superior to that at 30 mg/mL. Figure 1 compares the percent monomer in the 10 mg/mL eculizumab composition versus the percent monomer in the 30 mg/mL eculizumab composition after storage at 23 to 27°C over six months. Figure 1 clearly shows that the percent monomer of eculizumab decreases (and thus aggregate formation increases) significantly more quickly in the 30 mg/mL eculizumab composition than in the 10 mg/mL eculizumab composition. In addition, whereas the 10 mg/mL eculizumab composition remains sufficiently stable over the specification ("LSL") monomer level of 98.0% over the entire six-month period, the 30 mg/mL eculizumab composition falls below that stability threshold during the same time period.

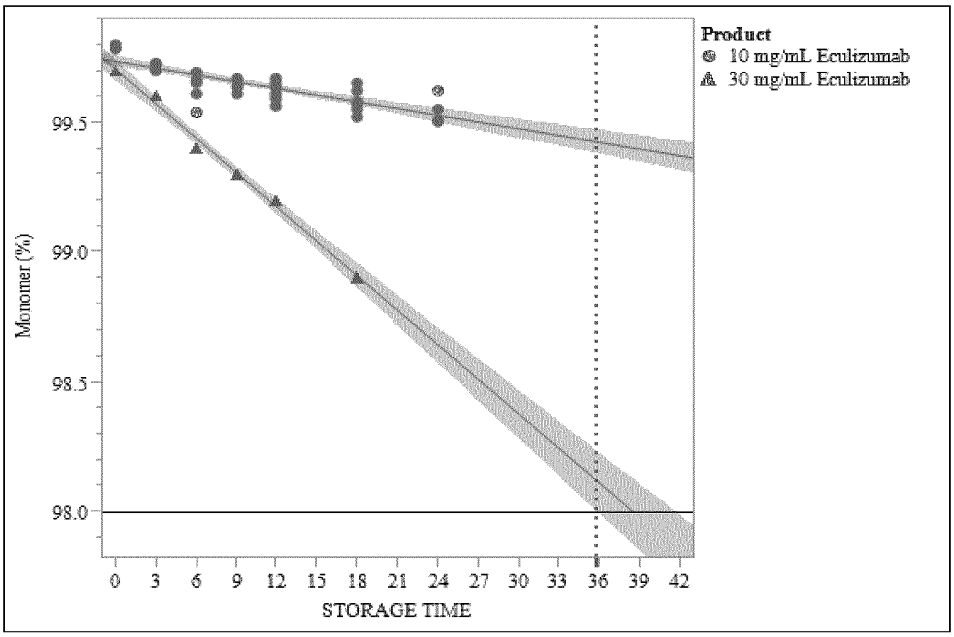
Figure 1. Percent monomer of eculizumab in 10 mg/mL eculizumab composition versus 30 mg/mL eculizumab composition over six months when stored at 23-27°C



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7. Similar trends are seen between the 10 mg/mL eculizumab composition and the 30 mg/mL eculizumab composition even at storage temperatures of 2 to 8°C. Figure 2 compares the percent monomer in the 10 mg/mL eculizumab composition versus the percent monomer in the 30 mg/mL eculizumab composition after storage at 2 to 8°C over 18 months. Again, as with the 23 to 27°C storage conditions, the percent monomer remaining in the 30 mg/mL eculizumab samples decreased much more rapidly than the percent monomer remaining in the 10 mg/mL eculizumab samples at 2 to 8°C over the 18-month period. In addition, the model generated from this data predicted that the 30 mg/mL eculizumab composition would fall below the acceptable 98.0% monomer level before 36 months of storage.

Figure 2. Percent monomer of eculizumab in 10 mg/mL eculizumab composition versus 30 mg/mL eculizumab composition over 18 months when stored at 2-8°C



8. Thus, these long-term stability studies demonstrate a decrease in monomer and an increase in aggregate formation for ALXN5100, the 30 mg/mL eculizumab concentration, throughout the stability program. In all instances the aggregate formation in the 30 mg/mL concentration was greater than what was observed in the 10 mg/mL concentration. These results are clearly demonstrated through 18 months at 2 to 8°C and are even more pronounced within 6 months at the higher temperature studies. These results suggest that the commercial formulation is optimal for an eculizumab concentration of 10 mg/mL but is not suitable when the eculizumab concentration is increased to 30 mg/mL.

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I believe and do hereby declare that the facts stated above are to the best of my knowledge true.

Dr. Dino Miano

17 Mar 2018
Date

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Exhibit 1

Dino C. Miano, Ph. D.
217 Sunset Ridge
Rocky Hill, CT 06067
(860) 563-5433 (Home)
(860) 983-6178 (Mobile)
dinomiano@sbcglobal.net

QUALIFICATION PROFILE

- Results-oriented professional with eighteen years' successful track record of innovation, management, in depth scientific and regulatory knowledge in analytical and pharmaceutical development in the field of biotherapeutics. Experience encompasses various stages of biologics including early and late phase development, regulatory submission for marketing authorization and life cycle management.

- Well established division-level leader that consistently drives vision toward successful and timely completion of projects, while focusing on translating goals into actionable strategies that support the business

COMPETENCIES

- Pharmaceutical (biological) development
  - Formulation development
  - Fill finish
  - Stability monitoring and trending
  - Device development
  - Analytical support of clinical operations
- Regulatory Compliance
  - CMC development
- Analytical Development
  - Method Validation & Transfer
  - GMP Testing & Compliance
  - Implementing new analytical technologies
  - Analytical support of manufacturing
- Strategic leadership and innovation
  - Cross functional leader

Accomplishments and Qualifications

- Assumed a key analytical role in the clinical development and global commercialization of Soliris® and Strensiq®. Led development and validation activities for commercial release and stability test methods.
- Lead Global Operations efforts in delivering clinical material from early stage through commercial. Lead cross functional teams consisting of manufacturing, supply chain, QA and CMC and working with clinical groups to ensure supply demands are consistently met
- Lead Global Analytical and Pharmaceutical Development groups in providing analytical support to the entire Alexion organization, including analytical development, validation and transfer, GMP testing of clinical products, process development support, formulation and device development, product characterization and stability to allow for expiry determination.
- Implemented GMP quality infrastructure in the Analytical Sciences laboratories to ensure testing compliance with FDA, EU and PMDA regulations.
- Lead formulation development of new, existing and commercial products for IV, IVT and subcutaneous administration.

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- Implement drug substance and drug product specifications for clinical products
- Generate analytical sections in support of all new and existing CMC submissions
- Lead the drug delivery and device development group in developing a strategy and business process to advance drug and device/combination products for existing commercial and clinical products.

Employment History

Alexion Pharmaceuticals, Inc., New Haven, CT

Executive Director, Global Analytical and Pharmaceutical Development **12/15-present**

- Lead global analytical support function for the entire organization for all commercial and clinical programs.
- Lead the global method technology transfers for Alexion's clinical and commercial products.
- Oversee compatibility assessments for materials used for delivery of product to patients during clinical studies.
- Represent Global Operations as lead for late stage process development, drug substance and drug product manufacturing, supply chain and CMC/IND submissions.
- Lead the device group in collaboration with internal and external partners for the development of delivery devices for Alexion products.

Senior Director, Analytical and Pharmaceutical Development **03/15-12/15**

- Led Early Stage Analytical and Product Characterization groups in supporting Research in efforts to move the best candidates into development.
- Led Analytical Sciences in method development, validation and transfer for new candidates and provide support to process development groups.
- Lead the stability program for all clinical products, including expiry determinations
- Led and aligned formulation development of new candidates with Clinical development and Target product profiles (TPP).
- Led Device Development and Drug Delivery group in identifying and integrating large volume bolus injector options for clinical and commercial development.
- Appointed CMC lead during Regulatory meetings with FDA, Health Canada, EMA and MFDS (F2F) to support CMC section of regulatory filings.

Director, Product Characterization **07/14-03/15**

- Led Global Operations Cross Functional Product Team in supporting second generation development of commercial product.
- Led device teams consisting of Clinical and Commercial Operations, Development, Regulatory and QA in evaluating large volume subcutaneous administration devices. Device included auto-injectors and larger wearables patch devices.
- Managed Mass Spectroscopy Characterization group. Involves the MS characterization of new and existing molecules to support CMC, Research and Development groups.
- Managed the Bio-Physical Characterization group. Involves directing the testing and collaboration of multiple groups to better understand new protein therapeutics
- Provided analytical support of new and existing CMC submissions and address inquiries from agencies

Director, Analytical Sciences **09/08-07/14**

- Managed the Operations group that provided GMP testing of clinical products and analytical testing to all development groups.
- Managed the Analytical Development group that provided method development, qualification, validation and transfer of analytical methods for new clinical products and existing commercial product to both QC and EU laboratories.
- Manage the Formulation Development group that identified stable formulations for new clinical products and determined stable subcutaneous formulations for both clinical and commercials products.
- Managed Stability program of clinical products that generated GMP stability protocols and reports, trended data and provided statistical analysis of trends to support expiry, and led deviations, LIR and OOS for GMP testing.
- Member of Specification committee to direct the generation or revision of specifications for clinical products

Associate Director, Analytical Sciences **05/03-08/08**

- Analytical Group
  - Provided analytical support of new and existing Soliris® CMC submissions
  - Supervised Analytical Operations laboratory in providing method development, qualification, validation, transfer and GMP testing to support clinical products.
- Purification Development group:
  - Managed PD studies in support of Process Validation. Studies included viral clearance, DNA clearance, process limits evaluation (PLE), resin re-use, and UF/DF membrane re-use.

Manager, Quality Control **08/99-05/03**

- Managed QC laboratories; maintained cGMP compliance with regards to current SOPs, validation/qualifications, and training.
- Coordinated studies, supervised investigations, generated reports and implemented systems with QA in response to OOS, invalid assays, aberrant trends/results.
- Represented area functions as required during audits by external agencies and internal QA department.

Senior Scientist, Quality Control **08/98-08/99**

- Developed and optimized RP-HPLC, SEC-HPLC, SDS-PAGE, and IEF methods for all protein based therapeutic product.

R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
A Johnson & Johnson Company

Scientist, Analytical Development **07/97-07/98**

- Developed HPLC assays for quantitation of becaplermin drug substance in alternative REGRANEX® formulations. Developed HPLC assays for determining content of preservatives in REGRANEX® formulations.

Scientist, Protein Development **01/97-07/97**

- Investigated modified roller bottle processes for the production of erythropoietin (rHuEPO), potentially increasing production/yields by 3-fold.
- Evaluated alternative purification processes for rHuEPO, involving HIC, IEC and Reverse phase chromatography.

Postdoctoral Research Scientist, Protein Development **10/95-01/97**

- Chemically modified rHuEPO using polyethylene glycol (PEG) to enhance in vivo erythropoiesis while prolonging circulatory half-life. Experiments were designed to specifically modify the carbohydrate (NANA) moieties of the glycoprotein with PEG while maintaining the native polypeptide conformation. Animal model analysis revealed successful prolonged in vivo biological activity.

University of Connecticut Health Center, Farmington, CT **01/89-08/90**

Research Scientist, Surgery Department

- Investigated/detected tissue derived inflammatory mediators released from injured corneas, urinary bladders and ischemic hearts. Modified Boyden chambers were employed to evaluate chemotactic response via neutrophil migration through 8 μm membranes.
- Investigated/synthesized cyclocreatine, a creatine analog, to determine inhibitory effects on the production and release of neutrophil chemotactic factors (NCF) in ischemic canines and rabbits myocardium.

EDUCATION

University of Massachusetts, Lowell, MA **09/90-10/95**

Ph. D. Chemistry/Biochemistry
Advisor: Dr. Edwin G.E. Jahngen
Thesis: "The Isolation and characterization of an ubiquitin-histone protein from *Dictyostelium discoideum AX3.*"

University of Hartford, Bloomfield, CT **09/85-05/89**

B.S. Chemistry/Biology
Research: "Corneal derived neutrophil chemotactic factors, intracellular synthesis and release."

PUBLICATIONS

Neill, A., Nowak, C., Patel, R., Ponniah, G., Gonzalez, N., **Miano, D**. and Liu, H., (2015) Characterization of recombinant monoclonal antibody charge variants using OFFGEL fractionator, weak anion exchange chromatography and mass spectrometry, *Analytical Chemistry*, **87**, 6204-6211

Ponniah, G., Nowak, C., Gonzalez, N., **Miano D**., and Liu, H., (2015) Detection and quantitation of low abundance oligosaccharides in recombinant monoclonal antibodies. *Analytical Chemistry*, **87**, 2718-2726

Peckham, N., Neil, F., and **Miano, D.C**., "Quantitative SDS-PAGE Analysis of a Recombinant Protein by Densitometry", IBC's Well Characterized Biologicals Poster Session, Washington D.C. (11/11/04)

Peckham, N., Bosley, K., and **Miano, D.C**., Adaptation of Complexometric Titration Method for Quantitation of EDTA in Biopharmaceutical Formulations to Microplate Format, IBC's Well Characterized Biologicals Poster Session, Washington D.C. (11/11/04).

Miano, D.C., Villalobos, A.P., Ghosh-Datidar, P., and Sharma, B. (1997), Chemical Modification of Recombinant Human Erythropoietin by Poly (ethylene glycol). Johnson and Johnson Chromatography Symposium, Princeton, NJ (abstract).

Miano, D.C. (1995) The Isolation and Characterization of a Ubiquitinylated Histone Protein in *Dictyostelium discoideum* AX3. Ph.D. Thesis, University of Massachusetts/Lowell.

Miano, D.C, and Jahngen, G.E. (1994) The Ubiquitinylation of Histones in *Dictyostelium discoideum* AX3 Chromatin May Be Linked to Replication, Transcription and Stress. Keystone Symposium, Tammarron, CO (abstract).

Elgebaly, S.A., **Miano, D.C.**, Ehler, W., Rahha,l R. and Elkerm, A. (1994) The Induction of Corneal Inflammation by Factors Derived From Hydrogen Peroxide Injured Corneas: Effect of Dexamethasone and Indomethacin. *Occ. Pharm.*, **10**, (1), 295-306.

Elgebaly, S.A., Allam, M.E., Houser, S., Hashmi, F., Forouhar, F. and **Miano, D.C.**, (1993) Cyclocreatine Inhibits Neutrophil Accumulation in the Myocardium of Canine Models in Coronary Artery Occlusion and Reperfusion. *J. Pharm. Exp. Ther.*, **266**, (3), 1670-1677.

Elgebaly, S.A., **Miano, D.C.**, Kreutzer, D.L. and Fishman, J.B., (1990) Cornea Derived Neutrophil Chemotactic Factors: Intracellular Synthesis and Release. *Curr. Eye Res.*, **9**, 839-845.

Elgebaly, S.A., **Miano, D.C.**, Ehler, W. and Rahhal, R. (1990) The Induction of Corneal Inflammation by Factors Derived From Hydrogen Peroxide Injured Corneas. ARVO, Sarasota, FL (abstract).

Elgebaly, S.A., **Miano, D.C.**, Kreutzer, D.L. and Fishman, J.B. (1989) Cornea Derived Neutrophil Chemotactic Factors. ARVO, Sarasota, FL (abstract).

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

Our STN: BL 125166/0

MAR 16 2007

Alexion Pharmaceuticals
Attention: Nancy Motola, Ph.D., RAC
Senior Vice President, Regulatory and Quality
352 Knotter Drive
Cheshire, CT 06410

Dear Dr. Motola:

We are issuing Department of Health and Human Services U.S. License No. 1743 to Alexion Pharmaceuticals, Inc., Cheshire, Connecticut, under the provisions of section 351(a) of the Public Health Service Act controlling the manufacture and sale of biological products. The license authorizes you to introduce or deliver for introduction into interstate commerce, those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are approved to manufacture the product eculizumab, which is indicated for the treatment of paroxysmal nocturnal hemoglobinuria to reduce hemolysis.

You are approved to manufacture eculizumab drug substance at Lonza Biologics, PLC in Portsmouth, NH. The final formulated product will be manufactured, filled, labeled, and packaged at Ben Venue Laboratories, Bedford, Ohio. You may label your product with the proprietary name Soliris™ and will market it in 300 mg vials.

The final printed labeling (FPL) must be identical to the enclosed labeling. Marketing product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for eculizumab drug product shall be 24 months from the date of manufacture when stored at 2-8°C. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for your drug substance shall be 18 months when stored at 2-8°C.

You currently are not required to submit samples of future lots of eculizumab to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

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You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of eculizumab, or in the manufacturing facilities.

We acknowledge your written commitments as described in your letter of February 22, 2007, March 12, 2007 and March 12, 2007 as outlined below:

Postmarketing Studies subject to reporting requirements of 21 CFR 601.70.

1. To evaluate long-term safety of eculizumab by analyzing outcomes in the Soliris Safety Registry for a time period of no less than five years. At the end of the five year period, a study report will be submitted to the Biological License Application (BLA) that describes the major safety findings from the registry program, including the specific items listed below and proposing labeling changes as appropriate. Additionally, annual interim reports will be submitted to the BLA, along with expedited reports as specified below. The protocol for addressing this PMC will be submitted to the IND by May 31, 2007, and the five year study report will be submitted by June 30, 2012. All patients within the registry will be followed for the occurrence of:

    a. Serious infections, defined as infections necessitating or prolonging hospitalization or resulting in death. Alexion commits to collecting follow-up information from these patients to assess the nature of the serious infection, the duration of hospitalization, the major features of the clinical course and the survival status. Expedited reporting (15 day telephone or facsimile Medwatch communication) will be provided for the occurrence of these serious infections.

    b. Malignancy, including the nature of the malignancy and the survival status of patients who develop a malignancy;

    c. Use of eculizumab among pediatric patients under 16 years of age, to include collection of eculizumab dosage information, as well as the same information being required for adult patients in the registry;

    d. Pregnancy, including the clinical course of each pregnancy and the detection of congenital abnormalities among babies born to the women exposed to eculizumab during the pregnancy.

    e. Thrombotic events, including the nature of the event, the clinical outcome as well as the anticoagulant management prior to and after the event.

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2. To submit a comprehensive description of the Soliris Guardian Program Risk Minimization Action Plan (RiskMAP), including all items listed below. Fulfillment of this post-marketing commitment will be contingent upon FDA concurrence upon the expectations of the Soliris Guardian Program. Submission of all items listed below will occur no later than May 18, 2007. The submitted information will include:

   a. A final version of the Soliris Guardian Program document that, in addition to any other items, provides information fully consistent with the approved prescribing information.

   b. A copy of all educational materials to be provided as part of the program, including but not limited to all components of the Soliris Starter Kit, the Drug Fact Sheet and patient-health care provider documents relating to the Soliris Safety Registry.

   c. A commitment to develop and submit a protocol (with the understanding that this protocol may need modification based upon FDA review findings) that uses surveys of health care providers and patients to assess compliance with the vaccination requirements as well as their knowledge of the risks of eculizumab and the need for vaccination.

   d. A copy of the Soliris Safety Registry protocol and any supportive documents to be provided to health care providers and patients. These documents may need modification based upon FDA review findings. The submitted protocol will indicate that the Registry will continue until Alexion receives written concurrence from FDA to terminate the registry. The protocol will include collection of the occurrence of the following events:

      i. Death;

      ii. Meningococcal vaccination (type and dates of all vaccinations);

      iii. eculizumab administration dates, at designated time points to establish initiation and termination of therapy as well as to correlate eculizumab administration with the fatalities and the events listed below:

         - All meningococcal infections causing sepsis or meningitis
         - Other serious infections
         - Malignancy, including the nature of the malignancy and the survival status of patients who develop a malignancy;
         - Use of eculizumab among pediatric patients under 16 years of age, to include collection of eculizumab dosage information, as well as the required information for adult patients in the registry;
         - Use of eculizumab by indication;

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- Pregnancy, including the clinical course of each pregnancy and the detection of congenital abnormalities among off-spring of the women exposed to eculizumab during a pregnancy and;
- Serious hemolysis, as defined by specific criteria.

e. A commitment to submit quarterly RiskMAP reports for the first year and annual reports thereafter (unless FDA provides written request for more frequent submissions) summarizing all information relating to the Soliris Guardian Program. The reports should include the following:

i. An analysis of all cases of the following, including a root cause analysis and factors that might have contributed to serious outcomes:
   - Meningococcal sepsis or meningitis, including the timing of all vaccinations relative to administration of Soliris
   - Other infections (with serious outcomes)
   - All deaths
   - Cases of serious hemolysis and all cases of hemolysis with serious outcomes

ii. Soliris use patterns including indication for use;

iii. Extent of compliance with RiskMAP requirements such as the percentage of patients that were vaccinated prior to receiving eculizumab and the percentage of patients that were re-vaccinated at 3-year or 10-year intervals (as applicable);

iv. Analysis of all cumulative data collected in the Soliris Safety Registry;

v. Results from all health care provider and patient surveys, including:

   - Any known data about patients or physicians who refused to participate in the surveys
   - Any known data about survey participants who are considered "lost" (drop-outs).

3. To conduct a randomized, controlled clinical study to assess the effects of anticoagulant withdrawal among PNH patients receiving eculizumab. This study will randomize at least 100 anticoagulated patients to either continue or discontinue anticoagulation therapy. The major outcomes will assess the safety of discontinuation of anticoagulant therapy while continuing eculizumab, especially with respect to providing important evidence regarding major bleeding and that this discontinuation does not increase the risk for occurrence of thrombotic events in these patients. A full study report and data from this study will be submitted to the BLA and may include a label revision, contingent upon the importance of the study results. The study protocol will be submitted to the investigational new drug application (IND) by June 30, 2007 and patient accrual completed no later than June 1, 2009. A final study report will be submitted no later than March 31, 2014.

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4. To develop a validated and quantitative assay for the measurement of human anti-human antibodies (HAHA) for the detection of antibody formation to eculizumab. This assay will assess potential immune responses to the whole eculizumab molecule. Description of the validated assay will be submitted to the BLA as a CBE 30 by July 9, 2008.

5. To develop a validated and sensitive assay for the measurement of neutralizing HAHA to eculizumab. Alternatively, Alexion commits to submit documentation to FDA demonstrating with due diligence that a suitable assay could not be feasibly developed and that the assessment of serum lactate dehydrogenase (LDH) is a sufficiently sensitive indicator of the presence of neutralizing antibodies. This information will be submitted to the BLA by July 9, 2008.

6. To utilize samples from the ongoing E05-001 Phase III extension study (approximately 170 patients for at least 2 years) as test samples for the new validated HAHA assays. Alexion will continue to obtain serum samples from those patients who transition from E05-001 to the Soliris Safety Registry, at intervals of no less than one year, and continue this collection process for an additional three years. Sample collection will cease during this additional three year period for patients who terminate eculizumab administration. Additionally, serum samples will be obtained based upon physician reports of suspected loss of eculizumab bioactivity, based upon unanticipated alterations in serum LDH concentrations. All serum samples will be assayed at least annually and the results provided within an annual report to the BLA. Clinical data, to include the results of serum LDH concentrations, will also be obtained from any patients who show evidence of antibody formation. The protocol describing Alexion's plan for responding to this commitment will be submitted by May 1, 2007 and the final study report submitted by January 31, 2011.

Postmarketing Studies not subject to reporting requirements of 21 CFR 601.70.

7. To revalidate the linearity and accuracy of the Osmolality method across the full specification range using a combination of product samples diluted to lower osmolality and product samples spiked with osmolality standards. The revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

8. To revalidate the linearity of the IEF method across a load range of 8 µg to 12 µg. The revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 supplement by August 31, 2007.

9. To revise the IEF method SOP to specify that the method is validated only for a 10 µg load. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

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10. To improve and revalidate the existing hemolytic assay. Improvements include increasing the number of sample replicates and qualifying the chicken erythrocytes reagent. The revised method SOP and revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

11. To develop a new quantitative biological activity assay to replace the existing hemolytic assay, or submit documentation to FDA demonstrating with due diligence that a suitable assay could not be feasibly developed. This information will be submitted to the BLA by February 29, 2008. Validation of the quantitative biological activity assay will be submitted by July 9, 2008.

12. To provide FDA with a completed drug substance and drug product container closure system leachables evaluation using end-of-shelf-life, long-term 2-8°C stability samples. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

13. To develop a suitable antifoam assay and subsequently confirm the removal of antifoam from the process stream at the Protein A chromatography pool on three drug substance batches. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application (BLA), STN BL 125166. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to your BLA, STN BL 125166. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Commitment Protocol**
- **Postmarketing Study Commitment Final Study Report**
- **Postmarketing Study Commitment Correspondence**
- **Annual Status Report of Postmarketing Commitment Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

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As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Study Commitments - Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cder/guidance/5569fnl.htm) for further information.

Under 21 CFR Part 208, we have determined that this product poses a serious and significant public health concern requiring the distribution of a Medication Guide. Eculizumab is a product for which patient labeling could help prevent serious adverse effects and inform the patient of serious risks relative to benefit that could affect their decisions to use, or continue to use, the product. Therefore, a Medication Guide is necessary for safe and effective use of this product and FDA hereby approves the draft Medication Guide you submitted March 15, 2007. Please note that:

- this Medication Guide must be reprinted at the end of the package insert or accompany it [21 CFR 201.57(c)(18)];
- you are responsible for ensuring that this Medication Guide is available for distribution to every patient who is dispensed a prescription for this product [21 CFR 208];
- the final printed Medication Guide distributed to patients must conform to all conditions described in 21 CFR 208.24, including a minimum of 10 point text; and
- you are responsible for ensuring that the label of each container or package includes a prominent and conspicuous instruction to authorized dispensers to provide a Medication Guide to each patient to whom the drug is dispensed, and states how the Medication Guide is provided.

Please submit within 30 days content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/oc/datacouncil/spl.html, that is identical in content to the enclosed labeling text dated March 16, 2007. Upon receipt and verification, we will transmit that version to the National Library of Medicine for public dissemination.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

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You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all final printed labeling at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for information regarding therapeutic biological products, including the addresses for submissions.

Sincerely,

Karen Weiss for

Richard Pazdur, M.D.
Director
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Enclosure: Package Insert
Carton and Vial Labeling
Medication Guide
Patient Safety Card

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Soliris ™ (eculizumab)

FULL PRESCRIBING INFORMATION

WARNING: SERIOUS MENINGOCOCCAL INFECTION

Soliris increases the risk of meningococcal infections (5.1)

- **Vaccinate patients with a meningococcal vaccine at least 2 weeks prior to receiving the first dose of Soliris; revaccinate according to current medical guidelines for vaccine use**
- **Monitor patients for early signs of meningococcal infections, evaluate immediately if infection is suspected, and treat with antibiotics if necessary.**

1 INDICATIONS AND USAGE

Soliris is indicated for the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH) to reduce hemolysis.

2 DOSAGE AND ADMINISTRATION

Patients must be administered a meningococcal vaccine at least two weeks prior to initiation of Soliris therapy and revaccinated according to current medical guidelines for vaccine use. [*see Warnings and Precautions (5.1)*].

2.1 Recommended Dosage Regimen

Soliris therapy consists of

- 600 mg every 7 days for the first 4 weeks, followed by
- 900 mg for the fifth dose 7 days later, then
- 900 mg every 14 days thereafter.

Soliris should be administered at the recommended dosage regimen time points, or within two days of these time points. [*see Warnings and Precautions (5.5)*]

2.2 Preparation for Administration

Soliris must be diluted to a final admixture concentration of 5 mg/mL using the following steps:

- Withdraw the required amount of Soliris from the vial into a sterile syringe.
- Transfer the recommended dose to an infusion bag.
- Dilute Soliris to a final concentration of 5 mg/mL by adding the appropriate amount (equal volume of diluent to drug volume) of 0.9% Sodium Chloride Injection, USP; 0.45% Sodium Chloride Injection, USP; 5% Dextrose in Water Injection, USP; or Ringer's Injection, USP to the infusion bag.

The final admixed Soliris 5 mg/mL infusion volume is 120 mL for 600 mg doses or 180 mL for 900 mg doses. Gently invert the infusion bag containing the diluted Soliris solution to ensure thorough mixing of the product and diluent. Discard any unused portion left in a vial, as the product contains no preservatives.

Prior to administration, the admixture should be allowed to adjust to room temperature [18°-25° C, 64-77° F]. The admixture must not be heated in a microwave or with any heat source other than ambient air temperature. The Soliris admixture should be inspected visually for particulate matter and discoloration prior to administration.

2.3 Administration

Do Not Administer As An Intravenous Push Or Bolus Injection

The Soliris admixture should be administered by intravenous infusion over 35 minutes via gravity feed, a syringe-type pump, or an infusion pump. Admixed solutions of Soliris are stable for 24 hours at 2-8° C (36-46° F) and at room temperature.

If an adverse reaction occurs during the administration of Soliris, the infusion may be slowed or stopped at the discretion of the physician. If the infusion is slowed, the total infusion time should not exceed two hours. Monitor the patient for at least one hour following completion of the infusion for signs or symptoms of an infusion reaction.

3 DOSAGE FORMS AND STRENGTHS

Soliris is supplied as 300 mg single-use vials each containing 30 mL of 10 mg/mL sterile, preservative-free eculizumab solution.

4 CONTRAINDICATIONS

Do not initiate Soliris therapy in patients:

- with unresolved serious *Neisseria meningitidis* infection.
- who are not currently vaccinated against *Neisseria meningitidis*.

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5 WARNINGS AND PRECAUTIONS

5.1 Serious Meningococcal Infections

The use of Soliris increases a patient's susceptibility to serious meningococcal infections (septicemia and/or meningitis). All patients without a history of prior meningococcal vaccination must receive the meningococcal vaccine at least 2 weeks prior to receiving the first dose of Soliris and revaccinated according to current medical guidelines for vaccine use. Quadravalent, conjugated meningococcal vaccines are strongly recommended. Vaccination may not prevent meningococcal infections.

All patients must be monitored for early signs and symptoms of meningococcal infections and evaluated immediately if an infection is suspected. Physicians should strongly consider discontinuation of Soliris during the treatment of serious meningococcal infections.

In clinical studies, 2 out of 196 PNH patients developed serious meningococcal infections while receiving treatment with Soliris; both had been vaccinated. [*see Adverse Reactions (6.1)*].

5.2 Other Infections

Soliris blocks terminal complement; therefore patients may have increased susceptibility to infections, especially with encapsulated bacteria. Use caution when administering Soliris to patients with any systemic infection.

5.3 Monitoring After Soliris Discontinuation

Since Soliris therapy increases the number of PNH cells [in study 1, the proportion of PNH RBCs increased among Soliris-treated patients by a median of 28% from baseline (range from -25% to 69%)], patients who discontinue treatment with Soliris may be at increased risk for serious hemolysis. Serious hemolysis is identified by serum LDH levels greater than the pre-treatment level, along with any of the following: greater than 25% absolute decrease in PNH clone size (in the absence of dilution due to transfusion) in one week or less; a hemoglobin level of <5 gm/dL or a decrease of >4 gm/dL in one week or less; angina; change in mental status; a 50% increase in serum creatinine level; or thrombosis. Monitor any patient who discontinues Soliris for at least 8 weeks to detect serious hemolysis and other reactions.

If serious hemolysis occurs after Soliris discontinuation, consider the following procedures/treatments: blood transfusion (packed RBCs), or exchange transfusion if the PNH RBCs are >50% of the total RBCs by flow cytometry; anticoagulation; corticosteroids; or reinstitution of Soliris.

In clinical studies, 16 of 196 PNH patients discontinued treatment with Soliris. Patients were followed for evidence of worsening hemolysis and no serious hemolysis was observed.

5.4 Thrombosis Prevention and Management

The effect of withdrawal of anticoagulant therapy during Soliris treatment has not been established. Therefore, treatment with Soliris should not alter anticoagulant management.

5.5 Laboratory Monitoring

Serum LDH levels increase during hemolysis and may assist in monitoring Soliris effects, including the response to discontinuation of therapy. In clinical studies, six patients achieved a reduction in serum LDH levels only after a decrease in the Soliris dosing interval from 14 to 12 days. All other patients achieved a reduction in serum LDH levels with the 14 day dosing interval [*see Clinical Pharmacology (12.2) and Clinical Studies (14)*].

5.6 Infusion Reactions

As with all protein products, administration of Soliris may result in infusion reactions, including anaphylaxis or other hypersensitivity reactions. In clinical trials, no PNH patients experienced an infusion reaction which required discontinuation of Soliris. Soliris administration should be interrupted in all patients experiencing severe infusion reactions and appropriate medical therapy administered.

6 ADVERSE REACTIONS

6.1 Clinical Trial Experience

Meningococcal infections are the most important adverse reactions experienced by patients receiving Soliris therapy. In PNH clinical studies, two patients experienced meningococcal sepsis. Both patients had previously received a meningococcal vaccine. In clinical studies among patients without PNH, meningococcal meningitis occurred in an unvaccinated patient [*see Warnings and Precautions (5.1)*].

The data described below reflect exposure to Soliris in 196 adult patients with PNH, age 18-85, of whom 55% were female. All had signs or symptoms of intravascular hemolysis. Soliris was studied in a placebo-controlled clinical study (in which 43 patients received Soliris and 44, placebo); a single arm clinical study and a long term extension study. 182 patients were exposed for greater than one year. All patients received the recommended Soliris dose regimen.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. Table 1 summarizes the adverse reactions that occurred at a numerically

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higher rate in the Soliris group than the placebo group and at a rate of 5% or more among patients treated with Soliris.

TABLE 1

ADVERSE REACTIONS REPORTED IN 5% OR MORE OF SOLIRIS TREATED PATIENTS AND GREATER THAN PLACEBO IN THE CONTROLLED CLINICAL STUDY

Reaction	Soliris N = 43 N (%)	Placebo N = 44 N (%)
Headache	19 (44)	12 (27)
Nasopharyngitis	10 (23)	8 (18)
Back pain	8 (19)	4 (9)
Nausea	7 (16)	5 (11)
Fatigue	5 (12)	1 (2)
Cough	5 (12)	4 (9)
Herpes simplex infections	3 (7)	0
Sinusitis	3 (7)	0
Respiratory tract infection	3 (7)	1 (2)
Constipation	3 (7)	2 (5)
Myalgia	3 (7)	1 (2)
Pain in extremity	3 (7)	1 (2)
Influenza-like illness	2 (5)	1 (2)

In the placebo-controlled clinical study, serious adverse reactions occurred among 4 (9%) patients receiving Soliris and 9 (21%) patients receiving placebo. The serious reactions included infections and progression of PNH. No deaths occurred in the study and no patients receiving Soliris experienced a thrombotic event; one thrombotic event occurred in a patient receiving placebo.

Among 193 patients with PNH treated with Soliris in the single arm, clinical study or the follow-up study, the adverse reactions were similar to those reported in the placebo-controlled clinical study. Serious adverse reactions occurred among 16% of the patients in these studies. The most common serious adverse reactions were: viral infection (2%), headache (2%), anemia (2%), and pyrexia (2%).

6.2 Immunogenicity

As with all proteins there is a potential for immunogenicity. Low titers of antibodies to Soliris were detected in 3/196 (2%) of all PNH patients treated with Soliris. No apparent correlation of antibody development to clinical response was observed. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to Soliris in an enzyme linked immunosorbent assay (ELISA) and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in the assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to Soliris with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

Drug interaction studies have not been performed with Soliris.

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8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C:

PNH is a serious illness. Pregnant women with PNH and their fetuses have high rates of morbidity and mortality during pregnancy and the postpartum period. There are no adequate and well-controlled studies of Soliris in pregnant women. Soliris, a recombinant IgG molecule (humanized anti-C5 antibody), is expected to cross the placenta. Animal studies using a mouse analogue of the Soliris molecule (murine anti-C5 antibody) showed increased rates of developmental abnormalities and an increased rate of dead and moribund offspring at doses 2-8 times the human dose. Soliris should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal reproduction studies were conducted in mice using doses of a murine anti-C5 antibody that approximated 2-4 times (low dose) and 4-8 times (high dose) the recommended human Soliris dose, based on a body weight comparison. When animal exposure to the antibody occurred in the time period from before mating until early gestation, no decrease in fertility or reproductive performance was observed. When maternal exposure to the antibody occurred during organogenesis, two cases of retinal dysplasia and one case of umbilical hernia were observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. When maternal exposure to the antibody occurred in the time period from implantation through weaning, a higher number of male offspring became moribund or died (1/25 controls, 2/25 low dose group, 5/25 high dose group). Surviving offspring had normal development and reproductive performance.

8.2 Labor and Delivery

No information is available on the effects of Soliris during labor and delivery.

8.3 Nursing Mothers

It is not known whether Soliris is secreted into human milk. IgG is excreted in human milk, so it is expected that Soliris will be present in human milk. However, published data suggest that breast milk antibodies do not enter the neonatal and infant circulation in substantial amounts. Caution should be exercised when Soliris is administered to a nursing woman. The unknown risks to the infant from gastrointestinal or limited systemic exposure to Soliris should be weighed against the known benefits of breastfeeding.

8.4 Pediatric Use

The safety and effectiveness of Soliris therapy in pediatric patients below the age of 18 have not been established.

8.5 Geriatric Use

In PNH studies, 15 patients 65 years of age or older were treated with Soliris. Although there were no apparent age-related differences observed in these studies, the number of patients aged 65 and over is not sufficient to determine whether they respond differently from younger patients.

10 OVERDOSAGE

No cases of Soliris overdose have been reported during clinical studies.

11 DESCRIPTION

Soliris is a formulation of eculizumab which is a recombinant humanized monoclonal $IgG_{2/4}\kappa$ antibody produced by murine myeloma cell culture and purified by standard bioprocess technology. Eculizumab contains human constant regions from human IgG2 sequences and human IgG4 sequences and murine complementarity-determining regions grafted onto the human framework light- and heavy-chain variable regions. Eculizumab is composed of two 448 amino acid heavy chains and two 214 amino acid light chains and has a molecular weight of approximately 148 kDa.

Soliris is a sterile, clear, colorless, preservative-free 10 mg/mL solution for intravenous infusion and is supplied in 30-mL single-use vials. The product is formulated at pH 7 and each vial contains 300 mg of eculizumab, 13.8 mg sodium phosphate monobasic, 53.4 mg sodium phosphate dibasic, 263.1 mg sodium chloride, 6.6 mg polysorbate 80 (vegetable origin) and Water for Injection, USP.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Eculizumab, the active ingredient in Soliris, is a monoclonal antibody that specifically binds to the complement protein C5 with high affinity, thereby inhibiting its cleavage to C5a and C5b and preventing the generation of the terminal complement complex C5b-9. Soliris inhibits terminal complement mediated intravascular hemolysis in PNH patients.

A genetic mutation in PNH patients leads to the generation of populations of abnormal RBCs (known as PNH cells) that are deficient in terminal complement inhibitors, rendering PNH RBCs sensitive to persistent terminal complement-mediated destruction. The destruction and loss of these PNH cells (intravascular hemolysis) results in low RBC counts (anemia), and also fatigue, difficulty in functioning, pain, dark urine, shortness of breath, and blood clots.

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12.2 Pharmacodynamics

In the placebo-controlled clinical study, Soliris when administered as recommended reduced hemolysis as shown by the reduction of serum LDH levels from 2200 ± 1034 U/L (mean ± SD) at baseline to 700 ± 388 U/L by week one and maintained the effect through the end of the study at week 26 (327 ± 433 U/L). In the single arm clinical study, Soliris maintained this effect through 52 weeks [*see Clinical Studies (14)*].

12.3 Pharmacokinetics

A population PK analysis with a standard 1-compartmental model was conducted on the multiple dose PK data from 40 PNH patients receiving the recommended Soliris regimen [*see Dosage and Administration (2.1)*]. In this model, the clearance of Soliris for a typical PNH patient weighing 70 kg was 22 mL/hr and the volume of distribution was 7.7 L. The half-life was 272 ± 82 hrs (mean ± SD). The mean observed peak and trough serum concentrations of Soliris by week 26 were 194 ± 76 mcg/mL and 97 ± 60 mcg/mL, respectively.

Studies have not been conducted to evaluate the PK of Soliris in special patient populations identified by gender, race, age (pediatric or geriatric), or the presence of renal or hepatic impairment.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been conducted to evaluate the carcinogenic and genotoxic potential of Soliris. Effects of Soliris upon fertility have not been studied in animals. Intravenous injections of male and female mice with a murine anti-C5 antibody at up to 4-8 times the equivalent of the clinical dose of Soliris had no adverse effects on mating or fertility.

14 CLINICAL STUDIES

The safety and efficacy of Soliris in PNH patients with hemolysis were assessed in a randomized, double-blind, placebo-controlled 26 week study (Study 1); PNH patients were also treated with Soliris in a single arm 52 week study (Study 2); and in a long term extension study. Patients received meningococcal vaccination prior to receipt of Soliris. In all studies, the dose of Soliris was 600 mg study drug every 7 ± 2 days for 4 weeks, followed by 900 mg 7 ± 2 days later, then 900 mg every 14 ± 2 days for the study duration. Soliris was administered as an intravenous infusion over 25 - 45 minutes.

Study 1:

PNH patients with at least four transfusions in the prior 12 months, flow cytometric confirmation of at least 10% PNH cells and platelet counts of at least 100,000/microliter were randomized to either Soliris (n = 43) or placebo (n = 44). Prior to randomization, all patients underwent an initial observation period to confirm the need for RBC transfusion and to identify the hemoglobin concentration (the "set-point") which would define each patient's hemoglobin stabilization and transfusion outcomes. The hemoglobin set-point was less than or equal to 9 g/dL in patients with symptoms and was less than or equal to 7 g/dL in patients without symptoms. Endpoints related to hemolysis included the numbers of patients achieving hemoglobin stabilization, the number of RBC units transfused, fatigue, and health-related quality of life. To achieve a designation of hemoglobin stabilization, a patient had to maintain a hemoglobin concentration above the hemoglobin set-point and avoid any RBC transfusion for the entire 26 week period. Hemolysis was monitored mainly by the measurement of serum LDH levels, and the proportion of PNH RBCs was monitored by flow cytometry. Patients receiving anticoagulants and systemic corticosteroids at baseline continued these medications.

Major baseline characteristics were balanced (see table 2).

TABLE 2
STUDY 1 PATIENT BASELINE CHARACTERISTICS

	Study 1	
Parameter	Placebo N = 44	Soliris N = 43
Mean age (SD)	38 (13)	42 (16)
Gender - female (%)	29 (66)	23 (54)
History of aplastic anemia or myelodysplastic syndrome (%)	12 (27)	8 (19)
Patients with history of thrombosis (events)	8 (11)	9 (16)
Concomitant anticoagulants (%)	20 (46)	24 (56)
Concomitant steroids/immunosuppressant	16 (36)	14 (33)

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Parameter	Study 1 Placebo N = 44	 Soliris N = 43
treatments (%)		
Packed RBC units transfused per patient in previous 12 months (median (Q1,Q3))	17 (14, 25)	18 (12, 24)
Mean hgb level (g/dL) at setpoint (SD)	8 (1)	8 (1)
Pre-treatment LDH levels (median, U/L)	2,234	2,032
Free hemoglobin at baseline (median, mg/dL)	46	41

Patients treated with Soliris had significantly reduced ($p < 0.001$) hemolysis resulting in improvements in anemia as indicated by increased hemoglobin stabilization and reduced need for RBC transfusions compared to placebo treated patients (see table 3). These effects were seen among patients within each of the three pre-study RBC transfusion strata (4 - 14 units; 15 - 25 units; > 25 units). After 3 weeks of Soliris treatment, patients reported less fatigue and improved health-related quality of life. Because of the study sample size and duration, the effects of Soliris on thrombotic events could not be determined.

TABLE 3
STUDY 1 RESULTS

	Placebo N = 44	Soliris N = 43
Percentage of patients with stabilized hemoglobin levels	0	49
Packed RBC units transfused per patient (median)	10	0
(range)	(2 - 21)	(0 - 16)
Transfusion avoidance (%)	0	51
LDH levels at end of study (median, U/L)	2,167	239
Free hemoglobin at end of study (median, mg/dL)	62	5

Study 2 and Extension Study:
PNH patients with at least one transfusion in the prior 24 months and at least 30,000 platelets/microliter received Soliris over a 52-week period. Concomitant medications included anti-thrombotic agents in 63% of the patients and systemic corticosteroids in 40% of the patients. Overall, 96 of the 97 enrolled patients completed the study (one patient died following a thrombotic event). A reduction in intravascular hemolysis as measured by serum LDH levels was sustained for the treatment period and resulted in a reduced need for RBC transfusion and less fatigue. 187 Soliris-treated PNH patients were enrolled in a long term extension study. All patients sustained a reduction in intravascular hemolysis over a total Soliris exposure time ranging from 10 to 54 months. There were fewer thrombotic events with Soliris treatment than during the same period of time prior to treatment. However, the majority of patients received concomitant anticoagulants; the effects of anticoagulant withdrawal during Soliris therapy was not studied [*see Warnings and Precautions (5.4)*].

16 HOW SUPPLIED / STORAGE AND HANDLING

Soliris (eculizumab) is supplied as 300 mg single-use vials containing 30 mL of 10 mg/mL sterile, preservative-free Soliris solution per vial.

Soliris vials must be stored in the original carton until time of use under refrigerated conditions at 2-8° C (36-46° F) and protected from light. Do not use beyond the expiration date stamped on the carton. Refer to *Dosage and Administration* (2) for information on the stability and storage of diluted solutions of Soliris.

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DO NOT FREEZE. DO NOT SHAKE.

NDC 25682-001-01 Single unit 300 mg carton: Contains one (1) 30 mL vial of Soliris (10 mg/mL).

17 PATIENT COUNSELING INFORMATION

See Medication Guide.

Prior to treatment, patients should fully understand the risks and benefits of Soliris, in particular the risk of meningococcal infection. Ensure that patients receive the Medication Guide.

Patients should be informed that they are required to receive a meningococcal vaccination at least 2 weeks prior to receiving the first dose of Soliris, if they have not previously been vaccinated. They are required to be revaccinated according to current medical guidelines for meningococcal vaccine use while on Soliris therapy. Patients should also be informed that vaccination may not prevent meningococcal infection. Patients should be educated about any of the signs and symptoms of meningococcal infection, and strongly advised to seek immediate medical attention if these signs or symptoms occur. These signs and symptoms are as follows:

- moderate to severe headache with nausea or vomiting
- moderate to severe headache and a fever
- moderate to severe headache with a stiff neck or stiff back
- fever of 103° F (39.4° C) or higher
- fever and a rash
- confusion
- severe muscle aches with flu-like symptoms, and eyes sensitive to light

Patients should be informed that they would be provided with the Patient Safety Card that they should carry with them at all times. This card describes symptoms which, if experienced, should prompt the patient to immediately seek medical evaluation.

Patients should be informed that there is a potential for serious hemolysis when Soliris is discontinued and that they will be monitored by their healthcare professional for at least 8 weeks following Soliris discontinuation.

Manufactured by:

Alexion Pharmaceuticals, Inc.

352 Knotter Drive

Cheshire, CT 06410 USA

US License Number 1743

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Patients receiving Soliris treatment should carry this card at all times.

INFORMATION FOR THE PATIENT

You should call your doctor (#_____) right away if you have any of these symptoms:

- **moderate to severe headache with nausea or vomiting**
- **moderate to severe headache and a fever**
- **moderate to severe headache with a stiff neck or stiff back**
- **fever of 103° F (39.4° C) or higher**
- **fever and a rash**
- **confusion**
- **severe muscle aches with flu-like symptoms, and eyes sensitive to light**

If you cannot reach your doctor right away, go to an emergency room and show them this card.

INFORMATION FOR THE PHYSICIAN

This patient was prescribed Soliris (eculizumab) therapy. Soliris is an antibody that inhibits terminal complement activation and prevents the formation of C5b-9 complex. Before beginning treatment, this patient should have received a meningococcal vaccine, but may still be susceptible to meningococcal infections.

For more information about Soliris, see the full prescribing information available at www.soliris.net\soliris.pdf, or call 1-888-SOLIRIS (1-888-765-4747)

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Food and Drug Administration
Center for Drug Evaluation and Research
Office of Biotechnology Products

FACSIMILE TRANSMITTAL SHEET

DATE: May 8, 2009

To: Mr. Arthur D. Edwards	**From:** LCDR Andrew Shiber Project Manager
Company: Alexion Pharmaceuticals Corporation	Office of Biotechnology Products
Fax number: 203-271-8191	**Fax number:** (301) 796-9743
Phone number: 203-272-2596	**Phone number:** (301) 796-4798

Subject: STN 125166/36

Total no. of pages including cover: 2

Comments: Please find attached the action letter for the above supplement for Soliris®(eculizumab).

Document to be mailed: X☐ YES ☐ NO

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

STN 125166-36

May 8, 2009

Alexion Pharmaceuticals
Attention: Mr. Arthur D. Edwards
Executive Director, Regulatory Affairs
352 Knotter Drive
Cheshire, CT 06410

Dear Mr. Edwards:

Your request to supplement your biologics license application for Soliris®(eculizumab), to extend the shelf-life of the drug product from 24 to 30 months when stored at 2 – 8 degrees Celsius, has been approved.

Please refer to http://www.fda.gov/cder/biologics/default.htm for information regarding therapeutic biological products, including the addresses for submissions.

This information will be included in your biologics license application file.

Sincerely,

Kathleen A. Clouse

Kathleen A. Clouse, Ph.D.
Director
Division of Monoclonal Antibodies
Office of Biotechnology Products
Office of Pharmaceutical Science
Center for Drug Evaluation and Research

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D20 Table 1 and D21 Table 1

Antibody	Type	Ab concentration mg/ml	Formulation
Satumomab pendetide	IgG1k (murine, conjugated to GYK-DTPA)	0.5	PBS
Indium-111 capromab pendeti	IgG1k (murine, conjugated to GYK-DTPA)	0.5	PBS
Muromomab-CD3	**IgG2a (murine)**	**1**	**Monobasic Na P, dibasic Na P, NaCl, PS80**
I-131 tositumab	**IgG2 gamma (murine)**	**1.1**	**Phosphate, povidone, maltose, NaCl, ascorbic acid**
Ibritumomab-Tiuxetan	IgG1k (murine, thiourea covalent linkage to Tiuxetan)	1.6	Na acetate, K P, NaCl
Cetuximab	IgG1k (chimeric human/mouse)	2	Dibasic Na P septahydrate, monobasic Na P monohydrate, NaCl
Abciximab	Fab, IgG1 (chimeric human/murine)	2	Na P, NaCl, PS80
Daclizimab	IgG1 (humanized)	5	Monobasic Na P monohydrate, dibasic Na P septahydrate, NaCl, PS80
Alemtuzumab	IgG1k (humanized)	10	Dibasic Na P, monobasic K P, NaCl, KCl, Na2EDTA, PS80
Ranibizumab	IgG1k (humanized)	10	His HCl, alpha-trehalose dihydrate, PS20
Rituximab	IgG1k (chimeric mouse/human)	10	Na citrate dihydrate, NaCl, PS80
Nofetumomab	Fab (murine)	10	PBS
Tositumomab	**IgG2 gamma (murine)**	**14**	**Phosphate, NaCl, maltose**
Natalizumab	**IgG4k (humanized)**	**20**	**Monobasic Na P, dibasic NaP septahydrate, NaCl, PS80**
Bevacizumab	IgG1 (humanized)	25	Monobasic Na P hydrate, dibasic Na P anhydrous, alpha-trehalose dihydrate, PS 20
Adalimumab	IgG1k (human)	50	Monobasic Na P dihydrate, dibasic Na P dihydrate, Na citrate, citric acid hydrate, NaCl, mannitol, PS80
Acrituomab	Murine Fab	powder	Stannous chloride, K Na tartrate tetrahydrate, Na acetate trihydrate, NaCl, glacial acetic acid, HCl, sucrose
Gemtuzumab ozogamicin	**IgG4k (conjugated with alicheamicin)**	**powder**	**Monobasic and dibasic Na P, dextran 40, sucrose, NaCl**
Basiliximab	IgG1k (chimeric)	powder	Monobasic K P, Na2HPO4, NaCl, sucrose, mannitol, glycine, PS80
Infliximab	IgG1 (chimeric human/murine)	powder	Monobasic Na P monohydrate, dibasic Na P dihydrate, sucrose, PS80
Trastuzumab	IgG1k (humanized)	powder	L-His HCl, L-His, alpha trehalose dihydrate, PS20 (benzyl alcohol as preservative)
Efalizumab*	IgG1 (humanized)	powder	L-His HCl monohydrate, L-His, sucrose, PS20
Palivizumab	IgG1k (humanized, CDR of murine Mab)	powder	His, Gly, mannitol
Omalizumab	IgG1k (humanized)	powder	L-His HCl, L-His, sucrose, PS20
Eculizumab	**IgG2/4**	**10 mg/ml solution**	**Na P, NaCl, PS80**

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EUROPEAN MEDICINES AGENCY
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An Agency of the European Union

28/11/2017

Medicine Name	Active Substance	Status	Authorisation date	Biosimilar	Product concentration mg/ml
Zevalin	ibritumomab tiuxetan	Authorised	16/01/2004	no	1.6
Dinutuximab beta EUSA (previously Dinutuximab beta Apeiron)	dinutuximab beta	Authorised	08/05/2017	no	4.5
Erbitux	cetuximab	Authorised	29/06/2004	no	5
Yervoy	ipilimumab	Authorised	13/07/2011	no	5
Stelara	ustekinumab	Authorised	16/01/2009	no	5
Lemtrada	alemtuzumab	Authorised	12/09/2013	no	10
Soliris	eculizumab	Authorised	20/06/2007	no	10
Opdivo	nivolumab	Authorised	19/06/2015	no	10
Lartruvo	olaratumab	Authorised	09/11/2016	no	10
Cyramza	ramucirumab	Authorised	19/12/2014	no	10
Lucentis	ranibizumab	Authorised	22/01/2007	no	10
Cinqaero	reslizumab	Authorised	16/08/2016	no	10
MabThera	rituximab	Authorised	02/06/1998	no	10
Portrazza	necitumumab	Authorised	15/02/2016	no	16
Bavencio	avelumab	Authorised	18/09/2017	no	20
Darzalex	daratumumab	Authorised	20/05/2016	no	20
Tysabri	natalizumab	Authorised	27/06/2006	no	20
Arzerra	ofatumumab	Authorised	19/04/2010	no	20
Vectibix	panitumumab	Authorised	03/12/2007	no	20
RoActemra	tocilizumab	Authorised	16/01/2009	no	20
Avastin	bevacizumab	Authorised	12/01/2005	no	25
Zinplava	bezlotoxumab	Authorised	18/01/2017	no	25

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Gazyvaro	obinutuzumab	Authorised	23/07/2014	no	25
Perjeta	pertuzumab	Authorised	04/03/2013	no	30
Humira	adalimumab	Authorised	08/09/2003	no	50
Praxbind	idarucizumab	Authorised	20/11/2015	no	50
Tecentriq	atezolizumab	Authorised	21/09/2017	no	60
Prolia	denosumab	Authorised	26/05/2010	no	60
Xgeva	denosumab	Authorised	13/07/2011	no	70
Taltz	ixekizumab	Authorised	25/04/2016	no	80
Simponi	golimumab	Authorised	01/10/2009	no	100
Kyntheum	brodalumab	Authorised	17/07/2017	no	140
Repatha	evolocumab	Authorised	17/07/2015	no	140
Zinbryta	daclizumab	Authorised	01/07/2016	no	150
Dupixent	dupilumab	Authorised	27/09/2017	no	150
Cimzia	certolizumab pegol	Authorised	01/10/2009	no	200
Kevzara	sarilumab	Authorised	23/06/2017	no	131.6 or 175
Praluent	alirocumab	Authorised	23/09/2015	no	75 or 150
Simulect	basiliximab	Authorised	09/10/1998	no	powder
Benlysta	belimumab	Authorised	13/07/2011	no	powder
Scintimun	besilesomab	Authorised	11/01/2010	no	powder
Blincyto	blinatumomab	Authorised	23/11/2015	no	powder
Adcetris	brentuximab vedotin	Authorised	25/10/2012	no	powder
Ilaris	canakinumab	Authorised	23/10/2009	no	powder
Empliciti	elotuzumab	Authorised	11/05/2016	no	powder
Remicade	infliximab	Authorised	13/08/1999	no	powder
Besponsa	inotuzumab ozogamicin	Authorised	29/06/2017	no	powder
Nucala	mepolizumab	Authorised	02/12/2015	no	powder
Xolair	omalizumab	Authorised	25/10/2005	no	powder
Synagis	palivizumab	Authorised	13/08/1999	no	powder
Keytruda	pembrolizumab	Authorised	17/07/2015	no	powder
Cosentyx	secukinumab	Authorised	15/01/2015	no	powder
Sylvant	siltuximab	Authorised	22/05/2014	no	powder
LeukoScan	sulesomab	Authorised	14/02/1997	no	powder
Herceptin	trastuzumab	Authorised	28/08/2000	no	powder
Kadcyla	trastuzumab emtansine	Authorised	15/11/2013	no	powder
Entyvio	vedolizumab	Authorised	22/05/2014	no	powder

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Consolidated List of Documents cited in opposition to EP2359834 B1				
Document Name	Consolidated Number	Patentee	Opponent 1 (Amgen)	Opponent 2 (Hoffman Eitle)
US 2005/191298 A1	D1		D1	D1
Hillmen *et al.*, (2003) *J. Hematol.* 121 (Suppl. 1): 87-95	D2		D2	D2
Hillmen *et al.*, (2004) *N. Engl. J. Med.* 350(6): 552-559	D3		D3	D3
Harding. (2004) *Drugs Future*. 29(7): 673-676	D4		D4	D4
US 2005/221382 A1	D5		D5	D5
Hill. (2005) *Clin. Adv. Hematol. Oncol.* 3(11): 849-50	D6		D6	D6
Morris *et al.*, (1992) *South. Med. J.* 85(19): 1030-1031	D7		D7	
Corvini *et al.*, (2004) *Ann. Allerg. Asth. Immunol.* 93(2): 200-205	D8		D8	
Sierra *et al.*, (1991) *NIPH Annals.* 14(2): 195-210	D9		D9	
US Pharmacopeia (USP29-NF24), page 2989	D10		D10	
Adis Data Information. (2007) *Drugs R. D.* 8(1): 61-68	D11		D11	
Hill *et al.*, (2005) *Blood.* 106(7): 2559-2565	D12		D12	
Thomas *et al.*, (1996) *Mol. Immunol.* 33(17/18): 1389-1401	D13		D13	D8
Mueller *et al.*, (1997) *Mol. Immunol.* 34(6): 441-452	D14		D14	
WO 97/11971 A1	D15		D15	
WO 2005/007809 A2	D16		D16	
US 6,355,245 B1	D17		D17	D7
Hillmen *et al.*, (2006) *N. Engl. J. Med.* 355(12): 1233-1243	D18		D18	
EMEA, CPMP, 20 February 2003, *Note for guidance on excipients, antioxidants and antimicrobial preservatives in the dossier for application of marketing authorization of a medicinal product.*	D19		D19	
Wang *et al.*, (2007) *J. Pharm. Sci.* 96(1): 1-26 (January 2007)	D20		D20	D10
Daugherty *et al.*, (2006) *Adv. Drug Del. Rev.* 58: 686-706	D21		D21	
US 2005/0271660 A1	D22		D22	
Expert Declaration by Dr Michael Treuheit	D23		D23	

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Soliris®(eculizumab) *Highlights of prescribing information.*	D24		D24	
US 2012/0230982 A1	D25		D25	
WO 2007/002571 A2	D26		D26	
Hill *et al.*, (2004) *Blood*. 104: 2823-2824	D27		D27	
US 2003/0202972 A1	D28			D9
US 6,171,586 B1	D29			D11
Maloney *et al.*, (1997) *Blood*. 90(6): 2188-2195	D30			D12
Commercial monoclonal antibodies 2006 (all modes of administration)	D31			Annex I
Commercial monoclonal antibodies 2006 (I.V. administration)	D32			Annex II
Declaration of Dr Miano	D33	D33		
Rouet *et al.*, (2014) *FEBS Letters*. 588: 269-277	D34	D34		
Summary of antibodies listed in D20/D21 Tables	D35	D35		
Graphical representation of D20 Table 2	D36	D36		
Summary of antibody products approved by EMA since filing date	D37	D37		
Extracts from Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991)	D38	D38		

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Acknowledgement of receipt

We hereby acknowledge receipt of the following submission by the proprietor:

Submission number	6164848
Application number	EP11001632.6
Patent number	EP2359834
Date of receipt	22 March 2018
Your reference	C411599EP
Proprietor	Alexion Pharmaceuticals, Inc
Title	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement

Documents submitted

package-data.xml	ep-opposition-data.xml
ep-oppo.pdf (2 p.)	OTHER-1.PDF\Consolidated List of Documents.PDF (2 p.)
OBSO3.PDF\Response to Notice of Opposition 22 Mar 18 C411599EP PXT_ALS.PDF (23 p.)	OTHER-2.PDF\Enclose Response to Oppositions C411599EP PXT ALS.PDF (1 p.)
Other-evidence-4.pdf\D36 Graphical Representation of D20 Table 2.pdf (1 p.)	Other-evidence-5.pdf\D34 Rouet et al.pdf (9 p.)
Other-evidence-3.pdf\D35 Summary of Antibodies listed in D20 and D21 Tables.pdf (1 p.)	Other-evidence-1.pdf\D33 Declaration of Dr Miano C411599EP.pdf (27 p.)
Other-evidence-6.pdf\D38 Kabat Exhibit for Response C411599EP.pdf (10 p.)	Other-evidence-2.pdf\D37 EMA Approved Antibodies C411599EP.pdf (2 p.)

Submitted by	CN=Amanda Simons 23469

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Date: 27.03.2018

Reference	Application No./Patent No.
198 441 u6/ne OPPO 02	11001632.6 - 1112 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

BRIEF COMMUNICATION

Subject:
- ☐ Your letter of
- ☐ Our telephone conversation of
- ☐ Communication of
- ☐

Enclosure(s):
- ☒ Letter from the proprietor of the patent of 22.03.2018
- ☐ Letter from the opponent of
- ☐ Copy (copies)

Communication:

☐ A letter from the opponent /proprietor was received on ..... . The documents specified as patent documents in this letter are available via the European Patent Register at **www.epo.org/register.** Should you wish to receive these documents in paper format, they will be supplied to you free of charge if specifically requested on receipt of this communication (OJ EPO 2009, 434).

☐

For the Opposition Division

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☐ **Registered letter**

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Submission in opposition proceedings

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- representing the proprietor(s):	Alexion Pharmaceuticals, Inc
Proprietor/representative's reference	C411599EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Reply of the patent proprietor to the notice(s) of opposition	Response to Notice of Opposition 22 Mar 18 C411599EP PXT_ALS.PDF	OBSO3.PDF
2	Any annexes (other than citation) to an opposition letter - Consolidated list of documents	Consolidated List of Documents.PDF	OTHER-1.PDF
3	Any annexes (other than citation) to an opposition letter - Cover letter	Enclose Response to Oppositions C411599EP PXT ALS.PDF	OTHER-2.PDF

Evidence filed subsequently:

D33	Other evidence	Declaration of Dr Miano C411599EP

		original file name: D33 Declaration of Dr Miano C411599EP.pdf attached as: Other-evidence-1.pdf
D34	Other evidence	Rouet et al Stability Engineering of the Human Antibody Repertoire original file name: D34 Rouet et al.pdf attached as: Other-evidence-5.pdf
D35	Other evidence	Summary of Antibodies listed in D20 and D21 Tables original file name: D35 Summary of Antibodies listed in D20 and D21 Tables.pdf attached as: Other-evidence-3.pdf
D36	Other evidence	Graphical Representation of D20 Table 2 original file name: D36 Graphical Representation of D20 Table 2.pdf attached as: Other-evidence-4.pdf
D37	Other evidence	EMA Approved Antibodies C411599EP original file name: D37 EMA Approved Antibodies C411599EP.pdf attached as: Other-evidence-2.pdf
D38	Other evidence	Extracts from Kabat et al., Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991) original file name: D38 Kabat Exhibit for Response C411599EP.pdf attached as: Other-evidence-6.pdf

Signatures

Place:	
Date:	**22 March 2018**
Signed by:	**Amanda Simons 23469**
Association:	**J A Kemp**
Representative name:	**/Amanda Simons/**
Capacity:	**(Representative)**

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BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
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22 March 2018

Dear Sirs

Opposition to European Patent No. 2359834
(Application No. 11001632.6)
ALEXION PHARMACEUTICALS, INC.
Our Ref: C411599EP PXT/ALS

In response to the Communication dated 13 September 2017, I attach the Patentee's response to the two Oppositions. Attached are:

- the Response;
- documents D33 to D38 as listed in section 2 of the response; and
- a consolidated list of documents D1 to D38.

The Patentee's requests are set out in section 1 of the response. Specifically, it is requested that the Opposition Division maintains the patent as granted. If the Opposition Division is minded to refuse this request, I request that oral proceedings be appointed. If oral proceedings are appointed, I shall speak in English and shall require interpretation into English should any other party speak in a language other than English.

Yours faithfully

Electronically Signed
DR PAMELA M TUXWORTH
ASSOCIATION NO. 811

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J A • K E M P

22 March 2018

Our Ref: C411599EP

RESPONSE TO NOTICE OF OPPOSITION

Patent No:	2359834	
Application No:	11001632.6	
Patentee:	Alexion Pharmaceuticals, Inc.	
Opponents:	(1)	Amgen Inc.
	(2)	Hoffmann Eitle

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1 REQUESTS

1.1 **Main Request**

1.1.1 The Patent Proprietor requests that the Opposition Division maintain the Patent as granted.

1.1.2 If the Opposition Division contemplates any decision other than the maintenance of the Patent as granted, then Oral Proceedings are requested. If Oral Proceedings are appointed, I shall speak in English. I shall require interpretation into English should any other party speak in a language other than English.

1.2 **Auxiliary Requests**

1.2.1 The Patent Proprietor requests the opportunity to file further claim requests if it is appropriate to do so during these Opposition Proceedings.

2 DOCUMENTS

2.1 The following documents are cited:

D33 Declaration of Dr Miano

D34 Rouet *et al*; FEBS Letters 588 (2014) 269-277

D35 Summary of antibodies listed in D20/D21 Tables

D36 Graphical representation of D20 Table 2

D37 Summary of antibody products approved by EMA since the filing date

D38 Extracts from Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991)

2.2 We also enclose a consolidated list of all of the documents cited by both Opponents, and including the additional documents mentioned above.

3 No Added Subject Matter

3.1 Opponent 1 (O1) argues that claim 1 includes added subject matter compared with the application as filed. O1's reasoning is that claim 1 of the patent as granted does not include the term "single unit dosage form" which is found both in the passage on page 5, lines 3 to 9 and that on page 22, lines 7 to 14 of the application as filed. O1 argues that this is presented as an essential element of the pharmaceutical composition and

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therefore its omission from claim 1 leads to inadmissible subject matter. Notably, Opponent 2 (O2) has not raised the same objection and makes no argument that the claim as granted includes added subject matter.

3.2 The Patentee disagrees with O1's position. Basis for the wording used in claim 1 of the patent as granted is provided at the following passages of the application as filed:

- Page 5, lines 9 to 10: *"In certain embodiments, the 300mg single-use dosage form comprises 30ml of a 10mg/ml sterile, preservative free solution."*
- Page 6, lines 21 to 23: *"In certain embodiments, the pharmaceutical composition comprises a 300mg single-use formulation of 30ml of a 10mg/ml sterile, preservative free solution."*
- Page 22, lines 11 to 13: *"In certain embodiments, the pharmaceutical composition comprises a 300mg single-use formulation of 30ml of a 10mg/ml sterile, preservative free solution."*

3.3 As is apparent from the above extracts from the application as filed, in none of these passages is the term "single-unit dosage form" presented as an essential feature. Accordingly, these passages are sufficient to provide basis for the wording used in claim 1 of the present patent. There is no addition of subject matter in contravention of Article 123 (2) EPC.

3.4 For completeness, it is noted that the Patentee disagrees that D10 supports O1's argument. D10 does not define either the terms "single-use" or "single-unit dosage form". For at least this reason, D10 is therefore not relevant to the interpretation of these terms.

4 Inventive Step: State of the Art

4.1 The inventive step of the claimed invention will be discussed with reference to the problem and solution approach in section 5 below. It is useful, however, firstly to provide a discussion of the situation in the art at the time the invention was made, in particular the general knowledge of the skilled person in the art in relation to eculizumab and formulating antibodies in general.

4.2 *Eculizumab and treatment of PNH*

4.2.1 Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematologic disease which results in PNH blood cells which have a partial or complete deficiency of GPI-anchored proteins. Anemia is a significant problem for PNH patients leading to an impaired quality of life which is disproportionate to the degree of anemia. Many PNH patients depend on blood transfusions to maintain adequate haemoglobin levels.

4.2.2 The nature of PNH is such that patients, once identified, need to start on treatment very quickly. Thus, the required drug, in the present case

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eculizumab, must be available and ready for use as soon as possible. It is therefore stored on site or at a location nearby (within a certain radius) such that it is essentially ready for use when a patient is identified.

4.2.3 PNH is, however, an ultra-rare disease, so the frequency with which patients are diagnosed is low. The person of skill in the art of PNH treatment would therefore be aware of the need for a long shelf-life product, in order that it can be stored where it is needed for a long period of time without loss of drug activity.

4.2.4 Eculizumab has been identified as a useful treatment for PNH and is the first antibody of its kind to be brought to market. Prior to eculizumab, the vast majority of antibodies brought to market were of IgG1 type, or occasionally IgG2 or IgG4. Eculizumab is none of these IgG types. Instead, appended to eculizumab's variable region is a unique, non-naturally occurring CH1-hinge-CH2-CH3 sequence. No analogously-engineered antibodies were even in development at the filing date of the patent and certainly none had been approved for clinical use. Even today there are no antibodies on the market with the same uniquely-engineered CH1-hinge-CH2-CH3 sequence as eculizumab.

4.3 *Formulation of Antibodies is a Complex Challenge*

4.3.1 A number of antibody drugs were in development at the time the present invention was made, and a small number had already reached the market. However, the issue of the formulation of antibody drugs was at the time regarded as a highly complex and unpredictable issue. As with other protein drugs, it is very important to maintain the integrity of the antibody and to minimize any possible degradation pathways. Therefore, in order to maintain activity of the antibody, both in storage and for administration, a balance is required between maintaining functionality of the antibody and minimizing the inevitable processes of protein degradation that occur in solution. Providing formulations in which antibodies maintain good stability, however, had proved to be a challenging problem.

4.3.2 These issues are discussed in D20 and D21, review articles published in 2007 and 2006 respectively. As Wang *et al* put it in the abstract of D20:

> *"Although antibodies share certain structural similarities, development of commercially viable antibody pharmaceuticals has not been straightforward because of their unique and somewhat unpredictable solution behaviour."*

4.3.3 The various different degradation pathways to which antibodies may be susceptible are described in the section beginning at page 8 onwards of D20. These include denaturation, aggregation, surface adsorption, disulphide bond formation/exchange, non-reducible cross-linking, deamidation, isomerisation, oxidation, formation of acidic or basic species, C-terminal clipping, fragmentation and Maillard reaction. The skilled person looking to formulate an antibody therefore has a multitude of

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different problems to consider, each of which will apply in different ways to each individual antibody.

4.3.4 Of course, it is not the case that only one degradation pathway will necessarily dominate for any one antibody. In the right-hand column, top paragraph of D20, Wang *et al* states that:

> *"In many cases, multiple degradation pathways can occur at the same time and the degradation mechanism may change depending on the stress conditions."*

4.3.5 Moreover, the strategies available to minimise the various degradation pathways may conflict with one another. For instance, deamidation, a major degradation pathway in antibodies, has been shown to have a strong pH-dependence, with less deamidation occurring at pH 4 compared to pH 7 (D20, page 12, right-hand column penultimate paragraph). In contrast, antibody precipitation can be a significant problem at low pH values. D20 reports (page 11, left-hand column first paragraph) that incubation of two mouse monoclonal antibodies (IgG2a and IgG1) at 37°C for 32 days caused precipitation at pH 3 and pH 4 solutions for both antibodies. It is therefore difficult to create a stable antibody formulation due to the numerous degradation pathways possible, and the potentially conflicting strategies available to the formulation chemist for dealing with each of them in the context of each individual antibody.

4.3.6 This issue is also highlighted in D21. In the passage set out below, the authors comment not only on this issue but also on the critical need to devise a formulation that remains compatible with delivery:

> *"In general, **successful antibody formulation designs are simply the best compromise of minimizing several competing degradation pathways**. One approach is to find a pH where the minimal amount of degradation events such as deamidation and oxidation will occur. Another is to identify a buffer system that can stabilize the formulation at that pH without damaging the antibody. One common tactic taken to reduce the rate of many of these chemical degradation events is to prepare lyophilized or freeze-dried antibody preparation but even these can be compromised by aggregation issues due to thermodynamic stress that occurs during freezing, drying and reconstitution. A number of protective excipients, mostly carbohydrates, can reduce the propensity of antibodies to aggregate during these stressful events. **Another issue is to produce a formulation that is compatible with delivery — neither hypotonic — nor hypertonic solutions are well-tolerated upon injection.**"* (D21, page 701 right-hand column, first full paragraph emphasis added)

4.4 *Each Antibody is Unique*

4.4.1 Despite the various structural similarities between some of the different antibody drugs available in 2007, different antibodies have significantly

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different behaviours and each requires completely separate study in terms of its formulation. Daugherty et al put it like this in D21:

> " *A priori, one might assume that by finding a stable formulation for one of these antibody drugs, that such a formulation would be good for most if not all, similar antibodies. If this were borne out by experience, there would be no need for a review such as this.* ***Instead, each antibody seems to have a unique personality relating to its requirements for stability****; a phenomenon derived from the fact that the small differences between these antibodies are focused on surface-exposed amino acid differences that stipulate antigen specificity. Thus, the interfacial surface of each antibody drug is unique and thus requires specific formulation components to provide maximal stability and retention of activity."* (D21, page 690, right-hand column, first paragraph, emphasis added)

4.4.2 This need to treat even very similar antibodies individually in terms of their stability characteristics is illustrated at page 693, left-hand column of D21. Here the authors describe a recombinant monoclonal antibody that recognises vascular endothelial growth factor (rhMab-VEGF) which was studied for its kinetic and thermodynamic aspects of antibody solution dimerization. The results are reported by Daugherty et al as follows:

> *"It was found that for this IgG1 class antibody, aggregation rates were greater in slightly alkaline (pH 7.5-8.5) compared to slightly acidic (pH 6.5-7.5) conditions. A high salt environment (1 M NaCl) also enhanced dimerization. .... One of the most striking aspects of these dimerization studies on rhMab-VEGF is that trastuzumab, another genetically engineered antibody with the same IgG1 framework and a 92% sequence homology with rhMab- VEGF, fails to demonstrate aggregation events under the same conditions."* (D21, page 693, left-hand column)

4.4.3 This example therefore illustrates that antibodies having very close structural similarity can behave in very different ways in terms of their degradation pathways. Strategies to provide stable antibody formulations therefore must be considered on a case by case basis for each individual antibody.

4.4.4 It is also notable that the vast majority of the discussion of both D20 and D21 relates to IgG1 antibodies. There is a brief discussion in the first paragraph of page 689 of D21 of engineered antibody molecules. D21 notes that such structurally-different antibodies could have very different properties. D21 goes on to state that:

> *"as these new approaches gain prominence, more information will be acquired about their unique requirements for successful formulation and delivery. At present, however, there is little information on formulation approaches for antibodies produced using these production processes."*

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4.4.5 Therefore, the authors of D21 acknowledge that experience gained in relation to IgG1 antibodies may well not be applicable at all to structurally different antibodies, for example the non-natural, uniquely-engineered structure of eculizumab.

4.5 *No Single Technique Provides Stability*

4.5.1 It can be seen from the discussion above that the formulation of antibody therapeutics was a highly complex field, multiple degradation pathways needed to be taken into account and potentially conflicting strategies were available to the skilled person for dealing with these degradation pathways. Further, each antibody must be individually studied, since small differences in antibody structure were known to lead to significant differences in antibody stability and degradation behaviour. It follows, therefore, that no single technique, no single group of excipients, and no single formulation strategy was available to the skilled person to provide a stable antibody formulation. This is evident from the variety of strategies discussed in the art. Not only do D20 and D21 themselves describe a wide variety of different strategies for effecting antibody stability, but this is also apparent from other prior art documents cited by the Opponents. For example:

- D28 describes a technique for providing stable lyophilised protein formulations using a lyoprotectant such as sucrose or trehalose (see paragraph 8). This is exemplified only in two specific antibodies, an anti-HER2 antibody and an anti-IGE antibody.

- In D29 a very different strategy is used in which the antibodies are not subject to lyophilisation but particular formulation pH and excipients are considered. This strategy was applied to two specific antibodies, an anti-CD18 antibody and an anti-CD20 antibody. Notably, D29 provides an entirely separate study of the suitable pH, buffer and excipients for each of these two separate antibodies.

4.6 *Antibody Concentrations Used in Earlier Formulations*

4.6.1 The Opponents have argued that Table 1 of D20 and Table 1 of D21 demonstrate that antibodies were commonly formulated at 10mg/ml in the art and that therefore this would be an obvious choice of formulation concentration for eculizumab. That 10mg/ml is not the obvious choice for eculizumab is explained in detail below with reference to the problem and solution approach. However, it is useful here to consider what, if any, information the skilled person could derive from the Tables of D20 and D21 when considering suitable formulations for any future antibody drug.

4.6.2 The enclosed table D35 lists each antibody of Table 1 of D20 and Table 1 of D21. The type of antibody is listed, as well as the antibody concentration (in the form in which the product is stored), together with details of the formulation. D35 therefore summarises the information provided in Table 1 of D20 and Table 1 of D21, whilst additionally providing the antibody type. IgG2 and IgG4 antibodies are highlighted in bold.

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4.6.3 A number of observations can be made based on D35 regarding the antibody products on the market before the filing date of the patent:

- The concentrations of products provided in liquid form range from 1 to 50mg/ml with a wide variety of different concentrations being used for different products.
- Those products that can be stored in solution form include five products having a concentration from 1 to 2 mg/ml; four products having a concentration of 10 mg/ml; and three products having a concentration of 20 mg/ml or above.
- Eight products are provided in lyophilised form.
- Half of the antibodies provided in solution form were formulated at concentrations of 5 mg/ml or less.
- The vast majority of antibody drugs which had reached the market before the filing date are IgG1 antibodies.

4.6.4 It is difficult, if not impossible, to derive any pattern in terms of antibody formulation based on the wide variety of formulations used for marketed drugs. The only possible conclusion is that since each antibody is unique for its binding specificity to an antigen, a unique formulation must be developed individually for each antibody. It certainly cannot be concluded that 10 mg/ml is a "typical" concentration for an antibody formulation. Some commercial antibodies were formulated at 10 mg/ml, but the majority were not and it is not possible to determine from the information in Table 1 of D20 and Table 1 of D22 why each formulation was selected. There is no indication as to whether other future antibodies might or might not be suitable for formulation at 10 mg/ml.

4.6.5 D35 also shows that the largest group of antibodies are lyophilised. Again, there is no indication in the Tables of D20 and D21 as to why these particular products are lyophilised for storage. However, the first paragraph of the left-hand column of page 18 of D20 hints that this could be due to instabilities:

> "*Like most proteins, some antibodies are not stable enough in a liquid form and lyophilized dosage forms will have to be considered*".

4.6.6 The wide variety of different formulation types, concentrations and the varying use of lyophilisation which is apparent from D35 demonstrates the unpredictability of antibody formulation. Each antibody needs separate consideration, and the behaviour of other antibodies clearly has not in the past led to any consistency in the way that these products are formulated. The same is true for any future antibody product: the evidence of Table 1 of D20 and Table 1 of D21 is no guide to a suitable formulation for eculizumab.

4.6.7 This is particularly the case given that eculizumab is a non-natural, uniquely-engineered antibody, whilst the vast majority of those antibodies which had reached the market by the filing date were IgG1 type

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antibodies. There are only five examples of non-IgG1 antibodies on the market before the filing date. These were formulated at 1 mg/ml, 1.1 mg/ml, 14 mg/ml, 20 mg/ml and in lyophilised form. The skilled person simply cannot predict from this information a suitable formulation for any future non-IgG1 product. It is certainly not possible to predict from this information a suitable formulation type for a product such as eculizumab. No antibody of this type had reached the market before the filing date.

4.6.8 As well as providing information on the formulation of marketed antibodies, D20 also provides information on antibody stability, including aggregation, in Table 2. Table 2 lists five antibodies where the percent aggregates following storage of a liquid formulation is provided. In D36, the Patentee has plotted the concentration of the formulation against the percent aggregates for these five examples. It is notable that there is no clear trend evident from this graph. Aggregate formation does not vary linearly as antibody concentration changes. There is no direct relationship between these two parameters and thus there are other factors at play in determining the degree of aggregation. In particular, the tendency towards aggregation is very different for different antibodies.

4.6.9 In summary, formulation of antibody drugs remains to this day a challenging topic. There is no single technique or set of excipients and buffer/pH conditions, which formulation experts have at their disposal, which is guaranteed to lead to stability of any particular antibody product. Rather, each antibody has a unique biochemical behaviour. The process of developing a suitable formulation for an antibody, which maintains its biological integrity and activity over time, is one which needs to be carried out individually for each new antibody product. This is particularly the case for an antibody having a novel type of structure, such as the uniquely-engineered structure of eculizumab.

5 Inventive Step: Problem and Solution Approach

5.1 Closest Prior Art

5.1.1 Both Opponents have suggested that D1 is a suitable prior art document. The problem and solution analysis below is accordingly based on D1 as the closest prior art.

5.2 Difference from D1

5.2.1 The claims of the patent differ from the disclosure of D1 in that:

- The formulation is a single use 300mg dosage form
- The concentration is 10 mg/ml
- The volume is 30ml
- The solution is preservative free

5.3 *Technical Problem to be solved*

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5.3.1 The patent aims to provide treatments for PNH. Any such treatment must, to ensure efficacy, be provided in a form which maintains its biological integrity and activity during any period of storage. This is clearly important so that the drug remains an effective treatment for PNH. The treatment must also be in a suitable form for a clinician to administer to a patient.

5.3.2 The specific pharmaceutical composition of eculizumab set out in claim 1 of the patent provides the ideal balance of these properties. The composition is easy for the clinician to use: it is a liquid formulation so there is no need for reconstitution which brings inherent inconvenience for clinical staff and risks of incomplete dissolution. Further, the composition provides a balance of (a) excellent long-term storage stability without loss of biological integrity, (b) minimising the bulk of the stored product, and (c) a suitable concentration for use to provide convenient administration in an acceptable infusion time. All of these factors make the product an excellent treatment for PNH, which has led to the approval of the claimed composition as a treatment for PNH by the regulatory bodies.

5.3.3 It is, of course, critical that the antibody maintains its biological integrity and activity in the period between manufacture and use. Aggregation is a common indicator of antibody stability, and accordingly, any formulation for the treatment of PNH needs to have a low incidence of aggregation on long-term storage. The enclosed declaration by Doctor Miano (D33) provides evidence of the long-term stability of eculizumab 10 mg/ml formulations, and the improved stability of these formulations compared with 30 mg/ml eculizumab formulations.

5.3.4 In paragraph 2 and Exhibits 2 and 3 of D33, Dr Miano provides evidence demonstrating that the eculizumab formulation at 10 mg/ml (i.e. the commercial formulation, Soliris®) is stable for a total period of 48 months. The product is stable for 18 months when stored as the drug substance, i.e. in bulk, and for a further 30 months when stored as the drug product in individual vials. Even over this long storage period, the product meets the commercial requirement that at least 98% monomer antibody remains.

5.3.5 Dr Miano also provides data demonstrating the improved stability of the 10 mg/ml formulation compared with a formulation which is identical in all respects, except that the eculizumab concentration is 30 mg/ml. The studies were carried out at 2 to 8°C and also at 23 to 27°C. As Dr Miano explains at paragraph 5 of D33, these higher temperature stability studies provide a good indication of effects which might be seen at 2 to 8°C over a longer period of time.

5.3.6 As is apparent from Figure 1 of D33, after only six months storage at 23 to 27°C the 30 mg/ml concentration led to an unacceptable level of aggregate formation, with the % monomer dropping to below the threshold of 98% which is required for release. In contrast, the 10 mg/ml concentration maintained over 99% monomer under the same storage conditions.

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5.3.7 Similar trends are seen in the studies at 2 to 8°C, where the % monomer retained after storage is significantly higher for the 10 mg/ml concentration compared with the 30 mg/ml concentration. Based on this data, Dr Miano concludes that the 30 mg/ml concentration would not meet the release standards after 36 months of storage.

5.3.8 Dr Miano's evidence therefore demonstrates that the 10 mg/ml composition of eculizumab provides extremely good long-term stability such that the antibody maintains its integrity and biological activity. It also demonstrates that the 10 mg/ml concentration of eculizumab provides improved stability compared with other concentrations.

5.3.9 The 10 mg/ml concentration combines this enhanced stability with ease of use for the clinician due to the low-volume liquid formulation. Firstly, because it is a liquid formulation, there is no need for reconstitution. Secondly, simple dilution of product required by the label conveniently provides the required 600 or 900 mg administration dosages. Thirdly, the 600 or 900 mg doses required by the patient can be administered via IV infusion of only 120 or 180 ml of solution (following dilution for administration). This relatively low volume enables the drug to be administered by IV infusion over relatively short periods of time and without the need for high infusion volumes which are undesirable for the patient. Finally, the low volume of product in the storage vials (30 mL per vial) minimises the volume which must be stored on site at each clinic.

5.3.10 The technical problem which is solved by the invention is therefore the provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion times and which is convenient to store.

5.3.11 The solution to this problem is the 300 mg single-use dosage form comprising 30 mL of a 10mg/ml preservative-free solution.

5.4 *Response to Opponents' Comments on Technical Solution*

5.4.1 The Opponents argue that the 10 mg/ml concentration of the formulation is an arbitrary selection. This is not the case, for the reasons set out above: the 10 mg/ml formulation shows enhanced maintenance of biological integrity following long-term storage, combined with low volumes for convenient dilution, administration and storage. The formulation as claimed is therefore not only extremely effective but is improved over alternative formulations of eculizumab.

5.4.2 Opponent 1 has filed a declaration by Dr Treuheit (D23) which is said to show the equivalence of differing concentrations of eculizumab. The evidence provided by Dr Treuheit does not show the equivalence of the different concentrations for the following reasons:

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- Dr Treuheit tested stability only over a 13 week period. This is significantly less than the 48-month stability demonstrated for the 10 mg/ml eculizumab product in D33. As the Opposition Division will clearly appreciate, where stability tests are carried out for a very short period of time, differences between the samples tested may not be detected, or may be very small.

- Even at the short period of time over which Dr Treuheit's test was conducted, an increase in formation of aggregates can be seen at elevated temperature for both the 30 mg/ml and 50 mg/ml formulations. These trends support the 30 mg/mL data presented in Dr. Miano's declaration on behalf of the Patentee.

5.4.3 As Dr Treuheit explains in paragraph 5 of his declaration, aggregation of the antibody was studied by size-exclusion ultra high-performance liquid chromatography (SE-UHPLC). The results are shown in Appendix A to his declaration. The lower panel of Appendix A shows that the 30 mg/ml and 50 mg/ml formulations resulted in a reduced percentage of intact antibody after 13 weeks. In both cases, the percent antibody remaining after this period of time was lower than at base line. However, the 10 mg/ml formulation did not show any reduction in percent antibody after 13 weeks even after storage at 25°C.

5.4.4 This result is in accordance with the longer 6 month study carried out by Dr Miano where the 30 mg/ml concentration had a reduced proportion of intact antibody as storage progressed compared with the 10 mg/ml concentration.

5.4.5 The SEC data in Appendix A of Dr Treuheit's declaration shows that the 30 mg/ml and 50 mg/ml formulations had close to 98% antibody remaining after 13 weeks of storage at 25°C. This antibody content falls below the release parameter for commercial lots of eculizumab, indicating that this drug would not be sufficiently stable over this period of time for clinical use.

5.4.6 Opponent 1 also comments on D25 which discloses formulations of anti-C5 antibodies, including eculizumab, of various concentrations. Opponent 1 highlights formulations of eculizumab at various concentrations which were found to be stable. However, the evidence of D25 does not provide any indication of the relative stability of these high concentration formulations compared to the claimed 10 mg/ml formulation. As is clear from the formulation flow chart depicted in the abstract of D25, the formulation is changed on moving from the 10 mg/ml phosphate-based solution to the higher concentration solutions. Thus, it is not possible to directly compare the 10 mg/ml solutions with the higher concentration solutions of D25. Indeed, the skilled person might assume that the higher concentration solutions of eculizumab needed particular treatment, with variation of buffers and excipients, since simply increasing the concentration of the 10 mg/ml solution did not lead to sufficient stability.

5.4.7 In summary, in light of D1,

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- The claimed subject matter provides a specific formulation for eculizumab which is a 300 mg single use dosage form comprising 30 mL of a 10 mg/ml preservative free solution.
- This formulation has extremely good storage stability (48 months at 2-8°C) which means that clinics can store the product for long periods of time and still rely on the biological activity of the product being suitable for treatment of PNH.
- The 10 mg/ml concentration has an *improved* storage stability compared with 30 mg/ml as demonstrated by Dr Miano (D33).
- The volume of the formulation is only 30 ml, such that the volume of product which needs to be stored and administered is low. This has benefits in terms of acceptable infusion volumes and times for the treatment of PNH as well as providing the ability to store eculizumab at various sites to ensure that PNH patients are able to quickly receive this critical therapy.
- The product can be stored in liquid form, avoiding risks of incomplete/incorrect reconstitution by a clinician and providing convenience of use in the treatment of patients having PNH.

5.4.8 The claimed subject matter therefore solves the technical problem of providing a composition of eculizumab which demonstrates the ideal balance of enhanced maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low volume liquid formulation.

5.5 *The Solution is Not Obvious From the Art: Overview*

5.5.1 As is set out in the Guidelines for Examination, the consideration of whether or not a claimed invention would have been obvious involves asking:

> *"whether there is any teaching in the prior art as a whole that **would** (not simply could, but would) have prompted the skilled person, faced with the objective technical problem, to modify or adapt the closest prior art while taking account of that teaching, thereby arriving at something falling within the terms of the claims, and thus achieving what the invention achieves (see **G-VII, 4**).*
>
> *In other words, the point is not whether the skilled person could have arrived at the invention by adapting or modifying the closest prior art, but whether he **would have done** so because the prior art incited him to do so in the hope of solving the objective technical problem or in expectation of some improvement or advantage (see **T 2/83**)."*

5.5.2 It is therefore necessary in the present case to consider whether the skilled person *would* have arrived at the claimed 300 mg preservative-free dosage form containing 30ml of a 10 mg/ml eculizumab solution, with a reasonable expectation that such a composition would provide the ideal balance of maintaining biological integrity of the antibody following storage in a low-

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volume liquid formulation (not merely whether he/she *could* have done so).

5.5.3 The Boards of Appeal have further held that it is necessary to consider whether a skilled person would have followed a particular course of action with a reasonable expectation of successfully solving the technical problem. A reasonable expectation of success has been distinguished from a "hope to succeed" or from a situation where it is "obvious to try" (T296/93). Rather, what is required is that the skilled person can predict, before starting a project, whether or not there is a realistic expectation of a successful conclusion to a project (i.e. of solving the technical problem.)

5.5.4 The Boards of Appeal have, in a few occasions, denied an inventive step on the basis that the skilled person is in a "try and see" situation, as mentioned by Opponent 1 (T333/97). However, a "try and see" situation is considered by the Boards to be limited to particular situations, i.e. where the prior art clearly envisages a specific feature, and the skilled person merely has to determine by routine tests whether such feature does indeed provide a desired effect. In T333/97, for instance, the skilled person is faced with the problem of providing a method for blocking in plants a given pathogenic target RNA strand activity. A specific, suitable method was known in E coli and all parties agreed that it would be obvious to try this specific method in plants. However, it was not known whether or not the method would succeed. A lack of inventive step was found since a single course of action, agreed to be the route obvious to the skilled person, merely had to be tested to see if it worked.

5.5.5 In the present case, as will be discussed further below, none of the documents cited by the Opponents gives the skilled person a reasonable expectation of achieving, in a formulation of eculizumab, good stability and low liquid volume via *any* type of formulation. There is certainly no suggestion that a liquid formulation of 30 ml at 10 mg/ml would provide the ideal balance of properties. It is also clear that the present situation is not a "try and see" situation. The prior art does not provide an indication of a single suitable formulation which could be tested to see if it is effective. Rather, a wide variety of different formulations are used for different antibodies. To determine a suitable composition for the uniquely- engineered eculizumab antibody, the skilled person would have to conduct a significant research program to fully investigate the stability and biological integrity of the antibody, as well as the various factors (and their interplay) affecting each.

5.5.6 Indeed, the Opponents largely seek to rely on references which relate to formulations of other antibodies. As discussed in detail above in Section 4, each antibody has individual properties in terms of its stability, even where structure is very similar. In the present case, eculizumab is a non-naturally occurring, non-IgG1 isotype and is therefore *not* structurally similar to the largely IgG1 antibodies referred to by the Opponents. No conclusions can therefore be drawn from prior art which refers to suitable formulations of different antibodies.

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5.6 *Claimed Subject Matter is Not Obvious over D1 combined with D19-D21*

5.6.1 In light of D1, the technical problem to be solved is to provide a composition for eculizumab which has the right balance of properties providing biological integrity following storage and a low volume liquid formulation for ease of storage and administration. The question in relation to inventive step is: would the skilled person have chosen a 30ml single use formulation of 10 mg/ml eculizumab in a preservative free solution, based on the teaching in D19 to D21, with a reasonable expectation of solving this problem?

5.6.2 In Section 4 above we discussed the knowledge in the art in relation to antibody formulations in D20 and D21. This can be summarised as follows:

- Formulation of antibodies was a complex area with a wide array of different stability considerations to be taken into account for each individual antibody.
- Each antibody is unique and needed separate consideration in terms of determining a suitable composition, even where antibodies were structurally similar.
- There was no single "common" formulation, or antibody concentration, which the skilled person would turn to first.
- The knowledge in the art was largely in relation to IgG1 antibodies. Non-IgG1 antibodies with non-naturally occurring, engineered constant regions were known to act differently, but little work had been done on formulation of such antibodies.
- A wide array of different formulations had been used for various antibodies on the market, giving no clear guidance as to preferable or even suitable formulations for future therapeutic molecules.

5.6.3 It is clear from the above, therefore, that the skilled person would not have known which concentration to use in a pharmaceutical composition of eculizumab. The skilled person would not have had any awareness as to how eculizumab would be affected by the wide variety of potential stability issues to consider. The skilled person would not have had any awareness of which of the many tools available to the formulation scientist (pH, excipients, antibody concentration, lyophilisation) would be effective in addressing the as yet unknown stability issues. The skilled person would certainly not have any indication that 30ml of a 10 mg/ml liquid composition of eculizumab would provide the combined benefits of improved biological integrity of the antibody on storage at up to 48 months and low volume liquid formulation for improved administration. Rather, a full research project would be needed to arrive at such a suitable and ideal composition.

5.6.4 Importantly, the skilled person would be aware that the knowledge about formulation in D20 and D21 largely relates to IgG1 isotype antibodies. However, as set out in D21, little information was available about engineered antibodies:

> *"A number of engineered antibody-like molecules are now being examined for therapeutic applications. Some engineered fragments,*

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such as diabodies and minibodies, are much smaller than intact, full length IgG1 and have very different properties of systemic distribution and clearance. Additionally, methods to prepare other antibody types such as dimeric secretory IgA and methods to prepare antibodies using non-mammalian cell systems, such as plants, from the eggs of chimeric chickens and from transgenic animals, are now being explored by academic and industrial groups. As these new approaches gain prominence, more information will be acquired about their unique requirements for successful formulation and delivery. At present, however, there is little information on formulation approaches for antibodies produced using these production processes." (D21, p689, LH column)

5.6.5 Eculizumab is an example of an engineered antibody having a unique, non-naturally-occurring CH1-hinge-CH2-CH3 structure. Given the unpredictability of behaviour even between IgG1-type antibodies, the skilled person would not have been able to use the information about IgG1 antibodies in the art to predict the behaviour of eculizumab. Indeed, the skilled person would not have been able to predict whether a liquid formulation would have been possible at all for this new type of antibody. There is certainly no reasonable expectation of successfully providing a formulation which maintains the biological integrity of the antibody, in a low volume liquid formulation, over a period of 48 months. The claimed subject matter is therefore not obvious from D1 in combination with D20 and D21.

5.6.6 D19 is also relied on by O1 to show that it is desirable to exclude preservatives for regulatory reasons. However, there is no suggestion in D19 that a 30 ml eculizumab formulation at a concentration of 10 mg/ml would have beneficial properties. D19 is not therefore relevant to solving the objective technical problem.

5.7 *Antibody Concentrations After The Filing Date*

5.7.1 The Opponents have indicated that 10 mg/ml was a typical antibody concentration and it would have been obvious for a skilled person developing a new antibody to use this concentration based on the common knowledge. If the Opponents are right in their assertion, it could be expected that antibodies developed after the filing date would generally have a concentration of 10 mg/ml. However, this is not the case.

5.7.2 D37 summarises data available from the European Medicines Association (EMA) website (http://www.ema.europa.eu). D37 provides a list of active antibodies (excluding biosimilars) which have been given EMA authorisation after the filing date of the patent. The nature of the product is also indicated, including the product concentration, where the product is provided as a solution.

5.7.3 As is evident from D37, only six out of 44 antibody products were approved having a formulation of a 10 mg/ml solution. It is quite clear, therefore, that other factors are considered by developers when selecting an

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appropriate concentration for a formulation of any new drug. 10 mg/ml is not an automatic choice. Indeed, as for antibodies formulated before the filing date, the largest group of products were provided in lyophilised form.

5.7.4 A recent review article on antibody formulation by Rouet *et al* (D34) confirms that, even well after the filing date of the present patent, the issue of providing a stable formulation for an antibody product remains a challenging problem. Even in 2014, Rouet considered that:

> "*Despite recent advances, the identification of suitable formulation conditions for a specific monoclonal antibody remains challenging and cannot be determined from its amino acid sequence. Indeed, a considerable proportion of human monoclonal antibody candidates fail formulation studies, often at relatively late pre-clinical stage, placing heavy burdens onto drug development pipelines.*" (Page 271, right-hand column, second paragraph).

5.7.5 In the first paragraph of the right-hand column of page 271, D34 gives the example of therapeutic monoclonal antibody cetuximab (Erbitux). Even the initial suggested formulation conditions of 2 mg/ml in phosphate buffer at pH 6.0 led to visible aggregates under mechanical stress and had to be reformulated. Notably, the new formulation was at a higher concentration – 5 mg/mL – but at a different pH and using different excipients.

5.8 *Claimed Subject Matter is Not Obvious over D1 Combined with D22*

5.8.1 O1 relies on D22 which relates to a nebulisable formulation. A critical factor in considering a suitable formulation of any drug is the manner in which it is administered. Clearly, a topically administered drug will be formulated very differently from an orally or parenterally administered drug. Similarly, the skilled person looking for a suitable formulation for the D1 treatments, which are administered by IV, would not consider nebulized formulations. Thus, the skilled person would not consider D22 when seeking suitable formulations in light of the D1 disclosure.

5.8.2 Moreover, even if the skilled person had considered D22, this would not have led him to the claimed solution. As O1 points out, D22 exemplifies at para [170] to [172] formulations described as eculizumab formulations at 30 mg/ml. However, this example would, if anything, lead the skilled person *away* from the claimed 10 mg/ml concentration. There is no hint or suggestion in D22 that 10 mg/ml would provide improved biological integrity of eculizumab following storage compared with 30 mg/ml. Indeed, there is no information whatsoever regarding stability in D22.

5.8.3 Moreover, a skilled person seeking a dosage form of eculizumab having a *low volume* would not consider reducing the concentration of the 30 mg/ml solution as this would inherently *increase* the volume of solution needed to provide the same drug content. This document would not, therefore, have led the skilled person to the claimed invention starting from D1.

5.9 *Claimed Subject Matter is Not Obvious over D1 combined with D29*

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5.9.1 Starting from D1, the skilled person is seeking a suitable formulation for a unique antibody having a non-naturally-occurring, engineered heavy chain. D29 would not be of assistance to the skilled person since it relates to very different antibodies, which the skilled person would understand to likely have very different stability properties.

5.9.2 D29 is primarily related to formulations for anti-CD18 and anti-CD20 antibodies. This is clear, for example, from column 2, lines 19 to 22 which state that: *"There is a need in the art for a stable aqueous pharmaceutical formulation comprising an antibody, such as an anti-CD18 or anti-CD20 antibody, which is suitable for therapeutic use"*. The only examples of specific formulations given in D29 are for anti-CD18 and anti-CD20 antibodies. Specifically, Example 1 relates to rhuMAb CD18 which is a $F(ab')_2$ molecule (column 24, line 42) and Example 2 relates to rhuMAb CD20.

5.9.3 Eculizumab, on the other hand, is not at all similar to the antibodies exemplified in D29. Eculizumab has a uniquely engineered CH1-hinge-CH2-CH3 sequence and is structurally remote from the antibodies described in D29. The skilled person would have no expectation that the formulation behaviour and stability of the anti-CD18 and anti-CD20 antibodies described in D29 would shed any light at all on a composition for eculizumab. The skilled person would not therefore consider combining D29 with D1.

5.9.4 Further, even if the skilled person had considered D29, the focus of the document is on the excipients used in the formulation. As set out in the summary of the invention at column 2, beginning at line 25, the invention of D29 suggests that a buffer providing a pH in the range 4.5 to 6.0, a surfactant and a polyol are useful in providing a formulation which is "*preferably* [...] stable at a temperature of 2-8°C for at least one year, and/or is stable at a temperature of about 30°C for at least one month" (emphasis added). Had the skilled person followed the suggestions of D29, they would have considered primarily the excipients in the formulation to achieve stability. The concentration is not mentioned in the summary of the invention at all. Further, they would not have expected to be able to achieve stability (of a very different antibody) for as long as 48 months, given D29's suggestion of 12 months.

5.9.5 D29 does make some reference to antibody concentration at column 22, as pointed out by O2. However, even D29 itself makes clear that in the actual selection of antibody concentration to use, other factors need to be taken into account. At column 22, line 10 onwards, D29 explains, for example, that the amount of antibody is determined by taking into account the desired dose volumes and mode(s) of administration. The actual concentrations used in the Examples of D1 are very different for the two antibodies: Example 1 uses 1 mg/ml, whereas Example 2 uses 40 mg/ml. Notably, neither is within the narrowest range of 2 to 10 mg/ml which O2 suggests would be the obvious choice of concentration for *any* antibody formulation.

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5.9.6 Based on this information, there is no hint that 10 mg/ml is an obvious choice for a skilled person. Indeed, a skilled person could just as easily consider 1 mg/ml, 40 mg/ml or any other concentration in the range of 1 to 50 mg/ml. There is certainly no suggestion that the specific concentration of 10 mg/ml, when used for an eculizumab formulation, would provide an ideal balance of maintaining biological integrity on storage and low volume liquid formulation.

5.9.7 In summary, D29 gives the skilled person no additional information than they would already be aware of from his common general knowledge with regard to antibody concentration. The skilled person knows that many antibodies (where liquid formulation is possible) may be formulated in the range of 1 mg/ml to 50 mg/ml, and that some of those are formulated in the range of 2 mg/ml to 10 mg/ml. But, the skilled person also knows that various considerations must be taken into account when selecting a suitable concentration for any particular antibody. The skilled person also knows that each antibody requires its own individual study into suitable formulations: concentrations of antibody in a given formulation that lead to stability for that one antibody may not do so for another antibody. This is borne out by the contrast between the concentrations used in Examples 1 and 2 of D29. D29 therefore does not lead the skilled person any closer to the present invention.

5.9.8 Finally, it is noted that O2 draws comparison between the commercial formulation of rituximab, an anti-CD20 antibody, and D29. This does not have any bearing on the scope of D29 itself, which suggests a different concentration of 40 mg/ml in Example 2. Moreover, given the significant structural differences between rituximab and eculizumab, the skilled person would have had no expectation that a formulation for rituximab would prove useful for eculizumab, let alone that it would provide a storage stable composition for at least 48 months.

5.10 *Definition of the technical problem*

5.10.1 The discussion above does, of course, make reference to stability data for eculizumab. The Guidelines for Examination in the European Patent Office confirm that any technical effect derivable from the application as filed can be used as a basis to reformulate the objective technical problem. The relevant passage in the Guidelines reads as follows (G-VII-5.2).

> *"As a matter of principle any effect provided by the invention may be used as a basis for the reformulation of the technical problem, as long as said effect is derivable from the application as filed (see T386/89).* ***It is also possible to rely on new effects submitted subsequently during the proceedings by the applicant, provided that the skilled person would recognise these effects as implied by or related to the technical problem initially suggested*** *(see G-VII, 11 and T184/82)".* (Emphasis added.)

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5.10.2 The technical effects at issue in the present case are in the maintenance of the biological integrity of the antibody following storage, and suitable volumes for storage and administration to a patient. These properties are clearly intimately connected with the underlying problem of providing a useful treatment for PNH. Unless the biological integrity of the antibody is maintained, the treatment cannot be expected to be efficacious. Similarly, the ability of the clinician to safely and effectively administer the drug to a patient is clearly connected to the ability to treat PNH.

5.10.3 As noted in the passage of the Guidelines set out above, effects which are related to the technical problem set out in the application as filed may be relied upon in the formulation of the technical problem. The relevant passage is set out at G-VII-11:

> *"Subsequently, the applicant submits new evidence which shows that the claimed composition exhibits an unexpected advantage in terms of low toxicity. In this case, it is allowable to reformulate the technical problem by including the aspect of toxicity,* ***since pharmaceutical activity and toxicity are related in the sense that the skilled person would always contemplate the two aspects together****".* (Emphasis added.)

5.10.4 It is therefore clearly acceptable to rely on the evidence set out above in the formulation of the objective technical problem for the purposes of a problem/solution analysis for inventive step.

5.11 *Inventive Step: Summary*

5.11.1 In summary, starting from D1, the technical problem which is solved by the present invention is the provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced stability and thus maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion times and which is convenient to store.

5.11.2 Based on D20 and D21, the skilled person would have been aware that formulation of antibodies is a complex area and that each antibody needs to be treated as its own unique formulation project. The skilled person would also have treated with scepticism any information in the prior art that was based on IgG1 antibodies given the non-IgG1, non-naturally occurring, engineered structure of eculizumab: the skilled person would not have any expectation that formulations which had been successful with previous antibodies would be useful in the case of eculizumab.

5.11.3 Moreover, based on D20 and D21, the skilled person would see that there is no clear starting place for considering antibody formulation and that a separate research program would be needed to find the ideal formulation for eculizumab. The result of that research program, carried out by the inventors, was the finding that 30 ml of a 10 mg/ml liquid formulation of eculizumab is surprisingly stable over a period of 48 months, having

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superior maintenance of biological integrity compared with other concentrations of eculizumab, whilst also providing a low volume of liquid formulation. The skilled person could not have had any reasonable expectation of this result in advance of carrying out the research.

5.11.4 Moreover, neither D22 nor D29 cited by the Opponents provides any assistance. D22 relates to a different type of formulation from D1 so would not be considered relevant. In any case, it directs the skilled person to inferior 30 mg/ml formulations. D29 relates primarily to the excipients used in antibody formulation, in particular anti-CD18 and anti-CD20 formulations. Again, it would give the skilled person no guidance for suitable compositions for eculizumab.

5.11.5 The claimed subject matter is therefore inventive over the art.

6 Sufficiency

6.1 The skilled person would be able to carry out the invention without undue burden.

6.2 There is no confusion as to what the heavy and light chain amino acid sequences of eculizumab are. The patent discloses the variable heavy and variable light chain regions of eculizumab (SEQ ID NO: 1 and SEQ ID NO: 3) and the full heavy chain and light chain sequences of eculizumab (SEQ ID NO: 2 and SEQ ID NO: 4).

6.3 The skilled person would immediately recognise, based on his common general knowledge, that SEQ ID NO: 4 (and also the light chain variable region sequence of SEQ ID NO: 3) includes the N-terminal leader sequence which is not present in the mature antibody.

6.4 It was well known in the art at the filing date of the application that a signal peptide is present at the N-terminus of immature heavy and light chain polypeptides. Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991) (D38), which is a database of biological sequences containing sequence alignments and is evidence of the skilled person's common general knowledge, shows the signal peptides of various human light chains.

6.5 The attached page 1 from Kabat *et al.* shows the 22-residue signal peptides of human kappa light chains. The first three residues are predominantly Met Asp Met and the last two residues are predominantly Arg Cys. In SEQ ID NO: 4 of the patent, the first three residues are Met Asp Met and residues 21 and 22 are Arg Cys. Therefore, the skilled person would recognise that SEQ ID NO: 4 is the amino acid sequence of the immature light chain that contains a signal peptide that is not present in the mature eculizumab antibody.

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6.6 Furthermore, the skilled person would know that the framework of human kappa light chains commonly starts with the residues Asp Ile Gln Met as shown in the Table at pages 103 to 108 of Kabat *et al*. Residues 23 to 26 of SEQ ID NO: 4 are Asp Ile Gln Met, which the skilled person would recognise as being the start of the mature light chain of eculizumab.

6.7 The existence of post-published D25 that includes the mature sequence of SEQ ID NO: 4 has no bearing on whether the disclosure of the patent is sufficient. The date for determining sufficiency of disclosure is the filing date of the patent. At any rate, D25 merely confirms what the skilled person would know from reading the patent in light of his common general knowledge, i.e. that SEQ ID NO: 4 contains the light chain signal peptide as well as the light chain sequence present in the mature antibody.

6.8 It should also be pointed out that the skilled person would produce eculizumab using cell-based recombinant methods, and not by artificially synthesising the polypeptide chains. The cells used to make eculizumab would process the light chain polypeptide sequence to remove the signal sequence during antibody production in the cells. Therefore, contrary to the assertion of O1, the skilled person would not use an antibody that is not eculizumab to treat PNH because he would not be able make an antibody that contains the whole sequence of SEQ ID NO:4.

6.9 Thus, the skilled person seeking to reproduce the claimed invention based on the disclosure in the patent of SEQ ID NOs: 2 and SEQ ID NO: 4 would be able to make and formulate eculizumab as required by claim 1 using routine methods known in the art.

6.10 The claimed subject matter is therefore sufficiently disclosed in the patent.

7 Summary

7.1 For the reasons discussed above, the patent does not contain added matter, is inventive over the art and is sufficient. The oppositions should therefore be dismissed and the patent maintained as granted.

Signed

A SIMONS P TUXWORTH

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Consolidated List of Documents cited in opposition to EP2359834 B1				
Document Name	Consolidated Number	Patentee	Opponent 1 (Amgen)	Opponent 2 (Hoffman Eitle)
US 2005/191298 A1	D1		D1	D1
Hillmen *et al.*, (2003) *J. Hematol.* 121 (Suppl. 1): 87-95	D2		D2	D2
Hillmen *et al.*, (2004) *N. Engl. J. Med.* 350(6): 552-559	D3		D3	D3
Harding. (2004) *Drugs Future*. 29(7): 673-676	D4		D4	D4
US 2005/221382 A1	D5		D5	D5
Hill. (2005) *Clin. Adv. Hematol. Oncol.* 3(11): 849-50	D6		D6	D6
Morris *et al.*, (1992) *South. Med. J.* 85(19): 1030-1031	D7		D7	
Corvini *et al.*, (2004) *Ann. Allerg. Asth. Immunol.* 93(2): 200-205	D8		D8	
Sierra *et al.*, (1991) *NIPH Annals.* 14(2): 195-210	D9		D9	
US Pharmacopeia (USP29-NF24), page 2989	D10		D10	
Adis Data Information. (2007) *Drugs R. D.* 8(1): 61-68	D11		D11	
Hill *et al.*, (2005) *Blood.* 106(7): 2559-2565	D12		D12	
Thomas *et al.*, (1996) *Mol. Immunol.* 33(17/18): 1389-1401	D13		D13	D8
Mueller *et al.*, (1997) *Mol. Immunol.* 34(6): 441-452	D14		D14	
WO 97/11971 A1	D15		D15	
WO 2005/007809 A2	D16		D16	
US 6,355,245 B1	D17		D17	D7
Hillmen *et al.*, (2006) *N. Engl. J. Med.* 355(12): 1233-1243	D18		D18	
EMEA, CPMP, 20 February 2003, *Note for guidance on excipients, antioxidants and antimicrobial preservatives in the dossier for application of marketing authorization of a medicinal product.*	D19		D19	
Wang *et al.*, (2007) *J. Pharm. Sci.* 96(1): 1-26 (January 2007)	D20		D20	D10
Daugherty *et al.*, (2006) *Adv. Drug Del. Rev.* 58: 686-706	D21		D21	
US 2005/0271660 A1	D22		D22	
Expert Declaration by Dr Michael Treuheit	D23		D23	

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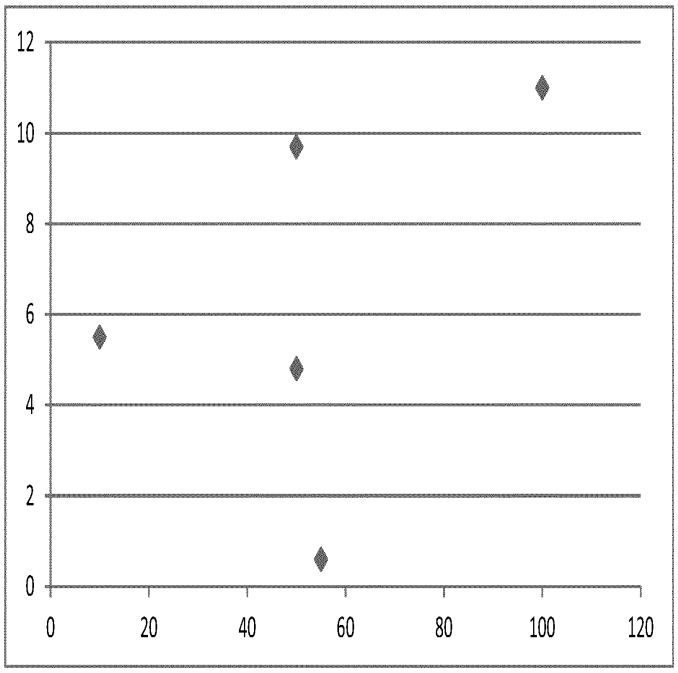
Soliris®(eculizumab) *Highlights of prescribing information.*	D24		D24	
US 2012/0230982 A1	D25		D25	
WO 2007/002571 A2	D26		D26	
Hill *et al.*, (2004) *Blood*. 104: 2823-2824	D27		D27	
US 2003/0202972 A1	D28			D9
US 6,171,586 B1	D29			D11
Maloney *et al.*, (1997) *Blood*. 90(6): 2188-2195	D30			D12
Commercial monoclonal antibodies 2006 (all modes of administration)	D31			Annex I
Commercial monoclonal antibodies 2006 (I.V. administration)	D32			Annex II
Declaration of Dr Miano	D33	D33		
Rouet *et al.*, (2014) *FEBS Letters*. 588: 269-277	D34	D34		
Summary of antibodies listed in D20/D21 Tables	D35	D35		
Graphical representation of D20 Table 2	D36	D36		
Summary of antibody products approved by EMA since filing date	D37	D37		
Extracts from Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991)	D38	D38		

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% Aggregation Versus Antibody Concentration

From Table 2 of D20 where concentration and % aggregates known)

Antibody	Concentration in Formulation (mg/mL)	% Aggregates
rhyMAbE25	10	5.5
Human anti-IL-8 (IgG2)	50	4.8
RhuMAb HER2	50	9.7
Human Anti-IL-8 (IgG2)	55	0.6
Anti-IL-8 (IgG2)	100	11



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Review

Stability engineering of the human antibody repertoire

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ABSTRACT

Human monoclonal antibodies often display limited thermodynamic and colloidal stabilities. This behavior hinders their production, and places limitations on the development of novel formulation conditions and therapeutic applications. Antibodies are highly diverse molecules, with much of the sequence variation observed within variable domain families and, in particular, their complementarity determining regions. This has complicated the development of comprehensive strategies for the stability engineering of the human antibody repertoire. Here we provide an overview of the field, and discuss recent advances in the development of robust and aggregation resistant antibody therapeutics.

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1. Introduction

The number of human monoclonal antibody candidates has increased rapidly in recent years, and now represent the largest single class of molecules entering clinical studies [1]. Despite the rapid growth of the antibody therapeutics market over the past twenty to thirty years, hurdles remain that limit their manufacture. Key factors relate to the variable and often limited stability of human antibodies, which negatively impact on many production processes including expression [2,3], purification [4] and formulation [5].

Antibodies are complex multidomain proteins and mechanisms governing their thermodynamic [6] and, in particular, colloidal [7] stability are not fully understood. Human antibodies of the commonly used immunoglobulin G (IgG) type consist of a total of twelve domains, which can be further divided into two chains (heavy and light) and variable (V_H, V_L) and constant (C_H1, C_H2, C_H3, C_L) domains (Fig. 1). Only limited diversity is observed among constant domains, with a total of four isotype (IgG1–4) and two light chain ([κ] kappa and [λ] lambda) classes expressed in humans. Consequently, isotype differences can explain only a limited proportion of the observed stability variation in the human antibody repertoire [8]. A higher proportion of sequence diversity is observed among antibody variable domains, which are assembled through genetic recombination of variable, diverse (V_H only) and joining segments (VDJ-recombination). Further diversity is then introduced into framework, and in particular complementarity determining regions (CDR), through somatic hypermutation, followed by clonal selection of the repertoire. As a result of these processes, the overwhelming majority of sequence variation within the human antibody repertoire is observed within the CDR regions of a limited range of human variable domain families. In this review, we summarize the influence of the observed antibody diversity on colloidal and thermodynamic stabilities and discuss recent advances to improve these properties through engineering approaches.

2. Protein aggregation

Aggregation is a complex process by which proteins can form alternative colloidal states, which are different from the native state, but otherwise energetically favorable [9,10]. It is generally believed that such aggregate species are predominantly formed via unfolded or partially unfolded states [11]. Protein aggregation is increasingly recognized as a problem affecting the manufacturability of human therapeutic antibodies, shelf life and efficacy [12]. Importantly, the presence of aggregates has also been linked to increased immunogenicity, with effects ranging from mild skin irritation to anaphylaxis [13].

Although it can be assumed that stabilization of the native state over alternative aggregate states has occurred during evolution [14], it is important to note that the production of monoclonal antibodies exposes these molecules to a wide range of non-physiological

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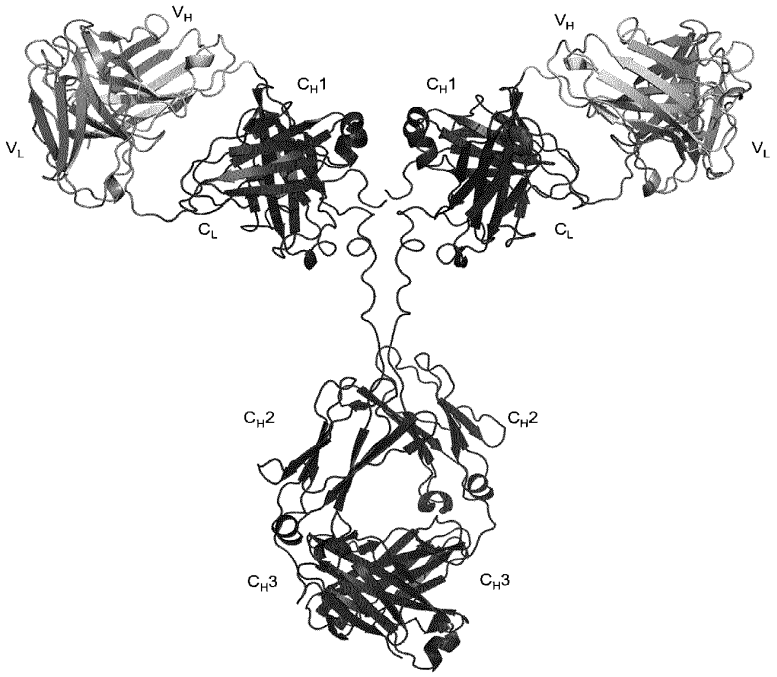


Fig. 1. Structure of the human IgG molecule. The molecule is formed by two heavy chains (consisting of V_H, C_H1, C_H2 and C_H3 domains) and two light chains (consisting of V_L and C_L). Figure was generated using PYMOL.

processes and conditions. These include recombinant expression, purification, concentration, viral inactivation, filtration, formulation, freeze/drying, transport and long-term storage. Throughout these steps the antibody molecule may encounter several stress factors that can dramatically increase its propensity to aggregate (including variations of temperature, pH, protein concentrations, ionic strength, exposure to air–water interfaces and mechanical stress). A further driver of protein aggregation has been a growing trend towards formulations that allow sub-cutaneous administration routes. This requires formulation at high protein concentrations (at around 100 mg/ml) in syringes for self-injection, which places increased demands on colloidal stability [15].

3. Stability of human antibody isotypes and constant domains

The four human IgG isotypes differ in their stabilities and biological functions, namely their potential to induce cellular killing through antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). While the human IgG1 isotype induces powerful ADCC and CDC responses, this is not the case for human IgG2, which is particularly suitable for applications where cellular killing is not required (such as neutralization of soluble ligands) [16]. Unlike the other human isotypes, IgG4 also has a naturally occurring potential to form a bispecific molecule [17] (it has been recently shown that this dual specificity can be grafted onto other isotypes [18]). Human IgG3 is not commonly used due to its longer hinge region, which renders it susceptible to proteolysis [19]. The vast majority of human antibody therapeutics currently in clinical practice and development are of the IgG1 isotype [16].

Conflicting evidence exists in respect to the colloidal stability of the various human IgG isotypes. A recent study of eleven different IgG1 and IgG2 antibodies concluded that, after high temperature storage, the IgG2 isotype is more prone to aggregation [20]. Similar findings were also obtained when subjecting these isotypes to high salt conditons [21] and from isotype switching studies [22]. The latter study confirmed that the human IgG1 isotype is less prone to aggregation when compared to IgG2 or IgG4. However, the authors also found that this isotype is more prone to fragmentation, especially at low pH, due to a non-enzymatic site in the upper hinge region. The hinge regions have also been implicated in the observed differences in aggregation propensity as the IgG2 hinge contains two additional cysteines in each heavy chain compared to IgG1, and is prone to the display of free cysteine residues [23,24]. Differences in the thermal stability of human constant domains have also been reported, with the C_H3 domains of human IgG1 and IgG2 displaying similar melting temperatures, much higher than what was observed for IgG4 [8]. In general, C_H3 domains exhibit more favorable biophysical properties than C_H2 domains [25]. This is reflected by melting temperatures of 8–10 °C higher than those observed for C_H2 domains [8,26].

Although human IgG1 therefore appears less aggregation prone than other isotypes, this is not universally the case. For example, a recent study found that the IgG1 isotype variant of an anti-LINGO-1 antibody exhibited a much higher propensity to aggregate than an IgG2 variant containing identical variable regions [27]. Studies such as these demonstrate that predicting the aggregation propensities of IgG molecules based on isotype can be difficult at the best of times. Moreover, evidence suggests that the aggregation propensity of human IgG molecules is greatly influenced by their variable domains [8,27,28]. This has the effect that antibodies with identical constant domains, but different variable domains, can vary widely in their stability profiles [5].

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4. Stability of human antibody variable domains

Human antibody variable domains can be grouped into several homologous families, which differ in their biophysical properties. A seminal study of consensus domains of human V_H and V_L families has revealed considerable differences of expression yields and thermodynamic stability [6]. In the case of human V_H domains, stabilities ranging from ΔG_{N-U} 14–53 kJ/mol were observed, with soluble expression yields ranging from 1–2.4 mg/l (Table 1). Intriguingly, Ewert et al. also reported that members of the (odd numbered) V_H 1, 3 and 5 families generally displayed superior biophysical properties in comparison with the (even numbered) 2, 4 and 6 families. These differences were observed for both thermodynamic stabilities and expression yield. In particular, the human V_H3 family was identified as uniquely stable and well expressed. This family is also among the most abundant in the human repertoire [29]. The overall stability trends were confirmed in a more recent study, which analyzed the effect of pairing of different human variable domain families on expression, thermal and colloidal stabilities of Fab fragments and IgG monoclonals [30].

More limited differences in biophysical properties were observed among human V_L families, with thermodynamic stabilities ranging from ΔG_{N-U} 15–24 kJ/mol for lambda and ΔG_{N-U} 29–35 kJ/mol for kappa [6]. Similarly, higher expression yields were observed for kappa variable light domains (5–17 mg/l) than for lambda domains (0.3–2 mg/l). The observed differences between antibody variable light domains were much less pronounced when pairing these with human V_H in an scFv format, while considerable differences between variable heavy domain families remained. Overall, the authors found the V_H/V_L combinations H3κ3, H1bκ3, H5κ3 and H3κ1 to be superior in respect to expression yield and thermodynamic stability [6]. However, despite the clear influence of variable domain family (and framework regions), the study also observed a strong influence of CDR sequence on biophysical properties. Indeed, initial attempts of the authors to express human V_H domain in isolation failed to yield protein (with the exception of V_H3). Only the grafting of a solubilizing CDR3 region allowed the soluble expression of human V_H1, and of V_H2, V_H4, V_H5 and V_H6 through refolding from inclusion bodies.

Although several of the human variable domain families are thermodynamically stable, they nevertheless often display poor colloidal stability and readily aggregate when heated above their melting temperatures. This behavior is exemplified by the V_H3 model domain DP47 [31] and by studies of synthetic antibody repertoires on phage, which have demonstrated that as few as one in a thousand VH domains resists heat induced aggregation [32].

Table 1
Biophysical properties of human antibody variable domains. Adapted from Ref. [6].

	Human family	Solubilizing CDR3 required	Expression yield	ΔG_{N-U}
V_H	1a	Y	+	+
	1b	Y	+	++
	2	Y	Refold	nd
	3	N	+	+++
	4	Y	Refold	nd
	5	Y	Refold	+
	6	Y	Refold	nd
V_L	κ1	N	+	++
	κ2	N	+++	++
	κ3	N	+++	+++
	κ4	N	++	-
	λ1	N	+	++
	λ2	N	+	+
	λ3	N	+	+

5. Increasing antibody stability through formulation

The most common method for controlling the biophysical properties of monoclonal antibodies and other biologics is through changes of buffer conditions and excipients. Formulation allows for the control of ionic strength, pH and the addition of excipient agents such as sugars or surfactants, which have been shown to stabilize monoclonal antibody preparations [33]. Although formulation can shield proteins from some chemical and physical challenges (including deamidation, oxidation, hydrolysis/fragmentation and isomerization), aggregation remains difficult to control [12]. Consequently, finding appropriate formulation conditions for an individual product is often an arduous process. This is exemplified by the therapeutic monoclonal antibody cetuximab (Erbitux). Its initial formulation conditions (2 mg/ml in phosphate buffer pH 6.0) generated visible aggregates under mechanical stress [34]. To address this issue, conditions had to be empirically optimized and were changed to 5 mg/ml in citrate buffer pH 5.5 with the addition of glycine and polysorbate 80 as stabilizers.

In recent years, more high-throughput techniques for formulation optimization have been developed [35], and stabilizers and excipients with more general applicability have been identified. Among these, arginine is commonly used to improve refolding yields [36,37], and more recently has been used in formulation to reduce heat-induced aggregation [38]. Another commonly used class of excipient molecules are polysorbates, which accumulate at the air–solvent interface and offer protection against shaking and other mechanical stresses [39]. Despite recent advances, the identification of suitable formulation conditions for a specific monoclonal antibody remains challenging and cannot be determined from its amino acid sequence. Indeed, a considerable proportion of human monoclonal antibody candidates fail formulation studies, often at relatively late pre-clinical stage, placing heavy burdens onto drug development pipelines [5].

6. Stability engineering of human antibody constant domains

Many efforts have been made to increase the stability of the different human antibody isotypes. Early work in this field focused on sequences analyses to predict stabilizing mutations. This approach allowed the identification of a set of substitutions (371K, 376G and 392L), which considerably increased thermal stability (from 76 °C to 86 °C) of the C_H3 domain [40]. The study was based on frequency analyses within 19 mammalian species to generate a consensus sequence. Structural approaches have also been utilized to increase stability of the C_H1/C_L interface [41]. More recently, it was shown that the introduction of additional intra-domain disulfide bridges in the human C_H3 domain resulted in the thermal stabilization of an isolated IgG1 Fc fragment [42] (Fig. 2). In particular, the introduction of a link between the N-terminal A strand (position 343) and the F strand (position 431) caused a 8 °C increase in the melting temperature, while a linkage between positions 375 and 396 resulted in a 4 °C increase (combined 13 °C). These findings were successfully applied to a HER2 specific Fcab molecule (Fc fragment with engineered binding loops) resulting in an increased in the thermal stability of 19 °C. A similar finding was observed for a human IgG1 C_H3 single domain, which could be stabilized by introduction of an intra-domain disulfide bridge [43]. The authors used a two-step approach, by initially introducing mutations that rendered the C_H3 domain monomeric, resulting in a overall low thermal stability (41 °C compared to 82 °C for the native dimeric form). Using structure-based design, two additional cysteines (at positions 343 and 431) were then introduced to generate a disulfide bridge, which increased the thermal stability to 76 °C (a

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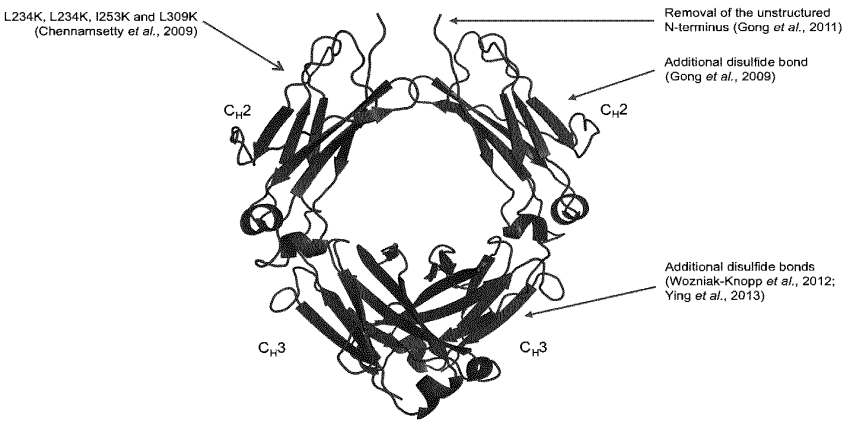


Fig. 2. Stability engineering of human constant domains.

35 °C increase) [43]. Similar strategies have been also reported for C_H2 domains [26]. Here the authors reported the engineering of an isolated human C_H2 domain, resulting in an increase in thermal stability of 20 °C (from 54 °C to 74 °C) [26]. More recently, the same group reported the stabilization of a C_H2 domain through removal of an unstructured region at the N-terminus. Combined with the intra-domain disulfide bond, this resulted in a further increase of melting temperature of 10 °C [44]. For all of the above studies, stability of constant domains was evaluated in the context of fragments or isolated domains.

Other studies have investigated the effects of such mutations on the stability of full-length human IgG. In the case of human IgG4, alteration of the disulfide bond network in the Fab region was shown to lead to an increase of thermal stability of between 3 and 7 °C [45]. This effect was achieved through removal of a cysteine at position 127 in the C_H1 domain, while an additional inter-domain disulfide bond between position 229 and C_L was introduced (similar to what is observed in human IgG1). Importantly, the authors reported that the introduced mutations did not affect antigen binding [45]. More recently, molecular dynamic simulations have been used to predict the spatial aggregation propensity (SAP) of human IgG molecules [46]. The authors later showed that by mutating identified surface-exposed hydrophobic patches within the hinge and Fc regions of an IgG, aggregation resistance and, to a more limited extent, thermodynamic stability could be improved [47].

7. Increased stability through charged fusion tags

In addition to the modification of constant domains, it was recently shown that the addition of charged fusion tags to the N-terminus of human variable domains could considerably improve aggregation propensity [48]. The authors noted that batches of the same IgG monoclonal expressed in yeast were less prone to aggregation than those expressed in mammalian cells. Mass spectrometry and sequencing analyses revealed the presence of a non-processed "EAEA" leader sequence within both heavy and light chains. In contrast to colloidal stability, the authors did not observe improved thermodynamic stability, and the effect seemed to be particularly pronounced for antibodies with a low global net charge. In addition to terminal fusion tags, a different study explored the internal use of a "DED" motif [49]. It was shown that the insertion of the tag into the CDR3 region of aggregation-prone V_H domain antibodies reduced their aggregation propensity, while reducing retention on gel filtration matrices.

8. Stability engineering of human antibody variable domains

8.1. Engineering of human V_H domains through mutation of the light chain interface

The poor biophysical properties of isolated human antibody variable domains are in marked contrast to those of the variable domains of camels and llamas [50]. Although these immunoglobulin domains (V_{HH}) display a relatively high level of sequence identity with human V_H domains, they are generally well expressed and soluble [51] and reversibly unfold after incubation at temperatures as high as 90 °C [52]. In addition to the observed differences in biophysical nature, camelid domains also display an important difference in chain structure, as they are naturally devoid of light chain partners [50]. Their single domain nature results in unique structural features, compared to human antibody V_H domains. These include CDR length and conformation, as well as framework residues. A particular feature of V_{HH} domains is their long flexible CDR3 loop capable of folding back on itself, thereby protecting an area forming the V_H/V_L interface in human antibodies. In addition, these naturally occurring single domains display a marked reduction in hydrophobicity of the former light chain interface, due to a limited number of changes in their respective germlines (Fig. 3). The most common and significant changes compared to humans, coined the "V_{HH} tetrad", are located at positions: G44Q, L45R/C, W47G/I and V37F/Y (human/camelid). The latter residue is nucleating a small core involving Y91, W103, R45 and other hydrophobic residues within the CDR3 loop [53]. Structural investigation of V_{HH} has also shown the CDR3 loop covering residue 37, thereby shielding hydrophobicity in an area corresponding to the light chain interface in human V_H [54]. In addition, camelid V_{HH} often contain an additional disulfide bridge between CDR1 and CDR3, which increases their thermal stability [55,56].

In order to improve the biophysical properties of human V_H domains, elements of the V_{HH} tetrad have been incorporated into human frameworks ("camelization") [57]. Although the introduced framework changes improved the solubility of human V_H, they also reduced expression levels, induced conformational changes, decreased thermodynamic stability and did not considerably improve the colloidal stability of the domains [58,59]. In contrast, combining the approach with the introduction of an additional disulfide bond between CDR1 and CDR3, yielded domains with high colloidal stability, although at the expense of antigen binding affinity [60].

As an alternative to camelid domains, shark single domain antibodies (V_{NAR}) could also potentially serve as a blueprint for improving

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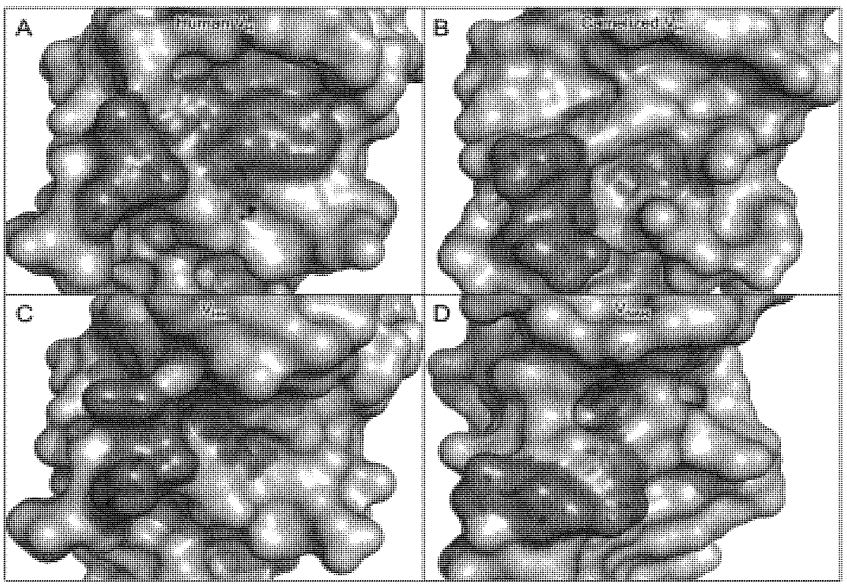


Fig. 3. Engineering of human VH domains through mutation of the light chain interface. Shown are surface representations of the light chain interface in human V_H (PDB 2VXS) and of the equivalent surfaces in camelized V_H (PDB 1VHP), camelid V_{HH} (PDB 1ZVH) and shark V_{NAR} domains (PDB 1T6V). Residues forming the camelid 'tetrad' at positions 37, 44, 45 and 47 are highlighted in green. Camelized V_H, V_{HH} and V_{NAR} domains display a considerable reduction in surface hydrophobicity in comparison to human V_H domains. Figure was generated using PYMOL.

the stability of human antibody V_H domains (although they are more distantly related to human domains [61]). As observed for camelid domains, shark V_{NAR} domains are characterized by a long protruding CDR3 [62]. The CDR loop is constrained by additional disulfide bonds, thereby folding over an area equivalent to the former V_H/V_L interface of human antibodies [61]. A recent study revealed that V_{NAR} domains are resistant to thermal denaturation, acidic denaturation, and lyophilisation denaturation and are capable of returning to their native state after thermal unfolding [63].

Rather than focusing on direct grafting of camelid residues, more recent approaches for the generation of autonomous single domains have focused on the identification of novel mutations. This has been possible due to the use of phage display based selection strategies and the use of protein A to select for folded domains (pA superantigen binds to folded human V_H3 domains, but not to unfolded or aggregated domains). Using this approach, an extensive mutagenesis study has identified hydrophilic substitutions within the V_H3/V_L interface [53]. The authors were able to demonstrate that their mutations (including H35G, Q39R, L45E, R50S) improved solubility, thermal refolding and melting temperature. Importantly, some of the mutations improved protein A binding (and presumably stability), independently of CDR3 sequence diversity. As CDR3 of V_H provides the majority of binding energy in antibody–antigen interactions, this indicated broad compatibility with antigen binding and suggested that the engineered frameworks could be used for the construction of stable repertoires of autonomous V_H domains.

8.2. Engineering of human V_H domains through CDR grafting and framework mutations

In addition to their use in the engineering of autonomous antibody single domains, homology-based approaches have also been used for the identification of mutations outside the interface regions, thereby retaining the ability of human V_H to interact with variable light domains. One of the earliest examples of such strategies relates to the use of CDR grafting for the improvement of biophysical properties. Developed by Jones and Winter in the mid-1980s, CDR grafting was initially used to reduce the immunogenicity of mouse monoclonals, by transplantation of murine CDR regions onto a human framework [64]. However, it was later shown that by grafting onto a stable and well-behaved framework (such as the Herceptin 4D5 [V_H3] framework) considerably improved expression levels and thermodynamic stability, while maintaining binding affinity of the parental antibody fragment [65]. A second approach is based on the use of consensus sequences to improve stability [66]. This approach has been applied to a range of protein scaffolds [67], and utilizes amino acid observed with high frequency within the repertoire [68]. This approach was also used in the construction of the HuCAL human antibody library, for which the authors have reported good expression yields [69]. More recent studies from the same group have identified additional framework mutations that transfer biophysical properties between human V_H3 and V_H6 families [70,71].

As an alternative to homology-based approaches, other studies have focused on the computational prediction of aggregation hotspots in antibody sequences. Early studies on peptides and proteins involved in neurodegenerative diseases have highlighted physicochemical properties that correlate with an increase in aggregate formation, namely hydrophobicity, β-sheet propensity and reduced net charge [72]. Algorithms have been developed to detect such aggregation-prone regions [73–76]. Two of these algorithms, TANGO and PAGE, have been used to identify potential aggregation-prone regions in commercial therapeutic antibodies, which are predominately located in variable domains, and particularly within CDR and adjacent framework residues [28].

8.3. Engineering of human variable domains through CDR mutations

The role of CDR residues in determining thermodynamic and in particular colloidal stability of human antibody variable domains is further highlighted by studies on the biophysical properties of the

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HEL4 model domain [31]. HEL4 was originally isolated through phage display selection from a synthetic CDR-only repertoire based on the human V_H3-23 DP47 germline [77]. As phage is remarkably resistant to chemical, proteolytic and thermal denaturation [78–80], the authors were able to heat the phage displayed antibody fragments at high temperature (80 °C), followed by cooling and binding to antigen (hen egg white lysozyme). The method thereby selected for domains that unfolded reversibly, and could be captured by binding to antigen or superantigen. Unlike other human V_H3 domains, HEL4 displayed highly favorable biophysical properties, including heat-refoldability, high expression levels in bacteria, and the absence of 'stickiness' on gel-filtration [31]. Indeed, it was later shown that aggregation resistance on phage is a general indicator of the solution behavior of human V_H domains [81]. In addition to HEL4, several other V_H domains, that were isolated using this method, showed a high level of resistance to aggregation. Intriguingly, despite their high level of colloidal stability, all of the domains actually had lower thermodynamic stabilities than the DP47 domain from which they had been derived from (ΔG_{N-U} = 15–20 kJ/mol vs 35 kJ/mol). In contrast, DP47 readily aggregated upon heating, further highlighting differences between thermodynamic and colloidal stability. As an alternative to heat-based selection on phage, a more recent study explored the use of pH stress (pH 3.2) for the selection of aggregation-resistant V_H domains [82]. In contrast to domains isolated after thermal denaturation, the selected V_H domains displayed increased thermodynamic stabilities and increased colloidal stability at low pH, reflecting the different denaturation conditions experienced during the selection process.

Although the denaturation method on phage was capable of selecting human V_H domains with CDR sequences that promoted a high level of aggregation resistance, it was also evident that such domains were rare within the repertoire. To increase the frequency of such clones, a human V_H library was constructed through combinatorial assembly of CDR regions that had been pre-selected for aggregation resistance in phage. Binders were successfully selected from this repertoire, which exhibited good antigen-binding properties together with favorable biophysical properties [32].

Despite clear improvements over other human V_H domains, detailed determinants of the observed aggregation resistance initially had remained unclear. As HEL4 and other clones had been selected from synthetic CDR-only repertoires, it was apparent that the

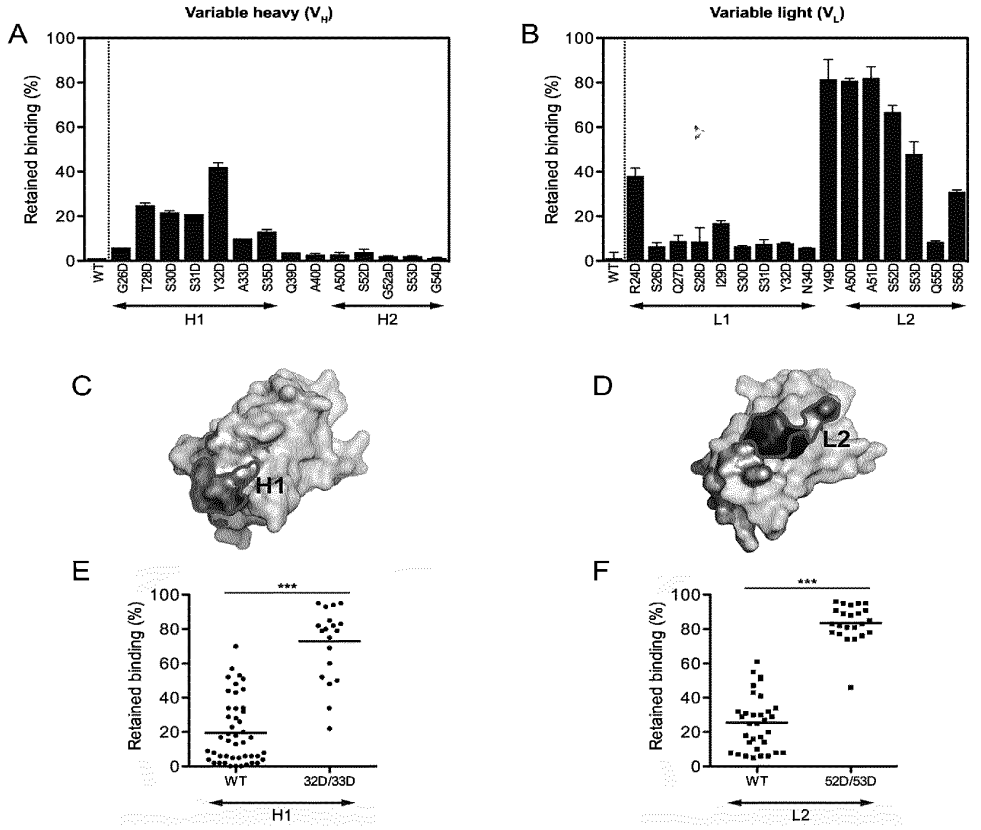


Fig. 4. Effect of mutations in human antibody V_H and V_L domains on aggregation resistance. Surface residues in variable heavy and light domains were targeted for substitution with aspartate. Aggregation resistance of the domains was determined by measuring binding to protein A and protein L superantigen after heating to 80 °C on phage. Complementarily determining regions are indicated (A) Mutations in human V_H. (B) Mutations in human V_L. Introduction of aspartate into CDR H1 and CDR L2 increases aggregation resistance of human variable domains up to 40-fold for V_H and up to 80-fold for V_L. (C) Mutations mapped onto the human V_H surface. (D) Mutations mapped onto the human V_L surface (blue: 100% retained superantigen binding, white: 0%). (E) Stability engineering of human V_H repertoire. (F) Stability engineering of human V_L repertoire. The mutant repertoires were generated through introduction of aspartate mutations in CDR H1 and CDR L2 (at positions 32/33 and 52/53, respectively). Introduction of the mutations significantly increases the aggregation resistance of human antibody repertoires, independent of sequence diversity at other CDR positions. Adapted from Ref. [7].

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exhibited biophysical properties were a result of changes in the complementarity determining regions, without any further influence of framework residues [31]. Crystal structure of the HEL4 model domain had indicated a potential effect of a serine to glycine mutation at position 35 [77]. Indeed, introduction of this mutation into DP47, resulted in increased solubility and reduced "stickiness" on gel-filtration. However, the mutant domain continued to readily aggregate when heated above its melting temperature. The authors also reported that many of the selected domains had a low isoelectric point (pI). Similar findings were also obtained in a second study, which reported that a low pI, together with the non-canonical inter-CDR disulfide linkages, contribute to improved biophysical properties human V_H domains [83]. Using transient heating to pre-select for proteins reversible thermal unfolding properties, the authors found that many of the isolated domains had pI values of less than six, although they also observed domains with values higher than eight. The authors concluded that the improved biophysical characteristics of these V_H domains were caused by a combination of global pI effects, together with an increase of stability through inter-CDR disulfide linkage (other studies by the same group demonstrated that, although the additional disulfide bridge significantly increased thermal stability and protease resistance [84], it also induced structural changes in human V_H as indicated by a marked reduction of protein A superantigen binding [85]). A global increase in charge was also observed in a different study, analyzing larger sets of V_H domain sequences after heat selection on phage, together with an overall reduction of hydrophobicity and a decrease in beta-sheet propensity [86].

More recently, an extensive mutagenesis study on phage has provided detailed insights into mutations that increase the colloidal stability of human V_H and V_L antibody domains [7]. Using superantigen binding after heat denaturation on phage as a mean to select for reversible unfolding, the investigators analyzed the effect of charged mutations at all CDR positions (excluding CDR3 to limit effects on antigen binding). This revealed that the introduction of negative charge (and in particular aspartate) at CDR-H1 positions (28, 30–33, 35) considerably increased the aggregation resistance of human V_H domains (up to 40-fold) (Fig. 4A). This is in excellent agreement with the observations for the model HEL4 domain, which contains negatively charged amino acids at a subset of the identified positions (as highlighted by CDR grafting studies [87–89]).

Intriguingly, this was not the case for human V_L domains, for which the introduction of mutations at equivalent positions had little effect. In contrast to what was observed for human V_H, increased aggregation resistance (up to 80-fold) was instead endowed by the introduction of asparate at CDR-L2 positions (49, 50–53, 56) (Fig. 4B). As observed in other studies [81], the improved properties on phage correlated with increased aggregation resistance in solution, improved expression yields and reduced retention on gel-filtration. Importantly, the observed increases in aggregation resistance were not only observed in model domains, but also when introducing CDR-H1 and CDR-L2 mutations into diverse antibody libraries [7] (Fig. 4E/F).

In addition, and unlike what had been observed for camelized and other engineered variable domains [53,59,85], crystal structures of mutant V_H and V_L domains showed no detectable conformational changes in either CDR conformations or the V_H/V_L interface. Taken together, these results indicated broad compatibility with high affinity antigen binding, which was confirmed by the introduction of CDR-H1 and CDR-L2 mutations into the antibody therapeutic Herceptin [7]. Further evidence for the influence of the identified mutations on colloidal stability has recently been reported in a study of an aggregation-prone human IgG1 monoclonal [90]. The authors found that the aspartic acid at position 49 of the V_L domain considerably reduced aggregation rate of the antibody during long-term stability studies (from 35% to 0.2% per month).

9. Conclusion – towards stable human antibody therapeutics

The increasing number of human monoclonals in development and clinical practice, together with a trend towards high concentration formulations for subcutaneous delivery places increasing demands on the stability of human antibody therapeutics. While the thermodynamic stability of human antibodies is relatively well studied and can be further increased through homology, disulfide and computational approaches [71,83,47], determinants of their colloidal stability are just beginning to emerge due to a combination of rational and high-throughput approaches on phage [7,31,46,91]. In particular, the recent availability of human V_L domains with high levels of aggregation resistance [7] will greatly assist in the generation of paired antibody fragments and IgG. Further advances in the generation of human antibody therapeutics with high thermodynamic and colloidal stability will not only improve the production and formulation of human antibody therapeutics, but may also open up new administration routes and therapeutic applications.

References

[1] Nelson, A.L., Dhimolea, E. and Reichert, J.M. (2010) Development trends for human monoclonal antibody therapeutics. Nat. Rev. Drug Discov. 9, 767–774.
[2] Finke, J.M., Roy, M., Zimm, B.H. and Jennings, P.A. (2000) Aggregation events occur prior to stable intermediate formation during refolding of interleukin 1beta. Biochemistry 39, 575–583.
[3] Baneyx, F. and Mujacic, M. (2004) Recombinant protein folding and misfolding in *Escherichia coli*. Nat. Biotechnol. 22, 1399–1408.
[4] Bondos, S.E. and Bicknell, A. (2003) Detection and prevention of protein aggregation before, during, and after purification. Anal. Biochem. 316, 223–231.
[5] Lowe, D., Dudgeon, K., Rouet, R., Schofield, P., Jermutus, L. and Christ, D. (2011) Aggregation, stability, and formulation of human antibody therapeutics. Adv. Protein Chem. Struct. Biol. 84, 41–61.
[6] Ewert, S., Huber, T., Honegger, A. and Pluckthun, A. (2003) Biophysical properties of human antibody variable domains. J. Mol. Biol. 325, 531–553.
[7] Dudgeon, K., Rouet, R., Kokmeijer, I., Schofield, P., Stolp, J., Langley, D., Stock, D. and Christ, D. (2012) General strategy for the generation of human antibody variable domains with increased aggregation resistance. Proc. Natl. Acad. Sci. USA 109, 10879–10884.
[8] Garber, E. and Demarest, S.J. (2007) A broad range of Fab stabilities within a host of therapeutic IgGs. Biochem. Biophys. Res. Commun. 355, 751–757.
[9] Wolynes, P.G., Onuchic, J.N. and Thirumalai, D. (1995) Navigating the folding routes. Science 267, 1619–1620.
[10] Sambashivan, S., Liu, Y., Sawaya, M.R., Gingery, M. and Eisenberg, D. (2005) Amyloid-like fibrils of ribonuclease A with three-dimensional domain-swapped and native-like structure. Nature 437, 266–269.
[11] Dobson, C.M. (2003) Protein folding and misfolding. Nature 426, 884–890.
[12] Wang, W. (2005) Protein aggregation and its inhibition in biopharmaceutics. Int. J. Pharm. 289, 1–30.
[13] Rosenberg, A.S. (2006) Effects of protein aggregates: an immunologic perspective. AAPS J. 8, E501–E507.
[14] Wright, C.F., Teichmann, S.A., Clarke, J. and Dobson, C.M. (2005) The importance of sequence diversity in the aggregation and evolution of proteins. Nature 438, 878–881.
[15] Shire, S.J. (2009) Formulation and manufacturability of biologics. Curr. Opin. Biotechnol. 20, 708–714.
[16] Salfeld, J.G. (2007) Isotype selection in antibody engineering. Nat. Biotechnol. 25, 1369–1372.
[17] Schuurman, J., Van Ree, R., Perdok, G.J., Van Doorn, H.R., Tan, K.Y. and Aalberse, R.C. (1999) Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Immunology 97, 693–698.
[18] Labrijn, A.F., Meesters, J.I., de Goeij, B.E., van den Bremer, E.T., Neijssen, J., van Kampen, M.D., Strumane, K., Verploegen, S., Kundu, A., Gramer, M.J., van Berkel, P.H., van de Winkel, J.G., Schuurman, J. and Parren, P.W. (2013) Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange. Proc. Natl. Acad. Sci. USA 110, 5145–5150.
[19] Carter, P.J. (2006) Potent antibody therapeutics by design. Nat. Rev. Immunol. 6, 343–357.
[20] Franey, H., Brych, S.R., Kolvenbach, C.G. and Rajan, R.S. (2010) Increased aggregation propensity of IgG2 subclass over IgG1: role of conformational changes and covalent character in isolated aggregates. Protein Sci. 19, 1601–1615.

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Page 6654

[21] Hari, S.B., Lau, H., Razinkov, V.I., Chen, S. and Latypov, R.F. (2010) Acid-induced aggregation of human monoclonal IgG1 and IgG2: molecular mechanism and the effect of solution composition. Biochemistry 49, 9328–9338.
[22] Ito, T. and Tsumoto, K. (2013) Effects of subclass change on the structural stability of chimeric, humanized, and human antibodies under thermal stress. Protein Sci. 22 (11), 1542–1551.
[23] Wypych, J., Li, M., Guo, A., Zhang, Z., Martinez, T., Allen, M.J., Fodor, S., Kelner, D.N., Flynn, G.C., Liu, Y.D., Bondarenko, P.V., Ricci, M.S., Dillon, T.M. and Balland, A. (2008) Human IgG2 antibodies display disulfide-mediated structural isoforms. J. Biol. Chem. 283, 16194–16205.
[24] Lacy, E.R., Baker, M. and Brigham-Burke, M. (2008) Free sulfhydryl measurement as an indicator of antibody stability. Anal. Biochem. 382, 66–68.
[25] Demarest, S.J. and Glaser, S.M. (2008) Antibody therapeutics, antibody engineering, and the merits of protein stability. Curr. Opin. Drug Discov. Devel. 11, 675–687.
[26] Gong, R., Vu, B.K., Feng, Y., Prieto, D.A., Dyba, M.A., Walsh, J.D., Prabakaran, P., Veenstra, T.D., Tarasov, S.G., Ishima, R. and Dimitrov, D.S. (2009) Engineered human antibody constant domains with increased stability. J. Biol. Chem. 284, 14203–14210.
[27] Pepinsky, R.B., Silvian, L., Berkowitz, S.A., Farrington, G., Lugovskoy, A., Walus, L., Eldredge, J., Capili, A., Mi, S., Graff, C. and Garber, E. (2010) Improving the solubility of anti-LINGO-1 monoclonal antibody Li33 by isotype switching and targeted mutagenesis. Protein Sci. 19, 954–966.
[28] Wang, X., Das, T.K., Singh, S.K. and Kumar, S. (2009) Potential aggregation prone regions in biotherapeutics: a survey of commercial monoclonal antibodies. MAbs 1, 254–267.
[29] Tomlinson, I.M., Walter, G., Marks, J.D., Llewelyn, M.B. and Winter, G. (1992) The repertoire of human germline V_H sequences reveals about fifty groups of V_H segments with different hypervariable loops. J. Mol. Biol. 227, 776–798.
[30] Tiller, T., Schuster, I., Deppe, D., Siegers, K., Strohner, R., Herrmann, T., Berenguer, M., Poujol, D., Stehle, J., Stark, Y., Heßling, M., Daubert, D., Felderer, K., Kaden, S., Kölln, J., Enzelberger, M. and Urlinger, S. (2013) A fully synthetic human Fab antibody library based on fixed V_H/V_L framework pairings with favorable biophysical properties. MAbs 5, 445–470.
[31] Jespers, L., Schon, O., Famm, K. and Winter, G. (2004) Aggregation-resistant domain antibodies selected on phage by heat denaturation. Nat. Biotechnol. 22, 1161–1165.
[32] Christ, D., Famm, K. and Winter, G. (2007) Repertoires of aggregation-resistant human antibody domains. Protein Eng. Des. Sel. 20, 413–416.
[33] Wang, W., Singh, S., Zeng, D.L., King, K. and Nema, S. (2007) Antibody structure, instability, and formulation. J. Pharm. Sci. 96, 1–26.
[34] Lahlou, A., Blanchet, B., Carvalho, M., Paul, M. and Astier, A. (2009) Mechanically-induced aggregation of the monoclonal antibody cetuximab. Ann. Pharm. Fr. 67, 340–352.
[35] Daugherty, A.L. and Mrsny, R.J. (2006) Formulation and delivery issues for monoclonal antibody therapeutics. Adv. Drug Deliv. Rev. 58, 686–706.
[36] Buchner, J. and Rudolph, R. (1991) Renaturation, purification and characterization of recombinant Fab-fragments produced in *Escherichia coli*. Biotechnology (NY) 9, 157–162.
[37] Lee, C.M., McGuire, H., Basten, A., King, C. and Christ, D. (2010) Expression, purification and characterization of recombinant interleukin-21. J. Immunol. Methods 362, 185–189.
[38] Arakawa, T., Tsumoto, K., Kita, Y., Chang, B. and Ejima, D. (2007) Biotechnology applications of amino acids in protein purification and formulations. Amino Acids 33, 587–605.
[39] Mahler, H.C., Senner, F., Maeder, K. and Mueller, R. (2009) Surface activity of a monoclonal antibody. J. Pharm. Sci. 98, 4525–4533.
[40] Demarest, S.J., Rogers, J. and Hansen, G. (2004) Optimization of the antibody C(H)3 domain by residue frequency analysis of IgG sequences. J. Mol. Biol. 335, 41–48.
[41] Teerinen, T., Valjakka, J., Rouvinen, J. and Takkinen, K. (2006) Structure-based stability engineering of the mouse IgG1 Fab fragment by modifying constant domains. J. Mol. Biol. 361, 687–697.
[42] Wozniak-Knopp, G., Stadlmann, J. and Ruker, F. (2012) Stabilisation of the Fc fragment of human IgG1 by engineered intradomain disulfide bonds. PLoS One 7, e30083.
[43] Ying, T., Chen, W., Feng, Y., Wang, Y., Gong, R. and Dimitrov, D.S. (2013) Engineered soluble monomeric IgG1 C_H3 domain: generation, mechanisms of function, and implications for design of biological therapeutics. J. Biol. Chem. 288, 25154–25164.
[44] Gong, R., Wang, Y., Feng, Y., Zhao, Q. and Dimitrov, D.S. (2011) Shortened engineered human antibody C_H2 domains: increased stability and binding to the human neonatal Fc receptor. J. Biol. Chem. 286, 27288–27293.
[45] Peters, S.J., Smales, C.M., Henry, A.J., Stephens, P.E., West, S. and Humphreys, D.P. (2012) Engineering an improved IgG4 molecule with reduced disulfide bond heterogeneity and increased Fab domain thermal stability. J. Biol. Chem. 287, 24525–24533.
[46] Chennamsetty, N., Helk, B., Voynov, V., Kayser, V. and Trout, B.L. (2009) Aggregation-prone motifs in human immunoglobulin G. J. Mol. Biol. 391, 404–413.
[47] Chennamsetty, N., Voynov, V., Kayser, V., Helk, B. and Trout, B.L. (2009) Design of therapeutic proteins with enhanced stability. Proc. Natl. Acad. Sci. USA 106, 11937–11942.
[48] Schaefer, J.V. and Pluckthun, A. (2012) Engineering aggregation resistance in IgG by two independent mechanisms: lessons from comparison of *Pichia pastoris* and mammalian cell expression. J. Mol. Biol. 417, 309–335.
[49] Perchiacca, J.M., Ladiwala, A.R., Bhattacharya, M. and Tessier, P.M. (2012) Aggregation-resistant domain antibodies engineered with charged mutations near the edges of the complementarity-determining regions. Protein Eng. Des. Sel. 25, 591–601.
[50] Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E.B., Bendahman, N. and Hamers, R. (1993) Naturally occurring antibodies devoid of light chains. Nature 363, 446–448.
[51] Arbabi Ghahroudi, M., Desmyter, A., Wyns, L., Hamers, R. and Muyldermans, S. (1997) Selection and identification of single domain antibody fragments from camel heavy-chain antibodies. FEBS Lett. 414, 521–526.
[52] van der Linden, R.H., Frenken, L.G., de Geus, B., Harmsen, M.M., Ruuls, R.C., Stok, W., de Ron, L., Wilson, S., Davis, P. and Verrips, C.T. (1999) Comparison of physical chemical properties of llama V_{HH} antibody fragments and mouse monoclonal antibodies. Biochim. Biophys. Acta 1431, 37–46.
[53] Barthelemy, P.A., Raab, H., Appleton, B.A., Bond, C.J., Wu, P., Wiesmann, C. and Sidhu, S.S. (2008) Comprehensive analysis of the factors contributing to the stability and solubility of autonomous human V_H domains. J. Biol. Chem. 283, 3639–3654.
[54] Vranken, W., Tolkatchev, D., Xu, P., Tanha, J., Chen, Z., Narang, S. and Ni, F. (2002) Solution structure of a llama single-domain antibody with hydrophobic residues typical of the V_H/V_L interface. Biochemistry 41, 8570–8579.
[55] Muyldermans, S., Atarhouch, T., Saldanha, J., Barbosa, J.A. and Hamers, R. (1994) Sequence and structure of V_H domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. Protein Eng. 7, 1129–1135.
[56] Ewert, S., Cambillau, C., Conrath, K. and Pluckthun, A. (2002) Biophysical properties of camelid V(HH) domains compared to those of human V(H)3 domains. Biochemistry 41, 3628–3636.
[57] Davies, J. and Riechmann, L. (1994) 'Camelising' human antibody fragments: NMR studies on V_H domains. FEBS Lett. 339, 285–290.
[58] Davies, J. and Riechmann, L. (1996) Single antibody domains as small recognition units: design and in vitro antigen selection of camelized, human VH domains with improved protein stability. Protein Eng. 9, 531–537.
[59] Riechmann, L. (1996) Rearrangement of the former V_L interface in the solution structure of a camelised, single antibody V_H domain. J. Mol. Biol. 259, 957–969.
[60] Tanha, J., Xu, P., Chen, Z., Ni, F., Kaplan, H., Narang, S.A. and MacKenzie, C.R. (2001) Optimal design features of camelized human single-domain antibody libraries. J. Biol. Chem. 276, 24774–24780.
[61] Roux, K.H., Greenberg, A.S., Greene, L., Strelets, L., Avila, D., McKinney, E.C. and Flajnik, M.F. (1998) Structural analysis of the nurse shark (new) antigen receptor (NAR): molecular convergence of NAR and unusual mammalian immunoglobulins. Proc. Natl. Acad. Sci. USA 95, 11804–11809.
[62] Greenberg, A.S., Avila, D., Hughes, M., Hughes, A., McKinney, E.C. and Flajnik, M.F. (1995) A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature 374, 168–173.
[63] Griffiths, K., Dolezal, O., Parisi, K., Angerosa, J., Dogovski, C., Barraclough, M., Sanalla, A., Casey, J., González, I., Perugini, M., Nuttall, S. and Foley, M. (2013) Shark variable new antigen receptor (VNAR) single domain antibody fragments: stability and diagnostic applications. Antibodies 2, 66–81.
[64] Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. and Winter, G. (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 321, 522–525.
[65] Jung, S. and Pluckthun, A. (1997) Improving in vivo folding and stability of a single-chain Fv antibody fragment by loop grafting. Protein Eng. 10, 959–966.
[66] Steipe, B., Schiller, B., Pluckthun, A. and Steinbacher, S. (1994) Sequence statistics reliably predict stabilizing mutations in a protein domain. J. Mol. Biol. 240, 188–192.
[67] Kohl, A., Binz, H.K., Forrer, P., Stumpp, M.T., Pluckthun, A. and Grutter, M.G. (2003) Designed to be stable: crystal structure of a consensus ankyrin repeat protein. Proc. Natl. Acad. Sci. USA 100, 1700–1705.
[68] Worn, A. and Pluckthun, A. (2001) Stability engineering of antibody single-chain Fv fragments. J. Mol. Biol. 305, 989–1010.
[69] Knappik, A., Ge, L., Honegger, A., Pack, P., Fischer, M., Wellnhofer, G., Hoess, A., Wolle, J., Pluckthun, A. and Virnekas, B. (2000) Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. J. Mol. Biol. 296, 57–86.
[70] Ewert, S., Honegger, A. and Pluckthun, A. (2003) Structure-based improvement of the biophysical properties of immunoglobulin VH domains with a generalizable approach. Biochemistry 42, 1517–1528.
[71] Schaefer, J.V. and Pluckthun, A. (2012) Transfer of engineered biophysical properties between different antibody formats and expression systems. Protein Eng. Des. Sel. 25, 485–506.
[72] Murphy, R.M. (2002) Peptide aggregation in neurodegenerative disease. Annu. Rev. Biomed. Eng. 4, 155–174.
[73] Kuhlman, B. and Baker, D. (2000) Native protein sequences are close to optimal for their structures. Proc. Natl. Acad. Sci. USA 97, 10383–10388.
[74] Fernandez-Escamilla, A.M., Rousseau, F., Schymkowitz, J. and Serrano, L. (2004) Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nat. Biotechnol. 22, 1302–1306.
[75] Pawar, A.P., Dubay, K.F., Zurdo, J., Chiti, F., Vendruscolo, M. and Dobson, C.M. (2005) Prediction of "aggregation-prone" and "aggregation-susceptible" regions in proteins associated with neurodegenerative diseases. J. Mol. Biol. 350, 379–392.

Samsung Bioepis Exhibit No. 1002
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[76] Tartaglia, G.G., Cavalli, A., Pellarin, R. and Caflisch, A. (2005) Prediction of aggregation rate and aggregation-prone segments in polypeptide sequences. Protein Sci. 14, 2723–2734.
[77] Jespers, L., Schon, O., James, L.C., Veprintsev, D. and Winter, G. (2004) Crystal structure of HEL4, a soluble, refoldable human V(H) single domain with a germ-line scaffold. J. Mol. Biol. 337, 893–903.
[78] Kristensen, P. and Winter, G. (1998) Proteolytic selection for protein folding using filamentous bacteriophages. Fold Des. 3, 321–328.
[79] Holliger, P., Riechmann, L. and Williams, R.L. (1999) Crystal structure of the two N-terminal domains of g3p from filamentous phage fd at 1.9 Å: evidence for conformational lability. J. Mol. Biol. 288, 649–657.
[80] Jermutus, L., Honegger, A., Schwesinger, F., Hanes, J. and Pluckthun, A. (2001) Tailoring in vitro evolution for protein affinity or stability. Proc. Natl. Acad. Sci. USA 98, 75–80.
[81] Dudgeon, K., Rouet, R. and Christ, D. (2013) Rapid prediction of expression and refolding yields using phage display. Protein Eng. Des. Sel. 26 (10), 671–674.
[82] Famm, K., Hansen, L., Christ, D. and Winter, G. (2008) Thermodynamically stable aggregation-resistant antibody domains through directed evolution. J. Mol. Biol. 376, 926–931.
[83] Arbabi-Ghahroudi, M., To, R., Gaudette, N., Hirama, T., Ding, W., MacKenzie, R. and Tanha, J. (2009) Aggregation-resistant VHs selected by in vitro evolution tend to have disulfide-bonded loops and acidic isoelectric points. Protein Eng. Des. Sel. 22, 59–66.
[84] Hussack, G., Hirama, T., Ding, W., Mackenzie, R. and Tanha, J. (2011) Engineered single-domain antibodies with high protease resistance and thermal stability. PLoS One 6, e28218.
[85] Kim, D.Y., Kandalaft, H., Ding, W., Ryan, S., van Faassen, H., Hirama, T., Foote, S.J., MacKenzie, R. and Tanha, J. (2012) Disulfide linkage engineering for improving biophysical properties of human V_H domains. Protein Eng. Des. Sel. 25, 581–589.
[86] Dudgeon, K., Famm, K. and Christ, D. (2009) Sequence determinants of protein aggregation in human V_H domains. Protein Eng. Des. Sel. 22, 217–220.
[87] Perchiacca, J.M., Bhattacharya, M. and Tessier, P.M. (2011) Mutational analysis of domain antibodies reveals aggregation hotspots within and near the complementarity determining regions. Proteins 79, 2637–2647.
[88] Lee, C.C., Perchiacca, J.M. and Tessier, P.M. (2013) Toward aggregation-resistant antibodies by design. Trends Biotechnol. 31 (11), 612–620.
[89] Christ, D. and Dudgeon, K. (2011) Modified variable domain molecules and methods for producing and using same. WO/2011/047442.
[90] Buchanan, A., Clementel, V., Woods, R., Harn, N., Bowen, M.A., Mo, W., Popovic, B., Bishop, S.M., Dall'Acqua, W., Minter, R., Jermutus, L. and Bedian, V. (2013) Engineering a therapeutic IgG molecule to address cysteinylation, aggregation and enhance thermal stability and expression. MAbs 5, 255–262.
[91] Lee, C.M., Iorno, N., Sierro, F. and Christ, D. (2007) Selection of human antibody fragments by phage display. Nat. Protoc. 2 (11), 3001–3008.

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D20 Table 1 and D21 Table 1

Antibody	Type	Ab concentration mg/ml	Formulation
Satumomab pendetide	IgG1k (murine, conjugated to GYK-DTPA)	0.5	PBS
Indium-111 capromab pendeti	IgG1k (murine, conjugated to GYK-DTPA)	0.5	PBS
Muromomab-CD3	**IgG2a (murine)**	**1**	**Monobasic Na P, dibasic Na P, NaCl, PS80**
I-131 tositumab	**IgG2 gamma (murine)**	**1.1**	**Phosphate, povidone, maltose, NaCl, ascorbic acid**
Ibritumomab-Tiuxetan	IgG1k (murine, thiourea covalent linkage to Tiuxetan)	1.6	Na acetate, K P, NaCl
Cetuximab	IgG1k (chimeric human/mouse)	2	Dibasic Na P septahydrate, monobasic Na P monohydrate, NaCl
Abciximab	Fab, IgG1 (chimeric human/murine)	2	Na P, NaCl, PS80
Daclizimab	IgG1 (humanized)	5	Monobasic Na P monohydrate, dibasic Na P septahydrate, NaCl, PS80
Alemtuzumab	IgG1k (humanized)	10	Dibasic Na P, monobasic K P, NaCl, KCl, Na2EDTA, PS80
Ranibizumab	IgG1k (humanized)	10	His HCl, alpha-trehalose dihydrate, PS20
Rituximab	IgG1k (chimeric mouse/human)	10	Na citrate dihydrate, NaCl, PS80
Nofetumomab	Fab (murine)	10	PBS
Tositumomab	**IgG2 gamma (murine)**	**14**	**Phosphate, NaCl, maltose**
Natalizumab	**IgG4k (humanized)**	**20**	**Monobasic Na P, dibasic NaP septahydrate, NaCl, PS80**
Bevacizumab	IgG1 (humanized)	25	Monobasic Na P hydrate, dibasic Na P anhydrous, alpha-trehalose dihydrate, PS 20
Adalimumab	IgG1k (human)	50	Monobasic Na P dihydrate, dibasic Na P dihydrate, Na citrate, citric acid hydrate, NaCl, mannitol, PS80
Acrituomab	Murine Fab	powder	Stannous chloride, K Na tartrate tetrahydrate, Na acetate trihydrate, NaCl, glacial acetic acid, HCl, sucrose
Gemtuzumab ozogamicin	**IgG4k (conjugated with alicheamicin)**	**powder**	**Monobasic and dibasic Na P, dextran 40, sucrose, NaCl**
Basiliximab	IgG1k (chimeric)	powder	Monobasic K P, Na2HPO4, NaCl, sucrose, mannitol, glycine, PS80
Infliximab	IgG1 (chimeric human/murine)	powder	Monobasic Na P monohydrate, dibasic Na P dihydrate, sucrose, PS80
Trastuzumab	IgG1k (humanized)	powder	L-His HCl, L-His, alpha trehalose dihydrate, PS20 (benzyl alcohol as preservative)
Efalizumab*	IgG1 (humanized)	powder	L-His HCl monohydrate, L-His, sucrose, PS20
Palivizumab	IgG1k (humanized, CDR of murine Mab)	powder	His, Gly, mannitol
Omalizumab	IgG1k (humanized)	powder	L-His HCl, L-His, sucrose, PS20
Eculizumab	**IgG2/4**	**10 mg/ml solution**	**Na P, NaCl, PS80**

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IN THE MATTER OF
European Patent No. EP 2 359 834

DECLARATION OF DR. DINO MIANO

I, Dr. Dino Miano, hereby declare as follows:

1. I am Dr. Dino Miano of Alexion Pharmaceuticals, Inc., 100 College St, New Haven, Connecticut, USA. I hold a Ph.D. degree in Chemistry and Biochemistry from the University of Massachusetts, and a Bachelor of Science degree in Chemistry and Biology from the University of Hartford. I have been employed by Alexion Pharmaceuticals for nearly 20 years, and I currently hold the post of Executive Director, Global Analytical & Pharmaceutical Development. Prior to this I was employed by R.W. Johnson Pharmaceutical Research Institute in Raritan, New Jersey for approximately two years. During my employment at Alexion my responsibilities and work have focused on analytical sciences and protein characterization. I have significant expertise in formulation development and analysis, which includes designing, performing and overseeing experiments that assess the impact of protein concentration, formulation, and storage conditions on the integrity and stability of a protein over time. A copy of my resume is attached as Exhibit 1.

2. The commercial formulation of Soliris®(eculizumab) consists of a concentration of 10 mg/mL of eculizumab in a buffer of 10 mM sodium phosphate, 150 mM sodium chloride and 0.02% polysorbate 80 at pH 7.0. The commercial formulation is satisfactorily stable for 18 months when it is stored as drug substance (i.e., in bulk in the commercial formulation) and for an additional 30 months when it is stored as a drug product (i.e., in 30 mL vials as it is supplied to consumers) providing a total of 48 months of stability. (See March 16, 2007 BLA Approval Letter from U.S. FDA, attached as Exhibit 2 (indicating the drug substance is stable for 18 months and the drug product is stable for 24 months at 2-8°C), and May 8, 2009 Letter from U.S. FDA, attached as Exhibit 3 (extending the shelf-life of the drug product from 24 to 30 months at 2-8°C after supplemental stability data was obtained).)

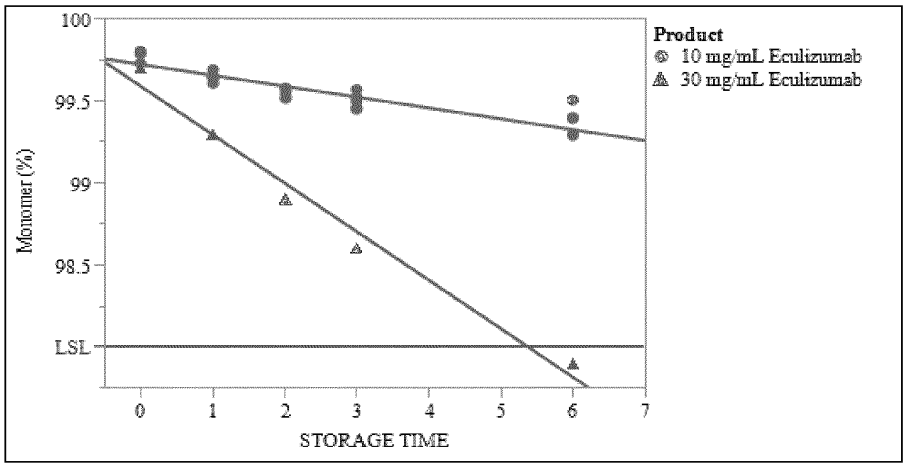
3. One of the stability parameters that the commercial formulation of eculizumab must meet in order to be deemed satisfactorily stable and thus suitable for release into the market is the percent of monomer antibody in the sample must be greater than or equal to 98.0%, which means that there must be less than or equal to 2.0% aggregates of the antibody, as determined via gel permeation high performance liquid chromatography. This release parameter for commercial lots of eculizumab ensures that the drug is satisfactorily stable for the designated storage period.

4. We generated a 30 mg/mL formulation of eculizumab (internally denoted "ALXN5100") that was identical to the commercial formulation of eculizumab, except that ALXN5100 has an eculizumab concentration of 30 mg/mL and the Soliris® commercial formulation has an eculizumab concentration of 10 mg/mL.

5. A batch of ALXN5100 was made under Good Manufacturing Practices (GMP) conditions, and stability studies on the batch were conducted by aliquotting samples into representative HyQ® CX5-14 Hyclone bags to simulate large-scale storage. The Hyclone bags were then stored horizontally at 2 to 8°C for at least 18 months and at 23 to 27°C for at least 6 months, which is how samples were stored for the stability program for the bulk drug substance for the commercial (10 mg/mL) formulation. Although the commercial formulation of eculizumab is to be stored at 2 to 8°C, when performing stability testing, researchers often store test samples at higher temperatures than commercial supplies are typically stored at, such as 23 to 27°C, because storage and stability testing at higher temperatures can provide a good indication of the stability of the formulation in a shorter test period of time. Gel Permeation High Performance Liquid Chromatography (GP-HPLC) was used to determine if there were any changes in monomer and aggregate drug at the two storage temperatures over time.

6. Figures 1 and 2 below demonstrate that the stability of eculizumab at 10 mg/mL is superior to that at 30 mg/mL. Figure 1 compares the percent monomer in the 10 mg/mL eculizumab composition versus the percent monomer in the 30 mg/mL eculizumab composition after storage at 23 to 27°C over six months. Figure 1 clearly shows that the percent monomer of eculizumab decreases (and thus aggregate formation increases) significantly more quickly in the 30 mg/mL eculizumab composition than in the 10 mg/mL eculizumab composition. In addition, whereas the 10 mg/mL eculizumab composition remains sufficiently stable over the specification ("LSL") monomer level of 98.0% over the entire six-month period, the 30 mg/mL eculizumab composition falls below that stability threshold during the same time period.

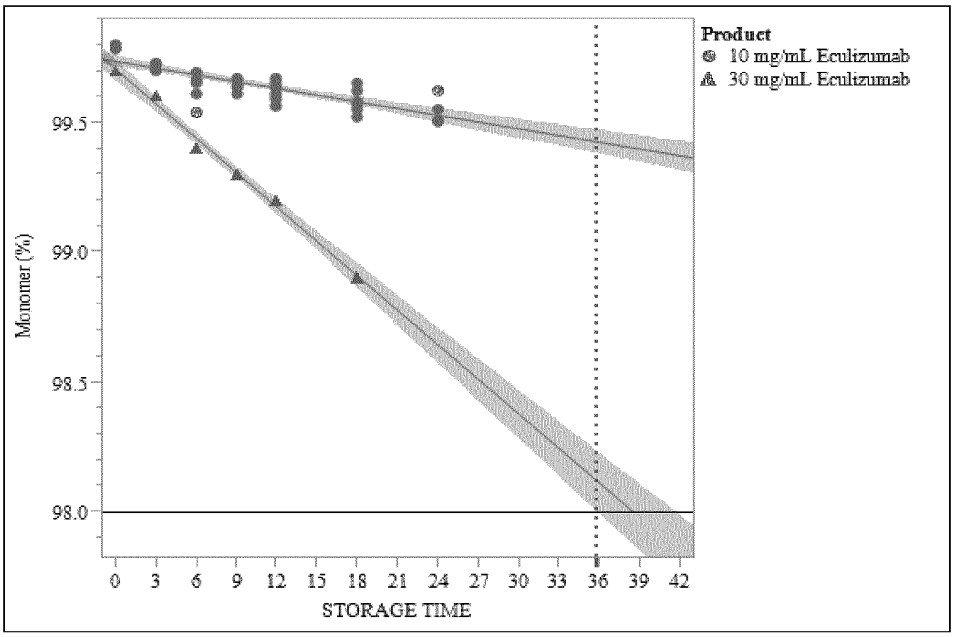
Figure 1. Percent monomer of eculizumab in 10 mg/mL eculizumab composition versus 30 mg/mL eculizumab composition over six months when stored at 23-27°C



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7. Similar trends are seen between the 10 mg/mL eculizumab composition and the 30 mg/mL eculizumab composition even at storage temperatures of 2 to 8°C. Figure 2 compares the percent monomer in the 10 mg/mL eculizumab composition versus the percent monomer in the 30 mg/mL eculizumab composition after storage at 2 to 8°C over 18 months. Again, as with the 23 to 27°C storage conditions, the percent monomer remaining in the 30 mg/mL eculizumab samples decreased much more rapidly than the percent monomer remaining in the 10 mg/mL eculizumab samples at 2 to 8°C over the 18-month period. In addition, the model generated from this data predicted that the 30 mg/mL eculizumab composition would fall below the acceptable 98.0% monomer level before 36 months of storage.

Figure 2. Percent monomer of eculizumab in 10 mg/mL eculizumab composition versus 30 mg/mL eculizumab composition over 18 months when stored at 2-8°C



8. Thus, these long-term stability studies demonstrate a decrease in monomer and an increase in aggregate formation for ALXN5100, the 30 mg/mL eculizumab concentration, throughout the stability program. In all instances the aggregate formation in the 30 mg/mL concentration was greater than what was observed in the 10 mg/mL concentration. These results are clearly demonstrated through 18 months at 2 to 8°C and are even more pronounced within 6 months at the higher temperature studies. These results suggest that the commercial formulation is optimal for an eculizumab concentration of 10 mg/mL but is not suitable when the eculizumab concentration is increased to 30 mg/mL.

I believe and do hereby declare that the facts stated above are to the best of my knowledge true.

Dr. Dino Miano

17 Mar 2018
Date

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Exhibit 1

Dino C. Miano, Ph. D.
217 Sunset Ridge
Rocky Hill, CT 06067
(860) 563-5433 (Home)
(860) 983-6178 (Mobile)
dinomiano@sbcglobal.net

QUALIFICATION PROFILE

- Results-oriented professional with eighteen years' successful track record of innovation, management, in depth scientific and regulatory knowledge in analytical and pharmaceutical development in the field of biotherapeutics. Experience encompasses various stages of biologics including early and late phase development, regulatory submission for marketing authorization and life cycle management.

- Well established division-level leader that consistently drives vision toward successful and timely completion of projects, while focusing on translating goals into actionable strategies that support the business

COMPETENCIES

- Pharmaceutical (biological) development
  - Formulation development
  - Fill finish
  - Stability monitoring and trending
  - Device development
  - Analytical support of clinical operations
- Regulatory Compliance
  - CMC development
- Analytical Development
  - Method Validation & Transfer
  - GMP Testing & Compliance
  - Implementing new analytical technologies
  - Analytical support of manufacturing
- Strategic leadership and innovation
  - Cross functional leader

Accomplishments and Qualifications

- Assumed a key analytical role in the clinical development and global commercialization of Soliris® and Strensiq®. Led development and validation activities for commercial release and stability test methods.
- Lead Global Operations efforts in delivering clinical material from early stage through commercial. Lead cross functional teams consisting of manufacturing, supply chain, QA and CMC and working with clinical groups to ensure supply demands are consistently met
- Lead Global Analytical and Pharmaceutical Development groups in providing analytical support to the entire Alexion organization, including analytical development, validation and transfer, GMP testing of clinical products, process development support, formulation and device development, product characterization and stability to allow for expiry determination.
- Implemented GMP quality infrastructure in the Analytical Sciences laboratories to ensure testing compliance with FDA, EU and PMDA regulations.
- Lead formulation development of new, existing and commercial products for IV, IVT and subcutaneous administration.

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- Implement drug substance and drug product specifications for clinical products
- Generate analytical sections in support of all new and existing CMC submissions
- Lead the drug delivery and device development group in developing a strategy and business process to advance drug and device/combination products for existing commercial and clinical products.

Employment History

Alexion Pharmaceuticals, Inc., New Haven, CT

Executive Director, Global Analytical and Pharmaceutical Development **12/15-present**

- Lead global analytical support function for the entire organization for all commercial and clinical programs.
- Lead the global method technology transfers for Alexion's clinical and commercial products.
- Oversee compatibility assessments for materials used for delivery of product to patients during clinical studies.
- Represent Global Operations as lead for late stage process development, drug substance and drug product manufacturing, supply chain and CMC/IND submissions.
- Lead the device group in collaboration with internal and external partners for the development of delivery devices for Alexion products.

Senior Director, Analytical and Pharmaceutical Development **03/15-12/15**

- Led Early Stage Analytical and Product Characterization groups in supporting Research in efforts to move the best candidates into development.
- Led Analytical Sciences in method development, validation and transfer for new candidates and provide support to process development groups.
- Lead the stability program for all clinical products, including expiry determinations
- Led and aligned formulation development of new candidates with Clinical development and Target product profiles (TPP).
- Led Device Development and Drug Delivery group in identifying and integrating large volume bolus injector options for clinical and commercial development.
- Appointed CMC lead during Regulatory meetings with FDA, Health Canada, EMA and MFDS (F2F) to support CMC section of regulatory filings.

Director, Product Characterization **07/14-03/15**

- Led Global Operations Cross Functional Product Team in supporting second generation development of commercial product.
- Led device teams consisting of Clinical and Commercial Operations, Development, Regulatory and QA in evaluating large volume subcutaneous administration devices. Device included auto-injectors and larger wearables patch devices.
- Managed Mass Spectroscopy Characterization group. Involves the MS characterization of new and existing molecules to support CMC, Research and Development groups.
- Managed the Bio-Physical Characterization group. Involves directing the testing and collaboration of multiple groups to better understand new protein therapeutics
- Provided analytical support of new and existing CMC submissions and address inquiries from agencies

Director, Analytical Sciences **09/08-07/14**

- Managed the Operations group that provided GMP testing of clinical products and analytical testing to all development groups.
- Managed the Analytical Development group that provided method development, qualification, validation and transfer of analytical methods for new clinical products and existing commercial product to both QC and EU laboratories.
- Manage the Formulation Development group that identified stable formulations for new clinical products and determined stable subcutaneous formulations for both clinical and commercials products.
- Managed Stability program of clinical products that generated GMP stability protocols and reports, trended data and provided statistical analysis of trends to support expiry, and led deviations, LIR and OOS for GMP testing.
- Member of Specification committee to direct the generation or revision of specifications for clinical products

Associate Director, Analytical Sciences **05/03-08/08**

- Analytical Group
  - Provided analytical support of new and existing Soliris® CMC submissions
  - Supervised Analytical Operations laboratory in providing method development, qualification, validation, transfer and GMP testing to support clinical products.
- Purification Development group:
  - Managed PD studies in support of Process Validation. Studies included viral clearance, DNA clearance, process limits evaluation (PLE), resin re-use, and UF/DF membrane re-use.

Manager, Quality Control **08/99-05/03**

- Managed QC laboratories; maintained cGMP compliance with regards to current SOPs, validation/qualifications, and training.
- Coordinated studies, supervised investigations, generated reports and implemented systems with QA in response to OOS, invalid assays, aberrant trends/results.
- Represented area functions as required during audits by external agencies and internal QA department.

Senior Scientist, Quality Control **08/98-08/99**

- Developed and optimized RP-HPLC, SEC-HPLC, SDS-PAGE, and IEF methods for all protein based therapeutic product.

R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
A Johnson & Johnson Company

Scientist, Analytical Development **07/97-07/98**

- Developed HPLC assays for quantitation of becaplermin drug substance in alternative REGRANEX® formulations. Developed HPLC assays for determining content of preservatives in REGRANEX® formulations.

Scientist, Protein Development **01/97-07/97**

- Investigated modified roller bottle processes for the production of erythropoietin (rHuEPO), potentially increasing production/yields by 3-fold.
- Evaluated alternative purification processes for rHuEPO, involving HIC, IEC and Reverse phase chromatography.

Postdoctoral Research Scientist, Protein Development **10/95-01/97**

- Chemically modified rHuEPO using polyethylene glycol (PEG) to enhance in vivo erythropoiesis while prolonging circulatory half-life. Experiments were designed to specifically modify the carbohydrate (NANA) moieties of the glycoprotein with PEG while maintaining the native polypeptide conformation. Animal model analysis revealed successful prolonged in vivo biological activity.

University of Connecticut Health Center, Farmington, CT **01/89-08/90**

Research Scientist, Surgery Department

- Investigated/detected tissue derived inflammatory mediators released from injured corneas, urinary bladders and ischemic hearts. Modified Boyden chambers were employed to evaluate chemotactic response via neutrophil migration through 8 μm membranes.
- Investigated/synthesized cyclocreatine, a creatine analog, to determine inhibitory effects on the production and release of neutrophil chemotactic factors (NCF) in ischemic canines and rabbits myocardium.

EDUCATION

University of Massachusetts, Lowell, MA **09/90-10/95**

Ph. D. Chemistry/Biochemistry
Advisor: Dr. Edwin G.E. Jahngen
Thesis: "The Isolation and characterization of an ubiquitin-histone protein from *Dictyostelium discoideum AX3.*"

University of Hartford, Bloomfield, CT **09/85-05/89**

B.S. Chemistry/Biology
Research: "Corneal derived neutrophil chemotactic factors, intracellular synthesis and release."

PUBLICATIONS

Neill, A., Nowak, C., Patel, R., Ponniah, G., Gonzalez, N., **Miano, D**. and Liu, H., (2015) Characterization of recombinant monoclonal antibody charge variants using OFFGEL fractionator, weak anion exchange chromatography and mass spectrometry, *Analytical Chemistry*, **87**, 6204-6211

Ponniah, G., Nowak, C., Gonzalez, N., **Miano D**., and Liu, H., (2015) Detection and quantitation of low abundance oligosaccharides in recombinant monoclonal antibodies. *Analytical Chemistry*, **87**, 2718-2726

Peckham, N., Neil, F., and **Miano, D.C**., "Quantitative SDS-PAGE Analysis of a Recombinant Protein by Densitometry", IBC's Well Characterized Biologicals Poster Session, Washington D.C. (11/11/04)

Peckham, N., Bosley, K., and **Miano, D.C**., Adaptation of Complexometric Titration Method for Quantitation of EDTA in Biopharmaceutical Formulations to Microplate Format, IBC's Well Characterized Biologicals Poster Session, Washington D.C. (11/11/04).

Miano, D.C., Villalobos, A.P., Ghosh-Datidar, P., and Sharma, B. (1997), Chemical Modification of Recombinant Human Erythropoietin by Poly (ethylene glycol). Johnson and Johnson Chromatography Symposium, Princeton, NJ (abstract).

Miano, D.C. (1995) The Isolation and Characterization of a Ubiquitinylated Histone Protein in *Dictyostelium discoideum* AX3. Ph.D. Thesis, University of Massachusetts/Lowell.

Miano, D.C, and Jahngen, G.E. (1994) The Ubiquitinylation of Histones in *Dictyostelium discoideum* AX3 Chromatin May Be Linked to Replication, Transcription and Stress. Keystone Symposium, Tammarron, CO (abstract).

Elgebaly, S.A., **Miano, D.C.**, Ehler, W., Rahha,l R. and Elkerm, A. (1994) The Induction of Corneal Inflammation by Factors Derived From Hydrogen Peroxide Injured Corneas: Effect of Dexamethasone and Indomethacin. *Occ. Pharm.*, **10**, (1), 295-306.

Elgebaly, S.A., Allam, M.E., Houser, S., Hashmi, F., Forouhar, F. and **Miano, D.C.**, (1993) Cyclocreatine Inhibits Neutrophil Accumulation in the Myocardium of Canine Models in Coronary Artery Occlusion and Reperfusion. *J. Pharm. Exp. Ther.*, **266**, (3), 1670-1677.

Elgebaly, S.A., **Miano, D.C.**, Kreutzer, D.L. and Fishman, J.B., (1990) Cornea Derived Neutrophil Chemotactic Factors: Intracellular Synthesis and Release. *Curr. Eye Res.*, **9**, 839-845.

Elgebaly, S.A., **Miano, D.C.**, Ehler, W. and Rahhal, R. (1990) The Induction of Corneal Inflammation by Factors Derived From Hydrogen Peroxide Injured Corneas. ARVO, Sarasota, FL (abstract).

Elgebaly, S.A., **Miano, D.C.**, Kreutzer, D.L. and Fishman, J.B. (1989) Cornea Derived Neutrophil Chemotactic Factors. ARVO, Sarasota, FL (abstract).

Exhibit 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

Our STN: BL 125166/0

MAR 16 2007

Alexion Pharmaceuticals
Attention: Nancy Motola, Ph.D., RAC
Senior Vice President, Regulatory and Quality
352 Knotter Drive
Cheshire, CT 06410

Dear Dr. Motola:

We are issuing Department of Health and Human Services U.S. License No. 1743 to Alexion Pharmaceuticals, Inc., Cheshire, Connecticut, under the provisions of section 351(a) of the Public Health Service Act controlling the manufacture and sale of biological products. The license authorizes you to introduce or deliver for introduction into interstate commerce, those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are approved to manufacture the product eculizumab, which is indicated for the treatment of paroxysmal nocturnal hemoglobinuria to reduce hemolysis.

You are approved to manufacture eculizumab drug substance at Lonza Biologics, PLC in Portsmouth, NH. The final formulated product will be manufactured, filled, labeled, and packaged at Ben Venue Laboratories, Bedford, Ohio. You may label your product with the proprietary name Soliris™ and will market it in 300 mg vials.

The final printed labeling (FPL) must be identical to the enclosed labeling. Marketing product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for eculizumab drug product shall be 24 months from the date of manufacture when stored at 2-8°C. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for your drug substance shall be 18 months when stored at 2-8°C.

You currently are not required to submit samples of future lots of eculizumab to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

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You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of eculizumab, or in the manufacturing facilities.

We acknowledge your written commitments as described in your letter of February 22, 2007, March 12, 2007 and March 12, 2007 as outlined below:

Postmarketing Studies subject to reporting requirements of 21 CFR 601.70.

1. To evaluate long-term safety of eculizumab by analyzing outcomes in the Soliris Safety Registry for a time period of no less than five years. At the end of the five year period, a study report will be submitted to the Biological License Application (BLA) that describes the major safety findings from the registry program, including the specific items listed below and proposing labeling changes as appropriate. Additionally, annual interim reports will be submitted to the BLA, along with expedited reports as specified below. The protocol for addressing this PMC will be submitted to the IND by May 31, 2007, and the five year study report will be submitted by June 30, 2012. All patients within the registry will be followed for the occurrence of:

    a. Serious infections, defined as infections necessitating or prolonging hospitalization or resulting in death. Alexion commits to collecting follow-up information from these patients to assess the nature of the serious infection, the duration of hospitalization, the major features of the clinical course and the survival status. Expedited reporting (15 day telephone or facsimile Medwatch communication) will be provided for the occurrence of these serious infections.

    b. Malignancy, including the nature of the malignancy and the survival status of patients who develop a malignancy;

    c. Use of eculizumab among pediatric patients under 16 years of age, to include collection of eculizumab dosage information, as well as the same information being required for adult patients in the registry;

    d. Pregnancy, including the clinical course of each pregnancy and the detection of congenital abnormalities among babies born to the women exposed to eculizumab during the pregnancy.

    e. Thrombotic events, including the nature of the event, the clinical outcome as well as the anticoagulant management prior to and after the event.

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2. To submit a comprehensive description of the Soliris Guardian Program Risk Minimization Action Plan (RiskMAP), including all items listed below. Fulfillment of this post-marketing commitment will be contingent upon FDA concurrence upon the expectations of the Soliris Guardian Program. Submission of all items listed below will occur no later than May 18, 2007. The submitted information will include:

   a. A final version of the Soliris Guardian Program document that, in addition to any other items, provides information fully consistent with the approved prescribing information.

   b. A copy of all educational materials to be provided as part of the program, including but not limited to all components of the Soliris Starter Kit, the Drug Fact Sheet and patient-health care provider documents relating to the Soliris Safety Registry.

   c. A commitment to develop and submit a protocol (with the understanding that this protocol may need modification based upon FDA review findings) that uses surveys of health care providers and patients to assess compliance with the vaccination requirements as well as their knowledge of the risks of eculizumab and the need for vaccination.

   d. A copy of the Soliris Safety Registry protocol and any supportive documents to be provided to health care providers and patients. These documents may need modification based upon FDA review findings. The submitted protocol will indicate that the Registry will continue until Alexion receives written concurrence from FDA to terminate the registry. The protocol will include collection of the occurrence of the following events:

      i. Death;

      ii. Meningococcal vaccination (type and dates of all vaccinations);

      iii. eculizumab administration dates, at designated time points to establish initiation and termination of therapy as well as to correlate eculizumab administration with the fatalities and the events listed below:

         - All meningococcal infections causing sepsis or meningitis
         - Other serious infections
         - Malignancy, including the nature of the malignancy and the survival status of patients who develop a malignancy;
         - Use of eculizumab among pediatric patients under 16 years of age, to include collection of eculizumab dosage information, as well as the required information for adult patients in the registry;
         - Use of eculizumab by indication;

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- Pregnancy, including the clinical course of each pregnancy and the detection of congenital abnormalities among off-spring of the women exposed to eculizumab during a pregnancy and;
- Serious hemolysis, as defined by specific criteria.

e. A commitment to submit quarterly RiskMAP reports for the first year and annual reports thereafter (unless FDA provides written request for more frequent submissions) summarizing all information relating to the Soliris Guardian Program. The reports should include the following:

i. An analysis of all cases of the following, including a root cause analysis and factors that might have contributed to serious outcomes:
   - Meningococcal sepsis or meningitis, including the timing of all vaccinations relative to administration of Soliris
   - Other infections (with serious outcomes)
   - All deaths
   - Cases of serious hemolysis and all cases of hemolysis with serious outcomes

ii. Soliris use patterns including indication for use;

iii. Extent of compliance with RiskMAP requirements such as the percentage of patients that were vaccinated prior to receiving eculizumab and the percentage of patients that were re-vaccinated at 3-year or 10-year intervals (as applicable);

iv. Analysis of all cumulative data collected in the Soliris Safety Registry;

v. Results from all health care provider and patient surveys, including:
   - Any known data about patients or physicians who refused to participate in the surveys
   - Any known data about survey participants who are considered "lost" (drop-outs).

3. To conduct a randomized, controlled clinical study to assess the effects of anticoagulant withdrawal among PNH patients receiving eculizumab. This study will randomize at least 100 anticoagulated patients to either continue or discontinue anticoagulation therapy. The major outcomes will assess the safety of discontinuation of anticoagulant therapy while continuing eculizumab, especially with respect to providing important evidence regarding major bleeding and that this discontinuation does not increase the risk for occurrence of thrombotic events in these patients. A full study report and data from this study will be submitted to the BLA and may include a label revision, contingent upon the importance of the study results. The study protocol will be submitted to the investigational new drug application (IND) by June 30, 2007 and patient accrual completed no later than June 1, 2009. A final study report will be submitted no later than March 31, 2014.

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4. To develop a validated and quantitative assay for the measurement of human anti-human antibodies (HAHA) for the detection of antibody formation to eculizumab. This assay will assess potential immune responses to the whole eculizumab molecule. Description of the validated assay will be submitted to the BLA as a CBE 30 by July 9, 2008.

5. To develop a validated and sensitive assay for the measurement of neutralizing HAHA to eculizumab. Alternatively, Alexion commits to submit documentation to FDA demonstrating with due diligence that a suitable assay could not be feasibly developed and that the assessment of serum lactate dehydrogenase (LDH) is a sufficiently sensitive indicator of the presence of neutralizing antibodies. This information will be submitted to the BLA by July 9, 2008.

6. To utilize samples from the ongoing E05-001 Phase III extension study (approximately 170 patients for at least 2 years) as test samples for the new validated HAHA assays. Alexion will continue to obtain serum samples from those patients who transition from E05-001 to the Soliris Safety Registry, at intervals of no less than one year, and continue this collection process for an additional three years. Sample collection will cease during this additional three year period for patients who terminate eculizumab administration. Additionally, serum samples will be obtained based upon physician reports of suspected loss of eculizumab bioactivity, based upon unanticipated alterations in serum LDH concentrations. All serum samples will be assayed at least annually and the results provided within an annual report to the BLA. Clinical data, to include the results of serum LDH concentrations, will also be obtained from any patients who show evidence of antibody formation. The protocol describing Alexion's plan for responding to this commitment will be submitted by May 1, 2007 and the final study report submitted by January 31, 2011.

Postmarketing Studies not subject to reporting requirements of 21 CFR 601.70.

7. To revalidate the linearity and accuracy of the Osmolality method across the full specification range using a combination of product samples diluted to lower osmolality and product samples spiked with osmolality standards. The revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

8. To revalidate the linearity of the IEF method across a load range of 8 µg to 12 µg. The revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 supplement by August 31, 2007.

9. To revise the IEF method SOP to specify that the method is validated only for a 10 µg load. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

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10. To improve and revalidate the existing hemolytic assay. Improvements include increasing the number of sample replicates and qualifying the chicken erythrocytes reagent. The revised method SOP and revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

11. To develop a new quantitative biological activity assay to replace the existing hemolytic assay, or submit documentation to FDA demonstrating with due diligence that a suitable assay could not be feasibly developed. This information will be submitted to the BLA by February 29, 2008. Validation of the quantitative biological activity assay will be submitted by July 9, 2008.

12. To provide FDA with a completed drug substance and drug product container closure system leachables evaluation using end-of-shelf-life, long-term 2-8°C stability samples. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

13. To develop a suitable antifoam assay and subsequently confirm the removal of antifoam from the process stream at the Protein A chromatography pool on three drug substance batches. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application (BLA), STN BL 125166. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to your BLA, STN BL 125166. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Commitment Protocol**
- **Postmarketing Study Commitment Final Study Report**
- **Postmarketing Study Commitment Correspondence**
- **Annual Status Report of Postmarketing Commitment Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

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As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Study Commitments - Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cder/guidance/5569fnl.htm) for further information.

Under 21 CFR Part 208, we have determined that this product poses a serious and significant public health concern requiring the distribution of a Medication Guide. Eculizumab is a product for which patient labeling could help prevent serious adverse effects and inform the patient of serious risks relative to benefit that could affect their decisions to use, or continue to use, the product. Therefore, a Medication Guide is necessary for safe and effective use of this product and FDA hereby approves the draft Medication Guide you submitted March 15, 2007. Please note that:

- this Medication Guide must be reprinted at the end of the package insert or accompany it [21 CFR 201.57(c)(18)];
- you are responsible for ensuring that this Medication Guide is available for distribution to every patient who is dispensed a prescription for this product [21 CFR 208];
- the final printed Medication Guide distributed to patients must conform to all conditions described in 21 CFR 208.24, including a minimum of 10 point text; and
- you are responsible for ensuring that the label of each container or package includes a prominent and conspicuous instruction to authorized dispensers to provide a Medication Guide to each patient to whom the drug is dispensed, and states how the Medication Guide is provided.

Please submit within 30 days content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/oc/datacouncil/spl.html, that is identical in content to the enclosed labeling text dated March 16, 2007. Upon receipt and verification, we will transmit that version to the National Library of Medicine for public dissemination.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

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You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all final printed labeling at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for information regarding therapeutic biological products, including the addresses for submissions.

Sincerely,

Karen Weiss for

Richard Pazdur, M.D.
Director
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Enclosure: Package Insert
Carton and Vial Labeling
Medication Guide
Patient Safety Card

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Soliris ™ (eculizumab)

FULL PRESCRIBING INFORMATION

WARNING: SERIOUS MENINGOCOCCAL INFECTION

Soliris increases the risk of meningococcal infections (5.1)

- **Vaccinate patients with a meningococcal vaccine at least 2 weeks prior to receiving the first dose of Soliris; revaccinate according to current medical guidelines for vaccine use**
- **Monitor patients for early signs of meningococcal infections, evaluate immediately if infection is suspected, and treat with antibiotics if necessary.**

1 INDICATIONS AND USAGE

Soliris is indicated for the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH) to reduce hemolysis.

2 DOSAGE AND ADMINISTRATION

Patients must be administered a meningococcal vaccine at least two weeks prior to initiation of Soliris therapy and revaccinated according to current medical guidelines for vaccine use. [*see Warnings and Precautions (5.1)*].

2.1 Recommended Dosage Regimen

Soliris therapy consists of:

- 600 mg every 7 days for the first 4 weeks, followed by
- 900 mg for the fifth dose 7 days later, then
- 900 mg every 14 days thereafter.

Soliris should be administered at the recommended dosage regimen time points, or within two days of these time points. [*see Warnings and Precautions (5.5)*]

2.2 Preparation for Administration

Soliris must be diluted to a final admixture concentration of 5 mg/mL using the following steps:

- Withdraw the required amount of Soliris from the vial into a sterile syringe.
- Transfer the recommended dose to an infusion bag.
- Dilute Soliris to a final concentration of 5 mg/mL by adding the appropriate amount (equal volume of diluent to drug volume) of 0.9% Sodium Chloride Injection, USP; 0.45% Sodium Chloride Injection, USP; 5% Dextrose in Water Injection, USP; or Ringer's Injection, USP to the infusion bag.

The final admixed Soliris 5 mg/mL infusion volume is 120 mL for 600 mg doses or 180 mL for 900 mg doses. Gently invert the infusion bag containing the diluted Soliris solution to ensure thorough mixing of the product and diluent. Discard any unused portion left in a vial, as the product contains no preservatives.

Prior to administration, the admixture should be allowed to adjust to room temperature [18°-25° C, 64-77° F]. The admixture must not be heated in a microwave or with any heat source other than ambient air temperature. The Soliris admixture should be inspected visually for particulate matter and discoloration prior to administration.

2.3 Administration

Do Not Administer As An Intravenous Push Or Bolus Injection

The Soliris admixture should be administered by intravenous infusion over 35 minutes via gravity feed, a syringe-type pump, or an infusion pump. Admixed solutions of Soliris are stable for 24 hours at 2-8° C (36-46° F) and at room temperature.

If an adverse reaction occurs during the administration of Soliris, the infusion may be slowed or stopped at the discretion of the physician. If the infusion is slowed, the total infusion time should not exceed two hours. Monitor the patient for at least one hour following completion of the infusion for signs or symptoms of an infusion reaction.

3 DOSAGE FORMS AND STRENGTHS

Soliris is supplied as 300 mg single-use vials each containing 30 mL of 10 mg/mL sterile, preservative-free eculizumab solution.

4 CONTRAINDICATIONS

Do not initiate Soliris therapy in patients:

- with unresolved serious *Neisseria meningitidis* infection.
- who are not currently vaccinated against *Neisseria meningitidis*.

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5 WARNINGS AND PRECAUTIONS

5.1 Serious Meningococcal Infections

The use of Soliris increases a patient's susceptibility to serious meningococcal infections (septicemia and/or meningitis). All patients without a history of prior meningococcal vaccination must receive the meningococcal vaccine at least 2 weeks prior to receiving the first dose of Soliris and revaccinated according to current medical guidelines for vaccine use. Quadravalent, conjugated meningococcal vaccines are strongly recommended. Vaccination may not prevent meningococcal infections.

All patients must be monitored for early signs and symptoms of meningococcal infections and evaluated immediately if an infection is suspected. Physicians should strongly consider discontinuation of Soliris during the treatment of serious meningococcal infections.

In clinical studies, 2 out of 196 PNH patients developed serious meningococcal infections while receiving treatment with Soliris; both had been vaccinated. [*see Adverse Reactions (6.1)*].

5.2 Other Infections

Soliris blocks terminal complement; therefore patients may have increased susceptibility to infections, especially with encapsulated bacteria. Use caution when administering Soliris to patients with any systemic infection.

5.3 Monitoring After Soliris Discontinuation

Since Soliris therapy increases the number of PNH cells [in study 1, the proportion of PNH RBCs increased among Soliris-treated patients by a median of 28% from baseline (range from -25% to 69%)], patients who discontinue treatment with Soliris may be at increased risk for serious hemolysis. Serious hemolysis is identified by serum LDH levels greater than the pre-treatment level, along with any of the following: greater than 25% absolute decrease in PNH clone size (in the absence of dilution due to transfusion) in one week or less; a hemoglobin level of <5 gm/dL or a decrease of >4 gm/dL in one week or less; angina; change in mental status; a 50% increase in serum creatinine level; or thrombosis. Monitor any patient who discontinues Soliris for at least 8 weeks to detect serious hemolysis and other reactions.

If serious hemolysis occurs after Soliris discontinuation, consider the following procedures/treatments: blood transfusion (packed RBCs), or exchange transfusion if the PNH RBCs are >50% of the total RBCs by flow cytometry; anticoagulation; corticosteroids; or reinstitution of Soliris.

In clinical studies, 16 of 196 PNH patients discontinued treatment with Soliris. Patients were followed for evidence of worsening hemolysis and no serious hemolysis was observed.

5.4 Thrombosis Prevention and Management

The effect of withdrawal of anticoagulant therapy during Soliris treatment has not been established. Therefore, treatment with Soliris should not alter anticoagulant management.

5.5 Laboratory Monitoring

Serum LDH levels increase during hemolysis and may assist in monitoring Soliris effects, including the response to discontinuation of therapy. In clinical studies, six patients achieved a reduction in serum LDH levels only after a decrease in the Soliris dosing interval from 14 to 12 days. All other patients achieved a reduction in serum LDH levels with the 14 day dosing interval [*see Clinical Pharmacology (12.2) and Clinical Studies (14)*].

5.6 Infusion Reactions

As with all protein products, administration of Soliris may result in infusion reactions, including anaphylaxis or other hypersensitivity reactions. In clinical trials, no PNH patients experienced an infusion reaction which required discontinuation of Soliris. Soliris administration should be interrupted in all patients experiencing severe infusion reactions and appropriate medical therapy administered.

6 ADVERSE REACTIONS

6.1 Clinical Trial Experience

Meningococcal infections are the most important adverse reactions experienced by patients receiving Soliris therapy. In PNH clinical studies, two patients experienced meningococcal sepsis. Both patients had previously received a meningococcal vaccine. In clinical studies among patients without PNH, meningococcal meningitis occurred in an unvaccinated patient [*see Warnings and Precautions (5.1)*].

The data described below reflect exposure to Soliris in 196 adult patients with PNH, age 18-85, of whom 55% were female. All had signs or symptoms of intravascular hemolysis. Soliris was studied in a placebo-controlled clinical study (in which 43 patients received Soliris and 44, placebo); a single arm clinical study and a long term extension study. 182 patients were exposed for greater than one year. All patients received the recommended Soliris dose regimen.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. Table 1 summarizes the adverse reactions that occurred at a numerically

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higher rate in the Soliris group than the placebo group and at a rate of 5% or more among patients treated with Soliris.

TABLE 1

ADVERSE REACTIONS REPORTED IN 5% OR MORE OF SOLIRIS TREATED PATIENTS AND GREATER THAN PLACEBO IN THE CONTROLLED CLINICAL STUDY

Reaction	Soliris N = 43 N (%)	Placebo N = 44 N (%)
Headache	19 (44)	12 (27)
Nasopharyngitis	10 (23)	8 (18)
Back pain	8 (19)	4 (9)
Nausea	7 (16)	5 (11)
Fatigue	5 (12)	1 (2)
Cough	5 (12)	4 (9)
Herpes simplex infections	3 (7)	0
Sinusitis	3 (7)	0
Respiratory tract infection	3 (7)	1 (2)
Constipation	3 (7)	2 (5)
Myalgia	3 (7)	1 (2)
Pain in extremity	3 (7)	1 (2)
Influenza-like illness	2 (5)	1 (2)

In the placebo-controlled clinical study, serious adverse reactions occurred among 4 (9%) patients receiving Soliris and 9 (21%) patients receiving placebo. The serious reactions included infections and progression of PNH. No deaths occurred in the study and no patients receiving Soliris experienced a thrombotic event; one thrombotic event occurred in a patient receiving placebo.

Among 193 patients with PNH treated with Soliris in the single arm, clinical study or the follow-up study, the adverse reactions were similar to those reported in the placebo-controlled clinical study. Serious adverse reactions occurred among 16% of the patients in these studies. The most common serious adverse reactions were: viral infection (2%), headache (2%), anemia (2%), and pyrexia (2%).

6.2 Immunogenicity

As with all proteins there is a potential for immunogenicity. Low titers of antibodies to Soliris were detected in 3/196 (2%) of all PNH patients treated with Soliris. No apparent correlation of antibody development to clinical response was observed. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to Soliris in an enzyme linked immunosorbent assay (ELISA) and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in the assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to Soliris with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

Drug interaction studies have not been performed with Soliris.

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8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C:

PNH is a serious illness. Pregnant women with PNH and their fetuses have high rates of morbidity and mortality during pregnancy and the postpartum period. There are no adequate and well-controlled studies of Soliris in pregnant women. Soliris, a recombinant IgG molecule (humanized anti-C5 antibody), is expected to cross the placenta. Animal studies using a mouse analogue of the Soliris molecule (murine anti-C5 antibody) showed increased rates of developmental abnormalities and an increased rate of dead and moribund offspring at doses 2-8 times the human dose. Soliris should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal reproduction studies were conducted in mice using doses of a murine anti-C5 antibody that approximated 2-4 times (low dose) and 4-8 times (high dose) the recommended human Soliris dose, based on a body weight comparison. When animal exposure to the antibody occurred in the time period from before mating until early gestation, no decrease in fertility or reproductive performance was observed. When maternal exposure to the antibody occurred during organogenesis, two cases of retinal dysplasia and one case of umbilical hernia were observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. When maternal exposure to the antibody occurred in the time period from implantation through weaning, a higher number of male offspring became moribund or died (1/25 controls, 2/25 low dose group, 5/25 high dose group). Surviving offspring had normal development and reproductive performance.

8.2 Labor and Delivery

No information is available on the effects of Soliris during labor and delivery.

8.3 Nursing Mothers

It is not known whether Soliris is secreted into human milk. IgG is excreted in human milk, so it is expected that Soliris will be present in human milk. However, published data suggest that breast milk antibodies do not enter the neonatal and infant circulation in substantial amounts. Caution should be exercised when Soliris is administered to a nursing woman. The unknown risks to the infant from gastrointestinal or limited systemic exposure to Soliris should be weighed against the known benefits of breastfeeding.

8.4 Pediatric Use

The safety and effectiveness of Soliris therapy in pediatric patients below the age of 18 have not been established.

8.5 Geriatric Use

In PNH studies, 15 patients 65 years of age or older were treated with Soliris. Although there were no apparent age-related differences observed in these studies, the number of patients aged 65 and over is not sufficient to determine whether they respond differently from younger patients.

10 OVERDOSAGE

No cases of Soliris overdose have been reported during clinical studies.

11 DESCRIPTION

Soliris is a formulation of eculizumab which is a recombinant humanized monoclonal $IgG_{2/4}\kappa$ antibody produced by murine myeloma cell culture and purified by standard bioprocess technology. Eculizumab contains human constant regions from human IgG2 sequences and human IgG4 sequences and murine complementarity-determining regions grafted onto the human framework light- and heavy-chain variable regions. Eculizumab is composed of two 448 amino acid heavy chains and two 214 amino acid light chains and has a molecular weight of approximately 148 kDa.

Soliris is a sterile, clear, colorless, preservative-free 10 mg/mL solution for intravenous infusion and is supplied in 30-mL single-use vials. The product is formulated at pH 7 and each vial contains 300 mg of eculizumab, 13.8 mg sodium phosphate monobasic, 53.4 mg sodium phosphate dibasic, 263.1 mg sodium chloride, 6.6 mg polysorbate 80 (vegetable origin) and Water for Injection, USP.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Eculizumab, the active ingredient in Soliris, is a monoclonal antibody that specifically binds to the complement protein C5 with high affinity, thereby inhibiting its cleavage to C5a and C5b and preventing the generation of the terminal complement complex C5b-9. Soliris inhibits terminal complement mediated intravascular hemolysis in PNH patients.

A genetic mutation in PNH patients leads to the generation of populations of abnormal RBCs (known as PNH cells) that are deficient in terminal complement inhibitors, rendering PNH RBCs sensitive to persistent terminal complement-mediated destruction. The destruction and loss of these PNH cells (intravascular hemolysis) results in low RBC counts (anemia), and also fatigue, difficulty in functioning, pain, dark urine, shortness of breath, and blood clots.

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12.2 Pharmacodynamics

In the placebo-controlled clinical study, Soliris when administered as recommended reduced hemolysis as shown by the reduction of serum LDH levels from 2200 ± 1034 U/L (mean ± SD) at baseline to 700 ± 388 U/L by week one and maintained the effect through the end of the study at week 26 (327 ± 433 U/L). In the single arm clinical study, Soliris maintained this effect through 52 weeks [*see Clinical Studies (14)*].

12.3 Pharmacokinetics

A population PK analysis with a standard 1-compartmental model was conducted on the multiple dose PK data from 40 PNH patients receiving the recommended Soliris regimen [*see Dosage and Administration (2.1)*]. In this model, the clearance of Soliris for a typical PNH patient weighing 70 kg was 22 mL/hr and the volume of distribution was 7.7 L. The half-life was 272 ± 82 hrs (mean ± SD). The mean observed peak and trough serum concentrations of Soliris by week 26 were 194 ± 76 mcg/mL and 97 ± 60 mcg/mL, respectively.

Studies have not been conducted to evaluate the PK of Soliris in special patient populations identified by gender, race, age (pediatric or geriatric), or the presence of renal or hepatic impairment.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been conducted to evaluate the carcinogenic and genotoxic potential of Soliris. Effects of Soliris upon fertility have not been studied in animals. Intravenous injections of male and female mice with a murine anti-C5 antibody at up to 4-8 times the equivalent of the clinical dose of Soliris had no adverse effects on mating or fertility.

14 CLINICAL STUDIES

The safety and efficacy of Soliris in PNH patients with hemolysis were assessed in a randomized, double-blind, placebo-controlled 26 week study (Study 1); PNH patients were also treated with Soliris in a single arm 52 week study (Study 2); and in a long term extension study. Patients received meningococcal vaccination prior to receipt of Soliris. In all studies, the dose of Soliris was 600 mg study drug every 7 ± 2 days for 4 weeks, followed by 900 mg 7 ± 2 days later, then 900 mg every 14 ± 2 days for the study duration. Soliris was administered as an intravenous infusion over 25 - 45 minutes.

Study 1:

PNH patients with at least four transfusions in the prior 12 months, flow cytometric confirmation of at least 10% PNH cells and platelet counts of at least 100,000/microliter were randomized to either Soliris (n = 43) or placebo (n = 44). Prior to randomization, all patients underwent an initial observation period to confirm the need for RBC transfusion and to identify the hemoglobin concentration (the "set-point") which would define each patient's hemoglobin stabilization and transfusion outcomes. The hemoglobin set-point was less than or equal to 9 g/dL in patients with symptoms and was less than or equal to 7 g/dL in patients without symptoms. Endpoints related to hemolysis included the numbers of patients achieving hemoglobin stabilization, the number of RBC units transfused, fatigue, and health-related quality of life. To achieve a designation of hemoglobin stabilization, a patient had to maintain a hemoglobin concentration above the hemoglobin set-point and avoid any RBC transfusion for the entire 26 week period. Hemolysis was monitored mainly by the measurement of serum LDH levels, and the proportion of PNH RBCs was monitored by flow cytometry. Patients receiving anticoagulants and systemic corticosteroids at baseline continued these medications.

Major baseline characteristics were balanced (see table 2).

TABLE 2
STUDY 1 PATIENT BASELINE CHARACTERISTICS

	Study 1	
Parameter	Placebo N = 44	Soliris N = 43
Mean age (SD)	38 (13)	42 (16)
Gender - female (%)	29 (66)	23 (54)
History of aplastic anemia or myelodysplastic syndrome (%)	12 (27)	8 (19)
Patients with history of thrombosis (events)	8 (11)	9 (16)
Concomitant anticoagulants (%)	20 (46)	24 (56)
Concomitant steroids/immunosuppressant	16 (36)	14 (33)

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Parameter	Study 1 Placebo N = 44	 Soliris N = 43
treatments (%)		
Packed RBC units transfused per patient in previous 12 months (median (Q1,Q3))	17 (14, 25)	18 (12, 24)
Mean hgb level (g/dL) at setpoint (SD)	8 (1)	8 (1)
Pre-treatment LDH levels (median, U/L)	2,234	2,032
Free hemoglobin at baseline (median, mg/dL)	46	41

Patients treated with Soliris had significantly reduced ($p < 0.001$) hemolysis resulting in improvements in anemia as indicated by increased hemoglobin stabilization and reduced need for RBC transfusions compared to placebo treated patients (see table 3). These effects were seen among patients within each of the three pre-study RBC transfusion strata (4 - 14 units; 15 - 25 units; > 25 units). After 3 weeks of Soliris treatment, patients reported less fatigue and improved health-related quality of life. Because of the study sample size and duration, the effects of Soliris on thrombotic events could not be determined.

TABLE 3
STUDY 1 RESULTS

	Placebo N = 44	Soliris N = 43
Percentage of patients with stabilized hemoglobin levels	0	49
Packed RBC units transfused per patient (median)	10	0
(range)	(2 - 21)	(0 - 16)
Transfusion avoidance (%)	0	51
LDH levels at end of study (median, U/L)	2,167	239
Free hemoglobin at end of study (median, mg/dL)	62	5

Study 2 and Extension Study:
PNH patients with at least one transfusion in the prior 24 months and at least 30,000 platelets/microliter received Soliris over a 52-week period. Concomitant medications included anti-thrombotic agents in 63% of the patients and systemic corticosteroids in 40% of the patients. Overall, 96 of the 97 enrolled patients completed the study (one patient died following a thrombotic event). A reduction in intravascular hemolysis as measured by serum LDH levels was sustained for the treatment period and resulted in a reduced need for RBC transfusion and less fatigue. 187 Soliris-treated PNH patients were enrolled in a long term extension study. All patients sustained a reduction in intravascular hemolysis over a total Soliris exposure time ranging from 10 to 54 months. There were fewer thrombotic events with Soliris treatment than during the same period of time prior to treatment. However, the majority of patients received concomitant anticoagulants; the effects of anticoagulant withdrawal during Soliris therapy was not studied [*see Warnings and Precautions (5.4)*].

16 HOW SUPPLIED / STORAGE AND HANDLING

Soliris (eculizumab) is supplied as 300 mg single-use vials containing 30 mL of 10 mg/mL sterile, preservative-free Soliris solution per vial.

Soliris vials must be stored in the original carton until time of use under refrigerated conditions at 2-8° C (36-46° F) and protected from light. Do not use beyond the expiration date stamped on the carton. Refer to *Dosage and Administration* (2) for information on the stability and storage of diluted solutions of Soliris.

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DO NOT FREEZE. DO NOT SHAKE.

NDC 25682-001-01 Single unit 300 mg carton: Contains one (1) 30 mL vial of Soliris (10 mg/mL).

17 PATIENT COUNSELING INFORMATION

See Medication Guide.

Prior to treatment, patients should fully understand the risks and benefits of Soliris, in particular the risk of meningococcal infection. Ensure that patients receive the Medication Guide.

Patients should be informed that they are required to receive a meningococcal vaccination at least 2 weeks prior to receiving the first dose of Soliris, if they have not previously been vaccinated. They are required to be revaccinated according to current medical guidelines for meningococcal vaccine use while on Soliris therapy. Patients should also be informed that vaccination may not prevent meningococcal infection. Patients should be educated about any of the signs and symptoms of meningococcal infection, and strongly advised to seek immediate medical attention if these signs or symptoms occur. These signs and symptoms are as follows:

- moderate to severe headache with nausea or vomiting
- moderate to severe headache and a fever
- moderate to severe headache with a stiff neck or stiff back
- fever of 103° F (39.4° C) or higher
- fever and a rash
- confusion
- severe muscle aches with flu-like symptoms, and eyes sensitive to light

Patients should be informed that they would be provided with the Patient Safety Card that they should carry with them at all times. This card describes symptoms which, if experienced, should prompt the patient to immediately seek medical evaluation.

Patients should be informed that there is a potential for serious hemolysis when Soliris is discontinued and that they will be monitored by their healthcare professional for at least 8 weeks following Soliris discontinuation.

Manufactured by:

Alexion Pharmaceuticals, Inc.

352 Knotter Drive

Cheshire, CT 06410 USA

US License Number 1743

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Patients receiving Soliris treatment should carry this card at all times.

INFORMATION FOR THE PATIENT

You should call your doctor (#_____) right away if you have any of these symptoms:

- **moderate to severe headache with nausea or vomiting**
- **moderate to severe headache and a fever**
- **moderate to severe headache with a stiff neck or stiff back**
- **fever of 103° F (39.4° C) or higher**
- **fever and a rash**
- **confusion**
- **severe muscle aches with flu-like symptoms, and eyes sensitive to light**

If you cannot reach your doctor right away, go to an emergency room and show them this card.

INFORMATION FOR THE PHYSICIAN

This patient was prescribed Soliris (eculizumab) therapy. Soliris is an antibody that inhibits terminal complement activation and prevents the formation of C5b-9 complex. Before beginning treatment, this patient should have received a meningococcal vaccine, but may still be susceptible to meningococcal infections.

For more information about Soliris, see the full prescribing information available at www.soliris.net\soliris.pdf, or call 1-888-SOLIRIS (1-888-765-4747)

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Food and Drug Administration
Center for Drug Evaluation and Research
Office of Biotechnology Products

FACSIMILE TRANSMITTAL SHEET

DATE: May 8, 2009

To: Mr. Arthur D. Edwards	**From:** LCDR Andrew Shiber Project Manager
Company: Alexion Pharmaceuticals Corporation	Office of Biotechnology Products
Fax number: 203-271-8191	**Fax number:** (301) 796-9743
Phone number: 203-272-2596	**Phone number:** (301) 796-4798

Subject: STN 125166/36

Total no. of pages including cover: 2

Comments: Please find attached the action letter for the above supplement for Soliris®(eculizumab).

Document to be mailed: X☐ YES ☐ NO

THIS DOCUMENT IS INTENDED ONLY FOR THE USE OF THE PARTY TO WHOM IT IS ADDRESSED AND MAY CONTAIN INFORMATION THAT IS PRIVILEGED, CONFIDENTIAL, AND PROTECTED FROM DISCLOSURE UNDER APPLICABLE LAW.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

STN 125166-36

May 8, 2009

Alexion Pharmaceuticals
Attention: Mr. Arthur D. Edwards
Executive Director, Regulatory Affairs
352 Knotter Drive
Cheshire, CT 06410

Dear Mr. Edwards:

Your request to supplement your biologics license application for Soliris®(eculizumab), to extend the shelf-life of the drug product from 24 to 30 months when stored at 2 – 8 degrees Celsius, has been approved.

Please refer to http://www.fda.gov/cder/biologics/default.htm for information regarding therapeutic biological products, including the addresses for submissions.

This information will be included in your biologics license application file.

Sincerely,

Kathleen A. Clouse

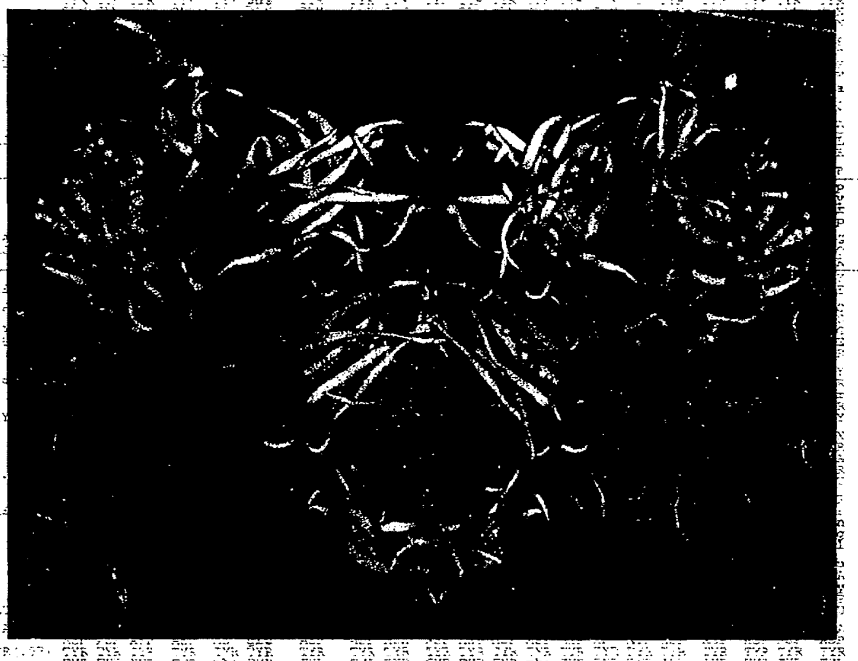
Kathleen A. Clouse, Ph.D.
Director
Division of Monoclonal Antibodies
Office of Biotechnology Products
Office of Pharmaceutical Science
Center for Drug Evaluation and Research

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Page 6684

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

VOL. I

FIFTH EDITION



1991

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

Samsung Bioepis Exhibit No. 1002
Page 6685

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

FIFTH EDITION

Tabulation and Analysis of
Amino Acid and Nucleic Acid Sequences of Precursors,
V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen,
T-Cell Surface Antigens, β_2-Microglobulins,
Major Histocompatibility Antigens, Thy-1, Complement,
C-Reactive Protein, Thymopoietin, Integrins, Post-gamma Globulin,
α_2-Macroglobulins, and Other Related Proteins

1991

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AND HUMAN SERVICES

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National Institutes of Health

NIH Publication No. 91-3242

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SIGNAL PEPTIDES OF HUMAN KAPPA LIGHT CHAINS

	INVARIANT RESIDUES	1 HK101 'CL	2 HK 137 'CL	3 VK1- Chr1 'CL	4 HuVH CAMP 'CL	5 VK1-2I 'CL	6 3D6 'CL	7 V108 'CL	8 Vb 'CL	9 Vb' 'CL	10 HK102 'CL	11 2A12 'CL	12 1H1 'CL	13 2C12 'CL	14 1B11 'CL	15 Vd 'CL	16 Va' 'CL	17 Ve 'CL	18 V13 'CL	19 GM 607 'CL	20 A3 'CL	21 RPMI 6410 'CL #	22 A2 'CL	23 A23 'CL
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-19		ARG	ARG	ARG	MET	ARG	ARG	ARG	ARG	MET	ARG	ARG				ARG	ARG	ARG	ARG		ARG	ARG	ARG	ARG
-18		VAL	VAL	ALA	GLY	ALA	VAL	VAL	VAL	VAL	VAL	VAL				VAL	VAL	VAL	VAL		LEU	LEU	LEU	LEU
-17		LEU	LEU	PRO	TRP	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO			PRO	PRO	PRO	PRO		PRO	PRO	PRO	LEU
-16		ALA	ALA	THR	SER	THR	ALA	ALA	ALA	ALA	ALA	ALA	ALA			ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA
-15		GLN	GLN	GLN	CYS	GLN	GLN	GLN	GLN	GLN	GLN	LEU	LEU			GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN
-14		LEU	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-13		LEU	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-12		GLY	GLY	GLY	LEU	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY			GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY
-11		LEU	LEU	LEU	PHE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-9		LEU	LEU	VAL	VAL	GLY	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		MET	MET	MET	MET
-8	LEU(.95)	LEU	LEU	LEU	ALA	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-7	TRP(.95)	CYS	CYS	TRP	THR	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP
-6		PHE	PHE	LEU	ALA	LEU	LEU	LEU	PHE	PHE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		VAL	VAL	ILE	VAL
-5		PRO	PRO	PRO	THR	PRO	PRO	ARG	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		SER	PRO	PRO	PRO
-4		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
-3		ALA	ALA	ALA	VAL	ALA	ALA	ALA	SER	SER	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	SER	SER	SER	SER	SER
-2		ARG	ARG	ARG	HIS	ARG	LYS	ARG	ARG	ARG	LYS	LYS	LYS	LYS	LYS	ARG	ARG	ARG	ARG	SER	SER	SER	SER	SER
-1		CYS	CYS	CYS	SER	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	GLY	GLY	GLY	ALA	GLY

	24 NG9 'CL (R) 'CL #	25 HIC HAH 14.1 'CL #	26 HAH 14.2 'CL #	27 HAH 16.1 'CL #	28 HAH NOV 'CL #	29 BE10 'CL #	30 TH3 'CL #	31 Humkv 325 'CL	32 EV1-15 'CL	33 IARC/ BL41 'CL	34 Humkv 305 'CL	35 LS1 'CL	36 LS2 'CL	37 LS4 'CL	38 LS5 'CL	39 LS6 'CL	40 LS7 'CL	41 LS8 'CL	42 Vg 'CL	43 Vh 'CL	44 CLL 'CL #	45 Hum kv328 'CL #	46 Hum ka31es 'CL #	47
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-19	GLU	GLU	GLU	GLU	GLU			GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU			
-18	THR	THR	THR	THR	THR			THR	THR	THR	THR	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA			
-17	PRO	PRO	PRO	PRO	PRO			PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO			
-16	ALA	ALA	ALA	ALA	ALA			ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA			
-15	GLN	GLN	GLN	GLN	GLN			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN			
-14	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	IEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			
-13	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			
-12	PHE	PHE	PHE	PHE	PHE			PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE			
-11	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			
-9	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			
-8	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			
-7	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP			
-6	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LSU	LEU	LEU	LEU	LEU	LEU	LEU			
-5	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO			
-4	VAL	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
-3	PRO	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
-2	SER	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
-1	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ARG	GLY	GLY	GLY

	48 GF4 /1.1 'CL	49 K- EV15 'CL	50 VKAPPA IV GERMLINE 'CL	51 PB17IV 'CL	52 FK-001 'CL #	53 WALKER 'CL	54 DAUDI 'CL	55 VJ1 'CL	56 HK100 'CL	57 Va 'CL	58 V2 'CL	59 V14 'CL	60 V52 'CL	61 V55 'CL	62 K- EVJK11 'CL	63 PSEUDO VKAPPA 24'CL	# OF SEQUENCES	# OF AMINO ACIDS
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-19	GLU	GLY	VAL	VAL	VAL	ARG	ARG	VAL	ARG	ARG	GLN	GLN	ARG		GLU	ARG	53	6
-18	ALA	SER	LEU	LEU	LEU	VAL	VAL	LEU	VAL	VAL	VAL	VAL	ALA		THR	CYS	53	7
-17	PRO	GLN	GLN	GLN	GLN	PRO	PRO	GLN	PRO	PRO	PRO	PRO	PRO		PRO	SER	54	5
-16	ALA	VAL	THR	THR	THR	ALA	ALA	THR	ALA	ALA	THR	THR	ALA		ALA	PRO	54	5
-15	HIS	HIS	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	HIS	54	4
-14	VAL	LEU	VAL	VAL	VAL	LEU	LEU	VAL	LEU	LEU	LEU	LEU	LEU		LEU	PHE	54	4
-13	LEU	LEU	PHE	PHE	PHE	LEU	LEU	PHE	LEU	LEU	LEU	LEU	LEU		LEU	LEU	54	3
-12	PHE	SER	ILE	ILE	ILE	GLY	GLY	ILE	GLY	GLY	GLY	GLY	GLY	GLY	PHE	GLU	55	6
-11	LEU	PHE	SER	SER	SER	LEU	LEU	SER	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	55	3
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	58	1
-9	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	VAL	VAL	LEU	PRO	LEU	VAL	58	5
-8	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	SER	LEU	PHE	58	4
-7	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	59	3
-6	LEU	ILE	ILE	ILE	ILE	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	PHE	LEU	ILE	59	5
-5	PRO	SER	SER	SER	SER	ARG	ARG	SER	SER	PRO	PRO	PRO	PRO	PRO	PRO	LEU	59	5
-4	GLU	ASP	GLY	GLY	GLY	GLY	ARG	GLY	GLY	GLY	GLY	GLY	GLY	ASP	GLY	GLU	63	5
-3	THR	THR	ALA	ALA	ALA	ALA	VAL	ALA	ALA	ALA	ALA	ALA	THR	THR	THR	VAL	63	5
-2	SER	ARG	TYR	TYR	TYR	ARG	ARG	TYR	ARG	ARG	ARG	ARG	ARG	ARG	THR	SER	63	6
-1	GLY	ALA	GLY	GLY	GLY	CYS	CYS	GLY	TYR	CYS	CYS	CYS	CYS	SER	GLY	GLY	63	6

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I

	INVARIANT RESIDUES	1 2C12 'CL #	2 1B11 'CL #	3 1H1 'CL #	4 2A12 'CL #	5 BR	6 DEN	7* 3D6 'CL	8 HK102 'CL #	9 EU	10 CAR	11 PA	12 HK 137 'CL #	13 RAU	14 KUE	15* HF2-1/17	16 Vb 'CL	17 Vb' 'CL	18 GAL (I)	19 CU (IOC)	20* WEA	21 WIL (=) #	22 WES	23 DAUDI 'CL	24 HK 134 'CL #
0		---	---	---	---	---	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	#
1		DASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
2	ILE(.98)	? ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
3		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
4		MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET
5	THR(.98)	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
6		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
7	SER(.99)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
8	PRO(.99)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
9	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
10		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	ser	THR	ser	ser	ser	ser	ser	ser	THR	ser	ser	ser
11		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	gln	LEU	val	val	LEU	LEU	LEU	LEU	val	LEU	LEU
12	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	pro	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
13		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
14		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
15	VAL(.96)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
16	GLY(.99)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
17		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP
18		SER	SER	SER	SER	thr	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg
19		ILE	ILE	ILE	ILE	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
20	THR(.96)	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
21	ILE(.95)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
22		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR	THR	THR	THR	THR
23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
24		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
25		ALA	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	pro	ALA	ALA	ALA
26		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	gly	SER
27		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	his	GLN
27A		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27B		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27C		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27D		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27E		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27F		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
28		SER	SER	SER	SER	SER	SER	SER	SER	SER	asn	SER	gly	SER	SER	gly	gly	gly	gly	thr	gly	ala	asp	asn	gly
29		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	phe	ILE	ILE	ILE
30		GLY	GLY	GLY	GLY	ser	ser	ser	ser	asn	ser	ser	ser	ser	asn	arg	ser	ser	arg	ser	arg	GLY	ser	thr	ser
31		ASN	ASN	ASN	gly	arg	arg	arg	ser	thr	ser	ser	ASN	ser	ile	ASN	ser	ser	ASN	ser	ASN	ser	his	ASN	ser
32		TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	ser	tyr	TRP	asp	TRP	TRP	asp	tyr	asp	TRP	TRP	phe	TRP
33	LEU(.95)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
34		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	gly	ALA	ALA	thr	asx	thr	ALA	ALA	ser	ALA
35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP
36		PHE	PHE	PHE	PHE	PHE	tyr	tyr	tyr	tyr	tyr	tyr	PHE	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr
37		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
38		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
39		LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS
40	PRO(.97)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ser	PRO	PRO
41		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu
42		LYS	LYS	LYS	LYS	LYS	glu	LYS	LYS	LYS	LYS	LYS	glu	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	thr	LYS	LYS	LYS
43		ALA	ALA	ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
45		ASN	ASN	ASN	lys	lys	lys	lys	lys	lys	lys	lys	lys	gln	lys	lys	lys	lys	ASX	lys	glu	lys	thr	lys	lys
46		VAL	VAL	VAL	VAL	leu	VAL	leu	leu	leu	VAL	leu	ser	VAL	leu	arg	leu	glu	leu	arg	leu	leu	leu	ser	ser
47	LEU(.98)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
48	ILE(.98)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	met	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
49		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
50		LYS	LYS	LYS	LYS	LYS	gly	LYS	asp	LYS	LYS	glu	ala	ala	LYS	ala	ala	ala	ala	gly	arg	ser	ala	ala	ala
51		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
52		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	SER	SER	SER	SER
53		ASN	ASN	ASN	ASN	thr	ASN	ser	ser	ser	ser	ser	ser	ser	thr	ser	ser	ser	ASN	ASX	ser	ser	ser	ASN	ser
54	LEU(.97)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
55		LYS	LYS	LYS	LYS	glu	glu	glu	glu	glu	glu	glu	glu	glu	pro	glu	gln	gln	gln	his	gln	gln	glu	gln	gln
56		ASN	ASN	ASN	lys	ser	ser	ser	ser	ser	ser	ser	ser	ser	thr	ser	ser	ser	ser	ser	ser	ser	ser	ASN	ser
57	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
58		ILE	ILE	ILE	ILE	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
59	PRO(.95)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
60	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
61	ARG(.97)	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
62	PHE(.96)	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
63		SER	SER	SER	SER	SER	SER	SER	SER	SER	ile	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
64	GLY(.96)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
65		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
66	GLY(.95)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
67		SER	SER	SER	SER	gha	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	ala	SER	SER	SER	SER	SER
68	GLY(.95)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
69		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR
70		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp	GLU	asp	asp	gln	GLU	asp	asp	GLU	asx	GLU	GLU	GLU	GLU	asp
71		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
72		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
73		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	phe	LEU	LEU	LEU	LEU	LEU
74		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
75		VAL	VAL	VAL	VAL	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile
76		ILE	ILE	ILE	ILE	asn	ser	ser	ser	ser	ser	ser	ser	ser	asn	ser	ser	asr	ser	ser	asn	ser	ser	ser	ser
77		ASN	ASN	ASN	ASN	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	gly	ser	ser	ser	ser	ser
78	LEU(.98)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
79		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
80		SER	SER	SER	SER	pro	SER	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
81		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	glu	glu	ASP	glu	glu	glu	glu	gla	glu	ASP	glu	glu	glu
82		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
83		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	val	PHE	PHE	PHE
84	ALA(.98)	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
85		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
86	TYR(.98)	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
87	TYR(.95)	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR
88	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
89		HIS	HIS	HIS	HIS	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	leu	gln	gln	leu	glx	leu	gln	gln	gln	gln
90		HIS	HIS	HIS	HIS	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	gln	gln	gln
91		ASN	ASN	ASN	ASN	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	ASN	tyr	his	ala	ala	gln	ser	tyr	ala	ala	ASN	tyr
92		ASP	ASP	ASP	glu	ASP	ASP	asn	asn	asn	asn	asn	asn	tyr	ser	asn	asn	asn	asn	tyr	ser	asn	his	tyr	asn
93		SER	SER	thr	thr	SER	SER	SER	SER	SER	thr	asn	SER	ile	arg	SER	SER	SER	SER	SER	arg	SER	asn	SER	SER
94		PHE	PHE	PHE	ile	PHE	PHE	tyr	tyr	asp	PHE	leu	tyr	thr	tyr	tyr	PHE	PHE	tyr	ser	PHE	tyr	val	PHE	tyr
95		SER	SER	SER	SER	pro	pro	SER	SER	---	---	pro	pro	pro	---	pro	pro	pro	pro	pro	pro	---	pro	SER	pro
95A		---	---	---	---	---	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	#
95B		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95C		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95D		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95E		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95F		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
96		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
97		THR	THR	THR	THR	THR	THR	THR	---	---	MET	THR	THR	---	SER	THR	THR	---	SER	THR	THR	---	THR	THR	---
98	PHE(.96)	PHE	PHE	PHE	PHE	PHE	PHE	PHE	---	PHE	PHE	PHE	---	PHE	PHE	PHE	---	---	PHE	PHE	PHE	PHE	PHE	PHE	---
99	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY	GLY	GLY	GLY	GLY	---
100		GLN	GLN	GLN	GLN	GLN	GLN	PRO	---	GLN	PRO	PRO	---	GLN	GLN	GLY	---	---	GLN	GLX	GLN	GLN	GLY	GLY	---
101	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY	GLY	GLY	GLY	GLY	---
102	THR(.98)	THR	THR	THR	THR	THR	THR	THR	---	THR	THR	THR	---	THR	THR	THR	---	---	THR	THR	THR	THR	THR	THR	---
103		LYS	LYS	LYS	LYS	LYS	LYS	LYS	---	LYS	LYS	LYS	---	ARG	LYS	LYS	---	---	LYS	ARG	LYS	LYS	THR	LYS	---
104		VAL	VAL	VAL	VAL	VAL	LEU	VAL	---	VAL	VAL	VAL	---	VAL	LEU	VAL	---	---	VAL	LEU	VAL	LEU	VAL	VAL	---
105		LEU	LEU	LEU	LEU	GLU	GLU	ASP	---	GLU	ASP	GLU	---	GLU	ASP	GLU	---	---	GLU	GLX	GLU	GLU	ASP	ASP	---
106		ILE	ILE	ILE	ILE	ILE	ILE	ILE	---	VAL	ILE	ILE	---	ILE	ILE	ILE	---	---	ILE	ILE	VAL	ILE	ILE	ASN	---
106A		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
107		LYS	LYS	LYS	LYS	LYS	LYS	LYS	---	LYS	LYS	LYS	---	LYS	LYS	LYS	---	---	LYS	LYS	LYS	LYS	LYS	LYS	---
108		ARG	ARG	ARG	ARG		ARG		---	GLY	ARG		---	ARG	ARG	ARG	---	---	ARG	ARG	ARG	ARG	ARG	---	---
109	THR						THR		---	THR	THR		---	THR	THR		---	---	THR	THR	THR	THR		---	---

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		25 Vd 'CL	26 WALKER 'CL	27 HK101 'CL #	28 Va' 'CL	29 VKI- Che1 'CL	30 AMYLOID BAN #	31 Ve 'CL	32 V13 'CL	33 DEE	34 WIL (-) #	35 AND #	36 V108 'CL #	37 VKI-ZI 'CL	38 REI	39 AU	40 ROY	41 BI	42* HuVH CAMP 'CL	43 AG #	44 SCH	45 MEV	46 KA	47* LAY	48 BEL	49 NI
	0	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ala	ASP	ASP	ala	val	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	arg	trp	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	4	leu	MET	MET	leu	MET	leu	MET	MET	MET	MET	MET	val	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	leu	MET
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
FR1	10	phe	ser	ser	ser	ser	ser	ser	leu	sec	THR	sec	ser	ser	ser	ser	pro	leu	leu	ser	ser	THR	sec	ser	ser	ser
	11	LEU	LEU	LEU	LEU	LEU	LEU	pha	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val	val	ALA	ALA	ALA
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr
	15	VAL	VAL	VAL	VAL	VAL	VAL	thr	thr	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLX
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	gly	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	18	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	SER	arg	arg	arg	arg	arg	arg	arg	arg	arg
	19	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	phe	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	leu
	22	THR	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	leu
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	24	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	gln	gln	gln	gln	lys	gln	gln	ARG	gln	gln	gln	glu
	25	ALA	ALA	ALA	ALA	ALA	ALA	ALA	met	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	26	SER	SER	arg	SER	SER	SER	SER	SER	gly	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	27	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN
	27A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	ser
	27B	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	val
CDR1	27C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	leu
	27D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	glu
	27E	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27F	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	28	gly	SER	gly	gly	gly	SER	gly	gly	SER	SER	SER	gly	gly	asp	asp	asp	asp	agn	asp	asp	SER	thr	asn	asx	SER
	29	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	val	val	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ser	val	val	ILE	gly
	30	ser	ser	ser	asn	ASP	tyr	ser	ser	asn	ser	GLY	ser	ser	asp	ser	ser	arg	asp	asn	arg	val	leu	asn	ser	asn
	31	asr	ASN	ser	ser	ASN	ser	ser	ser	lys	tyr	gly	ASN	ASN	lys	asp	ile	ASN	lys	his	lys	asp	ser	ala	lys	thr
	32	tyr	tyr	TRP	ala	asn	tyr	tyr	tyr	tyr	TRP	ser	gly	asn	tyr	tyr	phe	ser	tyr	tyr	his	tyr	tyr	tyr		phe
	33	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU
	34	ALA	asn	ALA	ALA	asn	ALA	ALA	ALA	asn	ALA	asn	ser	asn	asn	asn	asn	ile	asn	asn	asn	asn	asn	asn		ALA
	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP
	36	tyr	tyr	tyr	tyr	tyr	PHE	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr		tyr
	37	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	asp	GLN	GLN	GLN		GLN
	38	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN
	39	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	thr	LYS	LYS	LYS	LYS	$	LYS	LYS	LYS	LYS		LYS
	40	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO
	41	GLY	GLY	GLY	glu	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	$	GLY	GLY	GLY	GLY	GLY	lys
FR2	42	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	gln	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	leu	LYS	LYS
	43	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	pro	ALA	ALA
	44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	45	lys	lys	lys	lys	lys	lys	lys	glu	lys	ASN	ser	thr	lys	lys	lys	lys	lys	arg	lys	lys	lys	lys	glu	lys	lys
	46	leu	leu	leu	ser	leu	ser	leu	leu	VAL	leu	leu	leu	leu	leu	leu	phe	leu	leu	leu	leu	leu	leu	leu	leu	leu
	47	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	48	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	49	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR	phe	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR	TYR
	50	ala	ala	ala	asp	ala	asp	ala	ala	ala	gln	gly	ala	ala	glu	asp	asp	asp	asn	asp	gly	asp	ala	gly	asp	asp
	51	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
CDR2	52	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	glu	asn	SER	SER	SER	SER	SER	SER	SER
	53	thr	ser	ser	ser	ser	thr	thr	thr	ser	ile	ser	ser	ser	ASN	ASN	lys	ASN	ASN	ASN	thr	ASN	ser	thr	thr	ASN
	54	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	arg	LEU	LEU
	55	gln	gln	gln	glu	gln	gln	gln	gln	LYS	asp	arg	gln	gln	gln	glu	glu	glu	gln	glu	glu	gln	glu	LYS	glu	glu
	56	ser	ser	ser	ser	ser	ser	ser	ser	ser	ala	ser	ser	ser	ala	ser	ala	ile	thr	thr	ser	thr	ala	thr	thr	
	57	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	58	val	val	val	val	ILE	val	val	val	val	val	val	val	ILE	val	val	val	val	val	val	val	val	val	val	val	val
	59	PRO	thr	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	60	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	61	ARG	ARG	ARG	ARG	ARG	asn	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	62	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
	63	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	arg	SER	SER	SER	SER	SER	SER	SER	SER
	64	GLY	GLY	GLY	GLY	asp	GLY	GLY	GLY	GLY	GLY	GLY	GLY	asp	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	65	SER	SER	SER	SER	SER	SER	SER	SER	SER	asn	SER	SER	SER	SER	gly	thr	SER	SER	SER	gly	gln	SER	SER	SER	SER
	66	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ile	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	arg	GLY	GLY	GLY	GLY	GLY
	67	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	phe	SER	SER	SER	SER	SER	SER	SER	SER
	68	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu	GLY	GLY
	69	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	ala	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	70	GLU	asp	ssp	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	his	asp	asp	asp	asp	asp	asp	asx	asp	his	asp
	71	PHE	PHE	PHE	PHE	tyr	PHE	PHE	PHE	PHE	PHE	PHE	PHE	tyr	tyr	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
FR3	72	THR	THR	THR	THR	ile	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR
	73	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	phe	phe	phe	LEU	LEU	LEU	LEU	LEU	phe	phe	LEU	phe
	74	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR
	75	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile
	76	ssr	ser	ssr	ser	arg	ssr	ser	ser	ser	ssr	thr	ser	ser	ssr	ssr	ssr	ser	ssr	ssx	ser	ssr	ser	ser		ser
	77	ssr	ser	ssr	ser	ssr	ssr	cys	cys	gly	ssr	ssr	ssr	ssr	ssr	ssr	gly	thr	ssr	gly	thr	ssr	ssr	ser	ser	ser	gly
	78	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU	LEU
	79	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	leu	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLX
	80	pro	pro	pro	pro	pro	pro	SER	SER	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
	81	glu	glu	glu	glu	glu	glu	glu	glu	glu	ASP	glu	glu	glu	glu	glu	glu	glu	glu	glu	ASP	glx	glu	ala	glx	glx
	82	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	asn	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASX	ASX
	83	PHE	ser	PHE	PHE	PHE	PHE	PHE	PHE	PHE	ssr	PHE	val	PHE	ile	ile	ile	PHE	ile	ile	ile	PHE	PHE	ile	PHE	PHE
	84	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	gly	ALA	ALA	ALA	ALA	ALA
	85	THR	THR	THR	THR	THR	THR	THR	THR	THR	arg	ala	THR	ala	THR	THR	THR	THR	THR	THR	asn	THR	THR	THR	THR	val
	86	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYA	TYR	TYR	TYR
	87	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
	88	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	89	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	leu	gln	gln	gln	gln	gln	leu	gln	gln	gln	gln	gln	gln	gln
	90	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	glx
	91	leu	ser	tyr	phe	ser	tyr	tyr	tyr	ser	tyr	ssr	asp	ser	tyr	tyr	phe	tyr	his	tyr	tyr	ser	tyr	tyr	tyr	tyr
	92	ASP	tyr	asn	ASP	asn	tyr	tyr	tyr	asn	tyr	tyr	ASP	ASP	ile	ASP	ASP	tyr	ile	ASP	ASP	tyr	leu	asn	ASX	ASP
	93	SER	SER	SER	SER	SER	SER	SER	SER	thr	arg	SER	thr	SER	SER	tyr	asn	asn	SER	thr	asn	thr	asp	asn	his	thr
	94	tyr	thr	tyr	tyr	thr	tyr	tyr	PHE	thr	tyr	ssr	thr	thr	leu	leu	leu	leu	arg	leu	val	asn	leu	trp	PHE	leu
CDR3	95	pro	leu	pro	pro	pro	pro	pro	pro	pro	---	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
	95A	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	GLU	---	---	---	---	---
	95B	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95E	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95F	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	96	---	ILE	---	---	TYR	---	---	TYR	---	TRP	---	---	TYR	TRP	LEU	TYR	ARG	ARG	ILE	VAL	ARG	PRO	LEU	SER	
	97	---	THR	---	---	THR	---	---	THR	---	THR	---	---	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	
	98	---	PHE	---	---	PHE	---	---	PHE	PHE	PHE			PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	
	99	---	GLY	---	---	GLY	---	---	GLY	GLY	GLY			GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	
	100	---	GLN	---	---	GLN	---	---	PRO	GLN	GLN			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	VAL		
	101	---	GLY	---	---	GLY	---	---	GLY	GLY	GLY			GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		
	102	---	THR	---	---	THR	---	---	THR	THR	THR			THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	SER	
FR4	103	---	ARG	---	---	LYS	---	---	LYS	LYS	LYS			LYS	LYS	LYS	LYS	LYS	LYS	ARG	THR	LYS	LYS	GLU	LYS	
	104	---	LEU	---	---	#	---	---	VAL	LEU	VAL			LEU	VAL	VAL	LEU	VAL	LEU	VAL	VAL	VAL	VAL	VAL	VAL	
	105	---	GLU	---	---	#	---	---	GLU	GLU	GLU			GLN	GLU	ASP	GLU	GLU	GLU	GLY	ASP	ASP	GLU	GLU		
	106	---	ILE	---	---	ILE	---	---	MET	ILE	ILE			ILE	ILE	PHE	ILE	ILE	ILE	ASN	ILE	LEU	VAL	VAL	SER	
	106A	---	---	---	---	---	---	---	---	---	---			---	---	---	---	---	---	---	---	---	---	---	---	
	107	---	LYS	---	---	LYS	---	---	THR	LYS	ARG			THR	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	
	108	---	---	---	---	ARG	---	---	ARG	ARG				ARG	ARG	ARG	ARG	ARG	ARG	GLY	ARG	ARG	ARG		ARG	
	109	---	---	---	---	---	---	---	THR	THR				THR	THR	THR	THR		THR	THR		THR			THR	

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		50 RZ	51* Ni RSV19 VK #	52 HBJ 4	53 26 #	54 BJ 19 #	55 RFZ	56 HA	57 PAU	58 PSM	59 JBL #	60 ME	61 FRA	62 BJ 48	63 HEJ- 16/6	64 KFZ- 1/13B	65 HFZ- 18/2	66 GR'	67 G1 'CL #	68 HOM	69 KER	70 ESH IGG	71 ESH IGH	72* DAV	73* FIN	74 PW #	75 SHE	76 ADA
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	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	GLN	his	GLN	GLN
	4	MET	leu	MET	MET	leu	MET	MET	MET	MET	MET	leu	MET	MET	MET	MET	MET	MET		MET	MET	MET	MET	MET	leu	leu	val
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	PRO	SER	SER		SER	SER	SER	SER	SER	thr	SER	SER
F	10	ser	ser	THR	ser	ser	ser	ser	THR	ser	ser	ser	THR	ser	ser	ser	THR			ser	ser	ser	ser	ser	ser	ser	ser
R	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
1	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER			SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA			ALA	ALA	ALA	ALA	thr	thr	ALA	val
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	tyr	SER	SER	SER	SER	SER	SER			SER	SER	SER	val	val	SER	SER	pha
	15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL			VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY			GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP			ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP
	18	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg			arg	arg	arg	arg	arg	arg	arg	arg
	19	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val			val	val	val	val	val	val	val	val
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR			THR	THR	THR	THR	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	THR	ILE			ILE	ILE	ILE	ILE	ILE	val	phe	ILE
	22	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR			THR	THR	THR	THR	THR	arg	THR	THR
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS			CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	24	gln	ARG	ARG	gln	gln	gln	gln	ARG	gln	ARG	glx	ARG		ARG	ARG	ARG	ARG		gln	gln	ARG	ARG	asp	asp	ARG	glx	glx
	25	ALA	ser	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	26	asn	SER	SER	SER	SER	asn	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER		asp	SER	SER	SER	SER	SER	SER	SER
	27	GLN	GLN	GLN	GLN	GLN	GLN	---	GLN	GLN	GLN	GLX	GLX		GLN	GLN	GLN	arg		his	GLN	GLX	GLX	GLN	GLN	GLX	GLX	GLX
	27A	---	thr	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
	27B	---	leu	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
	27C	---	val	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
C	27D	---	his	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
D	27E	---	thr	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
R	27F	---	---	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
1	28	val	asp	SER	SER	asp	val	---	SER	asp	SER	asx	asx		gly	gly	gly	SER		asp	asp	SER	SER	asx	asx	SER	asx	asx
	29	ILE	gly	val	ILE	ILE	ILE	---	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	30	ser	asn	asn	asn	asn	ser	asp	ser	arg	ser	ser	asn		asp	arg	arg	ser		ser	lys	ser	ser	asx	asx	GLY	ser	
	31	lys	thr	ASN	lys	thr	lys	ASN	ser	ser	thr	ASX	ASX		ASN	ASN	ASN	ser		ASN		ser	ser	ser	ser		ASX	ASX
	32	ser	tyr	TRP	tyr	phe	ser	tyr	ser	tyr	phe	tyr	tyr		asp	asp	asp	TRP		tyr		tyr	tyr	TRP	TRP	tyr	tyr	tyr
	33	LEU	LEU	LEU	ala	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU		LEU		LEU	LEU	LEU	LEU	ser	LEU	LEU
	34	asn	glu	ALA	asn	asn	asn	asn	ALA	asn	#	asx	ALA		gly	gly	gly	ALA		asn		asx	asx	ile	ile	asx	asx	asx
	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP		TRP		TRP	TRP	TRP	TRP	TRP	TRP	TRP
	36	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr		tyr	tyr	tyr	tyr		PHE		tyr	tyr	tyr	tyr		tyr	tyr
	37	GLN	GLN	GLN	GLN	glu	GLN	GLN	GLX	GLN	GLN	GLX	GLX		GLN	GLN	GLN	GLN		GLN		GLX	GLX	GLN	GLN	GLN	his	GLX
	38	GLN	GLN	glu	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLX	GLX		GLN	GLN	GLN	gly		GLN				GLN	GLN	GLN	GLX	tyr
	39	arg	LYS	LYS	#	#	arg	LYS	LYS	LYS	LYS	LYS	LYS		LYS	LYS	LYS	arg		LYS				tyr	tyr	LYS	LYS	LYS
	40	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	gln	PRO	PRO	PRO		PRO	PRO	PRO	PRO		PRO				PRO	PRO		PRO	PRO
	41	GLY	GLY	GLY	#	#	GLY	GLY	GLY	GLY	GLY	GLY								GLY								
F	42	gln	LYS	glx	LYS	LYS	gln	arg	glx	LYS	LYS	glx																
R	43	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA														
2	44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO														
	45	lys	lys	lys	lys	lys	lys	lys	lys	lys	lys	lys		lys														
	46	leu	leu	leu	leu	leu	leu	leu	leu	leu	leu	val																
	47	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	met																
	48	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE		ILE																
	49	TYR	TYR	ser		TYR	TYR	TYR	TYR	TYR	TYR	TYR		gly														
	50	asp	arg	LYS		asp	asp	asp	glx	asp				asp														
	51	ALA	val	thr	asp	ALA	ALA	ALA		ALA				ALA														
C	52	SER	SER	SER	SER	SER	ala	phe		SER				SER														
D	53	ASN	ser	arg	ser	ASX	ASN							ser														
R	54	LEU	arg	LEU	LEU	LEU	LEU	LEU		LEU				LEU														
2	55	glu	phe	glu	glu	glu	glu	glu		glu				asx														
	56	ser	ser	arg	thr	thr		ala						ser														
	57	GLY	GLY	GLY	GLY	GLY	GLY	GLY						GLY														
	58	val	val	val	asx	val	val	val						val					ILE								
	59	PRO	PRO	PRO	PRO	glx	PRO	PRO						val					PRO								
	60	SER	SER	SER	SER	SER		SER						SER					SER								
	61	ARG	ARG	ARG	lys	ARG	ARG	ARG						ARG					ARG								
	62	PHE	PHE	PHE	val	PHE	PHE							gln					PHE								
	63	thr	SER	ala	SER	leu	thr							SER					SER								
	64	GLY	GLY	GLY	GLY	GLY	GLY							GLY					GLY								
	65	SER	SER	SER	SER	SER								SER					SER								
	66	GLY	GLY	GLY	GLY	asx	GLY							GLY					GLY								
	67	SER	SER		SER	phe								SER					SER								
	68	GLY	GLY		glu	asx								GLY					GLY								
	69	THR	THR		THR	THR								THR					THR								
	70	asp	asp		asp	lys								asp					GLU								
	71	PHE	PHE		val									pro					PHE								
F	72	phe	THR		THR									ser					THR								
R	73	LEU	pha		val									LEU					LEU								
3	74	THR	THR		asx									ala					THR								
	75	ile	ile		glx									val					VAL								
	76	ser	ser		ser									ser					ILE								
	77	ser	ser		ser									arg					ASN								
	78	LEU	LEU		LEU														LEU								
	79	arg	GLN		GLN														GLN								
	80	pro	pro		pro														GLU								
	81	glu	glu		glu														ASP								
	82	ASP	ASP		ASP														ASP								
	83	val	ile		ile														PHE								
	84	ALA	ALA		ALA														ALA								
	85	THR	THR		pro														THR								
	86	TYR	TYR		lys														TYR		TYR						
	87	phe	TYR																TYR		TYR						
	88	CYS	CYS																CYS		CYS						
	89	gln	phe		gln														HIS		gln						
	90	gln	gln		gln														HIS		gln						
	91	tyr	gly		arg	phe								gly					ASN		tyr						
	92	his	ser		ASP	ASP								glu					ASP		ASP						
	93	asn	his		asp	asp								SER					thr		asp						
C	94	leu	leu		leu	leu								asp					PHE		leu						
D	95	pro	pro		pro	pro								SER					SER		pro						
R	95A	---	---		---	---								---					---		---						
3	95B	---	---		---	---								---					---		---						
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	95D	---	---		---	---								---					---		---						
	95E	---	---		---	---								---					---		---						
	95F	---	---		---	---								---					---		---						
	96	LEU	ARG		TYR	TYR								ARG					TRP		PRO						
	97	THR	THR		THR	THR								THR					THR		THR						
	98	PHE	PHE		LEU	PHE								PHE					PHE		PHE						
	99	GLY	GLY		GLY	GLY								GLY					GLY		GLY						
	100	GLY	GLY		GLY	PRO								GLN					GLN		PRO						
	101	GLY	GLY		GLY	GLY								GLY					GLY		GLY						
	102	THR	THR		THR	THR								THR					TER		THR						
F	103	LYS	LYS		LYS	LYS								LYS					LYS		LYS						
R	104	VAL	VAL		VAL	VAL								VAL					VAL		VAL						
4	105	GLU	GLU		GLU	GLU								GLU					LEU		ASP						
	106	ILE	ILE		MET	LEU								VAL					ILE		LEU						
	106A	---			---	---								---					---		---						
	107	LYS			LYS	LYS								LYS					LYS		LYS						
	108																		ARG								
	109																										

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

	77 AMYLOID VIII-B	78 PAUL	79 RI #	80 NAT	81* LOW	82 AMYLOID ES305 #	83 DCC	84 BOL	85 AMYLOID X	86 SAC	87 BJ	88 GO	89 F-. GUI #	90 S- GUI #	91 HF6- 21/28	92 DIE	93 CAR A	94 TEI	95 MON	96 ALE	97 JOH	98 POT	99 CON	100 TRA	101* H-G2b
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1	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP
2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		val	ILE	ILE	val	ILE	ILE	ILE	ILE	GLN	ILE	ILE	ILE	ILE	ILE
3	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN		GLN	GLN	GLN	val	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
4	MET	MET	MET	MET	MET	leu	MET	ile	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET
5	THR	THR	THR	THR	THR	THR	THR	met	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
6	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLX	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	ala	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ala	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	leu	SER	SER	SER	SER	SER	ala	SER	SER	SER	SER
FR1 10	ser	THR	THR	ser	ser	ser	ser	ser	ser	ser		phe	ser	ser	ser	ser	ser	ser	THR		ser	ser	ser	ser	ser
11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU		val	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU
12	SER	SER	SER	SER	SER	SER	val	SER	SER	SER		SER	SER	SER	pro	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
13	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	ALA		ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
14	SER	SER	SER	SER	SER	SER	pro	SER	SER	SER		SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
17	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASX	ASP	ASP		ASX	ASX	ASX	gly	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
18	arg	arg	gly	arg	arg		leu	arg	arg	---	arg	arg	arg	arg	pro	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg
19	val	val	val	val	val	val	val	val	val	---	val	val	val	val	ala	val	val	val	val	val	val	val	val	val	val
20	THR	THR	THR	THR	THR	THR	THR	THR	ile	---	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
21	ILE	ILE	ILE	ILE	ILE	#	ILE	ILE	ILE	---	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	leu	ILE	ILE	ILE	ILE	ILE	ILE
22	THR	THR	ser	THR	THR	THR	THR	THR		---	THR	THR	THR	THR	ser	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR
23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	---	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS			
24	gln	ARG	ARG	glx	ala	gln	glx	ARG	glx	---	gln	ARG		ARG	ARG					ARG					
25	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	---	ALA	ALA	ALA	ALA	ser										
26	SER	SER	SER	SER	SER	SER	SER	SER		---	SER	SER			SER										
27	GLN	GLN	GLX	GLN	GLN	glu	GLX	GLX	GLX	---	GLN				GLN										
27A	---	---	---	---	---	---	---	---	---	---	---				ser										
27B	---	---	---	---	---	---	---	---	---	---	---				leu										
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27D	---	---	---	---	---	---	---	---	---	---	---				trp										
27E	---	---	---	---	---	---	---	---	---	---	---				ser										
27F	---	---	---	---	---	---	---	---	---	---	---				---										
28	asx	SER	SER	asp	asx	ala	asx	gly	asx	---	asp				asp										
29	ILE	ILE	ILE	ILE	ILE	#	ILE	ILE	ILE	---	ILE				gly										
30	GLY	ser	ser	asp	ser		ser			---	asn				arg										
31		ser		asp		ASN	ASX		pro	---	lys				ser										
32	tyr	ser		tyr		tyr	tyr		tyr	---					tyr										
33	LEU			val		LEU			LEU	---					LEU										
34				asn						---					glu										
35	TRP			TRP		TRP				---					TRP										
36						tyr			tyr	---					tyr										
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90										glx	gln														
91										glx	tyr														
92										ASX	glu														
93										ala	asn														
CDR3 94										ala	leu														
95										thr	pro														
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95D										---	---														
95E										---	---														
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96										ILE	TYR														
97										PRO															
98										ILE															
99										GLY															
100										GLY															
101										GLY															
102										THR															
FR4 103										LYS															
104										VAL															
105										ASX															
106										VAL															
106A										---															
107										LYS															
108										ARG															
109										THR															

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HUMAN KAPPA LIGET CHAINS SUBGROUP I (cont'd)

	102* K-G2	103 BRA	104 LUX	105* HEI	106 PAP	107* H-G2a	108 CRA	109 AMYLOID LEP	110* WAG	111 MOE	112 HBJ 1	113 AMYLOID 547	114 WEB	115 HBJ 10	116 LOD	117 BEN	118 MAA	119 GR	120 MUK	121 GM131 'CL #	122 AMYLOID 594	123* MAR	124 CL*
	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	---
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	---
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	leu	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	---
	4	MET	MET	leu	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET?		MET	leu	MET	MET		MET	leu	---
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	---
	6	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	---
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	---
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	---
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	---
FR1	10	ser	ser	phe	THR	ser	ser	ser	ser	THR	ser	ser	ser	ser	THR	ser	THR	ser	ser		ser	ser	---
	11	LEU	LEU	LEU	LEU	LEU	SER	LEU	LEU	LEU	LEU	LEU	val	LEU		LEU	LEU	LEU	LEU		LEU	LEU	---
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER				---
	13	ALA	val	ALA	ALA	val	ALA	ALA	SER	SER	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val					---
	14	SER	SER	SER	SER	SER	SER	SER	ALA	SER	SER	SER	SER	SER									---
	15	VAL	VAL	VAL	VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL	VAL								---
	16	GLY	GLY	GLY	GLY	GLY	GLY	arg	GLY	GLY	GLY												---
	17	ASP	glx	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP												---
	18	arg	arg	arg	arg	arg	arg	arg	arg														---
	19	val	val	val	val	val	val	val	val														---
	20	THR	THR	THR	THR	THR		THR	THR														---
	21	ILE	ILE	ILE		ILE	ILE	ILE															---
	22	THR	THR	THR		ala	THR	THR															---
			CYS																				
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	81																			glu			---
	82																			ASP			---
	83																			PHE			---
	84																			ALA			---
	85																			THR			---
	86																			TYR			---
	87																			TYR			---
	88																			CYS			---
	89																			gln			---
	90																			gln			---
	91																			his			---
	92																			ASP			ASP
	93																			his			ile
	94																			leu			PHE
	95																			pro			pro
CDR3	95A																			---			---
	95B																			---			---
	95C																			---			---
	95D																			---			---
	95E																			---			---
	95F																			---			---
	96																			LEU			GLY
	97																			THR			THR
	98																			PHE			PHE
	99																			GLY			GLY
	100																			GLY			GLN
	101																			GLY			GLY
	102																			THR			THR
FR4	103																			THR			LYS
	104																			VAL			VAL
	105																			GLU			GLU
	106																			MET			ILE
	106A																			---			---
	107																			LYS			LYS
	108																			ARG			ARG
	109																			THR			THR

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		125 AMYLOID	126 HBJ 6	127 AMYLOID MS #	128 PEN	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
	0	---	---	---	---	1	1	1(PCA)	
	1	ASP	ASP	ASP	ASP	125	3,4	122(ASP),118(ASP)	3.1,4.2
	2	ILE	ILE	#	ILE	123	2	120(ILE)	2.
	3	glu	GLN	GLN	GLN	123	7	116(GLN),112(GLN)	7.4,7.7
	4	MET	MET	MET	MET	124	4	109(MET)	4.6
	5	pro	THR	THR		124	3	122(THR)	3.
	6	GLN	GLN			123	1,2	123(GLN),116(GLN)	1.,2.1
	7	SER				121	2	120(SER)	2.
	8	PRO				121	2	120(PRO)	2.
	9	SER				121	4	117(SER)	4.1
FR1	10	ser				120	5	89(SER)	6.7
	11	LEU				119	4	111(LEU)	4.3
	12					118	3	115(SER)	3.1
	13					117	4	107(ALA)	4.4
	14					113	6	106(SER)	6.4
	15					113	3	109(VAL)	3.1
	16					109	2	108(GLY)	2.
	17					109	3,4	106(ASP),98(ASP)	3.1,4.4
	18					106	6	97(ARG)	6.6
	19					107	3	101(VAL)	3.2
	20					106	4	102(THR)	4.2
	21					104	4	99(ILE)	4.2
	22					104	6	95(THR)	6.6
	23					96	1	96(CYS)	1.
	24					88	6	57(ARG)	9.3
	25					88	6	82(ALA)	6.4
	26					85	4	79(SER)	4.3
	27					84	4	79(GLN),66(GLN)	4.3,5.1
	27A					3	2	2(SER)	
	27B					3	2	2(LEU)	
	27C					3	2	2(VAL)	
C	27D					3	3	1(+)	
D	27E					2	2	1(+)	
R	27F								
1	28					84	7	29(SER)	20.
	29					83	5	72(ILE)	5.8
	30					81	11	41(SER)	22.
	31					79	12	27(ASN),24(SER)	35.,39.
	32					79	9	34(TYR)	21.
	33					77	4	73(LEU)	4.2
	34					73	7,8	30(ALA)	17.,19.
	35					76	1	76(TRP)	1.
	36					74	2	66(TYR)	2.2
	37					73	4	68(GLN),61(GLN)	4.3,4.8
	38					71	4	68(GLN),63(GLN)	4.2,4.5
	39					68	4	62(LYS)	4.4
	40					70	3	68(PRO)	3.1
	41					58	3	54(GLY)	3.2
FR	42					60	6	49(LYS)	7.3
2	43					61	4	57(ALA)	4.3
	44					61	1	61(PRO)	1.
	45					61	7,8	46(LYS)	9.3,11.
	46					60	7	42(LEU)	10.
	47					59	2	58(LEU)	2.
	48					57	2	56(ILE)	2.
	49					59	4	55(TYR)	4.3
	50					59	9	19(ALA)	28.
	51					59	5	60(ALA)	5.9
C	52					58	6	53(SER)	6.6
D	53					57	6,7	26(SER)	13.,15.
R	54					58	2	56(LEU)	2.1
2	55					58	8,9	25(GLU)	19.,21.
	56					56	8	34(SER)	13.
	57					57	1	57(GLY)	1.
	58					58	3	50(VAL)	3.5
	59					57	4	54(PRO)	4.2
	60					57	1	57(SER)	1.
	61					58	3	56(ARG)	3.1
	62					57	3	55(PHE)	3.1
	63					57	6	50(SER)	6.8
	64					57	2	55(GLY)	2.1
	65					56	5	51(SER)	5.5
	66					57	4	54(GLY)	4.2
	67					55	3	51(SER)	3.2
	68					55	3	52(GLY)	3.2
	69					55	2	51(THR)	2.2
	70					55	5,6	33(ASP),31(ASP)	8.3,11.
	71					54	4	49(PHE)	4.4
FR	72					54	5	50(THR)	5.4
3	73					54	3	43(LEU)	3.8
	74					54	4	51(THR)	4.2
	75					54	3	47(ILE)	3.4
	76					53	5	43(SER)	6.2
	77					54	6	40(SER)	8.1
	78					54	2	53(LEU)	2.
	79					54	3,4	52(GLN),48(GLN)	3.1,4.5
	80					54	2	46(PRO)	2.3
	81					54	3,5	37(GLU),34(GLU)	4.4,7.9
	82					54	2	53(ASP),50(ASP)	2.,2.2
	83					54	4	40(PHE)	5.4
	84					54	2	53(ALA)	2.
	85					54	6	48(THR)	6.7
	86					56	2	55(TYR)	2.
	87					55	2	52(TYR)	2.1
	88					56	1	56(CYS)	1.
	89					57	4,5	46(GLN),43(GLN)	5.,6.6
	90					57	2,3	51(GLN),46(GLN)	2.2,3.7
	91					58	11,12	28(TYR)	23.,25.
	92					60	9	20(ASN),19(+)	27.,28.
	93					60	9	29(SER)	19.
	94					60	12	15(LEU)	48.
C	95					56	4	44(PRO)	5.1
D	95A					1	1	1(GLU)	
R	95B								
3	95C								
	95D								
	95E								
	95F								
	96					44	12	10(TRP)	53.
	97					43	4	39(THR)	4.4
	98					46	3	44(PHE)	3.1
	99					46	1	46(GLY)	1.
	100					46	4,5	28(GLN),27(GLN)	6.6,8.5
	101					46	1	46(GLY)	1.
	102					46	2	45(THR)	2.
FR	103					46	4	38(LYS)	4.8
4	104					45	2	36(VAL)	2.5
	105					45	4,5	29(GLU),28(GLU)	6.2,8.
	106					46	7	30(ILE)	11.
	106A								
	107					45	3	42(LYS)	3.2
	108					33	2	31(ARG)	2.1
	109					22	1	22(THR)	1.

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An Agency of the European Union

28/11/2017

Medicine Name	Active Substance	Status	Authorisation date	Biosimilar	Product concentration mg/ml
Zevalin	ibritumomab tiuxetan	Authorised	16/01/2004	no	1.6
Dinutuximab beta EUSA (previously Dinutuximab beta Apeiron)	dinutuximab beta	Authorised	08/05/2017	no	4.5
Erbitux	cetuximab	Authorised	29/06/2004	no	5
Yervoy	ipilimumab	Authorised	13/07/2011	no	5
Stelara	ustekinumab	Authorised	16/01/2009	no	5
Lemtrada	alemtuzumab	Authorised	12/09/2013	no	10
Soliris	eculizumab	Authorised	20/06/2007	no	10
Opdivo	nivolumab	Authorised	19/06/2015	no	10
Lartruvo	olaratumab	Authorised	09/11/2016	no	10
Cyramza	ramucirumab	Authorised	19/12/2014	no	10
Lucentis	ranibizumab	Authorised	22/01/2007	no	10
Cinqaero	reslizumab	Authorised	16/08/2016	no	10
MabThera	rituximab	Authorised	02/06/1998	no	10
Portrazza	necitumumab	Authorised	15/02/2016	no	16
Bavencio	avelumab	Authorised	18/09/2017	no	20
Darzalex	daratumumab	Authorised	20/05/2016	no	20
Tysabri	natalizumab	Authorised	27/06/2006	no	20
Arzerra	ofatumumab	Authorised	19/04/2010	no	20
Vectibix	panitumumab	Authorised	03/12/2007	no	20
RoActemra	tocilizumab	Authorised	16/01/2009	no	20
Avastin	bevacizumab	Authorised	12/01/2005	no	25
Zinplava	bezlotoxumab	Authorised	18/01/2017	no	25

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Gazyvaro	obinutuzumab	Authorised	23/07/2014	no	25
Perjeta	pertuzumab	Authorised	04/03/2013	no	30
Humira	adalimumab	Authorised	08/09/2003	no	50
Praxbind	idarucizumab	Authorised	20/11/2015	no	50
Tecentriq	atezolizumab	Authorised	21/09/2017	no	60
Prolia	denosumab	Authorised	26/05/2010	no	60
Xgeva	denosumab	Authorised	13/07/2011	no	70
Taltz	ixekizumab	Authorised	25/04/2016	no	80
Simponi	golimumab	Authorised	01/10/2009	no	100
Kyntheum	brodalumab	Authorised	17/07/2017	no	140
Repatha	evolocumab	Authorised	17/07/2015	no	140
Zinbryta	daclizumab	Authorised	01/07/2016	no	150
Dupixent	dupilumab	Authorised	27/09/2017	no	150
Cimzia	certolizumab pegol	Authorised	01/10/2009	no	200
Kevzara	sarilumab	Authorised	23/06/2017	no	131.6 or 175
Praluent	alirocumab	Authorised	23/09/2015	no	75 or 150
Simulect	basiliximab	Authorised	09/10/1998	no	powder
Benlysta	belimumab	Authorised	13/07/2011	no	powder
Scintimun	besilesomab	Authorised	11/01/2010	no	powder
Blincyto	blinatumomab	Authorised	23/11/2015	no	powder
Adcetris	brentuximab vedotin	Authorised	25/10/2012	no	powder
Ilaris	canakinumab	Authorised	23/10/2009	no	powder
Empliciti	elotuzumab	Authorised	11/05/2016	no	powder
Remicade	infliximab	Authorised	13/08/1999	no	powder
Besponsa	inotuzumab ozogamicin	Authorised	29/06/2017	no	powder
Nucala	mepolizumab	Authorised	02/12/2015	no	powder
Xolair	omalizumab	Authorised	25/10/2005	no	powder
Synagis	palivizumab	Authorised	13/08/1999	no	powder
Keytruda	pembrolizumab	Authorised	17/07/2015	no	powder
Cosentyx	secukinumab	Authorised	15/01/2015	no	powder
Sylvant	siltuximab	Authorised	22/05/2014	no	powder
LeukoScan	sulesomab	Authorised	14/02/1997	no	powder
Herceptin	trastuzumab	Authorised	28/08/2000	no	powder
Kadcyla	trastuzumab emtansine	Authorised	15/11/2013	no	powder
Entyvio	vedolizumab	Authorised	22/05/2014	no	powder

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Date
27.03.2018

Reference	Application No./Patent No.
AMG17203OP OPPO 01	11001632.6 - 1112 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

BRIEF COMMUNICATION

Subject:
- ☐ Your letter of
- ☐ Our telephone conversation of
- ☐ Communication of
- ☐

Enclosure(s):
- ☒ Letter from the proprietor of the patent of 22.03.2018
- ☐ Letter from the opponent of
- ☐ Copy (copies)

Communication:

☐ A letter from the opponent /proprietor was received on ..... . The documents specified as patent documents in this letter are available via the European Patent Register at **www.epo.org/register.** Should you wish to receive these documents in paper format, they will be supplied to you free of charge if specifically requested on receipt of this communication (OJ EPO 2009, 434).

☐

For the Opposition Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

☐ **Registered letter**

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Europäisches Patentamt
European Patent Office
Office européen des brevets

Submission in opposition proceedings

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- representing the proprietor(s):	Alexion Pharmaceuticals, Inc
Proprietor/representative's reference	C411599EP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Reply of the patent proprietor to the notice(s) of opposition	Response to Notice of Opposition 22 Mar 18 C411599EP PXT_ALS.PDF	OBSO3.PDF
2	Any annexes (other than citation) to an opposition letter - Consolidated list of documents	Consolidated List of Documents.PDF	OTHER-1.PDF
3	Any annexes (other than citation) to an opposition letter - Cover letter	Enclose Response to Oppositions C411599EP PXT ALS.PDF	OTHER-2.PDF

Evidence filed subsequently:

D33	Other evidence	Declaration of Dr Miano C411599EP

		original file name: D33 Declaration of Dr Miano C411599EP.pdf attached as: Other-evidence-1.pdf
D34	Other evidence	Rouet et al Stability Engineering of the Human Antibody Repertoire original file name: D34 Rouet et al.pdf attached as: Other-evidence-5.pdf
D35	Other evidence	Summary of Antibodies listed in D20 and D21 Tables original file name: D35 Summary of Antibodies listed in D20 and D21 Tables.pdf attached as: Other-evidence-3.pdf
D36	Other evidence	Graphical Representation of D20 Table 2 original file name: D36 Graphical Representation of D20 Table 2.pdf attached as: Other-evidence-4.pdf
D37	Other evidence	EMA Approved Antibodies C411599EP original file name: D37 EMA Approved Antibodies C411599EP.pdf attached as: Other-evidence-2.pdf
D38	Other evidence	Extracts from Kabat et al., Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991) original file name: D38 Kabat Exhibit for Response C411599EP.pdf attached as: Other-evidence-6.pdf

Signatures

Place:	
Date:	**22 March 2018**
Signed by:	**Amanda Simons 23469**
Association:	**J A Kemp**
Representative name:	**/Amanda Simons/**
Capacity:	**(Representative)**

Consolidated List of Documents cited in opposition to EP2359834 B1				
Document Name	Consolidated Number	Patentee	Opponent 1 (Amgen)	Opponent 2 (Hoffman Eitle)
US 2005/191298 A1	D1		D1	D1
Hillmen *et al.*, (2003) *J. Hematol.* 121 (Suppl. 1): 87-95	D2		D2	D2
Hillmen *et al.*, (2004) *N. Engl. J. Med.* 350(6): 552-559	D3		D3	D3
Harding. (2004) *Drugs Future*. 29(7): 673-676	D4		D4	D4
US 2005/221382 A1	D5		D5	D5
Hill. (2005) *Clin. Adv. Hematol. Oncol.* 3(11): 849-50	D6		D6	D6
Morris *et al.*, (1992) *South. Med. J.* 85(19): 1030-1031	D7		D7	
Corvini *et al.*, (2004) *Ann. Allerg. Asth. Immunol.* 93(2): 200-205	D8		D8	
Sierra *et al.*, (1991) *NIPH Annals.* 14(2): 195-210	D9		D9	
US Pharmacopeia (USP29-NF24), page 2989	D10		D10	
Adis Data Information. (2007) *Drugs R. D.* 8(1): 61-68	D11		D11	
Hill *et al.*, (2005) *Blood.* 106(7): 2559-2565	D12		D12	
Thomas *et al.*, (1996) *Mol. Immunol.* 33(17/18): 1389-1401	D13		D13	D8
Mueller *et al.*, (1997) *Mol. Immunol.* 34(6): 441-452	D14		D14	
WO 97/11971 A1	D15		D15	
WO 2005/007809 A2	D16		D16	
US 6,355,245 B1	D17		D17	D7
Hillmen *et al.*, (2006) *N. Engl. J. Med.* 355(12): 1233-1243	D18		D18	
EMEA, CPMP, 20 February 2003, *Note for guidance on excipients, antioxidants and antimicrobial preservatives in the dossier for application of marketing authorization of a medicinal product.*	D19		D19	
Wang *et al.*, (2007) *J. Pharm. Sci.* 96(1): 1-26 (January 2007)	D20		D20	D10
Daugherty *et al.*, (2006) *Adv. Drug Del. Rev.* 58: 686-706	D21		D21	
US 2005/0271660 A1	D22		D22	
Expert Declaration by Dr Michael Treuheit	D23		D23	

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Soliris®(eculizumab) *Highlights of prescribing information.*	D24		D24	
US 2012/0230982 A1	D25		D25	
WO 2007/002571 A2	D26		D26	
Hill *et al.*, (2004) *Blood*. 104: 2823-2824	D27		D27	
US 2003/0202972 A1	D28			D9
US 6,171,586 B1	D29			D11
Maloney *et al.*, (1997) *Blood*. 90(6): 2188-2195	D30			D12
Commercial monoclonal antibodies 2006 (all modes of administration)	D31			Annex I
Commercial monoclonal antibodies 2006 (I.V. administration)	D32			Annex II
Declaration of Dr Miano	D33	D33		
Rouet *et al.*, (2014) *FEBS Letters*. 588: 269-277	D34	D34		
Summary of antibodies listed in D20/D21 Tables	D35	D35		
Graphical representation of D20 Table 2	D36	D36		
Summary of antibody products approved by EMA since filing date	D37	D37		
Extracts from Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991)	D38	D38		

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J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

22 March 2018

Dear Sirs

Opposition to European Patent No. 2359834
(Application No. 11001632.6)
ALEXION PHARMACEUTICALS, INC.
Our Ref: C411599EP PXT/ALS

In response to the Communication dated 13 September 2017, I attach the Patentee's response to the two Oppositions. Attached are:

- the Response;
- documents D33 to D38 as listed in section 2 of the response; and
- a consolidated list of documents D1 to D38.

The Patentee's requests are set out in section 1 of the response. Specifically, it is requested that the Opposition Division maintains the patent as granted. If the Opposition Division is minded to refuse this request, I request that oral proceedings be appointed. If oral proceedings are appointed, I shall speak in English and shall require interpretation into English should any other party speak in a language other than English.

Yours faithfully

Electronically Signed
DR PAMELA M TUXWORTH
ASSOCIATION NO. 811

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J A • K E M P

22 March 2018

Our Ref: C411599EP

RESPONSE TO NOTICE OF OPPOSITION

Patent No:	2359834	
Application No:	11001632.6	
Patentee:	Alexion Pharmaceuticals, Inc.	
Opponents:	(1)	Amgen Inc.
	(2)	Hoffmann Eitle

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1 REQUESTS

1.1 **Main Request**

1.1.1 The Patent Proprietor requests that the Opposition Division maintain the Patent as granted.

1.1.2 If the Opposition Division contemplates any decision other than the maintenance of the Patent as granted, then Oral Proceedings are requested. If Oral Proceedings are appointed, I shall speak in English. I shall require interpretation into English should any other party speak in a language other than English.

1.2 **Auxiliary Requests**

1.2.1 The Patent Proprietor requests the opportunity to file further claim requests if it is appropriate to do so during these Opposition Proceedings.

2 DOCUMENTS

2.1 The following documents are cited:

D33 Declaration of Dr Miano

D34 Rouet *et al*; FEBS Letters 588 (2014) 269-277

D35 Summary of antibodies listed in D20/D21 Tables

D36 Graphical representation of D20 Table 2

D37 Summary of antibody products approved by EMA since the filing date

D38 Extracts from Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991)

2.2 We also enclose a consolidated list of all of the documents cited by both Opponents, and including the additional documents mentioned above.

3 No Added Subject Matter

3.1 Opponent 1 (O1) argues that claim 1 includes added subject matter compared with the application as filed. O1's reasoning is that claim 1 of the patent as granted does not include the term "single unit dosage form" which is found both in the passage on page 5, lines 3 to 9 and that on page 22, lines 7 to 14 of the application as filed. O1 argues that this is presented as an essential element of the pharmaceutical composition and

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therefore its omission from claim 1 leads to inadmissible subject matter. Notably, Opponent 2 (O2) has not raised the same objection and makes no argument that the claim as granted includes added subject matter.

3.2 The Patentee disagrees with O1's position. Basis for the wording used in claim 1 of the patent as granted is provided at the following passages of the application as filed:

- Page 5, lines 9 to 10: *"In certain embodiments, the 300mg single-use dosage form comprises 30ml of a 10mg/ml sterile, preservative free solution."*
- Page 6, lines 21 to 23: *"In certain embodiments, the pharmaceutical composition comprises a 300mg single-use formulation of 30ml of a 10mg/ml sterile, preservative free solution."*
- Page 22, lines 11 to 13: *"In certain embodiments, the pharmaceutical composition comprises a 300mg single-use formulation of 30ml of a 10mg/ml sterile, preservative free solution."*

3.3 As is apparent from the above extracts from the application as filed, in none of these passages is the term "single-unit dosage form" presented as an essential feature. Accordingly, these passages are sufficient to provide basis for the wording used in claim 1 of the present patent. There is no addition of subject matter in contravention of Article 123 (2) EPC.

3.4 For completeness, it is noted that the Patentee disagrees that D10 supports O1's argument. D10 does not define either the terms "single-use" or "single-unit dosage form". For at least this reason, D10 is therefore not relevant to the interpretation of these terms.

4 Inventive Step: State of the Art

4.1 The inventive step of the claimed invention will be discussed with reference to the problem and solution approach in section 5 below. It is useful, however, firstly to provide a discussion of the situation in the art at the time the invention was made, in particular the general knowledge of the skilled person in the art in relation to eculizumab and formulating antibodies in general.

4.2 *Eculizumab and treatment of PNH*

4.2.1 Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematologic disease which results in PNH blood cells which have a partial or complete deficiency of GPI-anchored proteins. Anemia is a significant problem for PNH patients leading to an impaired quality of life which is disproportionate to the degree of anemia. Many PNH patients depend on blood transfusions to maintain adequate haemoglobin levels.

4.2.2 The nature of PNH is such that patients, once identified, need to start on treatment very quickly. Thus, the required drug, in the present case

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eculizumab, must be available and ready for use as soon as possible. It is therefore stored on site or at a location nearby (within a certain radius) such that it is essentially ready for use when a patient is identified.

4.2.3 PNH is, however, an ultra-rare disease, so the frequency with which patients are diagnosed is low. The person of skill in the art of PNH treatment would therefore be aware of the need for a long shelf-life product, in order that it can be stored where it is needed for a long period of time without loss of drug activity.

4.2.4 Eculizumab has been identified as a useful treatment for PNH and is the first antibody of its kind to be brought to market. Prior to eculizumab, the vast majority of antibodies brought to market were of IgG1 type, or occasionally IgG2 or IgG4. Eculizumab is none of these IgG types. Instead, appended to eculizumab's variable region is a unique, non-naturally occurring CH1-hinge-CH2-CH3 sequence. No analogously-engineered antibodies were even in development at the filing date of the patent and certainly none had been approved for clinical use. Even today there are no antibodies on the market with the same uniquely-engineered CH1-hinge-CH2-CH3 sequence as eculizumab.

4.3 *Formulation of Antibodies is a Complex Challenge*

4.3.1 A number of antibody drugs were in development at the time the present invention was made, and a small number had already reached the market. However, the issue of the formulation of antibody drugs was at the time regarded as a highly complex and unpredictable issue. As with other protein drugs, it is very important to maintain the integrity of the antibody and to minimize any possible degradation pathways. Therefore, in order to maintain activity of the antibody, both in storage and for administration, a balance is required between maintaining functionality of the antibody and minimizing the inevitable processes of protein degradation that occur in solution. Providing formulations in which antibodies maintain good stability, however, had proved to be a challenging problem.

4.3.2 These issues are discussed in D20 and D21, review articles published in 2007 and 2006 respectively. As Wang *et al* put it in the abstract of D20:

> *"Although antibodies share certain structural similarities, development of commercially viable antibody pharmaceuticals has not been straightforward because of their unique and somewhat unpredictable solution behaviour."*

4.3.3 The various different degradation pathways to which antibodies may be susceptible are described in the section beginning at page 8 onwards of D20. These include denaturation, aggregation, surface adsorption, disulphide bond formation/exchange, non-reducible cross-linking, deamidation, isomerisation, oxidation, formation of acidic or basic species, C-terminal clipping, fragmentation and Maillard reaction. The skilled person looking to formulate an antibody therefore has a multitude of

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different problems to consider, each of which will apply in different ways to each individual antibody.

4.3.4 Of course, it is not the case that only one degradation pathway will necessarily dominate for any one antibody. In the right-hand column, top paragraph of D20, Wang *et al* states that:

> *"In many cases, multiple degradation pathways can occur at the same time and the degradation mechanism may change depending on the stress conditions."*

4.3.5 Moreover, the strategies available to minimise the various degradation pathways may conflict with one another. For instance, deamidation, a major degradation pathway in antibodies, has been shown to have a strong pH-dependence, with less deamidation occurring at pH 4 compared to pH 7 (D20, page 12, right-hand column penultimate paragraph). In contrast, antibody precipitation can be a significant problem at low pH values. D20 reports (page 11, left-hand column first paragraph) that incubation of two mouse monoclonal antibodies (IgG2a and IgG1) at 37°C for 32 days caused precipitation at pH 3 and pH 4 solutions for both antibodies. It is therefore difficult to create a stable antibody formulation due to the numerous degradation pathways possible, and the potentially conflicting strategies available to the formulation chemist for dealing with each of them in the context of each individual antibody.

4.3.6 This issue is also highlighted in D21. In the passage set out below, the authors comment not only on this issue but also on the critical need to devise a formulation that remains compatible with delivery:

> *"In general, **successful antibody formulation designs are simply the best compromise of minimizing several competing degradation pathways**. One approach is to find a pH where the minimal amount of degradation events such as deamidation and oxidation will occur. Another is to identify a buffer system that can stabilize the formulation at that pH without damaging the antibody. One common tactic taken to reduce the rate of many of these chemical degradation events is to prepare lyophilized or freeze-dried antibody preparation but even these can be compromised by aggregation issues due to thermodynamic stress that occurs during freezing, drying and reconstitution. A number of protective excipients, mostly carbohydrates, can reduce the propensity of antibodies to aggregate during these stressful events. **Another issue is to produce a formulation that is compatible with delivery — neither hypotonic — nor hypertonic solutions are well-tolerated upon injection.**"* (D21, page 701 right-hand column, first full paragraph emphasis added)

4.4 *Each Antibody is Unique*

4.4.1 Despite the various structural similarities between some of the different antibody drugs available in 2007, different antibodies have significantly

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different behaviours and each requires completely separate study in terms of its formulation. Daugherty et al put it like this in D21:

> " *A priori, one might assume that by finding a stable formulation for one of these antibody drugs, that such a formulation would be good for most if not all, similar antibodies. If this were borne out by experience, there would be no need for a review such as this.* ***Instead, each antibody seems to have a unique personality relating to its requirements for stability****; a phenomenon derived from the fact that the small differences between these antibodies are focused on surface-exposed amino acid differences that stipulate antigen specificity. Thus, the interfacial surface of each antibody drug is unique and thus requires specific formulation components to provide maximal stability and retention of activity."* (D21, page 690, right-hand column, first paragraph, emphasis added)

4.4.2 This need to treat even very similar antibodies individually in terms of their stability characteristics is illustrated at page 693, left-hand column of D21. Here the authors describe a recombinant monoclonal antibody that recognises vascular endothelial growth factor (rhMab-VEGF) which was studied for its kinetic and thermodynamic aspects of antibody solution dimerization. The results are reported by Daugherty et al as follows:

> *"It was found that for this IgG1 class antibody, aggregation rates were greater in slightly alkaline (pH 7.5-8.5) compared to slightly acidic (pH 6.5-7.5) conditions. A high salt environment (1 M NaCl) also enhanced dimerization. …. One of the most striking aspects of these dimerization studies on rhMab-VEGF is that trastuzumab, another genetically engineered antibody with the same IgG1 framework and a 92% sequence homology with rhMab- VEGF, fails to demonstrate aggregation events under the same conditions."* (D21, page 693, left-hand column)

4.4.3 This example therefore illustrates that antibodies having very close structural similarity can behave in very different ways in terms of their degradation pathways. Strategies to provide stable antibody formulations therefore must be considered on a case by case basis for each individual antibody.

4.4.4 It is also notable that the vast majority of the discussion of both D20 and D21 relates to IgG1 antibodies. There is a brief discussion in the first paragraph of page 689 of D21 of engineered antibody molecules. D21 notes that such structurally-different antibodies could have very different properties. D21 goes on to state that:

> "*as these new approaches gain prominence, more information will be acquired about their unique requirements for successful formulation and delivery. At present, however, there is little information on formulation approaches for antibodies produced using these production processes.*"

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4.4.5 Therefore, the authors of D21 acknowledge that experience gained in relation to IgG1 antibodies may well not be applicable at all to structurally different antibodies, for example the non-natural, uniquely-engineered structure of eculizumab.

4.5 *No Single Technique Provides Stability*

4.5.1 It can be seen from the discussion above that the formulation of antibody therapeutics was a highly complex field, multiple degradation pathways needed to be taken into account and potentially conflicting strategies were available to the skilled person for dealing with these degradation pathways. Further, each antibody must be individually studied, since small differences in antibody structure were known to lead to significant differences in antibody stability and degradation behaviour. It follows, therefore, that no single technique, no single group of excipients, and no single formulation strategy was available to the skilled person to provide a stable antibody formulation. This is evident from the variety of strategies discussed in the art. Not only do D20 and D21 themselves describe a wide variety of different strategies for effecting antibody stability, but this is also apparent from other prior art documents cited by the Opponents. For example:

- D28 describes a technique for providing stable lyophilised protein formulations using a lyoprotectant such as sucrose or trehalose (see paragraph 8). This is exemplified only in two specific antibodies, an anti-HER2 antibody and an anti-IGE antibody.

- In D29 a very different strategy is used in which the antibodies are not subject to lyophilisation but particular formulation pH and excipients are considered. This strategy was applied to two specific antibodies, an anti-CD18 antibody and an anti-CD20 antibody. Notably, D29 provides an entirely separate study of the suitable pH, buffer and excipients for each of these two separate antibodies.

4.6 *Antibody Concentrations Used in Earlier Formulations*

4.6.1 The Opponents have argued that Table 1 of D20 and Table 1 of D21 demonstrate that antibodies were commonly formulated at 10mg/ml in the art and that therefore this would be an obvious choice of formulation concentration for eculizumab. That 10mg/ml is not the obvious choice for eculizumab is explained in detail below with reference to the problem and solution approach. However, it is useful here to consider what, if any, information the skilled person could derive from the Tables of D20 and D21 when considering suitable formulations for any future antibody drug.

4.6.2 The enclosed table D35 lists each antibody of Table 1 of D20 and Table 1 of D21. The type of antibody is listed, as well as the antibody concentration (in the form in which the product is stored), together with details of the formulation. D35 therefore summarises the information provided in Table 1 of D20 and Table 1 of D21, whilst additionally providing the antibody type. IgG2 and IgG4 antibodies are highlighted in bold.

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4.6.3 A number of observations can be made based on D35 regarding the antibody products on the market before the filing date of the patent:

- The concentrations of products provided in liquid form range from 1 to 50mg/ml with a wide variety of different concentrations being used for different products.
- Those products that can be stored in solution form include five products having a concentration from 1 to 2 mg/ml; four products having a concentration of 10 mg/ml; and three products having a concentration of 20 mg/ml or above.
- Eight products are provided in lyophilised form.
- Half of the antibodies provided in solution form were formulated at concentrations of 5 mg/ml or less.
- The vast majority of antibody drugs which had reached the market before the filing date are IgG1 antibodies.

4.6.4 It is difficult, if not impossible, to derive any pattern in terms of antibody formulation based on the wide variety of formulations used for marketed drugs. The only possible conclusion is that since each antibody is unique for its binding specificity to an antigen, a unique formulation must be developed individually for each antibody. It certainly cannot be concluded that 10 mg/ml is a "typical" concentration for an antibody formulation. Some commercial antibodies were formulated at 10 mg/ml, but the majority were not and it is not possible to determine from the information in Table 1 of D20 and Table 1 of D22 why each formulation was selected. There is no indication as to whether other future antibodies might or might not be suitable for formulation at 10 mg/ml.

4.6.5 D35 also shows that the largest group of antibodies are lyophilised. Again, there is no indication in the Tables of D20 and D21 as to why these particular products are lyophilised for storage. However, the first paragraph of the left-hand column of page 18 of D20 hints that this could be due to instabilities:

> "*Like most proteins, some antibodies are not stable enough in a liquid form and lyophilized dosage forms will have to be considered*".

4.6.6 The wide variety of different formulation types, concentrations and the varying use of lyophilisation which is apparent from D35 demonstrates the unpredictability of antibody formulation. Each antibody needs separate consideration, and the behaviour of other antibodies clearly has not in the past led to any consistency in the way that these products are formulated. The same is true for any future antibody product: the evidence of Table 1 of D20 and Table 1 of D21 is no guide to a suitable formulation for eculizumab.

4.6.7 This is particularly the case given that eculizumab is a non-natural, uniquely-engineered antibody, whilst the vast majority of those antibodies which had reached the market by the filing date were IgG1 type

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antibodies. There are only five examples of non-IgG1 antibodies on the market before the filing date. These were formulated at 1 mg/ml, 1.1 mg/ml, 14 mg/ml, 20 mg/ml and in lyophilised form. The skilled person simply cannot predict from this information a suitable formulation for any future non-IgG1 product. It is certainly not possible to predict from this information a suitable formulation type for a product such as eculizumab. No antibody of this type had reached the market before the filing date.

4.6.8 As well as providing information on the formulation of marketed antibodies, D20 also provides information on antibody stability, including aggregation, in Table 2. Table 2 lists five antibodies where the percent aggregates following storage of a liquid formulation is provided. In D36, the Patentee has plotted the concentration of the formulation against the percent aggregates for these five examples. It is notable that there is no clear trend evident from this graph. Aggregate formation does not vary linearly as antibody concentration changes. There is no direct relationship between these two parameters and thus there are other factors at play in determining the degree of aggregation. In particular, the tendency towards aggregation is very different for different antibodies.

4.6.9 In summary, formulation of antibody drugs remains to this day a challenging topic. There is no single technique or set of excipients and buffer/pH conditions, which formulation experts have at their disposal, which is guaranteed to lead to stability of any particular antibody product. Rather, each antibody has a unique biochemical behaviour. The process of developing a suitable formulation for an antibody, which maintains its biological integrity and activity over time, is one which needs to be carried out individually for each new antibody product. This is particularly the case for an antibody having a novel type of structure, such as the uniquely-engineered structure of eculizumab.

5 Inventive Step: Problem and Solution Approach

5.1 Closest Prior Art

5.1.1 Both Opponents have suggested that D1 is a suitable prior art document. The problem and solution analysis below is accordingly based on D1 as the closest prior art.

5.2 Difference from D1

5.2.1 The claims of the patent differ from the disclosure of D1 in that:

- The formulation is a single use 300mg dosage form
- The concentration is 10 mg/ml
- The volume is 30ml
- The solution is preservative free

5.3 *Technical Problem to be solved*

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5.3.1 The patent aims to provide treatments for PNH. Any such treatment must, to ensure efficacy, be provided in a form which maintains its biological integrity and activity during any period of storage. This is clearly important so that the drug remains an effective treatment for PNH. The treatment must also be in a suitable form for a clinician to administer to a patient.

5.3.2 The specific pharmaceutical composition of eculizumab set out in claim 1 of the patent provides the ideal balance of these properties. The composition is easy for the clinician to use: it is a liquid formulation so there is no need for reconstitution which brings inherent inconvenience for clinical staff and risks of incomplete dissolution. Further, the composition provides a balance of (a) excellent long-term storage stability without loss of biological integrity, (b) minimising the bulk of the stored product, and (c) a suitable concentration for use to provide convenient administration in an acceptable infusion time. All of these factors make the product an excellent treatment for PNH, which has led to the approval of the claimed composition as a treatment for PNH by the regulatory bodies.

5.3.3 It is, of course, critical that the antibody maintains its biological integrity and activity in the period between manufacture and use. Aggregation is a common indicator of antibody stability, and accordingly, any formulation for the treatment of PNH needs to have a low incidence of aggregation on long-term storage. The enclosed declaration by Doctor Miano (D33) provides evidence of the long-term stability of eculizumab 10 mg/ml formulations, and the improved stability of these formulations compared with 30 mg/ml eculizumab formulations.

5.3.4 In paragraph 2 and Exhibits 2 and 3 of D33, Dr Miano provides evidence demonstrating that the eculizumab formulation at 10 mg/ml (i.e. the commercial formulation, Soliris®) is stable for a total period of 48 months. The product is stable for 18 months when stored as the drug substance, i.e. in bulk, and for a further 30 months when stored as the drug product in individual vials. Even over this long storage period, the product meets the commercial requirement that at least 98% monomer antibody remains.

5.3.5 Dr Miano also provides data demonstrating the improved stability of the 10 mg/ml formulation compared with a formulation which is identical in all respects, except that the eculizumab concentration is 30 mg/ml. The studies were carried out at 2 to 8°C and also at 23 to 27°C. As Dr Miano explains at paragraph 5 of D33, these higher temperature stability studies provide a good indication of effects which might be seen at 2 to 8°C over a longer period of time.

5.3.6 As is apparent from Figure 1 of D33, after only six months storage at 23 to 27°C the 30 mg/ml concentration led to an unacceptable level of aggregate formation, with the % monomer dropping to below the threshold of 98% which is required for release. In contrast, the 10 mg/ml concentration maintained over 99% monomer under the same storage conditions.

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5.3.7 Similar trends are seen in the studies at 2 to 8°C, where the % monomer retained after storage is significantly higher for the 10 mg/ml concentration compared with the 30 mg/ml concentration. Based on this data, Dr Miano concludes that the 30 mg/ml concentration would not meet the release standards after 36 months of storage.

5.3.8 Dr Miano's evidence therefore demonstrates that the 10 mg/ml composition of eculizumab provides extremely good long-term stability such that the antibody maintains its integrity and biological activity. It also demonstrates that the 10 mg/ml concentration of eculizumab provides improved stability compared with other concentrations.

5.3.9 The 10 mg/ml concentration combines this enhanced stability with ease of use for the clinician due to the low-volume liquid formulation. Firstly, because it is a liquid formulation, there is no need for reconstitution. Secondly, simple dilution of product required by the label conveniently provides the required 600 or 900 mg administration dosages. Thirdly, the 600 or 900 mg doses required by the patient can be administered via IV infusion of only 120 or 180 ml of solution (following dilution for administration). This relatively low volume enables the drug to be administered by IV infusion over relatively short periods of time and without the need for high infusion volumes which are undesirable for the patient. Finally, the low volume of product in the storage vials (30 mL per vial) minimises the volume which must be stored on site at each clinic.

5.3.10 The technical problem which is solved by the invention is therefore the provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion times and which is convenient to store.

5.3.11 The solution to this problem is the 300 mg single-use dosage form comprising 30 mL of a 10mg/ml preservative-free solution.

5.4 *Response to Opponents' Comments on Technical Solution*

5.4.1 The Opponents argue that the 10 mg/ml concentration of the formulation is an arbitrary selection. This is not the case, for the reasons set out above: the 10 mg/ml formulation shows enhanced maintenance of biological integrity following long-term storage, combined with low volumes for convenient dilution, administration and storage. The formulation as claimed is therefore not only extremely effective but is improved over alternative formulations of eculizumab.

5.4.2 Opponent 1 has filed a declaration by Dr Treuheit (D23) which is said to show the equivalence of differing concentrations of eculizumab. The evidence provided by Dr Treuheit does not show the equivalence of the different concentrations for the following reasons:

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- Dr Treuheit tested stability only over a 13 week period. This is significantly less than the 48-month stability demonstrated for the 10 mg/ml eculizumab product in D33. As the Opposition Division will clearly appreciate, where stability tests are carried out for a very short period of time, differences between the samples tested may not be detected, or may be very small.

- Even at the short period of time over which Dr Treuheit's test was conducted, an increase in formation of aggregates can be seen at elevated temperature for both the 30 mg/ml and 50 mg/ml formulations. These trends support the 30 mg/mL data presented in Dr. Miano's declaration on behalf of the Patentee.

5.4.3 As Dr Treuheit explains in paragraph 5 of his declaration, aggregation of the antibody was studied by size-exclusion ultra high-performance liquid chromatography (SE-UHPLC). The results are shown in Appendix A to his declaration. The lower panel of Appendix A shows that the 30 mg/ml and 50 mg/ml formulations resulted in a reduced percentage of intact antibody after 13 weeks. In both cases, the percent antibody remaining after this period of time was lower than at base line. However, the 10 mg/ml formulation did not show any reduction in percent antibody after 13 weeks even after storage at 25°C.

5.4.4 This result is in accordance with the longer 6 month study carried out by Dr Miano where the 30 mg/ml concentration had a reduced proportion of intact antibody as storage progressed compared with the 10 mg/ml concentration.

5.4.5 The SEC data in Appendix A of Dr Treuheit's declaration shows that the 30 mg/ml and 50 mg/ml formulations had close to 98% antibody remaining after 13 weeks of storage at 25°C. This antibody content falls below the release parameter for commercial lots of eculizumab, indicating that this drug would not be sufficiently stable over this period of time for clinical use.

5.4.6 Opponent 1 also comments on D25 which discloses formulations of anti-C5 antibodies, including eculizumab, of various concentrations. Opponent 1 highlights formulations of eculizumab at various concentrations which were found to be stable. However, the evidence of D25 does not provide any indication of the relative stability of these high concentration formulations compared to the claimed 10 mg/ml formulation. As is clear from the formulation flow chart depicted in the abstract of D25, the formulation is changed on moving from the 10 mg/ml phosphate-based solution to the higher concentration solutions. Thus, it is not possible to directly compare the 10 mg/ml solutions with the higher concentration solutions of D25. Indeed, the skilled person might assume that the higher concentration solutions of eculizumab needed particular treatment, with variation of buffers and excipients, since simply increasing the concentration of the 10 mg/ml solution did not lead to sufficient stability.

5.4.7 In summary, in light of D1,

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- The claimed subject matter provides a specific formulation for eculizumab which is a 300 mg single use dosage form comprising 30 mL of a 10 mg/ml preservative free solution.
- This formulation has extremely good storage stability (48 months at 2-8°C) which means that clinics can store the product for long periods of time and still rely on the biological activity of the product being suitable for treatment of PNH.
- The 10 mg/ml concentration has an *improved* storage stability compared with 30 mg/ml as demonstrated by Dr Miano (D33).
- The volume of the formulation is only 30 ml, such that the volume of product which needs to be stored and administered is low. This has benefits in terms of acceptable infusion volumes and times for the treatment of PNH as well as providing the ability to store eculizumab at various sites to ensure that PNH patients are able to quickly receive this critical therapy.
- The product can be stored in liquid form, avoiding risks of incomplete/incorrect reconstitution by a clinician and providing convenience of use in the treatment of patients having PNH.

5.4.8 The claimed subject matter therefore solves the technical problem of providing a composition of eculizumab which demonstrates the ideal balance of enhanced maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low volume liquid formulation.

5.5 *The Solution is Not Obvious From the Art: Overview*

5.5.1 As is set out in the Guidelines for Examination, the consideration of whether or not a claimed invention would have been obvious involves asking:

> *"whether there is any teaching in the prior art as a whole that **would** (not simply could, but would) have prompted the skilled person, faced with the objective technical problem, to modify or adapt the closest prior art while taking account of that teaching, thereby arriving at something falling within the terms of the claims, and thus achieving what the invention achieves (see **G-VII, 4**).*
>
> *In other words, the point is not whether the skilled person could have arrived at the invention by adapting or modifying the closest prior art, but whether he **would have done** so because the prior art incited him to do so in the hope of solving the objective technical problem or in expectation of some improvement or advantage (see **T 2/83**)."*

5.5.2 It is therefore necessary in the present case to consider whether the skilled person *would* have arrived at the claimed 300 mg preservative-free dosage form containing 30ml of a 10 mg/ml eculizumab solution, with a reasonable expectation that such a composition would provide the ideal balance of maintaining biological integrity of the antibody following storage in a low-

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volume liquid formulation (not merely whether he/she *could* have done so).

5.5.3 The Boards of Appeal have further held that it is necessary to consider whether a skilled person would have followed a particular course of action with a reasonable expectation of successfully solving the technical problem. A reasonable expectation of success has been distinguished from a "hope to succeed" or from a situation where it is "obvious to try" (T296/93). Rather, what is required is that the skilled person can predict, before starting a project, whether or not there is a realistic expectation of a successful conclusion to a project (i.e. of solving the technical problem.)

5.5.4 The Boards of Appeal have, in a few occasions, denied an inventive step on the basis that the skilled person is in a "try and see" situation, as mentioned by Opponent 1 (T333/97). However, a "try and see" situation is considered by the Boards to be limited to particular situations, i.e. where the prior art clearly envisages a specific feature, and the skilled person merely has to determine by routine tests whether such feature does indeed provide a desired effect. In T333/97, for instance, the skilled person is faced with the problem of providing a method for blocking in plants a given pathogenic target RNA strand activity. A specific, suitable method was known in E coli and all parties agreed that it would be obvious to try this specific method in plants. However, it was not known whether or not the method would succeed. A lack of inventive step was found since a single course of action, agreed to be the route obvious to the skilled person, merely had to be tested to see if it worked.

5.5.5 In the present case, as will be discussed further below, none of the documents cited by the Opponents gives the skilled person a reasonable expectation of achieving, in a formulation of eculizumab, good stability and low liquid volume via *any* type of formulation. There is certainly no suggestion that a liquid formulation of 30 ml at 10 mg/ml would provide the ideal balance of properties. It is also clear that the present situation is not a "try and see" situation. The prior art does not provide an indication of a single suitable formulation which could be tested to see if it is effective. Rather, a wide variety of different formulations are used for different antibodies. To determine a suitable composition for the uniquely- engineered eculizumab antibody, the skilled person would have to conduct a significant research program to fully investigate the stability and biological integrity of the antibody, as well as the various factors (and their interplay) affecting each.

5.5.6 Indeed, the Opponents largely seek to rely on references which relate to formulations of other antibodies. As discussed in detail above in Section 4, each antibody has individual properties in terms of its stability, even where structure is very similar. In the present case, eculizumab is a non-naturally occurring, non-IgG1 isotype and is therefore *not* structurally similar to the largely IgG1 antibodies referred to by the Opponents. No conclusions can therefore be drawn from prior art which refers to suitable formulations of different antibodies.

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5.6 *Claimed Subject Matter is Not Obvious over D1 combined with D19-D21*

5.6.1 In light of D1, the technical problem to be solved is to provide a composition for eculizumab which has the right balance of properties providing biological integrity following storage and a low volume liquid formulation for ease of storage and administration. The question in relation to inventive step is: would the skilled person have chosen a 30ml single use formulation of 10 mg/ml eculizumab in a preservative free solution, based on the teaching in D19 to D21, with a reasonable expectation of solving this problem?

5.6.2 In Section 4 above we discussed the knowledge in the art in relation to antibody formulations in D20 and D21. This can be summarised as follows:

- Formulation of antibodies was a complex area with a wide array of different stability considerations to be taken into account for each individual antibody.
- Each antibody is unique and needed separate consideration in terms of determining a suitable composition, even where antibodies were structurally similar.
- There was no single "common" formulation, or antibody concentration, which the skilled person would turn to first.
- The knowledge in the art was largely in relation to IgG1 antibodies. Non-IgG1 antibodies with non-naturally occurring, engineered constant regions were known to act differently, but little work had been done on formulation of such antibodies.
- A wide array of different formulations had been used for various antibodies on the market, giving no clear guidance as to preferable or even suitable formulations for future therapeutic molecules.

5.6.3 It is clear from the above, therefore, that the skilled person would not have known which concentration to use in a pharmaceutical composition of eculizumab. The skilled person would not have had any awareness as to how eculizumab would be affected by the wide variety of potential stability issues to consider. The skilled person would not have had any awareness of which of the many tools available to the formulation scientist (pH, excipients, antibody concentration, lyophilisation) would be effective in addressing the as yet unknown stability issues. The skilled person would certainly not have any indication that 30ml of a 10 mg/ml liquid composition of eculizumab would provide the combined benefits of improved biological integrity of the antibody on storage at up to 48 months and low volume liquid formulation for improved administration. Rather, a full research project would be needed to arrive at such a suitable and ideal composition.

5.6.4 Importantly, the skilled person would be aware that the knowledge about formulation in D20 and D21 largely relates to IgG1 isotype antibodies. However, as set out in D21, little information was available about engineered antibodies:

> *"A number of engineered antibody-like molecules are now being examined for therapeutic applications. Some engineered fragments,*

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such as diabodies and minibodies, are much smaller than intact, full length IgG1 and have very different properties of systemic distribution and clearance. Additionally, methods to prepare other antibody types such as dimeric secretory IgA and methods to prepare antibodies using non-mammalian cell systems, such as plants, from the eggs of chimeric chickens and from transgenic animals, are now being explored by academic and industrial groups. As these new approaches gain prominence, more information will be acquired about their unique requirements for successful formulation and delivery. At present, however, there is little information on formulation approaches for antibodies produced using these production processes." (D21, p689, LH column)

5.6.5 Eculizumab is an example of an engineered antibody having a unique, non-naturally-occurring CH1-hinge-CH2-CH3 structure. Given the unpredictability of behaviour even between IgG1-type antibodies, the skilled person would not have been able to use the information about IgG1 antibodies in the art to predict the behaviour of eculizumab. Indeed, the skilled person would not have been able to predict whether a liquid formulation would have been possible at all for this new type of antibody. There is certainly no reasonable expectation of successfully providing a formulation which maintains the biological integrity of the antibody, in a low volume liquid formulation, over a period of 48 months. The claimed subject matter is therefore not obvious from D1 in combination with D20 and D21.

5.6.6 D19 is also relied on by O1 to show that it is desirable to exclude preservatives for regulatory reasons. However, there is no suggestion in D19 that a 30 ml eculizumab formulation at a concentration of 10 mg/ml would have beneficial properties. D19 is not therefore relevant to solving the objective technical problem.

5.7 *Antibody Concentrations After The Filing Date*

5.7.1 The Opponents have indicated that 10 mg/ml was a typical antibody concentration and it would have been obvious for a skilled person developing a new antibody to use this concentration based on the common knowledge. If the Opponents are right in their assertion, it could be expected that antibodies developed after the filing date would generally have a concentration of 10 mg/ml. However, this is not the case.

5.7.2 D37 summarises data available from the European Medicines Association (EMA) website (http://www.ema.europa.eu). D37 provides a list of active antibodies (excluding biosimilars) which have been given EMA authorisation after the filing date of the patent. The nature of the product is also indicated, including the product concentration, where the product is provided as a solution.

5.7.3 As is evident from D37, only six out of 44 antibody products were approved having a formulation of a 10 mg/ml solution. It is quite clear, therefore, that other factors are considered by developers when selecting an

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appropriate concentration for a formulation of any new drug. 10 mg/ml is not an automatic choice. Indeed, as for antibodies formulated before the filing date, the largest group of products were provided in lyophilised form.

5.7.4 A recent review article on antibody formulation by Rouet *et al* (D34) confirms that, even well after the filing date of the present patent, the issue of providing a stable formulation for an antibody product remains a challenging problem. Even in 2014, Rouet considered that:

> *"Despite recent advances, the identification of suitable formulation conditions for a specific monoclonal antibody remains challenging and cannot be determined from its amino acid sequence. Indeed, a considerable proportion of human monoclonal antibody candidates fail formulation studies, often at relatively late pre-clinical stage, placing heavy burdens onto drug development pipelines."* (Page 271, right-hand column, second paragraph).

5.7.5 In the first paragraph of the right-hand column of page 271, D34 gives the example of therapeutic monoclonal antibody cetuximab (Erbitux). Even the initial suggested formulation conditions of 2 mg/ml in phosphate buffer at pH 6.0 led to visible aggregates under mechanical stress and had to be reformulated. Notably, the new formulation was at a higher concentration – 5 mg/mL – but at a different pH and using different excipients.

5.8 *Claimed Subject Matter is Not Obvious over D1 Combined with D22*

5.8.1 O1 relies on D22 which relates to a nebulisable formulation. A critical factor in considering a suitable formulation of any drug is the manner in which it is administered. Clearly, a topically administered drug will be formulated very differently from an orally or parenterally administered drug. Similarly, the skilled person looking for a suitable formulation for the D1 treatments, which are administered by IV, would not consider nebulized formulations. Thus, the skilled person would not consider D22 when seeking suitable formulations in light of the D1 disclosure.

5.8.2 Moreover, even if the skilled person had considered D22, this would not have led him to the claimed solution. As O1 points out, D22 exemplifies at para [170] to [172] formulations described as eculizumab formulations at 30 mg/ml. However, this example would, if anything, lead the skilled person *away* from the claimed 10 mg/ml concentration. There is no hint or suggestion in D22 that 10 mg/ml would provide improved biological integrity of eculizumab following storage compared with 30 mg/ml. Indeed, there is no information whatsoever regarding stability in D22.

5.8.3 Moreover, a skilled person seeking a dosage form of eculizumab having a *low volume* would not consider reducing the concentration of the 30 mg/ml solution as this would inherently *increase* the volume of solution needed to provide the same drug content. This document would not, therefore, have led the skilled person to the claimed invention starting from D1.

5.9 *Claimed Subject Matter is Not Obvious over D1 combined with D29*

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5.9.1 Starting from D1, the skilled person is seeking a suitable formulation for a unique antibody having a non-naturally-occurring, engineered heavy chain. D29 would not be of assistance to the skilled person since it relates to very different antibodies, which the skilled person would understand to likely have very different stability properties.

5.9.2 D29 is primarily related to formulations for anti-CD18 and anti-CD20 antibodies. This is clear, for example, from column 2, lines 19 to 22 which state that: *"There is a need in the art for a stable aqueous pharmaceutical formulation comprising an antibody, such as an anti-CD18 or anti-CD20 antibody, which is suitable for therapeutic use"*. The only examples of specific formulations given in D29 are for anti-CD18 and anti-CD20 antibodies. Specifically, Example 1 relates to rhuMAb CD18 which is a $F(ab')_2$ molecule (column 24, line 42) and Example 2 relates to rhuMAb CD20.

5.9.3 Eculizumab, on the other hand, is not at all similar to the antibodies exemplified in D29. Eculizumab has a uniquely engineered CH1-hinge-CH2-CH3 sequence and is structurally remote from the antibodies described in D29. The skilled person would have no expectation that the formulation behaviour and stability of the anti-CD18 and anti-CD20 antibodies described in D29 would shed any light at all on a composition for eculizumab. The skilled person would not therefore consider combining D29 with D1.

5.9.4 Further, even if the skilled person had considered D29, the focus of the document is on the excipients used in the formulation. As set out in the summary of the invention at column 2, beginning at line 25, the invention of D29 suggests that a buffer providing a pH in the range 4.5 to 6.0, a surfactant and a polyol are useful in providing a formulation which is "*preferably* [...] stable at a temperature of 2-8°C for at least one year, and/or is stable at a temperature of about 30°C for at least one month" (emphasis added). Had the skilled person followed the suggestions of D29, they would have considered primarily the excipients in the formulation to achieve stability. The concentration is not mentioned in the summary of the invention at all. Further, they would not have expected to be able to achieve stability (of a very different antibody) for as long as 48 months, given D29's suggestion of 12 months.

5.9.5 D29 does make some reference to antibody concentration at column 22, as pointed out by O2. However, even D29 itself makes clear that in the actual selection of antibody concentration to use, other factors need to be taken into account. At column 22, line 10 onwards, D29 explains, for example, that the amount of antibody is determined by taking into account the desired dose volumes and mode(s) of administration. The actual concentrations used in the Examples of D1 are very different for the two antibodies: Example 1 uses 1 mg/ml, whereas Example 2 uses 40 mg/ml. Notably, neither is within the narrowest range of 2 to 10 mg/ml which O2 suggests would be the obvious choice of concentration for *any* antibody formulation.

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5.9.6 Based on this information, there is no hint that 10 mg/ml is an obvious choice for a skilled person. Indeed, a skilled person could just as easily consider 1 mg/ml, 40 mg/ml or any other concentration in the range of 1 to 50 mg/ml. There is certainly no suggestion that the specific concentration of 10 mg/ml, when used for an eculizumab formulation, would provide an ideal balance of maintaining biological integrity on storage and low volume liquid formulation.

5.9.7 In summary, D29 gives the skilled person no additional information than they would already be aware of from his common general knowledge with regard to antibody concentration. The skilled person knows that many antibodies (where liquid formulation is possible) may be formulated in the range of 1 mg/ml to 50 mg/ml, and that some of those are formulated in the range of 2 mg/ml to 10 mg/ml. But, the skilled person also knows that various considerations must be taken into account when selecting a suitable concentration for any particular antibody. The skilled person also knows that each antibody requires its own individual study into suitable formulations: concentrations of antibody in a given formulation that lead to stability for that one antibody may not do so for another antibody. This is borne out by the contrast between the concentrations used in Examples 1 and 2 of D29. D29 therefore does not lead the skilled person any closer to the present invention.

5.9.8 Finally, it is noted that O2 draws comparison between the commercial formulation of rituximab, an anti-CD20 antibody, and D29. This does not have any bearing on the scope of D29 itself, which suggests a different concentration of 40 mg/ml in Example 2. Moreover, given the significant structural differences between rituximab and eculizumab, the skilled person would have had no expectation that a formulation for rituximab would prove useful for eculizumab, let alone that it would provide a storage stable composition for at least 48 months.

5.10 *Definition of the technical problem*

5.10.1 The discussion above does, of course, make reference to stability data for eculizumab. The Guidelines for Examination in the European Patent Office confirm that any technical effect derivable from the application as filed can be used as a basis to reformulate the objective technical problem. The relevant passage in the Guidelines reads as follows (G-VII-5.2).

> *"As a matter of principle any effect provided by the invention may be used as a basis for the reformulation of the technical problem, as long as said effect is derivable from the application as filed (see T386/89).* ***It is also possible to rely on new effects submitted subsequently during the proceedings by the applicant, provided that the skilled person would recognise these effects as implied by or related to the technical problem initially suggested*** *(see G-VII, 11 and T184/82)".* (Emphasis added.)

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5.10.2 The technical effects at issue in the present case are in the maintenance of the biological integrity of the antibody following storage, and suitable volumes for storage and administration to a patient. These properties are clearly intimately connected with the underlying problem of providing a useful treatment for PNH. Unless the biological integrity of the antibody is maintained, the treatment cannot be expected to be efficacious. Similarly, the ability of the clinician to safely and effectively administer the drug to a patient is clearly connected to the ability to treat PNH.

5.10.3 As noted in the passage of the Guidelines set out above, effects which are related to the technical problem set out in the application as filed may be relied upon in the formulation of the technical problem. The relevant passage is set out at G-VII-11:

> *"Subsequently, the applicant submits new evidence which shows that the claimed composition exhibits an unexpected advantage in terms of low toxicity. In this case, it is allowable to reformulate the technical problem by including the aspect of toxicity,* ***since pharmaceutical activity and toxicity are related in the sense that the skilled person would always contemplate the two aspects together****".* (Emphasis added.)

5.10.4 It is therefore clearly acceptable to rely on the evidence set out above in the formulation of the objective technical problem for the purposes of a problem/solution analysis for inventive step.

5.11 *Inventive Step: Summary*

5.11.1 In summary, starting from D1, the technical problem which is solved by the present invention is the provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced stability and thus maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion times and which is convenient to store.

5.11.2 Based on D20 and D21, the skilled person would have been aware that formulation of antibodies is a complex area and that each antibody needs to be treated as its own unique formulation project. The skilled person would also have treated with scepticism any information in the prior art that was based on IgG1 antibodies given the non-IgG1, non-naturally occurring, engineered structure of eculizumab: the skilled person would not have any expectation that formulations which had been successful with previous antibodies would be useful in the case of eculizumab.

5.11.3 Moreover, based on D20 and D21, the skilled person would see that there is no clear starting place for considering antibody formulation and that a separate research program would be needed to find the ideal formulation for eculizumab. The result of that research program, carried out by the inventors, was the finding that 30 ml of a 10 mg/ml liquid formulation of eculizumab is surprisingly stable over a period of 48 months, having

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superior maintenance of biological integrity compared with other concentrations of eculizumab, whilst also providing a low volume of liquid formulation. The skilled person could not have had any reasonable expectation of this result in advance of carrying out the research.

5.11.4 Moreover, neither D22 nor D29 cited by the Opponents provides any assistance. D22 relates to a different type of formulation from D1 so would not be considered relevant. In any case, it directs the skilled person to inferior 30 mg/ml formulations. D29 relates primarily to the excipients used in antibody formulation, in particular anti-CD18 and anti-CD20 formulations. Again, it would give the skilled person no guidance for suitable compositions for eculizumab.

5.11.5 The claimed subject matter is therefore inventive over the art.

6 Sufficiency

6.1 The skilled person would be able to carry out the invention without undue burden.

6.2 There is no confusion as to what the heavy and light chain amino acid sequences of eculizumab are. The patent discloses the variable heavy and variable light chain regions of eculizumab (SEQ ID NO: 1 and SEQ ID NO: 3) and the full heavy chain and light chain sequences of eculizumab (SEQ ID NO: 2 and SEQ ID NO: 4).

6.3 The skilled person would immediately recognise, based on his common general knowledge, that SEQ ID NO: 4 (and also the light chain variable region sequence of SEQ ID NO: 3) includes the N-terminal leader sequence which is not present in the mature antibody.

6.4 It was well known in the art at the filing date of the application that a signal peptide is present at the N-terminus of immature heavy and light chain polypeptides. Kabat *et al.*, Sequences of Proteins of Immunological Interest, US Dept, Health and Human Services (1991) (D38), which is a database of biological sequences containing sequence alignments and is evidence of the skilled person's common general knowledge, shows the signal peptides of various human light chains.

6.5 The attached page 1 from Kabat *et al.* shows the 22-residue signal peptides of human kappa light chains. The first three residues are predominantly Met Asp Met and the last two residues are predominantly Arg Cys. In SEQ ID NO: 4 of the patent, the first three residues are Met Asp Met and residues 21 and 22 are Arg Cys. Therefore, the skilled person would recognise that SEQ ID NO: 4 is the amino acid sequence of the immature light chain that contains a signal peptide that is not present in the mature eculizumab antibody.

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6.6 Furthermore, the skilled person would know that the framework of human kappa light chains commonly starts with the residues Asp Ile Gln Met as shown in the Table at pages 103 to 108 of Kabat *et al*. Residues 23 to 26 of SEQ ID NO: 4 are Asp Ile Gln Met, which the skilled person would recognise as being the start of the mature light chain of eculizumab.

6.7 The existence of post-published D25 that includes the mature sequence of SEQ ID NO: 4 has no bearing on whether the disclosure of the patent is sufficient. The date for determining sufficiency of disclosure is the filing date of the patent. At any rate, D25 merely confirms what the skilled person would know from reading the patent in light of his common general knowledge, i.e. that SEQ ID NO: 4 contains the light chain signal peptide as well as the light chain sequence present in the mature antibody.

6.8 It should also be pointed out that the skilled person would produce eculizumab using cell-based recombinant methods, and not by artificially synthesising the polypeptide chains. The cells used to make eculizumab would process the light chain polypeptide sequence to remove the signal sequence during antibody production in the cells. Therefore, contrary to the assertion of O1, the skilled person would not use an antibody that is not eculizumab to treat PNH because he would not be able make an antibody that contains the whole sequence of SEQ ID NO:4.

6.9 Thus, the skilled person seeking to reproduce the claimed invention based on the disclosure in the patent of SEQ ID NOs: 2 and SEQ ID NO: 4 would be able to make and formulate eculizumab as required by claim 1 using routine methods known in the art.

6.10 The claimed subject matter is therefore sufficiently disclosed in the patent.

7 Summary

7.1 For the reasons discussed above, the patent does not contain added matter, is inventive over the art and is sufficient. The oppositions should therefore be dismissed and the patent maintained as granted.

Signed

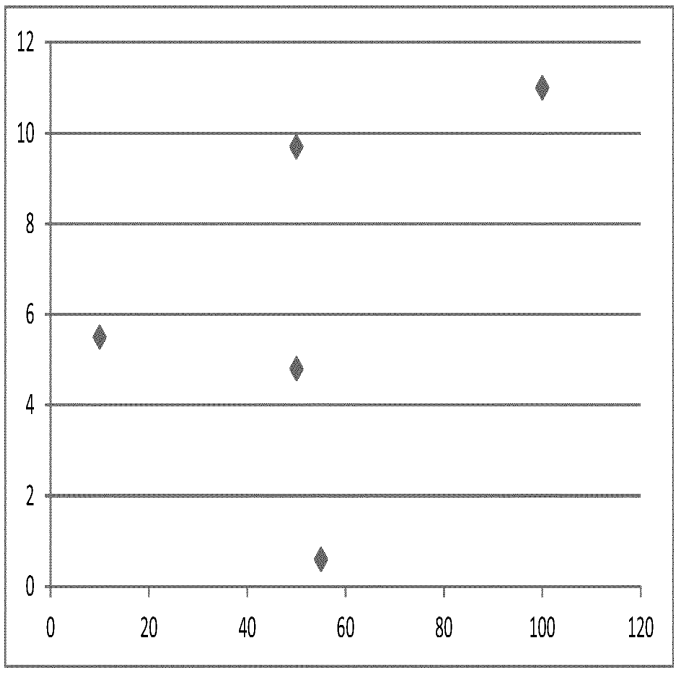
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% Aggregation Versus Antibody Concentration

From Table 2 of D20 where concentration and % aggregates known)

Antibody	Concentration in Formulation (mg/mL)	% Aggregates
rhyMAbE25	10	5.5
Human anti-IL-8 (IgG2)	50	4.8
RhuMAb HER2	50	9.7
Human Anti-IL-8 (IgG2)	55	0.6
Anti-IL-8 (IgG2)	100	11



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Review

Stability engineering of the human antibody repertoire

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ABSTRACT

Human monoclonal antibodies often display limited thermodynamic and colloidal stabilities. This behavior hinders their production, and places limitations on the development of novel formulation conditions and therapeutic applications. Antibodies are highly diverse molecules, with much of the sequence variation observed within variable domain families and, in particular, their complementarity determining regions. This has complicated the development of comprehensive strategies for the stability engineering of the human antibody repertoire. Here we provide an overview of the field, and discuss recent advances in the development of robust and aggregation resistant antibody therapeutics.

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1. Introduction

The number of human monoclonal antibody candidates has increased rapidly in recent years, and now represent the largest single class of molecules entering clinical studies [1]. Despite the rapid growth of the antibody therapeutics market over the past twenty to thirty years, hurdles remain that limit their manufacture. Key factors relate to the variable and often limited stability of human antibodies, which negatively impact on many production processes including expression [2,3], purification [4] and formulation [5].

Antibodies are complex multidomain proteins and mechanisms governing their thermodynamic [6] and, in particular, colloidal [7] stability are not fully understood. Human antibodies of the commonly used immunoglobulin G (IgG) type consist of a total of twelve domains, which can be further divided into two chains (heavy and light) and variable (V_H, V_L) and constant (C_H1, C_H2, C_H3, C_L) domains (Fig. 1). Only limited diversity is observed among constant domains, with a total of four isotype (IgG1–4) and two light chain ([κ] kappa and [λ] lambda) classes expressed in humans. Consequently, isotype differences can explain only a limited proportion of the observed stability variation in the human antibody repertoire [8]. A higher proportion of sequence diversity is observed among antibody variable domains, which are assembled through genetic recombination of variable, diverse (V_H only) and joining segments (VDJ-recombination). Further diversity is then introduced into framework, and in particular complementarity determining regions (CDR), through somatic hypermutation, followed by clonal selection of the repertoire. As a result of these processes, the overwhelming majority of sequence variation within the human antibody repertoire is observed within the CDR regions of a limited range of human variable domain families. In this review, we summarize the influence of the observed antibody diversity on colloidal and thermodynamic stabilities and discuss recent advances to improve these properties through engineering approaches.

2. Protein aggregation

Aggregation is a complex process by which proteins can form alternative colloidal states, which are different from the native state, but otherwise energetically favorable [9,10]. It is generally believed that such aggregate species are predominantly formed via unfolded or partially unfolded states [11]. Protein aggregation is increasingly recognized as a problem affecting the manufacturability of human therapeutic antibodies, shelf life and efficacy [12]. Importantly, the presence of aggregates has also been linked to increased immunogenicity, with effects ranging from mild skin irritation to anaphylaxis [13].

Although it can be assumed that stabilization of the native state over alternative aggregate states has occurred during evolution [14], it is important to note that the production of monoclonal antibodies exposes these molecules to a wide range of non-physiological

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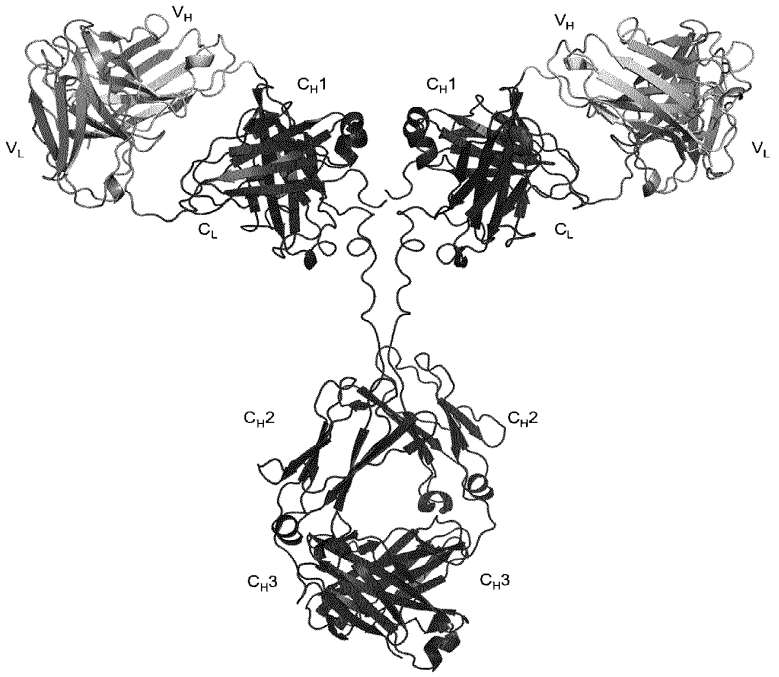


Fig. 1. Structure of the human IgG molecule. The molecule is formed by two heavy chains (consisting of V_H, C_H1, C_H2 and C_H3 domains) and two light chains (consisting of V_L and C_L). Figure was generated using PYMOL.

processes and conditions. These include recombinant expression, purification, concentration, viral inactivation, filtration, formulation, freeze/drying, transport and long-term storage. Throughout these steps the antibody molecule may encounter several stress factors that can dramatically increase its propensity to aggregate (including variations of temperature, pH, protein concentrations, ionic strength, exposure to air–water interfaces and mechanical stress). A further driver of protein aggregation has been a growing trend towards formulations that allow sub-cutaneous administration routes. This requires formulation at high protein concentrations (at around 100 mg/ml) in syringes for self-injection, which places increased demands on colloidal stability [15].

3. Stability of human antibody isotypes and constant domains

The four human IgG isotypes differ in their stabilities and biological functions, namely their potential to induce cellular killing through antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). While the human IgG1 isotype induces powerful ADCC and CDC responses, this is not the case for human IgG2, which is particularly suitable for applications where cellular killing is not required (such as neutralization of soluble ligands) [16]. Unlike the other human isotypes, IgG4 also has a naturally occurring potential to form a bispecific molecule [17] (it has been recently shown that this dual specificity can be grafted onto other isotypes [18]). Human IgG3 is not commonly used due to its longer hinge region, which renders it susceptible to proteolysis [19]. The vast majority of human antibody therapeutics currently in clinical practice and development are of the IgG1 isotype [16].

Conflicting evidence exists in respect to the colloidal stability of the various human IgG isotypes. A recent study of eleven different IgG1 and IgG2 antibodies concluded that, after high temperature storage, the IgG2 isotype is more prone to aggregation [20]. Similar findings were also obtained when subjecting these isotypes to high salt conditons [21] and from isotype switching studies [22]. The latter study confirmed that the human IgG1 isotype is less prone to aggregation when compared to IgG2 or IgG4. However, the authors also found that this isotype is more prone to fragmentation, especially at low pH, due to a non-enzymatic site in the upper hinge region. The hinge regions have also been implicated in the observed differences in aggregation propensity as the IgG2 hinge contains two additional cysteines in each heavy chain compared to IgG1, and is prone to the display of free cysteine residues [23,24]. Differences in the thermal stability of human constant domains have also been reported, with the C_H3 domains of human IgG1 and IgG2 displaying similar melting temperatures, much higher than what was observed for IgG4 [8]. In general, C_H3 domains exhibit more favorable biophysical properties than C_H2 domains [25]. This is reflected by melting temperatures of 8–10 °C higher than those observed for C_H2 domains [8,26].

Although human IgG1 therefore appears less aggregation prone than other isotypes, this is not universally the case. For example, a recent study found that the IgG1 isotype variant of an anti-LINGO-1 antibody exhibited a much higher propensity to aggregate than an IgG2 variant containing identical variable regions [27]. Studies such as these demonstrate that predicting the aggregation propensities of IgG molecules based on isotype can be difficult at the best of times. Moreover, evidence suggests that the aggregation propensity of human IgG molecules is greatly influenced by their variable domains [8,27,28]. This has the effect that antibodies with identical constant domains, but different variable domains, can vary widely in their stability profiles [5].

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4. Stability of human antibody variable domains

Human antibody variable domains can be grouped into several homologous families, which differ in their biophysical properties. A seminal study of consensus domains of human V_H and V_L families has revealed considerable differences of expression yields and thermodynamic stability [6]. In the case of human V_H domains, stabilities ranging from ΔG_{N-U} 14–53 kJ/mol were observed, with soluble expression yields ranging from 1–2.4 mg/l (Table 1). Intriguingly, Ewert et al. also reported that members of the (odd numbered) V_H 1, 3 and 5 families generally displayed superior biophysical properties in comparison with the (even numbered) 2, 4 and 6 families. These differences were observed for both thermodynamic stabilities and expression yield. In particular, the human V_H3 family was identified as uniquely stable and well expressed. This family is also among the most abundant in the human repertoire [29]. The overall stability trends were confirmed in a more recent study, which analyzed the effect of pairing of different human variable domain families on expression, thermal and colloidal stabilities of Fab fragments and IgG monoclonals [30].

More limited differences in biophysical properties were observed among human V_L families, with thermodynamic stabilities ranging from ΔG_{N-U} 15–24 kJ/mol for lambda and ΔG_{N-U} 29–35 kJ/mol for kappa [6]. Similarly, higher expression yields were observed for kappa variable light domains (5–17 mg/l) than for lambda domains (0.3–2 mg/l). The observed differences between antibody variable light domains were much less pronounced when pairing these with human V_H in an scFv format, while considerable differences between variable heavy domain families remained. Overall, the authors found the V_H/V_L combinations H3κ3, H1bκ3, H5κ3 and H3κ1 to be superior in respect to expression yield and thermodynamic stability [6]. However, despite the clear influence of variable domain family (and framework regions), the study also observed a strong influence of CDR sequence on biophysical properties. Indeed, initial attempts of the authors to express human V_H domain in isolation failed to yield protein (with the exception of V_H3). Only the grafting of a solubilizing CDR3 region allowed the soluble expression of human V_H1, and of V_H2, V_H4, V_H5 and V_H6 through refolding from inclusion bodies.

Although several of the human variable domain families are thermodynamically stable, they nevertheless often display poor colloidal stability and readily aggregate when heated above their melting temperatures. This behavior is exemplified by the V_H3 model domain DP47 [31] and by studies of synthetic antibody repertoires on phage, which have demonstrated that as few as one in a thousand VH domains resists heat induced aggregation [32].

Table 1
Biophysical properties of human antibody variable domains. Adapted from Ref. [6].

	Human family	Solubilizing CDR3 required	Expression yield	ΔG_{N-U}
V_H	1a	Y	+	+
	1b	Y	+	++
	2	Y	Refold	nd
	3	N	+	+++
	4	Y	Refold	nd
	5	Y	Refold	+
	6	Y	Refold	nd
V_L	κ1	N	+	++
	κ2	N	+++	++
	κ3	N	+++	+++
	κ4	N	++	-
	λ1	N	+	++
	λ2	N	+	+
	λ3	N	+	+

5. Increasing antibody stability through formulation

The most common method for controlling the biophysical properties of monoclonal antibodies and other biologics is through changes of buffer conditions and excipients. Formulation allows for the control of ionic strength, pH and the addition of excipient agents such as sugars or surfactants, which have been shown to stabilize monoclonal antibody preparations [33]. Although formulation can shield proteins from some chemical and physical challenges (including deamidation, oxidation, hydrolysis/fragmentation and isomerization), aggregation remains difficult to control [12]. Consequently, finding appropriate formulation conditions for an individual product is often an arduous process. This is exemplified by the therapeutic monoclonal antibody cetuximab (Erbitux). Its initial formulation conditions (2 mg/ml in phosphate buffer pH 6.0) generated visible aggregates under mechanical stress [34]. To address this issue, conditions had to be empirically optimized and were changed to 5 mg/ml in citrate buffer pH 5.5 with the addition of glycine and polysorbate 80 as stabilizers.

In recent years, more high-throughput techniques for formulation optimization have been developed [35], and stabilizers and excipients with more general applicability have been identified. Among these, arginine is commonly used to improve refolding yields [36,37], and more recently has been used in formulation to reduce heat-induced aggregation [38]. Another commonly used class of excipient molecules are polysorbates, which accumulate at the air–solvent interface and offer protection against shaking and other mechanical stresses [39]. Despite recent advances, the identification of suitable formulation conditions for a specific monoclonal antibody remains challenging and cannot be determined from its amino acid sequence. Indeed, a considerable proportion of human monoclonal antibody candidates fail formulation studies, often at relatively late pre-clinical stage, placing heavy burdens onto drug development pipelines [5].

6. Stability engineering of human antibody constant domains

Many efforts have been made to increase the stability of the different human antibody isotypes. Early work in this field focused on sequences analyses to predict stabilizing mutations. This approach allowed the identification of a set of substitutions (371K, 376G and 392L), which considerably increased thermal stability (from 76 °C to 86 °C) of the C_H3 domain [40]. The study was based on frequency analyses within 19 mammalian species to generate a consensus sequence. Structural approaches have also been utilized to increase stability of the C_H1/C_L interface [41]. More recently, it was shown that the introduction of additional intra-domain disulfide bridges in the human C_H3 domain resulted in the thermal stabilization of an isolated IgG1 Fc fragment [42] (Fig. 2). In particular, the introduction of a link between the N-terminal A strand (position 343) and the F strand (position 431) caused a 8 °C increase in the melting temperature, while a linkage between positions 375 and 396 resulted in a 4 °C increase (combined 13 °C). These findings were successfully applied to a HER2 specific Fcab molecule (Fc fragment with engineered binding loops) resulting in an increased in the thermal stability of 19 °C. A similar finding was observed for a human IgG1 C_H3 single domain, which could be stabilized by introduction of an intra-domain disulfide bridge [43]. The authors used a two-step approach, by initially introducing mutations that rendered the C_H3 domain monomeric, resulting in a overall low thermal stability (41 °C compared to 82 °C for the native dimeric form). Using structure-based design, two additional cysteines (at positions 343 and 431) were then introduced to generate a disulfide bridge, which increased the thermal stability to 76 °C (a

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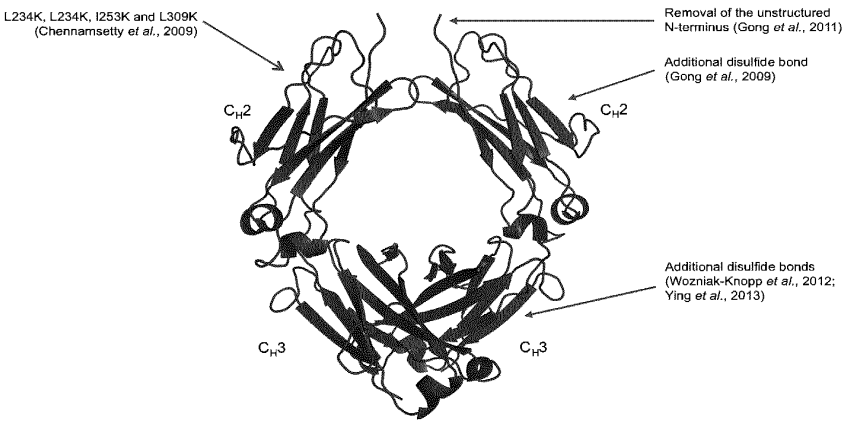


Fig. 2. Stability engineering of human constant domains.

35 °C increase) [43]. Similar strategies have been also reported for C_H2 domains [26]. Here the authors reported the engineering of an isolated human C_H2 domain, resulting in an increase in thermal stability of 20 °C (from 54 °C to 74 °C) [26]. More recently, the same group reported the stabilization of a C_H2 domain through removal of an unstructured region at the N-terminus. Combined with the intra-domain disulfide bond, this resulted in a further increase of melting temperature of 10 °C [44]. For all of the above studies, stability of constant domains was evaluated in the context of fragments or isolated domains.

Other studies have investigated the effects of such mutations on the stability of full-length human IgG. In the case of human IgG4, alteration of the disulfide bond network in the Fab region was shown to lead to an increase of thermal stability of between 3 and 7 °C [45]. This effect was achieved through removal of a cysteine at position 127 in the C_H1 domain, while an additional inter-domain disulfide bond between position 229 and C_L was introduced (similar to what is observed in human IgG1). Importantly, the authors reported that the introduced mutations did not affect antigen binding [45]. More recently, molecular dynamic simulations have been used to predict the spatial aggregation propensity (SAP) of human IgG molecules [46]. The authors later showed that by mutating identified surface-exposed hydrophobic patches within the hinge and Fc regions of an IgG, aggregation resistance and, to a more limited extent, thermodynamic stability could be improved [47].

7. Increased stability through charged fusion tags

In addition to the modification of constant domains, it was recently shown that the addition of charged fusion tags to the N-terminus of human variable domains could considerably improve aggregation propensity [48]. The authors noted that batches of the same IgG monoclonal expressed in yeast were less prone to aggregation than those expressed in mammalian cells. Mass spectrometry and sequencing analyses revealed the presence of a non-processed "EAEA" leader sequence within both heavy and light chains. In contrast to colloidal stability, the authors did not observe improved thermodynamic stability, and the effect seemed to be particularly pronounced for antibodies with a low global net charge. In addition to terminal fusion tags, a different study explored the internal use of a "DED" motif [49]. It was shown that the insertion of the tag into the CDR3 region of aggregation-prone V_H domain antibodies reduced their aggregation propensity, while reducing retention on gel filtration matrices.

8. Stability engineering of human antibody variable domains

8.1. Engineering of human V_H domains through mutation of the light chain interface

The poor biophysical properties of isolated human antibody variable domains are in marked contrast to those of the variable domains of camels and llamas [50]. Although these immunoglobulin domains (V_{HH}) display a relatively high level of sequence identity with human V_H domains, they are generally well expressed and soluble [51] and reversibly unfold after incubation at temperatures as high as 90 °C [52]. In addition to the observed differences in biophysical nature, camelid domains also display an important difference in chain structure, as they are naturally devoid of light chain partners [50]. Their single domain nature results in unique structural features, compared to human antibody V_H domains. These include CDR length and conformation, as well as framework residues. A particular feature of V_{HH} domains is their long flexible CDR3 loop capable of folding back on itself, thereby protecting an area forming the V_H/V_L interface in human antibodies. In addition, these naturally occurring single domains display a marked reduction in hydrophobicity of the former light chain interface, due to a limited number of changes in their respective germlines (Fig. 3). The most common and significant changes compared to humans, coined the "V_{HH} tetrad", are located at positions: G44Q, L45R/C, W47G/I and V37F/Y (human/camelid). The latter residue is nucleating a small core involving Y91, W103, R45 and other hydrophobic residues within the CDR3 loop [53]. Structural investigation of V_{HH} has also shown the CDR3 loop covering residue 37, thereby shielding hydrophobicity in an area corresponding to the light chain interface in human V_H [54]. In addition, camelid V_{HH} often contain an additional disulfide bridge between CDR1 and CDR3, which increases their thermal stability [55,56].

In order to improve the biophysical properties of human V_H domains, elements of the V_{HH} tetrad have been incorporated into human frameworks ("camelization") [57]. Although the introduced framework changes improved the solubility of human V_H, they also reduced expression levels, induced conformational changes, decreased thermodynamic stability and did not considerably improve the colloidal stability of the domains [58,59]. In contrast, combining the approach with the introduction of an additional disulfide bond between CDR1 and CDR3, yielded domains with high colloidal stability, although at the expense of antigen binding affinity [60].

As an alternative to camelid domains, shark single domain antibodies (V_{NAR}) could also potentially serve as a blueprint for improving

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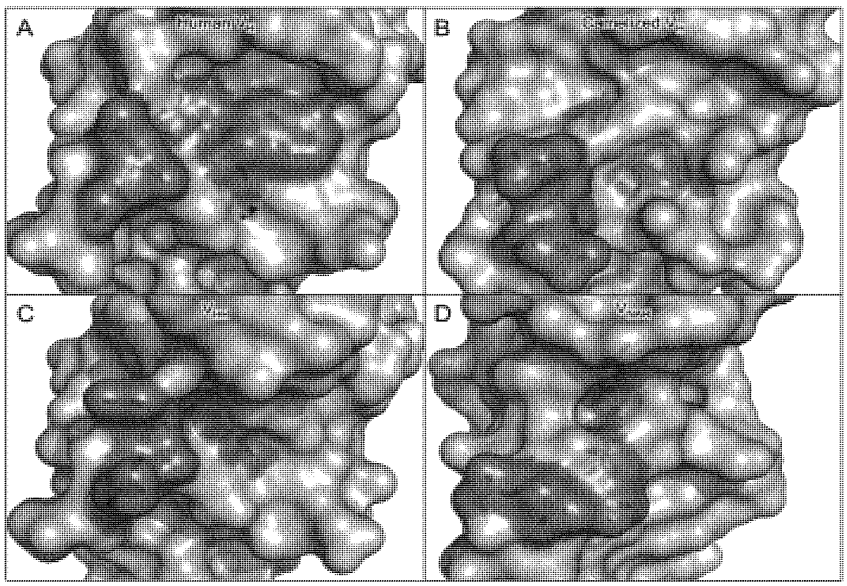


Fig. 3. Engineering of human VH domains through mutation of the light chain interface. Shown are surface representations of the light chain interface in human V_H (PDB 2VXS) and of the equivalent surfaces in camelized V_H (PDB 1VHP), camelid V_{HH} (PDB 1ZVH) and shark V_{NAR} domains (PDB 1T6V). Residues forming the camelid 'tetrad' at positions 37, 44, 45 and 47 are highlighted in green. Camelized V_H, V_{HH} and V_{NAR} domains display a considerable reduction in surface hydrophobicity in comparison to human V_H domains. Figure was generated using PYMOL.

the stability of human antibody V_H domains (although they are more distantly related to human domains [61]). As observed for camelid domains, shark V_{NAR} domains are characterized by a long protruding CDR3 [62]. The CDR loop is constrained by additional disulfide bonds, thereby folding over an area equivalent to the former V_H/V_L interface of human antibodies [61]. A recent study revealed that V_{NAR} domains are resistant to thermal denaturation, acidic denaturation, and lyophilisation denaturation and are capable of returning to their native state after thermal unfolding [63].

Rather than focusing on direct grafting of camelid residues, more recent approaches for the generation of autonomous single domains have focused on the identification of novel mutations. This has been possible due to the use of phage display based selection strategies and the use of protein A to select for folded domains (pA superantigen binds to folded human V_H3 domains, but not to unfolded or aggregated domains). Using this approach, an extensive mutagenesis study has identified hydrophilic substitutions within the V_H3/V_L interface [53]. The authors were able to demonstrate that their mutations (including H35G, Q39R, L45E, R50S) improved solubility, thermal refolding and melting temperature. Importantly, some of the mutations improved protein A binding (and presumably stability), independently of CDR3 sequence diversity. As CDR3 of V_H provides the majority of binding energy in antibody–antigen interactions, this indicated broad compatibility with antigen binding and suggested that the engineered frameworks could be used for the construction of stable repertoires of autonomous V_H domains.

8.2. Engineering of human V_H domains through CDR grafting and framework mutations

In addition to their use in the engineering of autonomous antibody single domains, homology-based approaches have also been used for the identification of mutations outside the interface regions, thereby retaining the ability of human V_H to interact with variable light domains. One of the earliest examples of such strategies relates to the use of CDR grafting for the improvement of biophysical properties. Developed by Jones and Winter in the mid-1980s, CDR grafting was initially used to reduce the immunogenicity of mouse monoclonals, by transplantation of murine CDR regions onto a human framework [64]. However, it was later shown that by grafting onto a stable and well-behaved framework (such as the Herceptin 4D5 [V_H3] framework) considerably improved expression levels and thermodynamic stability, while maintaining binding affinity of the parental antibody fragment [65]. A second approach is based on the use of consensus sequences to improve stability [66]. This approach has been applied to a range of protein scaffolds [67], and utilizes amino acid observed with high frequency within the repertoire [68]. This approach was also used in the construction of the HuCAL human antibody library, for which the authors have reported good expression yields [69]. More recent studies from the same group have identified additional framework mutations that transfer biophysical properties between human V_H3 and V_H6 families [70,71].

As an alternative to homology-based approaches, other studies have focused on the computational prediction of aggregation hotspots in antibody sequences. Early studies on peptides and proteins involved in neurodegenerative diseases have highlighted physicochemical properties that correlate with an increase in aggregate formation, namely hydrophobicity, β-sheet propensity and reduced net charge [72]. Algorithms have been developed to detect such aggregation-prone regions [73–76]. Two of these algorithms, TANGO and PAGE, have been used to identify potential aggregation-prone regions in commercial therapeutic antibodies, which are predominately located in variable domains, and particularly within CDR and adjacent framework residues [28].

8.3. Engineering of human variable domains through CDR mutations

The role of CDR residues in determining thermodynamic and in particular colloidal stability of human antibody variable domains is further highlighted by studies on the biophysical properties of the

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HEL4 model domain [31]. HEL4 was originally isolated through phage display selection from a synthetic CDR-only repertoire based on the human V_H3-23 DP47 germline [77]. As phage is remarkably resistant to chemical, proteolytic and thermal denaturation [78–80], the authors were able to heat the phage displayed antibody fragments at high temperature (80 °C), followed by cooling and binding to antigen (hen egg white lysozyme). The method thereby selected for domains that unfolded reversibly, and could be captured by binding to antigen or superantigen. Unlike other human V_H3 domains, HEL4 displayed highly favorable biophysical properties, including heat-refoldability, high expression levels in bacteria, and the absence of 'stickiness' on gel-filtration [31]. Indeed, it was later shown that aggregation resistance on phage is a general indicator of the solution behavior of human V_H domains [81]. In addition to HEL4, several other V_H domains, that were isolated using this method, showed a high level of resistance to aggregation. Intriguingly, despite their high level of colloidal stability, all of the domains actually had lower thermodynamic stabilities than the DP47 domain from which they had been derived from (ΔG_{N-U} = 15–20 kJ/mol vs 35 kJ/mol). In contrast, DP47 readily aggregated upon heating, further highlighting differences between thermodynamic and colloidal stability. As an alternative to heat-based selection on phage, a more recent study explored the use of pH stress (pH 3.2) for the selection of aggregation-resistant V_H domains [82]. In contrast to domains isolated after thermal denaturation, the selected V_H domains displayed increased thermodynamic stabilities and increased colloidal stability at low pH, reflecting the different denaturation conditions experienced during the selection process.

Although the denaturation method on phage was capable of selecting human V_H domains with CDR sequences that promoted a high level of aggregation resistance, it was also evident that such domains were rare within the repertoire. To increase the frequency of such clones, a human V_H library was constructed through combinatorial assembly of CDR regions that had been pre-selected for aggregation resistance in phage. Binders were successfully selected from this repertoire, which exhibited good antigen-binding properties together with favorable biophysical properties [32].

Despite clear improvements over other human V_H domains, detailed determinants of the observed aggregation resistance initially had remained unclear. As HEL4 and other clones had been selected from synthetic CDR-only repertoires, it was apparent that the

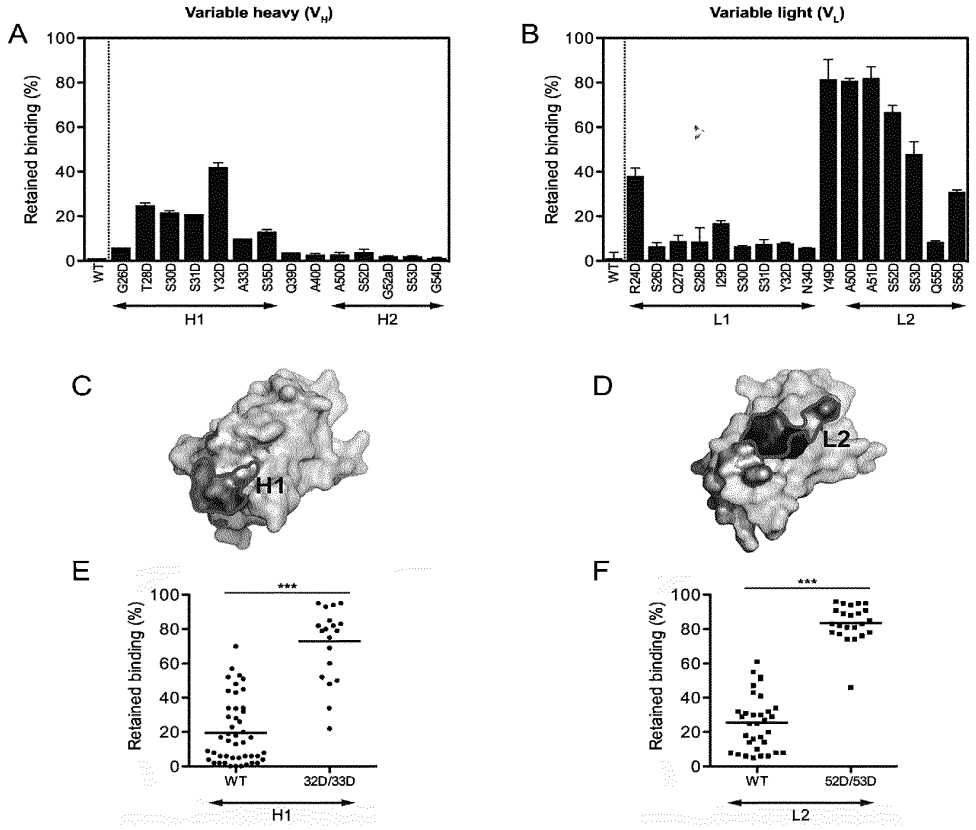


Fig. 4. Effect of mutations in human antibody V_H and V_L domains on aggregation resistance. Surface residues in variable heavy and light domains were targeted for substitution with aspartate. Aggregation resistance of the domains was determined by measuring binding to protein A and protein L superantigen after heating to 80 °C on phage. Complementarily determining regions are indicated (A) Mutations in human V_H. (B) Mutations in human V_L. Introduction of aspartate into CDR H1 and CDR L2 increases aggregation resistance of human variable domains up to 40-fold for V_H and up to 80-fold for V_L. (C) Mutations mapped onto the human V_H surface. (D) Mutations mapped onto the human V_L surface (blue: 100% retained superantigen binding, white: 0%). (E) Stability engineering of human V_H repertoire. (F) Stability engineering of human V_L repertoire. The mutant repertoires were generated through introduction of aspartate mutations in CDR H1 and CDR L2 (at positions 32/33 and 52/53, respectively). Introduction of the mutations significantly increases the aggregation resistance of human antibody repertoires, independent of sequence diversity at other CDR positions. Adapted from Ref. [7].

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exhibited biophysical properties were a result of changes in the complementarity determining regions, without any further influence of framework residues [31]. Crystal structure of the HEL4 model domain had indicated a potential effect of a serine to glycine mutation at position 35 [77]. Indeed, introduction of this mutation into DP47, resulted in increased solubility and reduced "stickiness" on gel-filtration. However, the mutant domain continued to readily aggregate when heated above its melting temperature. The authors also reported that many of the selected domains had a low isoelectric point (pI). Similar findings were also obtained in a second study, which reported that a low pI, together with the non-canonical inter-CDR disulfide linkages, contribute to improved biophysical properties human V_H domains [83]. Using transient heating to pre-select for proteins reversible thermal unfolding properties, the authors found that many of the isolated domains had pI values of less than six, although they also observed domains with values higher than eight. The authors concluded that the improved biophysical characteristics of these V_H domains were caused by a combination of global pI effects, together with an increase of stability through inter-CDR disulfide linkage (other studies by the same group demonstrated that, although the additional disulfide bridge significantly increased thermal stability and protease resistance [84], it also induced structural changes in human V_H as indicated by a marked reduction of protein A superantigen binding [85]). A global increase in charge was also observed in a different study, analyzing larger sets of V_H domain sequences after heat selection on phage, together with an overall reduction of hydrophobicity and a decrease in beta-sheet propensity [86].

More recently, an extensive mutagenesis study on phage has provided detailed insights into mutations that increase the colloidal stability of human V_H and V_L antibody domains [7]. Using superantigen binding after heat denaturation on phage as a mean to select for reversible unfolding, the investigators analyzed the effect of charged mutations at all CDR positions (excluding CDR3 to limit effects on antigen binding). This revealed that the introduction of negative charge (and in particular aspartate) at CDR-H1 positions (28, 30–33, 35) considerably increased the aggregation resistance of human V_H domains (up to 40-fold) (Fig. 4A). This is in excellent agreement with the observations for the model HEL4 domain, which contains negatively charged amino acids at a subset of the identified positions (as highlighted by CDR grafting studies [87–89]).

Intriguingly, this was not the case for human V_L domains, for which the introduction of mutations at equivalent positions had little effect. In contrast to what was observed for human V_H, increased aggregation resistance (up to 80-fold) was instead endowed by the introduction of asparate at CDR-L2 positions (49, 50–53, 56) (Fig. 4B). As observed in other studies [81], the improved properties on phage correlated with increased aggregation resistance in solution, improved expression yields and reduced retention on gel-filtration. Importantly, the observed increases in aggregation resistance were not only observed in model domains, but also when introducing CDR-H1 and CDR-L2 mutations into diverse antibody libraries [7] (Fig. 4E/F).

In addition, and unlike what had been observed for camelized and other engineered variable domains [53,59,85], crystal structures of mutant V_H and V_L domains showed no detectable conformational changes in either CDR conformations or the V_H/V_L interface. Taken together, these results indicated broad compatibility with high affinity antigen binding, which was confirmed by the introduction of CDR-H1 and CDR-L2 mutations into the antibody therapeutic Herceptin [7]. Further evidence for the influence of the identified mutations on colloidal stability has recently been reported in a study of an aggregation-prone human IgG1 monoclonal [90]. The authors found that the aspartic acid at position 49 of the V_L domain considerably reduced aggregation rate of the antibody during long-term stability studies (from 35% to 0.2% per month).

9. Conclusion – towards stable human antibody therapeutics

The increasing number of human monoclonals in development and clinical practice, together with a trend towards high concentration formulations for subcutaneous delivery places increasing demands on the stability of human antibody therapeutics. While the thermodynamic stability of human antibodies is relatively well studied and can be further increased through homology, disulfide and computational approaches [71,83,47], determinants of their colloidal stability are just beginning to emerge due to a combination of rational and high-throughput approaches on phage [7,31,46,91]. In particular, the recent availability of human V_L domains with high levels of aggregation resistance [7] will greatly assist in the generation of paired antibody fragments and IgG. Further advances in the generation of human antibody therapeutics with high thermodynamic and colloidal stability will not only improve the production and formulation of human antibody therapeutics, but may also open up new administration routes and therapeutic applications.

References

[1] Nelson, A.L., Dhimolea, E. and Reichert, J.M. (2010) Development trends for human monoclonal antibody therapeutics. Nat. Rev. Drug Discov. 9, 767–774.
[2] Finke, J.M., Roy, M., Zimm, B.H. and Jennings, P.A. (2000) Aggregation events occur prior to stable intermediate formation during refolding of interleukin 1beta. Biochemistry 39, 575–583.
[3] Baneyx, F. and Mujacic, M. (2004) Recombinant protein folding and misfolding in *Escherichia coli*. Nat. Biotechnol. 22, 1399–1408.
[4] Bondos, S.E. and Bicknell, A. (2003) Detection and prevention of protein aggregation before, during, and after purification. Anal. Biochem. 316, 223–231.
[5] Lowe, D., Dudgeon, K., Rouet, R., Schofield, P., Jermutus, L. and Christ, D. (2011) Aggregation, stability, and formulation of human antibody therapeutics. Adv. Protein Chem. Struct. Biol. 84, 41–61.
[6] Ewert, S., Huber, T., Honegger, A. and Pluckthun, A. (2003) Biophysical properties of human antibody variable domains. J. Mol. Biol. 325, 531–553.
[7] Dudgeon, K., Rouet, R., Kokmeijer, I., Schofield, P., Stolp, J., Langley, D., Stock, D. and Christ, D. (2012) General strategy for the generation of human antibody variable domains with increased aggregation resistance. Proc. Natl. Acad. Sci. USA 109, 10879–10884.
[8] Garber, E. and Demarest, S.J. (2007) A broad range of Fab stabilities within a host of therapeutic IgGs. Biochem. Biophys. Res. Commun. 355, 751–757.
[9] Wolynes, P.G., Onuchic, J.N. and Thirumalai, D. (1995) Navigating the folding routes. Science 267, 1619–1620.
[10] Sambashivan, S., Liu, Y., Sawaya, M.R., Gingery, M. and Eisenberg, D. (2005) Amyloid-like fibrils of ribonuclease A with three-dimensional domain-swapped and native-like structure. Nature 437, 266–269.
[11] Dobson, C.M. (2003) Protein folding and misfolding. Nature 426, 884–890.
[12] Wang, W. (2005) Protein aggregation and its inhibition in biopharmaceutics. Int. J. Pharm. 289, 1–30.
[13] Rosenberg, A.S. (2006) Effects of protein aggregates: an immunologic perspective. AAPS J. 8, E501–E507.
[14] Wright, C.F., Teichmann, S.A., Clarke, J. and Dobson, C.M. (2005) The importance of sequence diversity in the aggregation and evolution of proteins. Nature 438, 878–881.
[15] Shire, S.J. (2009) Formulation and manufacturability of biologics. Curr. Opin. Biotechnol. 20, 708–714.
[16] Salfeld, J.G. (2007) Isotype selection in antibody engineering. Nat. Biotechnol. 25, 1369–1372.
[17] Schuurman, J., Van Ree, R., Perdok, G.J., Van Doorn, H.R., Tan, K.Y. and Aalberse, R.C. (1999) Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Immunology 97, 693–698.
[18] Labrijn, A.F., Meesters, J.I., de Goeij, B.E., van den Bremer, E.T., Neijssen, J., van Kampen, M.D., Strumane, K., Verploegen, S., Kundu, A., Gramer, M.J., van Berkel, P.H., van de Winkel, J.G., Schuurman, J. and Parren, P.W. (2013) Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange. Proc. Natl. Acad. Sci. USA 110, 5145–5150.
[19] Carter, P.J. (2006) Potent antibody therapeutics by design. Nat. Rev. Immunol. 6, 343–357.
[20] Franey, H., Brych, S.R., Kolvenbach, C.G. and Rajan, R.S. (2010) Increased aggregation propensity of IgG2 subclass over IgG1: role of conformational changes and covalent character in isolated aggregates. Protein Sci. 19, 1601–1615.

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[21] Hari, S.B., Lau, H., Razinkov, V.I., Chen, S. and Latypov, R.F. (2010) Acid-induced aggregation of human monoclonal IgG1 and IgG2: molecular mechanism and the effect of solution composition. Biochemistry 49, 9328–9338.
[22] Ito, T. and Tsumoto, K. (2013) Effects of subclass change on the structural stability of chimeric, humanized, and human antibodies under thermal stress. Protein Sci. 22 (11), 1542–1551.
[23] Wypych, J., Li, M., Guo, A., Zhang, Z., Martinez, T., Allen, M.J., Fodor, S., Kelner, D.N., Flynn, G.C., Liu, Y.D., Bondarenko, P.V., Ricci, M.S., Dillon, T.M. and Balland, A. (2008) Human IgG2 antibodies display disulfide-mediated structural isoforms. J. Biol. Chem. 283, 16194–16205.
[24] Lacy, E.R., Baker, M. and Brigham-Burke, M. (2008) Free sulfhydryl measurement as an indicator of antibody stability. Anal. Biochem. 382, 66–68.
[25] Demarest, S.J. and Glaser, S.M. (2008) Antibody therapeutics, antibody engineering, and the merits of protein stability. Curr. Opin. Drug Discov. Devel. 11, 675–687.
[26] Gong, R., Vu, B.K., Feng, Y., Prieto, D.A., Dyba, M.A., Walsh, J.D., Prabakaran, P., Veenstra, T.D., Tarasov, S.G., Ishima, R. and Dimitrov, D.S. (2009) Engineered human antibody constant domains with increased stability. J. Biol. Chem. 284, 14203–14210.
[27] Pepinsky, R.B., Silvian, L., Berkowitz, S.A., Farrington, G., Lugovskoy, A., Walus, L., Eldredge, J., Capili, A., Mi, S., Graff, C. and Garber, E. (2010) Improving the solubility of anti-LINGO-1 monoclonal antibody Li33 by isotype switching and targeted mutagenesis. Protein Sci. 19, 954–966.
[28] Wang, X., Das, T.K., Singh, S.K. and Kumar, S. (2009) Potential aggregation prone regions in biotherapeutics: a survey of commercial monoclonal antibodies. MAbs 1, 254–267.
[29] Tomlinson, I.M., Walter, G., Marks, J.D., Llewelyn, M.B. and Winter, G. (1992) The repertoire of human germline V_H sequences reveals about fifty groups of V_H segments with different hypervariable loops. J. Mol. Biol. 227, 776–798.
[30] Tiller, T., Schuster, I., Deppe, D., Siegers, K., Strohner, R., Herrmann, T., Berenguer, M., Poujol, D., Stehle, J., Stark, Y., Heßling, M., Daubert, D., Felderer, K., Kaden, S., Kölln, J., Enzelberger, M. and Urlinger, S. (2013) A fully synthetic human Fab antibody library based on fixed V_H/V_L framework pairings with favorable biophysical properties. MAbs 5, 445–470.
[31] Jespers, L., Schon, O., Famm, K. and Winter, G. (2004) Aggregation-resistant domain antibodies selected on phage by heat denaturation. Nat. Biotechnol. 22, 1161–1165.
[32] Christ, D., Famm, K. and Winter, G. (2007) Repertoires of aggregation-resistant human antibody domains. Protein Eng. Des. Sel. 20, 413–416.
[33] Wang, W., Singh, S., Zeng, D.L., King, K. and Nema, S. (2007) Antibody structure, instability, and formulation. J. Pharm. Sci. 96, 1–26.
[34] Lahlou, A., Blanchet, B., Carvalho, M., Paul, M. and Astier, A. (2009) Mechanically-induced aggregation of the monoclonal antibody cetuximab. Ann. Pharm. Fr. 67, 340–352.
[35] Daugherty, A.L. and Mrsny, R.J. (2006) Formulation and delivery issues for monoclonal antibody therapeutics. Adv. Drug Deliv. Rev. 58, 686–706.
[36] Buchner, J. and Rudolph, R. (1991) Renaturation, purification and characterization of recombinant Fab-fragments produced in *Escherichia coli*. Biotechnology (NY) 9, 157–162.
[37] Lee, C.M., McGuire, H., Basten, A., King, C. and Christ, D. (2010) Expression, purification and characterization of recombinant interleukin-21. J. Immunol. Methods 362, 185–189.
[38] Arakawa, T., Tsumoto, K., Kita, Y., Chang, B. and Ejima, D. (2007) Biotechnology applications of amino acids in protein purification and formulations. Amino Acids 33, 587–605.
[39] Mahler, H.C., Senner, F., Maeder, K. and Mueller, R. (2009) Surface activity of a monoclonal antibody. J. Pharm. Sci. 98, 4525–4533.
[40] Demarest, S.J., Rogers, J. and Hansen, G. (2004) Optimization of the antibody C(H)3 domain by residue frequency analysis of IgG sequences. J. Mol. Biol. 335, 41–48.
[41] Teerinen, T., Valjakka, J., Rouvinen, J. and Takkinen, K. (2006) Structure-based stability engineering of the mouse IgG1 Fab fragment by modifying constant domains. J. Mol. Biol. 361, 687–697.
[42] Wozniak-Knopp, G., Stadlmann, J. and Ruker, F. (2012) Stabilisation of the Fc fragment of human IgG1 by engineered intradomain disulfide bonds. PLoS One 7, e30083.
[43] Ying, T., Chen, W., Feng, Y., Wang, Y., Gong, R. and Dimitrov, D.S. (2013) Engineered soluble monomeric IgG1 C_H3 domain: generation, mechanisms of function, and implications for design of biological therapeutics. J. Biol. Chem. 288, 25154–25164.
[44] Gong, R., Wang, Y., Feng, Y., Zhao, Q. and Dimitrov, D.S. (2011) Shortened engineered human antibody C_H2 domains: increased stability and binding to the human neonatal Fc receptor. J. Biol. Chem. 286, 27288–27293.
[45] Peters, S.J., Smales, C.M., Henry, A.J., Stephens, P.E., West, S. and Humphreys, D.P. (2012) Engineering an improved IgG4 molecule with reduced disulfide bond heterogeneity and increased Fab domain thermal stability. J. Biol. Chem. 287, 24525–24533.
[46] Chennamsetty, N., Helk, B., Voynov, V., Kayser, V. and Trout, B.L. (2009) Aggregation-prone motifs in human immunoglobulin G. J. Mol. Biol. 391, 404–413.
[47] Chennamsetty, N., Voynov, V., Kayser, V., Helk, B. and Trout, B.L. (2009) Design of therapeutic proteins with enhanced stability. Proc. Natl. Acad. Sci. USA 106, 11937–11942.
[48] Schaefer, J.V. and Pluckthun, A. (2012) Engineering aggregation resistance in IgG by two independent mechanisms: lessons from comparison of *Pichia pastoris* and mammalian cell expression. J. Mol. Biol. 417, 309–335.
[49] Perchiacca, J.M., Ladiwala, A.R., Bhattacharya, M. and Tessier, P.M. (2012) Aggregation-resistant domain antibodies engineered with charged mutations near the edges of the complementarity-determining regions. Protein Eng. Des. Sel. 25, 591–601.
[50] Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E.B., Bendahman, N. and Hamers, R. (1993) Naturally occurring antibodies devoid of light chains. Nature 363, 446–448.
[51] Arbabi Ghahroudi, M., Desmyter, A., Wyns, L., Hamers, R. and Muyldermans, S. (1997) Selection and identification of single domain antibody fragments from camel heavy-chain antibodies. FEBS Lett. 414, 521–526.
[52] van der Linden, R.H., Frenken, L.G., de Geus, B., Harmsen, M.M., Ruuls, R.C., Stok, W., de Ron, L., Wilson, S., Davis, P. and Verrips, C.T. (1999) Comparison of physical chemical properties of llama V_{HH} antibody fragments and mouse monoclonal antibodies. Biochim. Biophys. Acta 1431, 37–46.
[53] Barthelemy, P.A., Raab, H., Appleton, B.A., Bond, C.J., Wu, P., Wiesmann, C. and Sidhu, S.S. (2008) Comprehensive analysis of the factors contributing to the stability and solubility of autonomous human V_H domains. J. Biol. Chem. 283, 3639–3654.
[54] Vranken, W., Tolkatchev, D., Xu, P., Tanha, J., Chen, Z., Narang, S. and Ni, F. (2002) Solution structure of a llama single-domain antibody with hydrophobic residues typical of the V_H/V_L interface. Biochemistry 41, 8570–8579.
[55] Muyldermans, S., Atarhouch, T., Saldanha, J., Barbosa, J.A. and Hamers, R. (1994) Sequence and structure of V_H domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. Protein Eng. 7, 1129–1135.
[56] Ewert, S., Cambillau, C., Conrath, K. and Pluckthun, A. (2002) Biophysical properties of camelid V(HH) domains compared to those of human V(H)3 domains. Biochemistry 41, 3628–3636.
[57] Davies, J. and Riechmann, L. (1994) 'Camelising' human antibody fragments: NMR studies on V_H domains. FEBS Lett. 339, 285–290.
[58] Davies, J. and Riechmann, L. (1996) Single antibody domains as small recognition units: design and in vitro antigen selection of camelized, human VH domains with improved protein stability. Protein Eng. 9, 531–537.
[59] Riechmann, L. (1996) Rearrangement of the former V_L interface in the solution structure of a camelised, single antibody V_H domain. J. Mol. Biol. 259, 957–969.
[60] Tanha, J., Xu, P., Chen, Z., Ni, F., Kaplan, H., Narang, S.A. and MacKenzie, C.R. (2001) Optimal design features of camelized human single-domain antibody libraries. J. Biol. Chem. 276, 24774–24780.
[61] Roux, K.H., Greenberg, A.S., Greene, L., Strelets, L., Avila, D., McKinney, E.C. and Flajnik, M.F. (1998) Structural analysis of the nurse shark (new) antigen receptor (NAR): molecular convergence of NAR and unusual mammalian immunoglobulins. Proc. Natl. Acad. Sci. USA 95, 11804–11809.
[62] Greenberg, A.S., Avila, D., Hughes, M., Hughes, A., McKinney, E.C. and Flajnik, M.F. (1995) A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature 374, 168–173.
[63] Griffiths, K., Dolezal, O., Parisi, K., Angerosa, J., Dogovski, C., Barraclough, M., Sanalla, A., Casey, J., González, I., Perugini, M., Nuttall, S. and Foley, M. (2013) Shark variable new antigen receptor (VNAR) single domain antibody fragments: stability and diagnostic applications. Antibodies 2, 66–81.
[64] Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. and Winter, G. (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 321, 522–525.
[65] Jung, S. and Pluckthun, A. (1997) Improving in vivo folding and stability of a single-chain Fv antibody fragment by loop grafting. Protein Eng. 10, 959–966.
[66] Steipe, B., Schiller, B., Pluckthun, A. and Steinbacher, S. (1994) Sequence statistics reliably predict stabilizing mutations in a protein domain. J. Mol. Biol. 240, 188–192.
[67] Kohl, A., Binz, H.K., Forrer, P., Stumpp, M.T., Pluckthun, A. and Grutter, M.G. (2003) Designed to be stable: crystal structure of a consensus ankyrin repeat protein. Proc. Natl. Acad. Sci. USA 100, 1700–1705.
[68] Worn, A. and Pluckthun, A. (2001) Stability engineering of antibody single-chain Fv fragments. J. Mol. Biol. 305, 989–1010.
[69] Knappik, A., Ge, L., Honegger, A., Pack, P., Fischer, M., Wellnhofer, G., Hoess, A., Wolle, J., Pluckthun, A. and Virnekas, B. (2000) Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. J. Mol. Biol. 296, 57–86.
[70] Ewert, S., Honegger, A. and Pluckthun, A. (2003) Structure-based improvement of the biophysical properties of immunoglobulin VH domains with a generalizable approach. Biochemistry 42, 1517–1528.
[71] Schaefer, J.V. and Pluckthun, A. (2012) Transfer of engineered biophysical properties between different antibody formats and expression systems. Protein Eng. Des. Sel. 25, 485–506.
[72] Murphy, R.M. (2002) Peptide aggregation in neurodegenerative disease. Annu. Rev. Biomed. Eng. 4, 155–174.
[73] Kuhlman, B. and Baker, D. (2000) Native protein sequences are close to optimal for their structures. Proc. Natl. Acad. Sci. USA 97, 10383–10388.
[74] Fernandez-Escamilla, A.M., Rousseau, F., Schymkowitz, J. and Serrano, L. (2004) Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nat. Biotechnol. 22, 1302–1306.
[75] Pawar, A.P., Dubay, K.F., Zurdo, J., Chiti, F., Vendruscolo, M. and Dobson, C.M. (2005) Prediction of "aggregation-prone" and "aggregation-susceptible" regions in proteins associated with neurodegenerative diseases. J. Mol. Biol. 350, 379–392.

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[76] Tartaglia, G.G., Cavalli, A., Pellarin, R. and Caflisch, A. (2005) Prediction of aggregation rate and aggregation-prone segments in polypeptide sequences. Protein Sci. 14, 2723–2734.
[77] Jespers, L., Schon, O., James, L.C., Veprintsev, D. and Winter, G. (2004) Crystal structure of HEL4, a soluble, refoldable human V(H) single domain with a germ-line scaffold. J. Mol. Biol. 337, 893–903.
[78] Kristensen, P. and Winter, G. (1998) Proteolytic selection for protein folding using filamentous bacteriophages. Fold Des. 3, 321–328.
[79] Holliger, P., Riechmann, L. and Williams, R.L. (1999) Crystal structure of the two N-terminal domains of g3p from filamentous phage fd at 1.9 Å: evidence for conformational lability. J. Mol. Biol. 288, 649–657.
[80] Jermutus, L., Honegger, A., Schwesinger, F., Hanes, J. and Pluckthun, A. (2001) Tailoring in vitro evolution for protein affinity or stability. Proc. Natl. Acad. Sci. USA 98, 75–80.
[81] Dudgeon, K., Rouet, R. and Christ, D. (2013) Rapid prediction of expression and refolding yields using phage display. Protein Eng. Des. Sel. 26 (10), 671–674.
[82] Famm, K., Hansen, L., Christ, D. and Winter, G. (2008) Thermodynamically stable aggregation-resistant antibody domains through directed evolution. J. Mol. Biol. 376, 926–931.
[83] Arbabi-Ghahroudi, M., To, R., Gaudette, N., Hirama, T., Ding, W., MacKenzie, R. and Tanha, J. (2009) Aggregation-resistant VHs selected by in vitro evolution tend to have disulfide-bonded loops and acidic isoelectric points. Protein Eng. Des. Sel. 22, 59–66.
[84] Hussack, G., Hirama, T., Ding, W., Mackenzie, R. and Tanha, J. (2011) Engineered single-domain antibodies with high protease resistance and thermal stability. PLoS One 6, e28218.
[85] Kim, D.Y., Kandalaft, H., Ding, W., Ryan, S., van Faassen, H., Hirama, T., Foote, S.J., MacKenzie, R. and Tanha, J. (2012) Disulfide linkage engineering for improving biophysical properties of human V_H domains. Protein Eng. Des. Sel. 25, 581–589.
[86] Dudgeon, K., Famm, K. and Christ, D. (2009) Sequence determinants of protein aggregation in human V_H domains. Protein Eng. Des. Sel. 22, 217–220.
[87] Perchiacca, J.M., Bhattacharya, M. and Tessier, P.M. (2011) Mutational analysis of domain antibodies reveals aggregation hotspots within and near the complementarity determining regions. Proteins 79, 2637–2647.
[88] Lee, C.C., Perchiacca, J.M. and Tessier, P.M. (2013) Toward aggregation-resistant antibodies by design. Trends Biotechnol. 31 (11), 612–620.
[89] Christ, D. and Dudgeon, K. (2011) Modified variable domain molecules and methods for producing and using same. WO/2011/047442.
[90] Buchanan, A., Clementel, V., Woods, R., Harn, N., Bowen, M.A., Mo, W., Popovic, B., Bishop, S.M., Dall'Acqua, W., Minter, R., Jermutus, L. and Bedian, V. (2013) Engineering a therapeutic IgG molecule to address cysteinylation, aggregation and enhance thermal stability and expression. MAbs 5, 255–262.
[91] Lee, C.M., Iorno, N., Sierro, F. and Christ, D. (2007) Selection of human antibody fragments by phage display. Nat. Protoc. 2 (11), 3001–3008.

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D20 Table 1 and D21 Table 1

Antibody	Type	Ab concentration mg/ml	Formulation
Satumomab pendetide	IgG1k (murine, conjugated to GYK-DTPA)	0.5	PBS
Indium-111 capromab pendeti	IgG1k (murine, conjugated to GYK-DTPA)	0.5	PBS
Muromomab-CD3	**IgG2a (murine)**	**1**	**Monobasic Na P, dibasic Na P, NaCl, PS80**
I-131 tositumab	**IgG2 gamma (murine)**	**1.1**	**Phosphate, povidone, maltose, NaCl, ascorbic acid**
Ibritumomab-Tiuxetan	IgG1k (murine, thiourea covalent linkage to Tiuxetan)	1.6	Na acetate, K P, NaCl
Cetuximab	IgG1k (chimeric human/mouse)	2	Dibasic Na P septahydrate, monobasic Na P monohydrate, NaCl
Abciximab	Fab, IgG1 (chimeric human/murine)	2	Na P, NaCl, PS80
Daclizimab	IgG1 (humanized)	5	Monobasic Na P monohydrate, dibasic Na P septahydrate, NaCl, PS80
Alemtuzumab	IgG1k (humanized)	10	Dibasic Na P, monobasic K P, NaCl, KCl, Na2EDTA, PS80
Ranibizumab	IgG1k (humanized)	10	His HCl, alpha-trehalose dihydrate, PS20
Rituximab	IgG1k (chimeric mouse/human)	10	Na citrate dihydrate, NaCl, PS80
Nofetumomab	Fab (murine)	10	PBS
Tositumomab	**IgG2 gamma (murine)**	**14**	**Phosphate, NaCl, maltose**
Natalizumab	**IgG4k (humanized)**	**20**	**Monobasic Na P, dibasic NaP septahydrate, NaCl, PS80**
Bevacizumab	IgG1 (humanized)	25	Monobasic Na P hydrate, dibasic Na P anhydrous, alpha-trehalose dihydrate, PS 20
Adalimumab	IgG1k (human)	50	Monobasic Na P dihydrate, dibasic Na P dihydrate, Na citrate, citric acid hydrate, NaCl, mannitol, PS80
Acrituomab	Murine Fab	powder	Stannous chloride, K Na tartrate tetrahydrate, Na acetate trihydrate, NaCl, glacial acetic acid, HCl, sucrose
Gemtuzumab ozogamicin	**IgG4k (conjugated with alicheamicin)**	**powder**	**Monobasic and dibasic Na P, dextran 40, sucrose, NaCl**
Basiliximab	IgG1k (chimeric)	powder	Monobasic K P, Na2HPO4, NaCl, sucrose, mannitol, glycine, PS80
Infliximab	IgG1 (chimeric human/murine)	powder	Monobasic Na P monohydrate, dibasic Na P dihydrate, sucrose, PS80
Trastuzumab	IgG1k (humanized)	powder	L-His HCl, L-His, alpha trehalose dihydrate, PS20 (benzyl alcohol as preservative)
Efalizumab*	IgG1 (humanized)	powder	L-His HCl monohydrate, L-His, sucrose, PS20
Palivizumab	IgG1k (humanized, CDR of murine Mab)	powder	His, Gly, mannitol
Omalizumab	IgG1k (humanized)	powder	L-His HCl, L-His, sucrose, PS20
Eculizumab	**IgG2/4**	**10 mg/ml solution**	**Na P, NaCl, PS80**

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IN THE MATTER OF
European Patent No. EP 2 359 834

DECLARATION OF DR. DINO MIANO

I, Dr. Dino Miano, hereby declare as follows:

1. I am Dr. Dino Miano of Alexion Pharmaceuticals, Inc., 100 College St, New Haven, Connecticut, USA. I hold a Ph.D. degree in Chemistry and Biochemistry from the University of Massachusetts, and a Bachelor of Science degree in Chemistry and Biology from the University of Hartford. I have been employed by Alexion Pharmaceuticals for nearly 20 years, and I currently hold the post of Executive Director, Global Analytical & Pharmaceutical Development. Prior to this I was employed by R.W. Johnson Pharmaceutical Research Institute in Raritan, New Jersey for approximately two years. During my employment at Alexion my responsibilities and work have focused on analytical sciences and protein characterization. I have significant expertise in formulation development and analysis, which includes designing, performing and overseeing experiments that assess the impact of protein concentration, formulation, and storage conditions on the integrity and stability of a protein over time. A copy of my resume is attached as Exhibit 1.

2. The commercial formulation of Soliris®(eculizumab) consists of a concentration of 10 mg/mL of eculizumab in a buffer of 10 mM sodium phosphate, 150 mM sodium chloride and 0.02% polysorbate 80 at pH 7.0. The commercial formulation is satisfactorily stable for 18 months when it is stored as drug substance (i.e., in bulk in the commercial formulation) and for an additional 30 months when it is stored as a drug product (i.e., in 30 mL vials as it is supplied to consumers) providing a total of 48 months of stability. (See March 16, 2007 BLA Approval Letter from U.S. FDA, attached as Exhibit 2 (indicating the drug substance is stable for 18 months and the drug product is stable for 24 months at 2-8°C), and May 8, 2009 Letter from U.S. FDA, attached as Exhibit 3 (extending the shelf-life of the drug product from 24 to 30 months at 2-8°C after supplemental stability data was obtained).)

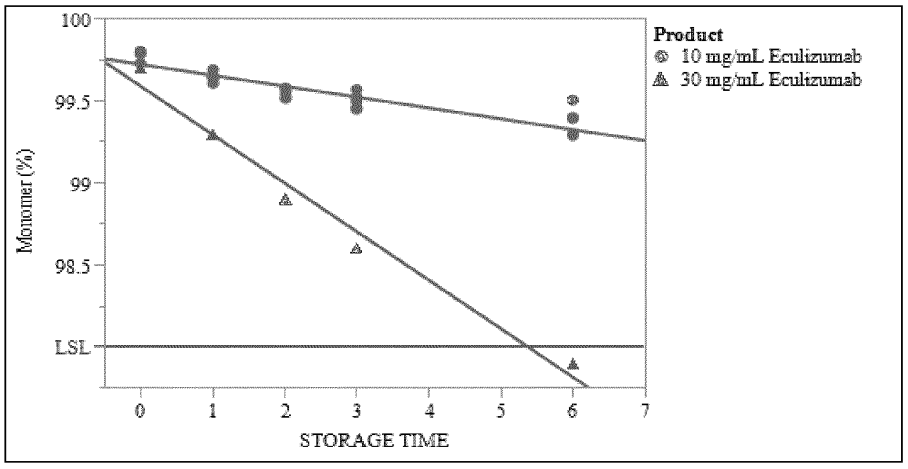
3. One of the stability parameters that the commercial formulation of eculizumab must meet in order to be deemed satisfactorily stable and thus suitable for release into the market is the percent of monomer antibody in the sample must be greater than or equal to 98.0%, which means that there must be less than or equal to 2.0% aggregates of the antibody, as determined via gel permeation high performance liquid chromatography. This release parameter for commercial lots of eculizumab ensures that the drug is satisfactorily stable for the designated storage period.

4. We generated a 30 mg/mL formulation of eculizumab (internally denoted "ALXN5100") that was identical to the commercial formulation of eculizumab, except that ALXN5100 has an eculizumab concentration of 30 mg/mL and the Soliris® commercial formulation has an eculizumab concentration of 10 mg/mL.

5. A batch of ALXN5100 was made under Good Manufacturing Practices (GMP) conditions, and stability studies on the batch were conducted by aliquotting samples into representative HyQ® CX5-14 Hyclone bags to simulate large-scale storage. The Hyclone bags were then stored horizontally at 2 to 8°C for at least 18 months and at 23 to 27°C for at least 6 months, which is how samples were stored for the stability program for the bulk drug substance for the commercial (10 mg/mL) formulation. Although the commercial formulation of eculizumab is to be stored at 2 to 8°C, when performing stability testing, researchers often store test samples at higher temperatures than commercial supplies are typically stored at, such as 23 to 27°C, because storage and stability testing at higher temperatures can provide a good indication of the stability of the formulation in a shorter test period of time. Gel Permeation High Performance Liquid Chromatography (GP-HPLC) was used to determine if there were any changes in monomer and aggregate drug at the two storage temperatures over time.

6. Figures 1 and 2 below demonstrate that the stability of eculizumab at 10 mg/mL is superior to that at 30 mg/mL. Figure 1 compares the percent monomer in the 10 mg/mL eculizumab composition versus the percent monomer in the 30 mg/mL eculizumab composition after storage at 23 to 27°C over six months. Figure 1 clearly shows that the percent monomer of eculizumab decreases (and thus aggregate formation increases) significantly more quickly in the 30 mg/mL eculizumab composition than in the 10 mg/mL eculizumab composition. In addition, whereas the 10 mg/mL eculizumab composition remains sufficiently stable over the specification ("LSL") monomer level of 98.0% over the entire six-month period, the 30 mg/mL eculizumab composition falls below that stability threshold during the same time period.

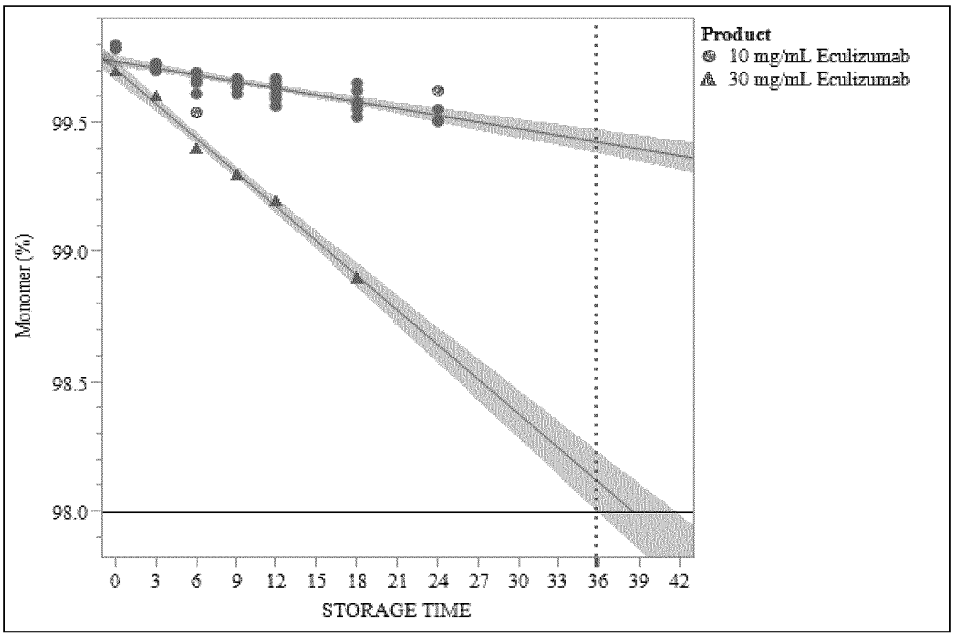
Figure 1. Percent monomer of eculizumab in 10 mg/mL eculizumab composition versus 30 mg/mL eculizumab composition over six months when stored at 23-27°C



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7. Similar trends are seen between the 10 mg/mL eculizumab composition and the 30 mg/mL eculizumab composition even at storage temperatures of 2 to 8°C. Figure 2 compares the percent monomer in the 10 mg/mL eculizumab composition versus the percent monomer in the 30 mg/mL eculizumab composition after storage at 2 to 8°C over 18 months. Again, as with the 23 to 27°C storage conditions, the percent monomer remaining in the 30 mg/mL eculizumab samples decreased much more rapidly than the percent monomer remaining in the 10 mg/mL eculizumab samples at 2 to 8°C over the 18-month period. In addition, the model generated from this data predicted that the 30 mg/mL eculizumab composition would fall below the acceptable 98.0% monomer level before 36 months of storage.

Figure 2. Percent monomer of eculizumab in 10 mg/mL eculizumab composition versus 30 mg/mL eculizumab composition over 18 months when stored at 2-8°C



8. Thus, these long-term stability studies demonstrate a decrease in monomer and an increase in aggregate formation for ALXN5100, the 30 mg/mL eculizumab concentration, throughout the stability program. In all instances the aggregate formation in the 30 mg/mL concentration was greater than what was observed in the 10 mg/mL concentration. These results are clearly demonstrated through 18 months at 2 to 8°C and are even more pronounced within 6 months at the higher temperature studies. These results suggest that the commercial formulation is optimal for an eculizumab concentration of 10 mg/mL but is not suitable when the eculizumab concentration is increased to 30 mg/mL.

I believe and do hereby declare that the facts stated above are to the best of my knowledge true.

Dr. Dino Miano

17 Mar 2018
Date

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Exhibit 1

Dino C. Miano, Ph. D.
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QUALIFICATION PROFILE

- Results-oriented professional with eighteen years' successful track record of innovation, management, in depth scientific and regulatory knowledge in analytical and pharmaceutical development in the field of biotherapeutics. Experience encompasses various stages of biologics including early and late phase development, regulatory submission for marketing authorization and life cycle management.

- Well established division-level leader that consistently drives vision toward successful and timely completion of projects, while focusing on translating goals into actionable strategies that support the business

COMPETENCIES

- Pharmaceutical (biological) development
  - Formulation development
  - Fill finish
  - Stability monitoring and trending
  - Device development
  - Analytical support of clinical operations
- Regulatory Compliance
  - CMC development
- Analytical Development
  - Method Validation & Transfer
  - GMP Testing & Compliance
  - Implementing new analytical technologies
  - Analytical support of manufacturing
- Strategic leadership and innovation
  - Cross functional leader

Accomplishments and Qualifications

- Assumed a key analytical role in the clinical development and global commercialization of Soliris® and Strensiq®. Led development and validation activities for commercial release and stability test methods.
- Lead Global Operations efforts in delivering clinical material from early stage through commercial. Lead cross functional teams consisting of manufacturing, supply chain, QA and CMC and working with clinical groups to ensure supply demands are consistently met
- Lead Global Analytical and Pharmaceutical Development groups in providing analytical support to the entire Alexion organization, including analytical development, validation and transfer, GMP testing of clinical products, process development support, formulation and device development, product characterization and stability to allow for expiry determination.
- Implemented GMP quality infrastructure in the Analytical Sciences laboratories to ensure testing compliance with FDA, EU and PMDA regulations.
- Lead formulation development of new, existing and commercial products for IV, IVT and subcutaneous administration.

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- Implement drug substance and drug product specifications for clinical products
- Generate analytical sections in support of all new and existing CMC submissions
- Lead the drug delivery and device development group in developing a strategy and business process to advance drug and device/combination products for existing commercial and clinical products.

Employment History

Alexion Pharmaceuticals, Inc., New Haven, CT

Executive Director, Global Analytical and Pharmaceutical Development **12/15-present**

- Lead global analytical support function for the entire organization for all commercial and clinical programs.
- Lead the global method technology transfers for Alexion's clinical and commercial products.
- Oversee compatibility assessments for materials used for delivery of product to patients during clinical studies.
- Represent Global Operations as lead for late stage process development, drug substance and drug product manufacturing, supply chain and CMC/IND submissions.
- Lead the device group in collaboration with internal and external partners for the development of delivery devices for Alexion products.

Senior Director, Analytical and Pharmaceutical Development **03/15-12/15**

- Led Early Stage Analytical and Product Characterization groups in supporting Research in efforts to move the best candidates into development.
- Led Analytical Sciences in method development, validation and transfer for new candidates and provide support to process development groups.
- Lead the stability program for all clinical products, including expiry determinations
- Led and aligned formulation development of new candidates with Clinical development and Target product profiles (TPP).
- Led Device Development and Drug Delivery group in identifying and integrating large volume bolus injector options for clinical and commercial development.
- Appointed CMC lead during Regulatory meetings with FDA, Health Canada, EMA and MFDS (F2F) to support CMC section of regulatory filings.

Director, Product Characterization **07/14-03/15**

- Led Global Operations Cross Functional Product Team in supporting second generation development of commercial product.
- Led device teams consisting of Clinical and Commercial Operations, Development, Regulatory and QA in evaluating large volume subcutaneous administration devices. Device included auto-injectors and larger wearables patch devices.
- Managed Mass Spectroscopy Characterization group. Involves the MS characterization of new and existing molecules to support CMC, Research and Development groups.
- Managed the Bio-Physical Characterization group. Involves directing the testing and collaboration of multiple groups to better understand new protein therapeutics
- Provided analytical support of new and existing CMC submissions and address inquiries from agencies

Director, Analytical Sciences **09/08-07/14**

- Managed the Operations group that provided GMP testing of clinical products and analytical testing to all development groups.
- Managed the Analytical Development group that provided method development, qualification, validation and transfer of analytical methods for new clinical products and existing commercial product to both QC and EU laboratories.
- Manage the Formulation Development group that identified stable formulations for new clinical products and determined stable subcutaneous formulations for both clinical and commercials products.
- Managed Stability program of clinical products that generated GMP stability protocols and reports, trended data and provided statistical analysis of trends to support expiry, and led deviations, LIR and OOS for GMP testing.
- Member of Specification committee to direct the generation or revision of specifications for clinical products

Associate Director, Analytical Sciences **05/03-08/08**

- Analytical Group
  - Provided analytical support of new and existing Soliris® CMC submissions
  - Supervised Analytical Operations laboratory in providing method development, qualification, validation, transfer and GMP testing to support clinical products.
- Purification Development group:
  - Managed PD studies in support of Process Validation. Studies included viral clearance, DNA clearance, process limits evaluation (PLE), resin re-use, and UF/DF membrane re-use.

Manager, Quality Control **08/99-05/03**

- Managed QC laboratories; maintained cGMP compliance with regards to current SOPs, validation/qualifications, and training.
- Coordinated studies, supervised investigations, generated reports and implemented systems with QA in response to OOS, invalid assays, aberrant trends/results.
- Represented area functions as required during audits by external agencies and internal QA department.

Senior Scientist, Quality Control **08/98-08/99**

- Developed and optimized RP-HPLC, SEC-HPLC, SDS-PAGE, and IEF methods for all protein based therapeutic product.

R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
A Johnson & Johnson Company

Scientist, Analytical Development **07/97-07/98**

- Developed HPLC assays for quantitation of becaplermin drug substance in alternative REGRANEX® formulations. Developed HPLC assays for determining content of preservatives in REGRANEX® formulations.

Scientist, Protein Development **01/97-07/97**

- Investigated modified roller bottle processes for the production of erythropoietin (rHuEPO), potentially increasing production/yields by 3-fold.
- Evaluated alternative purification processes for rHuEPO, involving HIC, IEC and Reverse phase chromatography.

Postdoctoral Research Scientist, Protein Development **10/95-01/97**

- Chemically modified rHuEPO using polyethylene glycol (PEG) to enhance in vivo erythropoiesis while prolonging circulatory half-life. Experiments were designed to specifically modify the carbohydrate (NANA) moieties of the glycoprotein with PEG while maintaining the native polypeptide conformation. Animal model analysis revealed successful prolonged in vivo biological activity.

University of Connecticut Health Center, Farmington, CT **01/89-08/90**

Research Scientist, Surgery Department

- Investigated/detected tissue derived inflammatory mediators released from injured corneas, urinary bladders and ischemic hearts. Modified Boyden chambers were employed to evaluate chemotactic response via neutrophil migration through 8 μm membranes.
- Investigated/synthesized cyclocreatine, a creatine analog, to determine inhibitory effects on the production and release of neutrophil chemotactic factors (NCF) in ischemic canines and rabbits myocardium.

EDUCATION

University of Massachusetts, Lowell, MA **09/90-10/95**

Ph. D. Chemistry/Biochemistry
Advisor: Dr. Edwin G.E. Jahngen
Thesis: "The Isolation and characterization of an ubiquitin-histone protein from *Dictyostelium discoideum AX3.*"

University of Hartford, Bloomfield, CT **09/85-05/89**

B.S. Chemistry/Biology
Research: "Corneal derived neutrophil chemotactic factors, intracellular synthesis and release."

PUBLICATIONS

Neill, A., Nowak, C., Patel, R., Ponniah, G., Gonzalez, N., **Miano, D**. and Liu, H., (2015) Characterization of recombinant monoclonal antibody charge variants using OFFGEL fractionator, weak anion exchange chromatography and mass spectrometry, *Analytical Chemistry*, **87**, 6204-6211

Ponniah, G., Nowak, C., Gonzalez, N., **Miano D**., and Liu, H., (2015) Detection and quantitation of low abundance oligosaccharides in recombinant monoclonal antibodies. *Analytical Chemistry*, **87**, 2718-2726

Peckham, N., Neil, F., and **Miano, D.C**., "Quantitative SDS-PAGE Analysis of a Recombinant Protein by Densitometry", IBC's Well Characterized Biologicals Poster Session, Washington D.C. (11/11/04)

Peckham, N., Bosley, K., and **Miano, D.C**., Adaptation of Complexometric Titration Method for Quantitation of EDTA in Biopharmaceutical Formulations to Microplate Format, IBC's Well Characterized Biologicals Poster Session, Washington D.C. (11/11/04).

Miano, D.C., Villalobos, A.P., Ghosh-Datidar, P., and Sharma, B. (1997), Chemical Modification of Recombinant Human Erythropoietin by Poly (ethylene glycol). Johnson and Johnson Chromatography Symposium, Princeton, NJ (abstract).

Miano, D.C. (1995) The Isolation and Characterization of a Ubiquitinylated Histone Protein in *Dictyostelium discoideum* AX3. Ph.D. Thesis, University of Massachusetts/Lowell.

Miano, D.C, and Jahngen, G.E. (1994) The Ubiquitinylation of Histones in *Dictyostelium discoideum* AX3 Chromatin May Be Linked to Replication, Transcription and Stress. Keystone Symposium, Tammarron, CO (abstract).

Elgebaly, S.A., **Miano, D.C.**, Ehler, W., Rahha,l R. and Elkerm, A. (1994) The Induction of Corneal Inflammation by Factors Derived From Hydrogen Peroxide Injured Corneas: Effect of Dexamethasone and Indomethacin. *Occ. Pharm.*, **10**, (1), 295-306.

Elgebaly, S.A., Allam, M.E., Houser, S., Hashmi, F., Forouhar, F. and **Miano, D.C.**, (1993) Cyclocreatine Inhibits Neutrophil Accumulation in the Myocardium of Canine Models in Coronary Artery Occlusion and Reperfusion. *J. Pharm. Exp. Ther.*, **266**, (3), 1670-1677.

Elgebaly, S.A., **Miano, D.C.**, Kreutzer, D.L. and Fishman, J.B., (1990) Cornea Derived Neutrophil Chemotactic Factors: Intracellular Synthesis and Release. *Curr. Eye Res.*, **9**, 839-845.

Elgebaly, S.A., **Miano, D.C.**, Ehler, W. and Rahhal, R. (1990) The Induction of Corneal Inflammation by Factors Derived From Hydrogen Peroxide Injured Corneas. ARVO, Sarasota, FL (abstract).

Elgebaly, S.A., **Miano, D.C.**, Kreutzer, D.L. and Fishman, J.B. (1989) Cornea Derived Neutrophil Chemotactic Factors. ARVO, Sarasota, FL (abstract).

Exhibit 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

Our STN: BL 125166/0

MAR 16 2007

Alexion Pharmaceuticals
Attention: Nancy Motola, Ph.D., RAC
Senior Vice President, Regulatory and Quality
352 Knotter Drive
Cheshire, CT 06410

Dear Dr. Motola:

We are issuing Department of Health and Human Services U.S. License No. 1743 to Alexion Pharmaceuticals, Inc., Cheshire, Connecticut, under the provisions of section 351(a) of the Public Health Service Act controlling the manufacture and sale of biological products. The license authorizes you to introduce or deliver for introduction into interstate commerce, those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are approved to manufacture the product eculizumab, which is indicated for the treatment of paroxysmal nocturnal hemoglobinuria to reduce hemolysis.

You are approved to manufacture eculizumab drug substance at Lonza Biologics, PLC in Portsmouth, NH. The final formulated product will be manufactured, filled, labeled, and packaged at Ben Venue Laboratories, Bedford, Ohio. You may label your product with the proprietary name Soliris™ and will market it in 300 mg vials.

The final printed labeling (FPL) must be identical to the enclosed labeling. Marketing product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for eculizumab drug product shall be 24 months from the date of manufacture when stored at 2-8°C. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for your drug substance shall be 18 months when stored at 2-8°C.

You currently are not required to submit samples of future lots of eculizumab to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

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You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of eculizumab, or in the manufacturing facilities.

We acknowledge your written commitments as described in your letter of February 22, 2007, March 12, 2007 and March 12, 2007 as outlined below:

Postmarketing Studies subject to reporting requirements of 21 CFR 601.70.

1. To evaluate long-term safety of eculizumab by analyzing outcomes in the Soliris Safety Registry for a time period of no less than five years. At the end of the five year period, a study report will be submitted to the Biological License Application (BLA) that describes the major safety findings from the registry program, including the specific items listed below and proposing labeling changes as appropriate. Additionally, annual interim reports will be submitted to the BLA, along with expedited reports as specified below. The protocol for addressing this PMC will be submitted to the IND by May 31, 2007, and the five year study report will be submitted by June 30, 2012. All patients within the registry will be followed for the occurrence of:

    a. Serious infections, defined as infections necessitating or prolonging hospitalization or resulting in death. Alexion commits to collecting follow-up information from these patients to assess the nature of the serious infection, the duration of hospitalization, the major features of the clinical course and the survival status. Expedited reporting (15 day telephone or facsimile Medwatch communication) will be provided for the occurrence of these serious infections.

    b. Malignancy, including the nature of the malignancy and the survival status of patients who develop a malignancy;

    c. Use of eculizumab among pediatric patients under 16 years of age, to include collection of eculizumab dosage information, as well as the same information being required for adult patients in the registry;

    d. Pregnancy, including the clinical course of each pregnancy and the detection of congenital abnormalities among babies born to the women exposed to eculizumab during the pregnancy.

    e. Thrombotic events, including the nature of the event, the clinical outcome as well as the anticoagulant management prior to and after the event.

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2. To submit a comprehensive description of the Soliris Guardian Program Risk Minimization Action Plan (RiskMAP), including all items listed below. Fulfillment of this post-marketing commitment will be contingent upon FDA concurrence upon the expectations of the Soliris Guardian Program. Submission of all items listed below will occur no later than May 18, 2007. The submitted information will include:

   a. A final version of the Soliris Guardian Program document that, in addition to any other items, provides information fully consistent with the approved prescribing information.

   b. A copy of all educational materials to be provided as part of the program, including but not limited to all components of the Soliris Starter Kit, the Drug Fact Sheet and patient-health care provider documents relating to the Soliris Safety Registry.

   c. A commitment to develop and submit a protocol (with the understanding that this protocol may need modification based upon FDA review findings) that uses surveys of health care providers and patients to assess compliance with the vaccination requirements as well as their knowledge of the risks of eculizumab and the need for vaccination.

   d. A copy of the Soliris Safety Registry protocol and any supportive documents to be provided to health care providers and patients. These documents may need modification based upon FDA review findings. The submitted protocol will indicate that the Registry will continue until Alexion receives written concurrence from FDA to terminate the registry. The protocol will include collection of the occurrence of the following events:

      i. Death;

      ii. Meningococcal vaccination (type and dates of all vaccinations);

      iii. eculizumab administration dates, at designated time points to establish initiation and termination of therapy as well as to correlate eculizumab administration with the fatalities and the events listed below:

         - All meningococcal infections causing sepsis or meningitis
         - Other serious infections
         - Malignancy, including the nature of the malignancy and the survival status of patients who develop a malignancy;
         - Use of eculizumab among pediatric patients under 16 years of age, to include collection of eculizumab dosage information, as well as the required information for adult patients in the registry;
         - Use of eculizumab by indication;

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- Pregnancy, including the clinical course of each pregnancy and the detection of congenital abnormalities among off-spring of the women exposed to eculizumab during a pregnancy and;
- Serious hemolysis, as defined by specific criteria.

e. A commitment to submit quarterly RiskMAP reports for the first year and annual reports thereafter (unless FDA provides written request for more frequent submissions) summarizing all information relating to the Soliris Guardian Program. The reports should include the following:

i. An analysis of all cases of the following, including a root cause analysis and factors that might have contributed to serious outcomes:
- Meningococcal sepsis or meningitis, including the timing of all vaccinations relative to administration of Soliris
- Other infections (with serious outcomes)
- All deaths
- Cases of serious hemolysis and all cases of hemolysis with serious outcomes

ii. Soliris use patterns including indication for use;

iii. Extent of compliance with RiskMAP requirements such as the percentage of patients that were vaccinated prior to receiving eculizumab and the percentage of patients that were re-vaccinated at 3-year or 10-year intervals (as applicable);

iv. Analysis of all cumulative data collected in the Soliris Safety Registry;

v. Results from all health care provider and patient surveys, including:

- Any known data about patients or physicians who refused to participate in the surveys
- Any known data about survey participants who are considered "lost" (drop-outs).

3. To conduct a randomized, controlled clinical study to assess the effects of anticoagulant withdrawal among PNH patients receiving eculizumab. This study will randomize at least 100 anticoagulated patients to either continue or discontinue anticoagulation therapy. The major outcomes will assess the safety of discontinuation of anticoagulant therapy while continuing eculizumab, especially with respect to providing important evidence regarding major bleeding and that this discontinuation does not increase the risk for occurrence of thrombotic events in these patients. A full study report and data from this study will be submitted to the BLA and may include a label revision, contingent upon the importance of the study results. The study protocol will be submitted to the investigational new drug application (IND) by June 30, 2007 and patient accrual completed no later than June 1, 2009. A final study report will be submitted no later than March 31, 2014.

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4. To develop a validated and quantitative assay for the measurement of human anti-human antibodies (HAHA) for the detection of antibody formation to eculizumab. This assay will assess potential immune responses to the whole eculizumab molecule. Description of the validated assay will be submitted to the BLA as a CBE 30 by July 9, 2008.

5. To develop a validated and sensitive assay for the measurement of neutralizing HAHA to eculizumab. Alternatively, Alexion commits to submit documentation to FDA demonstrating with due diligence that a suitable assay could not be feasibly developed and that the assessment of serum lactate dehydrogenase (LDH) is a sufficiently sensitive indicator of the presence of neutralizing antibodies. This information will be submitted to the BLA by July 9, 2008.

6. To utilize samples from the ongoing E05-001 Phase III extension study (approximately 170 patients for at least 2 years) as test samples for the new validated HAHA assays. Alexion will continue to obtain serum samples from those patients who transition from E05-001 to the Soliris Safety Registry, at intervals of no less than one year, and continue this collection process for an additional three years. Sample collection will cease during this additional three year period for patients who terminate eculizumab administration. Additionally, serum samples will be obtained based upon physician reports of suspected loss of eculizumab bioactivity, based upon unanticipated alterations in serum LDH concentrations. All serum samples will be assayed at least annually and the results provided within an annual report to the BLA. Clinical data, to include the results of serum LDH concentrations, will also be obtained from any patients who show evidence of antibody formation. The protocol describing Alexion's plan for responding to this commitment will be submitted by May 1, 2007 and the final study report submitted by January 31, 2011.

Postmarketing Studies not subject to reporting requirements of 21 CFR 601.70.

7. To revalidate the linearity and accuracy of the Osmolality method across the full specification range using a combination of product samples diluted to lower osmolality and product samples spiked with osmolality standards. The revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

8. To revalidate the linearity of the IEF method across a load range of 8 µg to 12 µg. The revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 supplement by August 31, 2007.

9. To revise the IEF method SOP to specify that the method is validated only for a 10 µg load. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

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10. To improve and revalidate the existing hemolytic assay. Improvements include increasing the number of sample replicates and qualifying the chicken erythrocytes reagent. The revised method SOP and revalidation plan will be submitted in advance to FDA for review and endorsement. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

11. To develop a new quantitative biological activity assay to replace the existing hemolytic assay, or submit documentation to FDA demonstrating with due diligence that a suitable assay could not be feasibly developed. This information will be submitted to the BLA by February 29, 2008. Validation of the quantitative biological activity assay will be submitted by July 9, 2008.

12. To provide FDA with a completed drug substance and drug product container closure system leachables evaluation using end-of-shelf-life, long-term 2-8°C stability samples. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

13. To develop a suitable antifoam assay and subsequently confirm the removal of antifoam from the process stream at the Protein A chromatography pool on three drug substance batches. This information will be submitted to the BLA as a CBE 30 by August 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application (BLA), STN BL 125166. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to your BLA, STN BL 125166. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Commitment Protocol**
- **Postmarketing Study Commitment Final Study Report**
- **Postmarketing Study Commitment Correspondence**
- **Annual Status Report of Postmarketing Commitment Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

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As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Study Commitments - Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cder/guidance/5569fnl.htm) for further information.

Under 21 CFR Part 208, we have determined that this product poses a serious and significant public health concern requiring the distribution of a Medication Guide. Eculizumab is a product for which patient labeling could help prevent serious adverse effects and inform the patient of serious risks relative to benefit that could affect their decisions to use, or continue to use, the product. Therefore, a Medication Guide is necessary for safe and effective use of this product and FDA hereby approves the draft Medication Guide you submitted March 15, 2007. Please note that:

- this Medication Guide must be reprinted at the end of the package insert or accompany it [21 CFR 201.57(c)(18)];
- you are responsible for ensuring that this Medication Guide is available for distribution to every patient who is dispensed a prescription for this product [21 CFR 208];
- the final printed Medication Guide distributed to patients must conform to all conditions described in 21 CFR 208.24, including a minimum of 10 point text; and
- you are responsible for ensuring that the label of each container or package includes a prominent and conspicuous instruction to authorized dispensers to provide a Medication Guide to each patient to whom the drug is dispensed, and states how the Medication Guide is provided.

Please submit within 30 days content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/oc/datacouncil/spl.html, that is identical in content to the enclosed labeling text dated March 16, 2007. Upon receipt and verification, we will transmit that version to the National Library of Medicine for public dissemination.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

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You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all final printed labeling at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for information regarding therapeutic biological products, including the addresses for submissions.

Sincerely,

Karen Weiss for

Richard Pazdur, M.D.
Director
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Enclosure: Package Insert
Carton and Vial Labeling
Medication Guide
Patient Safety Card

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Soliris ™ (eculizumab)

FULL PRESCRIBING INFORMATION

WARNING: SERIOUS MENINGOCOCCAL INFECTION

Soliris increases the risk of meningococcal infections (5.1)

- **Vaccinate patients with a meningococcal vaccine at least 2 weeks prior to receiving the first dose of Soliris; revaccinate according to current medical guidelines for vaccine use**
- **Monitor patients for early signs of meningococcal infections, evaluate immediately if infection is suspected, and treat with antibiotics if necessary.**

1 INDICATIONS AND USAGE

Soliris is indicated for the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH) to reduce hemolysis.

2 DOSAGE AND ADMINISTRATION

Patients must be administered a meningococcal vaccine at least two weeks prior to initiation of Soliris therapy and revaccinated according to current medical guidelines for vaccine use. [*see Warnings and Precautions (5.1)*].

2.1 Recommended Dosage Regimen

Soliris therapy consists of:

- 600 mg every 7 days for the first 4 weeks, followed by
- 900 mg for the fifth dose 7 days later, then
- 900 mg every 14 days thereafter.

Soliris should be administered at the recommended dosage regimen time points, or within two days of these time points. [*see Warnings and Precautions (5.5)*]

2.2 Preparation for Administration

Soliris must be diluted to a final admixture concentration of 5 mg/mL using the following steps:

- Withdraw the required amount of Soliris from the vial into a sterile syringe.
- Transfer the recommended dose to an infusion bag.
- Dilute Soliris to a final concentration of 5 mg/mL by adding the appropriate amount (equal volume of diluent to drug volume) of 0.9% Sodium Chloride Injection, USP; 0.45% Sodium Chloride Injection, USP; 5% Dextrose in Water Injection, USP; or Ringer's Injection, USP to the infusion bag.

The final admixed Soliris 5 mg/mL infusion volume is 120 mL for 600 mg doses or 180 mL for 900 mg doses. Gently invert the infusion bag containing the diluted Soliris solution to ensure thorough mixing of the product and diluent. Discard any unused portion left in a vial, as the product contains no preservatives.

Prior to administration, the admixture should be allowed to adjust to room temperature [18°-25° C, 64-77° F]. The admixture must not be heated in a microwave or with any heat source other than ambient air temperature. The Soliris admixture should be inspected visually for particulate matter and discoloration prior to administration.

2.3 Administration

Do Not Administer As An Intravenous Push Or Bolus Injection

The Soliris admixture should be administered by intravenous infusion over 35 minutes via gravity feed, a syringe-type pump, or an infusion pump. Admixed solutions of Soliris are stable for 24 hours at 2-8° C (36-46° F) and at room temperature.

If an adverse reaction occurs during the administration of Soliris, the infusion may be slowed or stopped at the discretion of the physician. If the infusion is slowed, the total infusion time should not exceed two hours. Monitor the patient for at least one hour following completion of the infusion for signs or symptoms of an infusion reaction.

3 DOSAGE FORMS AND STRENGTHS

Soliris is supplied as 300 mg single-use vials each containing 30 mL of 10 mg/mL sterile, preservative-free eculizumab solution.

4 CONTRAINDICATIONS

Do not initiate Soliris therapy in patients:

- with unresolved serious *Neisseria meningitidis* infection.
- who are not currently vaccinated against *Neisseria meningitidis*.

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5 WARNINGS AND PRECAUTIONS

5.1 Serious Meningococcal Infections

The use of Soliris increases a patient's susceptibility to serious meningococcal infections (septicemia and/or meningitis). All patients without a history of prior meningococcal vaccination must receive the meningococcal vaccine at least 2 weeks prior to receiving the first dose of Soliris and revaccinated according to current medical guidelines for vaccine use. Quadravalent, conjugated meningococcal vaccines are strongly recommended. Vaccination may not prevent meningococcal infections.

All patients must be monitored for early signs and symptoms of meningococcal infections and evaluated immediately if an infection is suspected. Physicians should strongly consider discontinuation of Soliris during the treatment of serious meningococcal infections.

In clinical studies, 2 out of 196 PNH patients developed serious meningococcal infections while receiving treatment with Soliris; both had been vaccinated. [*see Adverse Reactions (6.1)*].

5.2 Other Infections

Soliris blocks terminal complement; therefore patients may have increased susceptibility to infections, especially with encapsulated bacteria. Use caution when administering Soliris to patients with any systemic infection.

5.3 Monitoring After Soliris Discontinuation

Since Soliris therapy increases the number of PNH cells [in study 1, the proportion of PNH RBCs increased among Soliris-treated patients by a median of 28% from baseline (range from -25% to 69%)], patients who discontinue treatment with Soliris may be at increased risk for serious hemolysis. Serious hemolysis is identified by serum LDH levels greater than the pre-treatment level, along with any of the following: greater than 25% absolute decrease in PNH clone size (in the absence of dilution due to transfusion) in one week or less; a hemoglobin level of <5 gm/dL or a decrease of >4 gm/dL in one week or less; angina; change in mental status; a 50% increase in serum creatinine level; or thrombosis. Monitor any patient who discontinues Soliris for at least 8 weeks to detect serious hemolysis and other reactions.

If serious hemolysis occurs after Soliris discontinuation, consider the following procedures/treatments: blood transfusion (packed RBCs), or exchange transfusion if the PNH RBCs are >50% of the total RBCs by flow cytometry; anticoagulation; corticosteroids; or reinstitution of Soliris.

In clinical studies, 16 of 196 PNH patients discontinued treatment with Soliris. Patients were followed for evidence of worsening hemolysis and no serious hemolysis was observed.

5.4 Thrombosis Prevention and Management

The effect of withdrawal of anticoagulant therapy during Soliris treatment has not been established. Therefore, treatment with Soliris should not alter anticoagulant management.

5.5 Laboratory Monitoring

Serum LDH levels increase during hemolysis and may assist in monitoring Soliris effects, including the response to discontinuation of therapy. In clinical studies, six patients achieved a reduction in serum LDH levels only after a decrease in the Soliris dosing interval from 14 to 12 days. All other patients achieved a reduction in serum LDH levels with the 14 day dosing interval [*see Clinical Pharmacology (12.2) and Clinical Studies (14)*].

5.6 Infusion Reactions

As with all protein products, administration of Soliris may result in infusion reactions, including anaphylaxis or other hypersensitivity reactions. In clinical trials, no PNH patients experienced an infusion reaction which required discontinuation of Soliris. Soliris administration should be interrupted in all patients experiencing severe infusion reactions and appropriate medical therapy administered.

6 ADVERSE REACTIONS

6.1 Clinical Trial Experience

Meningococcal infections are the most important adverse reactions experienced by patients receiving Soliris therapy. In PNH clinical studies, two patients experienced meningococcal sepsis. Both patients had previously received a meningococcal vaccine. In clinical studies among patients without PNH, meningococcal meningitis occurred in an unvaccinated patient [*see Warnings and Precautions (5.1)*].

The data described below reflect exposure to Soliris in 196 adult patients with PNH, age 18-85, of whom 55% were female. All had signs or symptoms of intravascular hemolysis. Soliris was studied in a placebo-controlled clinical study (in which 43 patients received Soliris and 44, placebo); a single arm clinical study and a long term extension study. 182 patients were exposed for greater than one year. All patients received the recommended Soliris dose regimen.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. Table 1 summarizes the adverse reactions that occurred at a numerically

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higher rate in the Soliris group than the placebo group and at a rate of 5% or more among patients treated with Soliris.

TABLE 1

ADVERSE REACTIONS REPORTED IN 5% OR MORE OF SOLIRIS TREATED PATIENTS AND GREATER THAN PLACEBO IN THE CONTROLLED CLINICAL STUDY

Reaction	Soliris N = 43 N (%)	Placebo N = 44 N (%)
Headache	19 (44)	12 (27)
Nasopharyngitis	10 (23)	8 (18)
Back pain	8 (19)	4 (9)
Nausea	7 (16)	5 (11)
Fatigue	5 (12)	1 (2)
Cough	5 (12)	4 (9)
Herpes simplex infections	3 (7)	0
Sinusitis	3 (7)	0
Respiratory tract infection	3 (7)	1 (2)
Constipation	3 (7)	2 (5)
Myalgia	3 (7)	1 (2)
Pain in extremity	3 (7)	1 (2)
Influenza-like illness	2 (5)	1 (2)

In the placebo-controlled clinical study, serious adverse reactions occurred among 4 (9%) patients receiving Soliris and 9 (21%) patients receiving placebo. The serious reactions included infections and progression of PNH. No deaths occurred in the study and no patients receiving Soliris experienced a thrombotic event; one thrombotic event occurred in a patient receiving placebo.

Among 193 patients with PNH treated with Soliris in the single arm, clinical study or the follow-up study, the adverse reactions were similar to those reported in the placebo-controlled clinical study. Serious adverse reactions occurred among 16% of the patients in these studies. The most common serious adverse reactions were: viral infection (2%), headache (2%), anemia (2%), and pyrexia (2%).

6.2 Immunogenicity

As with all proteins there is a potential for immunogenicity. Low titers of antibodies to Soliris were detected in 3/196 (2%) of all PNH patients treated with Soliris. No apparent correlation of antibody development to clinical response was observed. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to Soliris in an enzyme linked immunosorbent assay (ELISA) and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in the assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to Soliris with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

Drug interaction studies have not been performed with Soliris.

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8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C:

PNH is a serious illness. Pregnant women with PNH and their fetuses have high rates of morbidity and mortality during pregnancy and the postpartum period. There are no adequate and well-controlled studies of Soliris in pregnant women. Soliris, a recombinant IgG molecule (humanized anti-C5 antibody), is expected to cross the placenta. Animal studies using a mouse analogue of the Soliris molecule (murine anti-C5 antibody) showed increased rates of developmental abnormalities and an increased rate of dead and moribund offspring at doses 2-8 times the human dose. Soliris should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal reproduction studies were conducted in mice using doses of a murine anti-C5 antibody that approximated 2-4 times (low dose) and 4-8 times (high dose) the recommended human Soliris dose, based on a body weight comparison. When animal exposure to the antibody occurred in the time period from before mating until early gestation, no decrease in fertility or reproductive performance was observed. When maternal exposure to the antibody occurred during organogenesis, two cases of retinal dysplasia and one case of umbilical hernia were observed among 230 offspring born to mothers exposed to the higher antibody dose; however, the exposure did not increase fetal loss or neonatal death. When maternal exposure to the antibody occurred in the time period from implantation through weaning, a higher number of male offspring became moribund or died (1/25 controls, 2/25 low dose group, 5/25 high dose group). Surviving offspring had normal development and reproductive performance.

8.2 Labor and Delivery

No information is available on the effects of Soliris during labor and delivery.

8.3 Nursing Mothers

It is not known whether Soliris is secreted into human milk. IgG is excreted in human milk, so it is expected that Soliris will be present in human milk. However, published data suggest that breast milk antibodies do not enter the neonatal and infant circulation in substantial amounts. Caution should be exercised when Soliris is administered to a nursing woman. The unknown risks to the infant from gastrointestinal or limited systemic exposure to Soliris should be weighed against the known benefits of breastfeeding.

8.4 Pediatric Use

The safety and effectiveness of Soliris therapy in pediatric patients below the age of 18 have not been established.

8.5 Geriatric Use

In PNH studies, 15 patients 65 years of age or older were treated with Soliris. Although there were no apparent age-related differences observed in these studies, the number of patients aged 65 and over is not sufficient to determine whether they respond differently from younger patients.

10 OVERDOSAGE

No cases of Soliris overdose have been reported during clinical studies.

11 DESCRIPTION

Soliris is a formulation of eculizumab which is a recombinant humanized monoclonal $IgG_{2/4}\kappa$ antibody produced by murine myeloma cell culture and purified by standard bioprocess technology. Eculizumab contains human constant regions from human IgG2 sequences and human IgG4 sequences and murine complementarity-determining regions grafted onto the human framework light- and heavy-chain variable regions. Eculizumab is composed of two 448 amino acid heavy chains and two 214 amino acid light chains and has a molecular weight of approximately 148 kDa.

Soliris is a sterile, clear, colorless, preservative-free 10 mg/mL solution for intravenous infusion and is supplied in 30-mL single-use vials. The product is formulated at pH 7 and each vial contains 300 mg of eculizumab, 13.8 mg sodium phosphate monobasic, 53.4 mg sodium phosphate dibasic, 263.1 mg sodium chloride, 6.6 mg polysorbate 80 (vegetable origin) and Water for Injection, USP.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Eculizumab, the active ingredient in Soliris, is a monoclonal antibody that specifically binds to the complement protein C5 with high affinity, thereby inhibiting its cleavage to C5a and C5b and preventing the generation of the terminal complement complex C5b-9. Soliris inhibits terminal complement mediated intravascular hemolysis in PNH patients.

A genetic mutation in PNH patients leads to the generation of populations of abnormal RBCs (known as PNH cells) that are deficient in terminal complement inhibitors, rendering PNH RBCs sensitive to persistent terminal complement-mediated destruction. The destruction and loss of these PNH cells (intravascular hemolysis) results in low RBC counts (anemia), and also fatigue, difficulty in functioning, pain, dark urine, shortness of breath, and blood clots.

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12.2 Pharmacodynamics

In the placebo-controlled clinical study, Soliris when administered as recommended reduced hemolysis as shown by the reduction of serum LDH levels from 2200 ± 1034 U/L (mean ± SD) at baseline to 700 ± 388 U/L by week one and maintained the effect through the end of the study at week 26 (327 ± 433 U/L). In the single arm clinical study, Soliris maintained this effect through 52 weeks [*see Clinical Studies (14)*].

12.3 Pharmacokinetics

A population PK analysis with a standard 1-compartmental model was conducted on the multiple dose PK data from 40 PNH patients receiving the recommended Soliris regimen [*see Dosage and Administration (2.1)*]. In this model, the clearance of Soliris for a typical PNH patient weighing 70 kg was 22 mL/hr and the volume of distribution was 7.7 L. The half-life was 272 ± 82 hrs (mean ± SD). The mean observed peak and trough serum concentrations of Soliris by week 26 were 194 ± 76 mcg/mL and 97 ± 60 mcg/mL, respectively.

Studies have not been conducted to evaluate the PK of Soliris in special patient populations identified by gender, race, age (pediatric or geriatric), or the presence of renal or hepatic impairment.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been conducted to evaluate the carcinogenic and genotoxic potential of Soliris. Effects of Soliris upon fertility have not been studied in animals. Intravenous injections of male and female mice with a murine anti-C5 antibody at up to 4-8 times the equivalent of the clinical dose of Soliris had no adverse effects on mating or fertility.

14 CLINICAL STUDIES

The safety and efficacy of Soliris in PNH patients with hemolysis were assessed in a randomized, double-blind, placebo-controlled 26 week study (Study 1); PNH patients were also treated with Soliris in a single arm 52 week study (Study 2); and in a long term extension study. Patients received meningococcal vaccination prior to receipt of Soliris. In all studies, the dose of Soliris was 600 mg study drug every 7 ± 2 days for 4 weeks, followed by 900 mg 7 ± 2 days later, then 900 mg every 14 ± 2 days for the study duration. Soliris was administered as an intravenous infusion over 25 - 45 minutes.

Study 1:

PNH patients with at least four transfusions in the prior 12 months, flow cytometric confirmation of at least 10% PNH cells and platelet counts of at least 100,000/microliter were randomized to either Soliris (n = 43) or placebo (n = 44). Prior to randomization, all patients underwent an initial observation period to confirm the need for RBC transfusion and to identify the hemoglobin concentration (the "set-point") which would define each patient's hemoglobin stabilization and transfusion outcomes. The hemoglobin set-point was less than or equal to 9 g/dL in patients with symptoms and was less than or equal to 7 g/dL in patients without symptoms. Endpoints related to hemolysis included the numbers of patients achieving hemoglobin stabilization, the number of RBC units transfused, fatigue, and health-related quality of life. To achieve a designation of hemoglobin stabilization, a patient had to maintain a hemoglobin concentration above the hemoglobin set-point and avoid any RBC transfusion for the entire 26 week period. Hemolysis was monitored mainly by the measurement of serum LDH levels, and the proportion of PNH RBCs was monitored by flow cytometry. Patients receiving anticoagulants and systemic corticosteroids at baseline continued these medications.

Major baseline characteristics were balanced (see table 2).

TABLE 2
STUDY 1 PATIENT BASELINE CHARACTERISTICS

	Study 1	
Parameter	Placebo N = 44	Soliris N = 43
Mean age (SD)	38 (13)	42 (16)
Gender - female (%)	29 (66)	23 (54)
History of aplastic anemia or myelodysplastic syndrome (%)	12 (27)	8 (19)
Patients with history of thrombosis (events)	8 (11)	9 (16)
Concomitant anticoagulants (%)	20 (46)	24 (56)
Concomitant steroids/immunosuppressant	16 (36)	14 (33)

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Parameter	Study 1 Placebo N = 44	 Soliris N = 43
treatments (%)		
Packed RBC units transfused per patient in previous 12 months (median (Q1,Q3))	17 (14, 25)	18 (12, 24)
Mean hgb level (g/dL) at setpoint (SD)	8 (1)	8 (1)
Pre-treatment LDH levels (median, U/L)	2,234	2,032
Free hemoglobin at baseline (median, mg/dL)	46	41

Patients treated with Soliris had significantly reduced ($p < 0.001$) hemolysis resulting in improvements in anemia as indicated by increased hemoglobin stabilization and reduced need for RBC transfusions compared to placebo treated patients (see table 3). These effects were seen among patients within each of the three pre-study RBC transfusion strata (4 - 14 units; 15 - 25 units; > 25 units). After 3 weeks of Soliris treatment, patients reported less fatigue and improved health-related quality of life. Because of the study sample size and duration, the effects of Soliris on thrombotic events could not be determined.

TABLE 3
STUDY 1 RESULTS

	Placebo N = 44	Soliris N = 43
Percentage of patients with stabilized hemoglobin levels	0	49
Packed RBC units transfused per patient (median)	10	0
(range)	(2 - 21)	(0 - 16)
Transfusion avoidance (%)	0	51
LDH levels at end of study (median, U/L)	2,167	239
Free hemoglobin at end of study (median, mg/dL)	62	5

Study 2 and Extension Study:
PNH patients with at least one transfusion in the prior 24 months and at least 30,000 platelets/microliter received Soliris over a 52-week period. Concomitant medications included anti-thrombotic agents in 63% of the patients and systemic corticosteroids in 40% of the patients. Overall, 96 of the 97 enrolled patients completed the study (one patient died following a thrombotic event). A reduction in intravascular hemolysis as measured by serum LDH levels was sustained for the treatment period and resulted in a reduced need for RBC transfusion and less fatigue. 187 Soliris-treated PNH patients were enrolled in a long term extension study. All patients sustained a reduction in intravascular hemolysis over a total Soliris exposure time ranging from 10 to 54 months. There were fewer thrombotic events with Soliris treatment than during the same period of time prior to treatment. However, the majority of patients received concomitant anticoagulants; the effects of anticoagulant withdrawal during Soliris therapy was not studied [*see Warnings and Precautions (5.4)*].

16 HOW SUPPLIED / STORAGE AND HANDLING

Soliris (eculizumab) is supplied as 300 mg single-use vials containing 30 mL of 10 mg/mL sterile, preservative-free Soliris solution per vial.

Soliris vials must be stored in the original carton until time of use under refrigerated conditions at 2-8° C (36-46° F) and protected from light. Do not use beyond the expiration date stamped on the carton. Refer to *Dosage and Administration* (2) for information on the stability and storage of diluted solutions of Soliris.

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DO NOT FREEZE. DO NOT SHAKE.

NDC 25682-001-01 Single unit 300 mg carton: Contains one (1) 30 mL vial of Soliris (10 mg/mL).

17 PATIENT COUNSELING INFORMATION

See Medication Guide.

Prior to treatment, patients should fully understand the risks and benefits of Soliris, in particular the risk of meningococcal infection. Ensure that patients receive the Medication Guide.

Patients should be informed that they are required to receive a meningococcal vaccination at least 2 weeks prior to receiving the first dose of Soliris, if they have not previously been vaccinated. They are required to be revaccinated according to current medical guidelines for meningococcal vaccine use while on Soliris therapy. Patients should also be informed that vaccination may not prevent meningococcal infection. Patients should be educated about any of the signs and symptoms of meningococcal infection, and strongly advised to seek immediate medical attention if these signs or symptoms occur. These signs and symptoms are as follows:

- moderate to severe headache with nausea or vomiting
- moderate to severe headache and a fever
- moderate to severe headache with a stiff neck or stiff back
- fever of 103° F (39.4° C) or higher
- fever and a rash
- confusion
- severe muscle aches with flu-like symptoms, and eyes sensitive to light

Patients should be informed that they would be provided with the Patient Safety Card that they should carry with them at all times. This card describes symptoms which, if experienced, should prompt the patient to immediately seek medical evaluation.

Patients should be informed that there is a potential for serious hemolysis when Soliris is discontinued and that they will be monitored by their healthcare professional for at least 8 weeks following Soliris discontinuation.

Manufactured by:

Alexion Pharmaceuticals, Inc.

352 Knotter Drive

Cheshire, CT 06410 USA

US License Number 1743

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Patients receiving Soliris treatment should carry this card at all times.

INFORMATION FOR THE PATIENT

You should call your doctor (#_____) right away if you have any of these symptoms:

- **moderate to severe headache with nausea or vomiting**
- **moderate to severe headache and a fever**
- **moderate to severe headache with a stiff neck or stiff back**
- **fever of 103° F (39.4° C) or higher**
- **fever and a rash**
- **confusion**
- **severe muscle aches with flu-like symptoms, and eyes sensitive to light**

If you cannot reach your doctor right away, go to an emergency room and show them this card.

INFORMATION FOR THE PHYSICIAN

This patient was prescribed Soliris (eculizumab) therapy. Soliris is an antibody that inhibits terminal complement activation and prevents the formation of C5b-9 complex. Before beginning treatment, this patient should have received a meningococcal vaccine, but may still be susceptible to meningococcal infections.

For more information about Soliris, see the full prescribing information available at www.soliris.net\soliris.pdf, or call 1-888-SOLIRIS (1-888-765-4747)

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Food and Drug Administration
Center for Drug Evaluation and Research
Office of Biotechnology Products

FACSIMILE TRANSMITTAL SHEET

DATE: May 8, 2009

To: Mr. Arthur D. Edwards	**From:** LCDR Andrew Shiber Project Manager
Company: Alexion Pharmaceuticals Corporation	Office of Biotechnology Products
Fax number: 203-271-8191	**Fax number:** (301) 796-9743
Phone number: 203-272-2596	**Phone number:** (301) 796-4798

Subject: STN 125166/36

Total no. of pages including cover: 2

Comments: Please find attached the action letter for the above supplement for Soliris®(eculizumab).

Document to be mailed: X☐ YES ☐ NO

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

STN 125166-36

May 8, 2009

Alexion Pharmaceuticals
Attention: Mr. Arthur D. Edwards
Executive Director, Regulatory Affairs
352 Knotter Drive
Cheshire, CT 06410

Dear Mr. Edwards:

Your request to supplement your biologics license application for Soliris®(eculizumab), to extend the shelf-life of the drug product from 24 to 30 months when stored at 2 – 8 degrees Celsius, has been approved.

Please refer to http://www.fda.gov/cder/biologics/default.htm for information regarding therapeutic biological products, including the addresses for submissions.

This information will be included in your biologics license application file.

Sincerely,

Kathleen A. Clouse

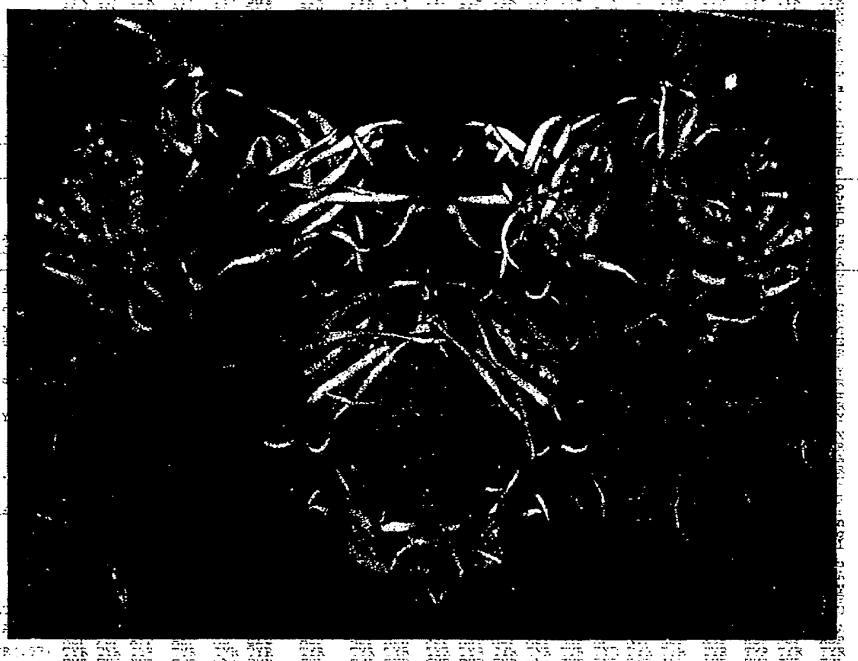
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Director
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Office of Pharmaceutical Science
Center for Drug Evaluation and Research

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SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

VOL. I

FIFTH EDITION



1991

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

FIFTH EDITION

Tabulation and Analysis of
Amino Acid and Nucleic Acid Sequences of Precursors,
V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen,
T-Cell Surface Antigens, β_2-Microglobulins,
Major Histocompatibility Antigens, Thy-1, Complement,
C-Reactive Protein, Thymopoietin, Integrins, Post-gamma Globulin,
α_2-Macroglobulins, and Other Related Proteins

1991

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U.S. DEPARTMENT OF HEALTH
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NIH Publication No. 91-3242

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SIGNAL PEPTIDES OF HUMAN KAPPA LIGHT CHAINS

	INVARIANT RESIDUES	1 HK101 'CL	2 HK 137 'CL	3 VKI- Chr1 'CL	4 HuVH CAMP 'CL	5 VKI-2I 'CL	6 3D6 'CL	7 V108 'CL	8 Vb 'CL	9 Vb' 'CL	10 HK102 'CL	11 2A12 'CL	12 1H1 'CL	13 2C12 'CL	14 1B11 'CL	15 Vd 'CL	16 Va' 'CL	17 Ve 'CL	18 V13 'CL	19 GM 607 'CL	20 A3 'CL	21 RPMI 6410 'CL #	22 A2 'CL	23 A23 'CL
-29		---	---	---	---		---	---	---	---	---	---				---	---	---	---		---	---	---	---
-28		---	---	---	---		---	---	---	---	---	---				---	---	---	---		---	---	---	---
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-21		ASP	ASP	---	---	ASP	ASP	GLU	ASP	ASP	MET	ASP				ASP	ASP	---	ASP		---	---	---	---
-20	MET(.98)	MET	MET	MET	---	VAL	MET	MET	MET	MET	MET	MET				MET	NET	MET	MET		MET	MET	MET	MET
-19		ARG	ARG	ARG	MET	ARG	ARG	ARG	ARG	MET	ARG	ARG				ARG	ARG	ARG	ARG		ARG	ARG	ARG	ARG
-18		VAL	VAL	ALA	GLY	ALA	VAL	VAL	VAL	VAL	VAL	VAL				VAL	VAL	VAL	VAL		LEU	LEU	LEU	LEU
-17		LEU	LEU	PRO	TRP	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO			PRO	ERO	PRO	PRO		PRO	PRO	PRO	LEU
-16		ALA	ALA	THR	SER	THR	ALA	ALA	ALA	ALA	ALA	ALA	ALA			ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA
-15		GLN	GLN	GLN	CYS	GLN	GLN	GLN	GLN	GLN	GLN	LEU	LEU			GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN
-14		LEU	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	IEU	LEU	LEU		LEU	LEU	LEU	LEU
-13		LEU	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-12		GLY	GLY	GLY	LEU	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY			GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY
-11		LEU	LEU	LEU	PHE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	IEU	LEU	LEU		LEU	LEU	LEU	LEU
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-9		LEU	LEU	VAL	VAL	GLY	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		MET	MET	MET	MET
-8	LEU(.95)	LEU	LEU	LEU	ALA	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
-7	TRP(.95)	CYS	CYS	TRP	THR	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP
-6		PHE	PHE	LEU	ALA	LEU	LEU	LEU	PHE	PHE	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		VAL	VAL	ILE	VAL
-5		PRO	PRO	PRO	THR	PRO	PRO	ARG	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		SER	PRO	PRO	PRO
-4		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
-3		ALA	ALA	ALA	VAL	ALA	ALA	ALA	SER	SER	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	SER	SER	SER	SER	SER
-2		ARG	ARG	ARG	HIS	ARG	LYS	ARG	ARG	ARG	LYS	LYS	LYS	LYS	LYS	ARG	ARG	ARG	ARG	SER	SER	SER	SER	SER
-1		CYS	CYS	CYS	SER	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	GLY	GLY	GLY	ALA	GLY

	24 HG9 'CL (R) 'CL #	25 HIC HAH 14.1 'CL #	26 HAH 14.2 'CL #	27 HAH 16.1 'CL #	28 NOV BE10 'CL #	29 30 TH3 'CL #	31 Humkv 325 'CL	32 33 EV1-15 'CL	34 IARC/ BL41 'CL	35 Humkv 305 'CL	36 LS1 'CL	37 LS2 'CL	38 LS4 'CL	39 LS5 'CL	40 LS6 'CL	41 LS7 'CL	42 LS8 'CL	43 Vg 'CL	44 Vh 'CL	45 CLL 'CL #	46 Hum kv328 'CL #	47 Hum ka31es 'CL #
-29	---	---	---	---			---	---	---	---	---	---	---	---	---	---	---	---	---	---		
-28	---	---	---	---			---	---	---	---	---	---	---	---	---	---	---	---	---	---		
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-21	---	---	---	---			---	---	---	---	---	---	---	---	---	---	---	---	---	---		
-20	MET	MET	MET	MET			MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET		
-19	GLU	GLU	GLU	GLU			GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU		
-18	THR	THR	THR	THR			THR	THR	THR	THR	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		
-17	PRO	PRO	PRO	PRO			PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		
-16	ALA	ALA	ALA	ALA			ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		
-15	GLN	GLN	GLN	GLN			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		
-14	LEU	LEU	LEU	LEU			LEU	LEU	LEU	IEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-13	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-12	PHE	PHE	PHE	PHE			PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE		
-11	LEU	LEU	LEU	LEU			LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-9	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-8	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
-7	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		
-6	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LSU	LEU	LEU	LEU	LEU	LEU	LEU		
-5	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		
-4	VAL	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
-3	PRO	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
-2	SER	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
-1	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ARG	GLY	GLY	GLY

	48 GF4 /1.1 'CL	49 K- EV15 'CL	50 VKAPPA IV GERMLINE 'CL	51 PB17IV 'CL	52 FK-001 'CL #	53 WALKER 'CL	54 DAUDI 'CL	55 VJ1 'CL	56 HK100 'CL	57 Va 'CL	58 V2 'CL	59 V14 'CL	60 V52 'CL	61 V55 'CL	62 K- EVJK11 'CL	63 PSEUDO VKAPPA 24'CL	# OF SEQUENCES	# OF AMINO ACIDS
-29	---	---	---	---	---	---	---	---	---	---	---	---	---		---	---		
-28	---	---	---	---	---	---	---	---	---	---	---	---	---		---	---		
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-23	---	---	---	---	---	---	---	---	---	---	---	---	---		---	---		
-22	---	---	---	---	---	MET	MET	---	MET	MET	---	---	MET		---	---	17	2
-21	---	---	---	---	---	ASP	ASP	---	ASP	ASP	---	---	ASP		---	---	17	2
-20	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET		MET	MET	52	2
-19	GLU	GLY	VAL	VAL	VAL	ARG	ARG	VAL	ARG	ARG	GLN	GLN	ARG		GLU	ARG	53	6
-18	ALA	SER	LEU	LEU	LEU	VAL	VAL	LEU	VAL	VAL	VAL	VAL	ALA		THR	CYS	53	7
-17	PRO	GLN	GLN	GLN	GLN	PRO	PRO	GLN	PRO	PRO	PRO	PRO	PRO		PRO	SER	54	5
-16	ALA	VAL	THR	THR	THR	ALA	ALA	THR	ALA	ALA	THR	THR	ALA		ALA	PRO	54	5
-15	HIS	HIS	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	HIS	54	4
-14	VAL	LEU	VAL	VAL	VAL	LEU	LEU	VAL	LEU	LEU	LEU	LEU	LEU		LEU	PHE	54	4
-13	LEU	LEU	PHE	PHE	PHE	LEU	LEU	PHE	LEU	LEU	LEU	LEU	LEU		LEU	LEU	54	3
-12	PHE	SER	ILE	ILE	ILE	GLY	GLY	ILE	GLY	GLY	GLY	GLY	GLY	GLY	PHE	GLU	55	6
-11	LEU	PHE	SER	SER	SER	LEU	LEU	SER	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	55	3
-10	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	58	1
-9	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	VAL	VAL	LEU	PRO	LEU	VAL	58	5
-8	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	SER	LEU	PHE	58	4
-7	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	59	3
-6	LEU	ILE	ILE	ILE	ILE	LEU	LEU	ILE	LEU	LEU	LEU	LEU	LEU	PHE	LEU	ILE	59	5
-5	PRO	SER	SER	SER	SER	ARG	ARG	SER	SER	PRO	PRO	PRO	PRO	PRO	PRO	LEU	59	5
-4	GLU	ASP	GLY	GLY	GLY	GLY	ARG	GLY	GLY	GLY	GLY	GLY	GLY	ASP	GLY	GLU	63	5
-3	THR	THR	ALA	ALA	ALA	ALA	VAL	ALA	ALA	ALA	ALA	ALA	THR	THR	THR	VAL	63	5
-2	SER	ARG	TYR	TYR	TYR	ARG	ARG	TYR	ARG	ARG	ARG	ARG	ARG	ARG	THR	SER	63	6
-1	GLY	ALA	GLY	GLY	GLY	CYS	CYS	GLY	TYR	CYS	CYS	CYS	CYS	SER	GLY	GLY	63	6

Samsung Bioepis Exhibit No. 1002
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HUMAN KAPPA LIGHT CHAINS SUBGROUP I

	INVARIANT RESIDUES	1 2C12 'CL #	2 1B11 'CL #	3 1H1 'CL #	4 2A12 'CL #	5 BR	6 DEN	7* 3D6 'CL	8 HK102 'CL #	9 EU	10 CAR	11 PA	12 HK 137 'CL #	13 RAU	14 KUE	15* HF2-1/17	16 Vb 'CL	17 Vb' 'CL	18 GAL (I)	19 CU (IOC)	20* WEA	21 WIL (=) #	22 WES	23 DAUDI 'CL	24 HK 134 'CL #
0		---	---	---	---	---	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	#
1		DASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
2	ILE(.98)	? ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
3		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
4		MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET
5	THR(.98)	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
6		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
7	SER(.99)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
8	PRO(.99)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
9	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
10		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	ser	THR	ser	ser	ser	ser	ser	ser	THR	ser	ser	ser
11		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	gln	LEU	val	val	LEU	LEU	LEU	LEU	val	LEU	LEU
12	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	pro	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
13		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
14		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
15	VAL(.96)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
16	GLY(.99)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
17		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP
18		SER	SER	SER	SER	thr	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg
19		ILE	ILE	ILE	ILE	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
20	THR(.96)	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
21	ILE(.95)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
22		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR	THR	THR	THR	THR
23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
24		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
25		ALA	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	pro	ALA	ALA	ALA
26		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	gly	SER
27		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	his	GLN
27A		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27B		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27C		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27D		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27E		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
27F		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
28		SER	SER	SER	SER	SER	SER	SER	SER	SER	asn	SER	gly	SER	SER	gly	gly	gly	gly	thr	gly	ala	asp	asn	gly
29		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	phe	ILE	ILE	ILE
30		GLY	GLY	GLY	GLY	ser	ser	ser	ser	asn	ser	ser	ser	ser	asn	arg	ser	ser	arg	ser	arg	GLY	ser	thr	ser
31		ASN	ASN	ASN	gly	arg	arg	arg	ser	thr	ser	ser	ASN	ser	ile	ASN	ser	ser	ASN	ser	ASN	ser	his	ASN	ser
32		TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	ser	tyr	TRP	asp	TRP	TRP	asp	tyr	asp	TRP	TRP	phe	TRP
33	LEU(.95)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
34		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	gly	ALA	ALA	thr	asx	thr	ALA	ALA	ser	ALA
35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP
36		PHE	PHE	PHE	PHE	PHE	tyr	tyr	tyr	tyr	tyr	tyr	PHE	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr
37		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
38		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
39		LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS
40	PRO(.97)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ser	PRO	PRO
41		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu
42		LYS	LYS	LYS	LYS	LYS	glu	LYS	LYS	LYS	LYS	LYS	glu	LYS	LYS	LYS	LYS	LYS	LYS	LYS	thr	LYS	LYS	LYS	LYS
43		ALA	ALA	ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	lys	lys	lys	lys	gln	lys	lys	lys	lys	ASX	lys	glu	lys	thr	lys	lys
45		ASN	ASN	ASN	ASN	lys	lys	lys	lys	leu	leu	leu	leu	leu	leu	leu	leu	leu	glu	leu	leu	leu	leu	leu	ser
46		VAL	VAL	VAL	VAL	leu	VAL	leu	leu	leu	leu	VAL	leu	ser	VAL	leu	arg	leu	leu	leu	arg	leu	leu	leu	ser
47	LEU(.98)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
48	ILE(.98)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	met	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
49		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
50		LYS	LYS	LYS	LYS	LYS	gly	LYS	asp	LYS	LYS	glu	ala	ala	LYS	ala	ala	ala	ala	gly	arg	ser	ala	ala	ala
51		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
52		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	SER	SER	SER	SER
53		ASN	ASN	ASN	ASN	thr	ASN	ser	ser	ser	ser	ser	ser	thr	ser	ser	ser	ser	ASN	ASX	ser	ser	ser	ASN	ser
54	LEU(.97)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
55		LYS	LYS	LYS	LYS	glu	glu	glu	glu	glu	glu	glu	glu	pro	glu	gln	gln	gln	gln	his	gln	gln	glu	gln	gln
56		ASN	ASN	ASN	lys	ser	ser	ser	ser	ser	ser	ser	ser	thr	ser	ser	ser	ser	ser	ser	ser	ser	ser	ASN	ser
57	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
58		ILE	ILE	ILE	ILE	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val
59	PRO(.95)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
60	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
61	ARG(.97)	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
62	PHE(.96)	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
63		SER	SER	SER	SER	SER	SER	SER	SER	SER	ile	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
64	GLY(.96)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
65		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
66	GLY(.95)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
67		SER	SER	SER	SER	gha	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	ala	SER	SER	SER	SER	SER
68	GLY(.95)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
69		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR
70		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp	GLU	asp	asp	gln	GLU	asp	asp	GLU	asx	GLU	GLU	GLU	GLU	asp
71		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
72		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
73		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	phe	LEU	LEU	LEU	LEU	LEU
74		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
75		VAL	VAL	VAL	VAL	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile
76		ILE	ILE	ILE	ILE	asn	ser	ser	ser	ser	ser	ser	ser	ser	asn	ser	ser	asr	ser	ser	asn	ser	ser	ser	ser
77		ASN	ASN	ASN	ASN	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	gly	ser	ser	ser	ser	ser
78	LEU(.98)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
79		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
80		SER	SER	SER	SER	pro	SER	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
81		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	glu	glu	ASP	glu	glu	glu	glu	glx	glu	ASP	glu	glu	glu
82		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
83		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	val	PHE	PHE	PHE
84	ALA(.98)	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
85		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
86	TYR(.98)	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
87	TYR(.95)	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR
88	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
89		HIS	HIS	HIS	HIS	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	leu	gln	gln	leu	glx	leu	gln	gln	gln	gln
90		HIS	HIS	HIS	HIS	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	gln	gln	gln
91		ASN	ASN	ASN	ASN	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	ASN	tyr	his	ala	ala	gln	ser	tyr	ala	ala	ASN	tyr
92		ASP	ASP	ASP	glu	ASP	ASP	asn	asn	asn	asn	asn	asn	tyr	ser	asn	asn	asn	asn	tyr	ser	asn	his	tyr	asn
93		SER	SER	thr	thr	SER	SER	SER	SER	SER	thr	asn	SER	ile	arg	SER	SER	SER	SER	SER	SER	arg	SER	asn	SER
94		PHE	PHE	PHE	ile	PHE	PHE	tyr	tyr	asp	PHE	leu	tyr	thr	tyr	tyr	PHE	PHE	tyr	ser	PHE	tyr	val	PHE	tyr
95		SER	SER	SER	SER	pro	pro	SER	SER	---	---	pro	pro	pro	---	pro	pro	pro	pro	pro	pro	---	pro	SER	pro
95A		---	---	---	---	---	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	#
95B		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95C		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95D		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95E		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
95F		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
96		---	---	---	---	TRP	TYR	---	---	LYS	PHE	TRP	---	THR	TYR	LEU	---	---	ARG	THR	TRP	---	LEU	PHE	---
97		THR	THR	THR	THR	THR	THR	---	---	MET	THR	THR	---	SER	THR	THR	---	---	SER	THR	THR	---	THR	THR	---
98	PHE(.96)	PHE	PHE	PHE	PHE	PHE	PHE	PHE	---	PHE	PHE	PHE	---	PHE	PHE	PHE	---	---	PHE	PHE	PHE	PHE	PHE	PHE	---
99	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY	GLY	GLY	GLY	GLY	---
100		GLN	GLN	GLN	GLN	GLN	GLN	PRO	---	GLN	PRO	PRO	---	GLN	GLN	GLY	---	---	GLN	GLX	GLN	GLN	GLY	GLY	---
101	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY	GLY	GLY	GLY	GLY	---
102	THR(.98)	THR	THR	THR	THR	THR	THR	THR	---	THR	THR	THR	---	THR	THR	THR	---	---	THR	THR	THR	THR	THR	THR	---
103		LYS	LYS	LYS	LYS	LYS	LYS	LYS	---	LYS	LYS	LYS	---	ARG	LYS	LYS	---	---	LYS	ARG	LYS	LYS	THR	LYS	---
104		VAL	VAL	VAL	VAL	VAL	LEU	VAL	---	VAL	VAL	VAL	---	VAL	LEU	VAL	---	---	VAL	LEU	VAL	LEU	VAL	VAL	---
105		LEU	LEU	LEU	LEU	GLU	GLU	ASP	---	GLU	ASP	GLU	---	GLU	ASP	GLU	---	---	GLU	GLX	GLU	GLU	ASP	ASP	---
106		ILE	ILE	ILE	ILE	ILE	ILE	ILE	---	VAL	ILE	ILE	---	ILE	ILE	ILE	---	---	ILE	ILE	VAL	ILE	ILE	ASN	---
106A		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
107		LYS	LYS	LYS	LYS	LYS	LYS	LYS	---	LYS	LYS	LYS	---	LYS	LYS	LYS	---	---	LYS	LYS	LYS	LYS	LYS	LYS	---
108		ARG	ARG	ARG	ARG		ARG		---	GLY	ARG		---	ARG	ARG	ARG	---	---	ARG	ARG	ARG	ARG	ARG	---	---
109	THR						THR		---	THR	THR		---	THR	THR		---	---	THR	THR	THR	THR		---	---

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		25 Vd 'CL	26 WALKER 'CL	27 HK101 'CL #	28 Va' 'CL	29 VKI-Che1 'CL	30 AMYLOID BAN #	31 Ve 'CL	32 V13 'CL	33 DEE	34 WIL (-) #	35 AND #	36 V108 'CL #	37 VKI-ZI 'CL	38 REI	39 AU	40 ROY	41 BI	42* HuVH CAMP 'CL	43 AG #	44 SCH	45 MEV	46 KA	47* LAY	48 BEL	49 NI
	0	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ala	ASP	ASP	ala	val	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	arg	trp	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	4	leu	MET	MET	leu	MET	leu	MET	MET	MET	MET	MET	val	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	leu	MET
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
FR1	10	phe	ser	ser	ser	ser	ser	ser	leu	sec	THR	sec	ser	ser	ser	ser	ser	pro	ser	sec	ser	ser	sec	ser	ser
	11	LEU	LEU	LEU	LEU	LEU	LEU	phe	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val	val	ALA	ALA
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr
	15	VAL	VAL	VAL	VAL	VAL	VAL	thr	thr	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	gly	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	18	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	SER	arg	arg	arg	arg	arg	arg	arg
	19	val	val	val	val	val	val	val	val	val	val	val	val	gly	val	val	val	val	val	val	val	val	val	val	val
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	phe	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	leu
	22	THR	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ILE	leu
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	24	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	gln	gln	gln	gln	lys	gln	gln	ARG	glu	gln	glu
	25	ALA	ALA	ALA	ALA	ALA	ALA	ALA	met	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	26	SER	SER	arg	SER	SER	SER	SER	SER	gly	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	27	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN
	27A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	ser
	27B	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	val
CDR1	27C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	leu
	27D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	glu
	27E	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27F	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	28	gly	SER	gly	gly	gly	SER	gly	gly	SER	SER	SER	gly	gly	asp	asp	asp	asp	agn	asp	asp	SER	thr	asn	asx	SER
	29	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	val	val	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ser	val	val	ILE	gly
	30	ser	ser	ser	asn	ASP	tyr	ser	ser	ser	ser	GLY	ser	ser	ile	ser	ser	arg	asp	asn	asp	val	leu	asn	ser	asn
	31	asr	ASN	ser	ser	ASN	ser	ser	ser	lys	tyr	gly	ASN	ASN	lys	asp	ile	ASN	lys	his	lys	asp	ser	ala	lys	thr
	32	tyr	tyr	TRP	ala	asn	tyr	tyr	tyr	tyr	TRP	ser	gly	asn	tyr	tyr	phe	ser	tyr	tyr	his	tyr	tyr	tyr		phe
	33	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU
	34	ALA	asn	ALA	ALA	asn	ALA	ALA	ALA	asn	ALA	asn	ser	asn	asn	asn	asn	ile	asn	asn	asn	asn	asn	asn		ALA
	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP
	36	tyr	tyr	tyr	tyr	tyr	PHE	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr		tyr
	37	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	asp	GLN	GLN	GLN		GLN
	38	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN
	39	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	thr	LYS	LYS	LYS	LYS	§	LYS	LYS	LYS	LYS		LYS
	40	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO
	41	GLY	GLY	GLY	glu	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	§	GLY	GLY	GLY	GLY	GLY	lys
FR2	42	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	gln	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	leu	LYS	LYS
	43	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	thr	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	pro	ALA	
	44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	
	45	lys	lys	lys	lys	lys	lys	lys	glu	lys	ASN	ser	thr	lys	lys	lys	lys	lys	lys	arg	lys	lys	glu	lys	lys	
	46	leu	leu	ser	ser	leu	ser	leu	leu	VAL	leu	leu	leu	leu	leu	leu	phe	leu	leu	leu	leu	leu	leu	leu	leu	
	47	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	
	48	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	
	49	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR	phe	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR	TYR
	50	ala	ala	ala	asp	ala	asp	ala	ala	ala	gln	gly	ala	ala	glu	asp	asp	asp	asn	asp	gly	asp	ala	gly	asp	asp
	51	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
CDR2	52	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	glu	asn	SER	SER	SER	SER	SER	SER	SER
	53	thr	ser	ser	ser	ser	thr	thr	thr	ser	ile	ser	ser	ser	ASN	ASN	lys	ASN	ASN	ASN	thr	ASN	ser	thr	thr	ASN
	54	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	arg	LEU	LEU
	55	gln	gln	gln	glu	gln	gln	gln	gln	LYS	asp	arg	gln	gln	gln	glu	glu	glu	gln	glu	glu	gln	glu	lys	LYS	glu
	56	ser	ser	ser	ser	ser	ser	ser	ser	ser	ala	ser	ser	ser	ala	ser	ala	ile	thr	thr	ser	thr	ala	thr	thr	
	57	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	58	val	val	val	val	ILE	val	val	val	val	val	val	val	ILE	val	val	val	val	val	val	val	val	val	val	val	val
	59	PRO	thr	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	60	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	61	ARG	ARG	ARG	ARG	ARG	asn	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	62	PRO	PRO	PRO	PRO	PRO	SER	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	63	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	arg	SER	SER	SER	SER	SER	SER	SER	SER
	64	GLY	GLY	GLY	GLY	asp	GLY	GLY	GLY	GLY	GLY	GLY	GLY	asp	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	65	SER	SER	SER	SER	SER	SER	SER	SER	SER	asn	SER	SER	SER	SER	gly	thr	SER	SER	SER	gly	gln	SER	SER	SER	SER
	66	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ile	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	arg	GLY	GLY	GLY	GLY	GLY
	67	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	phe	SER	SER	SER	SER	SER	LEU	SER	SER
	68	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	glu	GLY	GLY
	69	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	ala	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	70	GLU	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	asp	his	asp	asp	asp	asp	asp	asp	asx	asp	his	asp
	71	PHE	PHE	PHE	PHE	tyr	PHE	PHE	PHE	PHE	PHE	PHE	PHE	tyr	tyr	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
FR3	72	THR	THR	THR	THR	ile	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR
	73	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	phe	phe	phe	LEU	LEU	LEU	LEU	LEU	phe	phe	LEU	phe
	74	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR
	75	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile	ile
	76	ser	ser	ser	ser	arg	ser	ser	ser	ser	ser	thr	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser	ser		ser	
	77	ser	ser	ser	ser	ser	ser	cys	gly	ser	ser	ser	ser	ser	ser	ser	gly	thr	ser	ser	gly	thr	ser	ser	ser	gly
	78	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU
	79	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	leu	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLX	
	80	pro	pro	pro	pro	pro	pro	SER	SER	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	
	81	glu	glu	glu	glu	glu	glu	glu	glu	glu	ASP	glu	glu	glu	glu	glu	glu	glu	glu	glu	ASP	glx	glu	ala	glx	
	82	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	asn	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASX	
	83	PHE	ser	PHE	PHE	PHE	PHE	PHE	PHE	PHE	ser	PHE	val	PHE	ile	ile	ile	PHE	ile	ile	PHE	PHE	PHE	PHE	PHE	PHE
	84	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	gly	ALA	ALA	ALA	ALA	ALA
	85	THR	THR	THR	THR	THR	THR	THR	THR	THR	arg	ala	THR	ala	THR	THR	THR	THR	THR	THR	asn	THR	THR	THR	val	
	86	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
	87	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR
	88	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	89	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	leu	gln	gln	gln	gln	gln	leu	gln	gln	gln	gln	gln	gln	gln
	90	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	gln	glx	gln	glx
	91	leu	ser	tyr	phe	ser	tyr	tyr	tyr	ser	tyr	ser	asp	ser	tyr	tyr	phe	tyr	his	tyr	tyr	ser	tyr	tyr	tyr	tyr
	92	asn	tyr	asn	ASP	asn	tyr	tyr	tyr	asn	tyr	tyr	ASP	ASP	ASP	ASP	ASP	tyr	ile	ASP	ASP	tyr	leu	asn	ASX	ASP
	93	SER	SER	SER	SER	SER	SER	SER	SER	thr	arg	SER	thr	SER	SER	tyr	asn	asn	SER	thr	asn	thr	asp	asn	his	thr
	94	tyr	thr	tyr	tyr	thr	tyr	tyr	PHE	thr	tyr	ser	thr	thr	leu	leu	leu	leu	arg	leu	val	asn	leu	trp	PHE	leu
CDR3	95	pro	leu	pro	pro	pro	pro	pro	pro	pro	---	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro	pro
	95A	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	GLU	---	---	---	---	---
	95B	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95D	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95E	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	95F	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	96	---	ILE	---	---	TYR	---	---	TYR	---	TRP	---	---	TYR	TRP	LEU	TYR	ARG	ARG	ILE	VAL	ARG	PRO	LEU	SER	
	97	---	THR	---	---	THR	---	---	THR	---	THR	---	---	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	
	98	---	PHE	---	---	PHE	---	---	PHE	PHE	PHE			PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	
	99	---	GLY	---	---	GLY	---	---	GLY	GLY	GLY			GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	
	100	---	GLN	---	---	GLN	---	---	PRO	GLN	GLN			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	VAL		
	101	---	GLY	---	---	GLY	---	---	GLY	GLY	GLY			GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		
	102	---	THR	---	---	THR	---	---	THR	THR	THR			THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	SER	
FR4	103	---	ARG	---	---	LYS	---	---	LYS	LYS	LYS			LYS	LYS	LYS	LYS	LYS	LYS	ARG	THR	LYS	LYS	GLU	LYS	
	104	---	LEU	---	---	#	---	---	VAL	LEU	VAL			LEU	VAL	VAL	LEU	VAL	LEU	VAL	VAL	VAL	VAL	VAL	VAL	
	105	---	GLU	---	---	#	---	---	GLU	GLU	GLU			GLN	GLU	ASP	GLU	GLU	GLU	GLU	ASP	ASP	GLU	GLU		
	106	---	ILE	---	---	ILE	---	---	MET	ILE	ILE			ILE	ILE	PHE	ILE	ILE	ILE	ASN	ILE	LEU	VAL	VAL	SER	
	106A	---	---	---	---	---	---	---	---	---	---			---	---	---	---	---	---	---	---	---	---	---	---	
	107	---	LYS	---	---	LYS	---	---	THR	LYS	ARG			THR	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	
	108	---	---	---	---	ARG	---	---	ARG	ARG				ARG	ARG	ARG	ARG	ARG	ARG	GLY	ARG	ARG	ARG		ARG	
	109	---	---	---	---	---	---	---	THR	THR				THR	THR	THR	THR		THR	THR		THR			THR	

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		50 RZ	51* Ni RSV19 VK #	52 HBJ 4	53 26 #	54 BJ 19 #	55 RFZ	56 HA	57 PAU	58 PSM	59 JBL #	60 ME	61 FRA	62 BJ 48	63 HEJ- 16/6	64 KFZ- 1/13B	65 HFZ- 18/2	66 GR'	67 G1 'CL #	68 HOM	69 KER	70 ESH IGG	71 ESH IGH	72* DAV	73* FIN	74 PW #	75 SHE	76 ADA
	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	GLN	his	GLN	GLN
	4	MET	leu	MET	MET	leu	MET	MET	MET	MET	MET	leu	MET	MET	MET	MET	MET	MET		MET	MET	MET	MET	MET	MET	leu	val
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	thr	SER
F	10	ser	ser	THR	ser	ser	ser	ser	THR	ser	ser	ser	THR	ser	ser	ser	THR	ser		ser	ser	ser	ser	ser	ser	ser	ser
R	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
1	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA	thr	thr	ALA	val
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	tyr	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	val	val	SER	pha
	15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP
	18	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg	arg		arg	arg	arg	arg	arg	arg	arg	arg
	19	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val	val		val	ILE	val	val	val	val	val	val
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	THR	ILE	ILE		ILE	ILE	ILE	ILE	ILE	val	phe	ILE
	22	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	arg	THR	THR
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	24	gln	ARG	ARG	gln	gln	gln	gln	ARG	gln	ARG	glx	ARG		ARG	ARG	ARG	ARG		gln	gln	ARG	ARG	asp	asp	ARG	glx	glx
	25	ALA	ser	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	26	asn	SER	SER	SER	SER	asn	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER		asp	SER	SER	SER	SER	SER	SER	SER
	27	GLN	GLN	GLN	GLN	GLN	GLN	---	GLN	GLN	GLN	GLX	GLX		GLN	GLN	GLN	arg		his	GLN	GLX	GLX	GLN	GLN	GLX	GLX	GLX
	27A	---	thr	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
	27B	---	leu	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
	27C	---	val	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
C	27D	---	his	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
D	27E	---	thr	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
R	27F	---	---	---	---	---	---	---	---	---	---	---	---		---	---	---	---		---	---	---	---	---	---	---	---	---
1	28	val	asp	SER	SER	asp	val	---	SER	asp	SER	asx	asx		gly	gly	gly	SER		asp	asp	SER	SER	asx	asx	SER	asx	asx
	29	ILE	gly	val	ILE	ILE	ILE	---	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	30	ser	asn	asn	asn	asn	ser	asp	ser	arg	ser	ser	asn		asp	arg	arg	ser		ser	lys	ser	ser	asx	asx	GLY	ser	
	31	lys	thr	ASN	lys	thr	lys	ASN	ser	ser	thr	ASX	ASX		ASN	ASN	ASN	ser		ASN		ser	ser	ser	ser		ASX	ASX
	32	ser	tyr	TRP	tyr	phe	ser	tyr	ser	tyr	phe	tyr	tyr		asp	asp	asp	TRP		tyr		tyr	tyr	TRP	TRP	tyr	tyr	tyr
	33	LEU	LEU	LEU	ala	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU		LEU		LEU	LEU	LEU	LEU	ser	LEU	LEU
	34	asn	glu	ALA	asn	asn	asn	asn	ALA	asn	#	asx	ALA		gly	gly	gly	ALA		asn		asx	asx	ile	ile	asx	asx	asx
	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP		TRP		TRP	TRP	TRP	TRP	TRP	TRP	TRP
	36	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr	tyr		tyr	tyr	tyr	tyr		PHE		tyr	tyr	tyr	tyr		tyr	tyr
	37	GLN	GLN	GLN	glu	glu	GLN	GLN	GLX	GLN	GLN	GLX	GLX		GLN	GLN	GLN	GLN		GLN		GLX	GLX	GLN	GLN	GLN	his	GLX
	38	GLN	GLN	glu	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLX	GLX		GLN	GLN	GLN	gly		GLN				GLN	GLN	GLN	GLX	tyr
	39	arg	LYS	LYS	#	#	arg	LYS	LYS	LYS	LYS	LYS	LYS		LYS	LYS	LYS	arg		LYS				tyr	tyr	LYS	LYS	LYS
	40	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	gln	PRO	PRO	PRO		PRO	PRO	PRO	PRO		PRO				PRO	PRO		PRO	PRO
	41	GLY	GLY	GLY	#	#	GLY	GLY	GLY	GLY	GLY	GLY								GLY								
F	42	gln	LYS	glx	LYS	LYS	gln	arg	glx	LYS	LYS	glx																
R	43	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA														
2	44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO														
	45	lys	lys	lys	lys	lys	lys	lys	lys	lys	lys	lys		lys														
	46	leu	leu	leu	leu	leu	leu	leu	leu	leu	leu	VAL																
	47	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	met																
	48	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE		ILE																
	49	TYR	TYR	ser		TYR	TYR	TYR	TYR	TYR	TYR	TYR		gly														
	50	asp	arg	LYS		asp	asp	asp	glx	asp				asp														
	51	ALA	val	thr	asp	ALA	ALA	ALA		ALA				ALA														
C	52	SER	SER	SER	SER	SER	ala	phe		SER				SER														
D	53	ASN	ser	arg	ser	ASX	ASN							ser														
R	54	LEU	arg	LEU	LEU	LEU	LEU	LEU		LEU				LEU														
2	55	glu	phe	glu	glu	glu	glu	glu		glu				asx														
	56	ser	ser	arg	thr	thr		ala						ser														
	57	GLY	GLY	GLY	GLY	GLY	GLY	GLY						GLY														
	58	val	val	val	asx	val	val	val						val					ILE								
	59	PRO	PRO	PRO	PRO	glx	PRO	PRO						val					PRO								
	60	SER	SER	SER	SER	SER		SER						SER					SER								
	61	ARG	ARG	ARG	lys	ARG	ARG	ARG						ARG					ARG								
	62	PHE	PHE	PHE	val	PHE	PHE							gln					PHE								
	63	thr	SER	ala	SER	leu	thr							SER					SER								
	64	GLY	GLY	GLY	GLY	GLY	GLY							GLY					GLY								
	65	SER	SER	SER	SER	SER								SER					SER								
	66	GLY	GLY	GLY	GLY	asx	GLY							GLY					GLY								
	67	SER	SER		SER	phe								SER					SER								
	68	GLY	GLY		glu	asx								GLY					GLY								
	69	THR	THR		THR	THR								THR					THR								
	70	asp	asp		asp	lys								asp					GLU								
	71	PHE	PHE		val									pro					PHE								
F	72	phe	THR		THR									ser					THR								
R	73	LEU	pha		val									LEU					LEU								
3	74	THR	THR		asx									ala					THR								
	75	ile	ile		glx									VAL					VAL								
	76	ser	ser		ser									ser					ILE								
	77	ser	ser		ser									arg					ASN								
	78	LEU	LEU		LEU														LEU								
	79	arg	GLN		GLN														GLN								
	80	pro	pro		pro														SER								
	81	glu	glu		glu														ASP								
	82	ASP	ASP		ASP														ASP								
	83	val	ile		ile														PHE								
	84	ALA	ALA		ALA														ALA								
	85	THR	THR		pro														THR								
	86	TYR	TYR		lys														TYR		TYR						
	87	phe	TYR																TYR		TYR						
	88	CYS	CYS																CYS		CYS						
	89	gln	phe		gln														HIS		gln						
	90	gln	gln		gln														HIS		gln						
	91	tyr	gly		arg	phe								gly					ASN		tyr						
	92	his	ser		ASP	ASP								glu					ASP		ASP						
	93	asn	his		asp	asp								SER					thr		asp						
C	94	leu	leu		leu	leu								asp					PHE		leu						
D	95	pro	pro		pro	pro								SER					SER		pro						
R	95A	---	---		---	---								---					---		---						
3	95B	---	---		---	---								---					---		---						
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	96	LEU	ARG		TYR	TYR								ARG					TRP		PRO						
	97	THR	THR		THR	THR								THR					THR		THR						
	98	PHE	PHE		LEU	PHE								PHE					PHE		PHE						
	99	GLY	GLY		GLY	GLY								GLY					GLY		GLY						
	100	GLY	GLY		GLY	PRO								GLN					GLN		PRO						
	101	GLY	GLY		GLY	GLY								GLY					GLY		GLY						
	102	THR	THR		THR	THR								THR					TER		THR						
F	103	LYS	LYS		LYS	LYS								LYS					LYS		LYS						
R	104	VAL	VAL		VAL	VAL								VAL					VAL		VAL						
4	105	GLU	GLU		GLU	GLU								GLU					LEU		ASP						
	106	ILE	ILE		MET	LEU								VAL					ILE		LEU						
	106A	---			---	---								---					---		---						
	107	LYS			LYS	LYS								LYS					LYS		LYS						
	108																		ARG								
	109																										

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		77 AMYLOID VIII-B	78 PAUL	79 RI #	80 NAT	81* LOW	82 AMYLOID ES305 #	83 DCC	84 BOL	85 AMYLOID X	86 SAC	87 BJ	88 GO	89 F-GUI #	90 S-GUI #	91 HF6-21/28	92 DIE	93 CAR A	94 TEI	95 MON	96 ALE	97 JOH	98 POT	99 CON	100 TRA	101* H-G2b
	0	---	---	pca	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		val	ILE	ILE	val	ILE	ILE	ILE	ILE	GLX	ILE	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	MET	GLN	GLN	GLN	val	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN
	4	MET	MET	MET	MET	MET	leu	MET	ile	MET	MET		MET	MET	MET	MET	MET	MET	MET	MET		MET	MET	MET	MET
	5	THR	THR	THR	THR	THR	THR	THR	met	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLX	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	ala	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ala	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	leu	SER	SER	SER	SER	SER	ala	SER	SER	SER
FR1	10	ser	THR	THR	ser	ser	ser	ser	ser	ser	ser		phe	ser	ser	ser	ser	ser	ser	THR		ser	ser	ser	ser
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU		val	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	val	SER	SER	SER		SER	SER	SER	pro	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	ALA		ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	14	SER	SER	SER	SER	SER	SER	pro	SER	SER	SER		SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER
	15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASX	ASP	ASP		ASX	ASX	ASX	gly	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	18	arg	arg	gly	arg	arg		leu	arg	arg	---	arg	arg	arg	arg	pro	arg	arg	arg	arg	arg	arg	arg	arg	arg
	19	val	val	val	val	val	val	val	val	val	---	val	val	val	val	ala	val	val	val	val	leu	ile	val	val	val
	20	THR	THR	THR	THR	THR	THR	THR	THR	ile	---	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	#	ILE	ILE	ILE	---	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	leu	ILE	ILE	ILE	ILE
	22	THR	THR	ser	THR	THR	THR	THR	THR		---	THR	THR	THR	THR	ser	THR	THR	THR	THR	THR	ser	THR	THR	THR
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	---	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS	CYS		
	24	gln	ARG	ARG	glx	ala	gln	glx	ARG	glx	---	gln	ARG		ARG	ARG					ARG				
	25	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	---	ALA	ALA	ALA	ALA	ser									
	26	SER	SER	SER	SER	SER	SER	SER	SER		---	SER	SER			SER									
	27	GLN	GLN	GLX	GLN	GLN	glu	GLX	GLX	GLX	---	GLN				GLN									
	27A	---	---	---	---	---	---	---	---	---	---	---				ser									
	27B	---	---	---	---	---	---	---	---	---	---	---				leu									
CDR1	27C	---	---	---	---	---	---	---	---	---	---	---				val									
	27D	---	---	---	---	---	---	---	---	---	---	---				trp									
	27E	---	---	---	---	---	---	---	---	---	---	---				ser									
	27F	---	---	---	---	---	---	---	---	---	---	---				---									
	28	asx	SER	SER	asp	asx	ala	asx	gly	asx	---	asp				asp									
	29	ILE	ILE	ILE	ILE	ILE	#	ILE	ILE	ILE	---	ILE				gly									
	30	GLY		ser	ser	ser		ser			---	asn				asx									
	31			ser	asp		ASN	ASX		pro	---	lys				ser									
	32	tyr	ser		tyr		tyr	tyr		tyr	---					tyr									
	33	LEU			val		LEU			LEU	---					LEU									
	34				asn						---					glu									
	35	TRP			TRP		TRP				---					TRP									
	36						tyr			tyr	---					tyr									
	37										---					GLN									
	38										---					GLN									
	39										---					LYS									
	40										---					PRO									
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	89										glx	gln													
	90										glx	gln													
	91										glx	tyr													
	92										ASX	glu													
	93										ala	asn													
CDR3	94										ala	leu													
	95										thr	pro													
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	97										PRO														
	98										ILE														
	99										GLY														
	100										GLY														
	101										GLY														
FR4	102										THR														
	103										LYS														
	104										VAL														
	105										ASX														
	106										VAL														
	106A										---														
	107										LYS														
	108										ARG														
	109										THR														

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HUMAN KAPPA LIGET CHAINS SUBGROUP I (cont'd)

		102* K-G2	103 BRA	104 LUX	105* HEI	106 PAP	107* H-G2a	108 CRA	109 AMYLOID LEP	110* WAG	111 MOE	112 HBJ 1	113 AMYLOID 547	114 WEB	115 HBJ 10	116 LOD	117 BEN	118 MAA	119 GR	120 MUK	121 GM131 'CL #	122 AMYLOID 594	123* MAR	124 CL*
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	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	---
	2	ILE	ILE	ILE	ILE	ILE	ILE	GLN	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	---
	3	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	leu	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	---
	4	MET	MET	leu	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET?		MET	leu	MET	MET		MET	leu	---
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	---
	6	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	---
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	---
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	---
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	---
FR1	10	ser	ser	phe	THR	ser	ser	ser	ser	ser	THR	ser	ser	ser	ser	THR	ser	THR	ser	ser		ser	ser	---
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU		LEU	LEU	LEU	LEU		LEU	LEU	---
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER				---
	13	ALA	val	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val					---
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER									---
	15	VAL	VAL	VAL	VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL		VAL								---
	16	GLY	GLY	GLY	GLY	GLY	GLY	arg	GLY	GLY		GLY												---
	17	ASP	glx	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP												---
	18	arg	arg	arg	arg	arg	arg	arg	arg															---
	19	val	val	val	val	val	val	val	val															---
	20	THR	THR	THR	THR	THR		THR	TER															---
	21	ILE	ILE	ILE		ILE	ILE	ILE																---
	22	THR	THR	THR		ala	THR	THR																---
	23		CYS																					---
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CDR1	27C																							---
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FR2	42																							---
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CDR2	53																							---
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	81																				glu			---
	82																				ASP			---
	83																				PHE			---
	84																				ALA			---
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	89																				gln			---
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	91																				his			---
	92																				ASP			ASP
	93																				his			ile
	94																				leu			PHE
	95																				pro			pro
CDR3	95A																				---			---
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	96																				LEU			GLY
	97																				THR			THR
	98																				PHE			PHE
	99																				GLY			GLY
	100																				GLY			GLN
	101																				GLY			GLY
	102																				THR			THR
FR4	103																				THR			LYS
	104																				VAL			VAL
	105																				GLU			GLU
	106																				MET			ILE
	106A																				---			---
	107																				LYS			LYS
	108																				ARG			ARG
	109																				THR			THR

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HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		125 AMYLOID	126 HBJ 6	127 AMYLOID MS #	128 PEN	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
	0	---	---	---	---	1	1	1(PCA)	
	1	ASP	ASP	ASP	ASP	125	3,4	122(ASP),118(ASP)	3.1,4.2
	2	ILE	ILE	#	ILE	123	2	120(ILE)	2.
	3	glu	GLN	GLN	GLN	123	7	116(GLN),112(GLN)	7.4,7.7
	4	MET	MET	MET	MET	124	4	109(MET)	4.6
	5	pro	THR	THR		124	3	122(THR)	3.
	6	GLN	GLN			123	1,2	123(GLN),116(GLN)	1.,2.1
	7	SER				121	2	120(SER)	2.
	8	PRO				121	2	120(PRO)	2.
	9	SER				121	4	117(SER)	4.1
FR1	10	ser				120	5	89(SER)	6.7
	11	LEU				119	4	111(LEU)	4.3
	12					118	3	115(SER)	3.1
	13					117	4	107(ALA)	4.4
	14					113	6	106(SER)	6.4
	15					113	3	109(VAL)	3.1
	16					109	2	108(GLY)	2.
	17					109	3,4	106(ASP),98(ASP)	3.1,4.4
	18					106	6	97(ARG)	6.6
	19					107	3	101(VAL)	3.2
	20					106	4	102(THR)	4.2
	21					104	4	99(ILE)	4.2
	22					104	6	95(THR)	6.6
	23					96	1	96(CYS)	1.
	24					88	6	57(ARG)	9.3
	25					88	6	82(ALA)	6.4
	26					85	4	79(SER)	4.3
	27					84	4	79(GLN),66(GLN)	4.3,5.1
	27A					3	2	2(SER)	
	27B					3	2	2(LEU)	
	27C					3	2	2(VAL)	
CDR1	27D					3	3	1(+)	
	27E					2	2	1(+)	
	27F								
	28					84	7	29(SER)	20.
	29					83	5	72(ILE)	5.8
	30					81	11	41(SER)	22.
	31					79	12	27(ASN),24(SER)	35.,39.
	32					79	9	34(TYR)	21.
	33					77	4	73(LEU)	4.2
	34					73	7,8	30(ALA)	17.,19.
	35					76	1	76(TRP)	1.
	36					74	2	66(TYR)	2.2
	37					73	4	68(GLN),61(GLN)	4.3,4.8
	38					71	4	68(GLN),63(GLN)	4.2,4.5
	39					68	4	62(LYS)	4.4
	40					70	3	68(PRO)	3.1
	41					58	3	54(GLY)	3.2
FR2	42					60	6	49(LYS)	7.3
	43					61	4	57(ALA)	4.3
	44					61	1	61(PRO)	1.
	45					61	7,8	46(LYS)	9.3,11.
	46					60	7	42(LEU)	10.
	47					59	2	58(LEU)	2.
	48					57	2	56(ILE)	2.
	49					59	4	55(TYR)	4.3
	50					59	9	19(ALA)	28.
	51					59	5	60(ALA)	5.9
CDR2	52					58	6	53(SER)	6.6
	53					57	6,7	26(SER)	13.,15.
	54					58	2	56(LEU)	2.1
	55					58	8,9	25(GLU)	19.,21.
	56					56	8	34(SER)	13.
	57					57	1	57(GLY)	1.
	58					58	3	50(VAL)	3.5
	59					57	4	54(PRO)	4.2
	60					57	1	57(SER)	1.
	61					58	3	56(ARG)	3.1
	62					57	3	55(PHE)	3.1
	63					57	6	50(SER)	6.8
	64					57	2	55(GLY)	2.1
	65					56	5	51(SER)	5.5
	66					57	4	54(GLY)	4.2
	67					55	3	51(SER)	3.2
	68					55	3	52(GLY)	3.2
	69					55	2	51(THR)	2.2
	70					55	5,6	33(ASP),31(ASP)	8.3,11.
	71					54	4	49(PHE)	4.4
FR3	72					54	5	50(THR)	5.4
	73					54	3	43(LEU)	3.8
	74					54	4	51(THR)	4.2
	75					54	3	47(ILE)	3.4
	76					53	5	43(SER)	6.2
	77					54	6	40(SER)	8.1
	78					54	2	53(LEU)	2.
	79					54	3,4	52(GLN),48(GLN)	3.1,4.5
	80					54	2	46(PRO)	2.3
	81					54	3,5	37(GLU),34(GLU)	4.4,7.9
	82					54	2	53(ASP),50(ASP)	2.,2.2
	83					54	4	40(PHE)	5.4
	84					54	2	53(ALA)	2.
	85					54	6	48(THR)	6.7
	86					56	2	55(TYR)	2.
	87					55	2	52(TYR)	2.1
	88					56	1	56(CYS)	1.
	89					57	4,5	46(GLN),43(GLN)	5.,6.6
	90					57	2,3	51(GLN),46(GLN)	2.2,3.7
	91					58	11,12	28(TYR)	23.,25.
	92					60	9	20(ASN),19(+)	27.,28.
	93					60	9	29(SER)	19.
	94					60	12	15(LEU)	48.
	95					56	4	44(PRO)	5.1
CDR3	95A					1	1	1(GLU)	
	95B								
	95C								
	95D								
	95E								
	95F								
	96					44	12	10(TRP)	53.
	97					43	4	39(THR)	4.4
	98					46	3	44(PHE)	3.1
	99					46	1	46(GLY)	1.
	100					46	4,5	28(GLN),27(GLN)	6.6,8.5
	101					46	1	46(GLY)	1.
	102					46	2	45(THR)	2.
FR4	103					46	4	38(LYS)	4.8
	104					45	2	36(VAL)	2.5
	105					45	4,5	29(GLU),28(GLU)	6.2,8.
	106					46	7	30(ILE)	11.
	106A								
	107					45	3	42(LYS)	3.2
	108					33	2	31(ARG)	2.1
	109					22	1	22(THR)	1.

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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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An Agency of the European Union

28/11/2017

Medicine Name	Active Substance	Status	Authorisation date	Biosimilar	Product concentration mg/ml
Zevalin	ibritumomab tiuxetan	Authorised	16/01/2004	no	1.6
Dinutuximab beta EUSA (previously Dinutuximab beta Apeiron)	dinutuximab beta	Authorised	08/05/2017	no	4.5
Erbitux	cetuximab	Authorised	29/06/2004	no	5
Yervoy	ipilimumab	Authorised	13/07/2011	no	5
Stelara	ustekinumab	Authorised	16/01/2009	no	5
Lemtrada	alemtuzumab	Authorised	12/09/2013	no	10
Soliris	eculizumab	Authorised	20/06/2007	no	10
Opdivo	nivolumab	Authorised	19/06/2015	no	10
Lartruvo	olaratumab	Authorised	09/11/2016	no	10
Cyramza	ramucirumab	Authorised	19/12/2014	no	10
Lucentis	ranibizumab	Authorised	22/01/2007	no	10
Cinqaero	reslizumab	Authorised	16/08/2016	no	10
MabThera	rituximab	Authorised	02/06/1998	no	10
Portrazza	necitumumab	Authorised	15/02/2016	no	16
Bavencio	avelumab	Authorised	18/09/2017	no	20
Darzalex	daratumumab	Authorised	20/05/2016	no	20
Tysabri	natalizumab	Authorised	27/06/2006	no	20
Arzerra	ofatumumab	Authorised	19/04/2010	no	20
Vectibix	panitumumab	Authorised	03/12/2007	no	20
RoActemra	tocilizumab	Authorised	16/01/2009	no	20
Avastin	bevacizumab	Authorised	12/01/2005	no	25
Zinplava	bezlotoxumab	Authorised	18/01/2017	no	25

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Gazyvaro	obinutuzumab	Authorised	23/07/2014	no	25
Perjeta	pertuzumab	Authorised	04/03/2013	no	30
Humira	adalimumab	Authorised	08/09/2003	no	50
Praxbind	idarucizumab	Authorised	20/11/2015	no	50
Tecentriq	atezolizumab	Authorised	21/09/2017	no	60
Prolia	denosumab	Authorised	26/05/2010	no	60
Xgeva	denosumab	Authorised	13/07/2011	no	70
Taltz	ixekizumab	Authorised	25/04/2016	no	80
Simponi	golimumab	Authorised	01/10/2009	no	100
Kyntheum	brodalumab	Authorised	17/07/2017	no	140
Repatha	evolocumab	Authorised	17/07/2015	no	140
Zinbryta	daclizumab	Authorised	01/07/2016	no	150
Dupixent	dupilumab	Authorised	27/09/2017	no	150
Cimzia	certolizumab pegol	Authorised	01/10/2009	no	200
Kevzara	sarilumab	Authorised	23/06/2017	no	131.6 or 175
Praluent	alirocumab	Authorised	23/09/2015	no	75 or 150
Simulect	basiliximab	Authorised	09/10/1998	no	powder
Benlysta	belimumab	Authorised	13/07/2011	no	powder
Scintimun	besilesomab	Authorised	11/01/2010	no	powder
Blincyto	blinatumomab	Authorised	23/11/2015	no	powder
Adcetris	brentuximab vedotin	Authorised	25/10/2012	no	powder
Ilaris	canakinumab	Authorised	23/10/2009	no	powder
Empliciti	elotuzumab	Authorised	11/05/2016	no	powder
Remicade	infliximab	Authorised	13/08/1999	no	powder
Besponsa	inotuzumab ozogamicin	Authorised	29/06/2017	no	powder
Nucala	mepolizumab	Authorised	02/12/2015	no	powder
Xolair	omalizumab	Authorised	25/10/2005	no	powder
Synagis	palivizumab	Authorised	13/08/1999	no	powder
Keytruda	pembrolizumab	Authorised	17/07/2015	no	powder
Cosentyx	secukinumab	Authorised	15/01/2015	no	powder
Sylvant	siltuximab	Authorised	22/05/2014	no	powder
LeukoScan	sulesomab	Authorised	14/02/1997	no	powder
Herceptin	trastuzumab	Authorised	28/08/2000	no	powder
Kadcyla	trastuzumab emtansine	Authorised	15/11/2013	no	powder
Entyvio	vedolizumab	Authorised	22/05/2014	no	powder

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European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Dörries, Hans Ulrich
df-mp
Fünf Höfe
Theatinerstrasse 16
80333 München
ALLEMAGNE

Date
05.07.2018

Reference	Application No./Patent No.
AMG17203OP OPPO 01	11001632.6 - 1112 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

Summons to attend oral proceedings pursuant to Rule 115(1) EPC

You are hereby summoned to attend oral proceedings arranged in connection with the above-mentioned European patent.

The matters to be discussed are set out in the communication accompanying this summons (EPO Form 2906).

The oral proceedings, which will be public, will take place before the opposition division

on 21.01.19 at 09.00 hrs in Room 1653 at the EPO,
Grasserstr. 2, PschorrHöfe, D-80335 München

No changes to the date of the oral proceedings can be made, except on serious grounds (see OJ EPO 1/2009, 68). If you do not appear as summoned, the oral proceedings may continue without you (R. 115(2) EPC).

Your attention is drawn to Rule 4 EPC, regarding the language of the oral proceedings, and to the Special edition No. 3 OJ EPO 2007, 128, concerning the filing of authorisations for company employees and lawyers acting as representatives before the EPO.

The final date for making written submissions and/or amendments (R. 116 EPC) is 21.11.18.

You are requested to report in good time beforehand to the porter in the EPO foyer. Room 1657 is available as waiting room.

Parking is available in the underground car park, accessible only via the entrance "Grasserstrasse 2/6". On presentation of the summons to oral proceedings at the porters' lodge in the main foyer "Bayerstrasse", the parking ticket will be revoked.

1st Examiner:	2nd Examiner:	Chairman:
Trifilieff-Riolo S	Durrenberger A	Albayrak T

For the Opposition Division

Annexes:
Confirmation of receipt (Form 2936)
Rule 4 EPC (EPC Form 2043)
Communication (EPO Form 2906)

Registered letter with advice of delivery to EPO postal service: 28.06.18

Samsung Bioepis Exhibit No. 1002
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European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
05.07.2018

Reference	Application No./Patent No.
C411599EP PXT	11001632.6 - 1112 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

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Trifilieff-Riolo S	Durrenberger A	Albayrak T

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Hoffmann Eitle
Patent- und Rechtsanwälte PartmbB
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81925 München
ALLEMAGNE

Date
05.07.2018

Reference	Application No./Patent No.
198 441 u6/ne OPPO 02	11001632.6 - 1112 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

Summons to attend oral proceedings pursuant to Rule 115(1) EPC

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1st Examiner:	2nd Examiner:	Chairman:
Trifilieff-Riolo S	Durrenberger A	Albayrak T

For the Opposition Division

Annexes:
Confirmation of receipt (Form 2936)
Rule 4 EPC (EPC Form 2043)
Communication (EPO Form 2906)

Registered letter with advice of delivery to EPO postal service: 28.06.18

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European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

Application No.: 11 001 632.6

Patent No.: EP-B-2 359 834

Preparation for oral proceedings - Instructions to Support Service

Oral proceedings are to be held in connection with the above patent application

1. The matters to be discussed are set out in the annex (Form 2906)

2. Dispatch the summons using Form 2008/2310 and Form 2906 for the parties to attend on:

Day 21.01.2019 Time 09:00

Archibus

1653

Conference Room booked

ORAL 01, 02, 03 and 05 coded

Date Initials

md 28.05.18

2.1 If no room is available, notify the division on Form 2088

2.2 Parties' submissions in preparation for the oral proceedings, if any, should be made no later than

2 month(s)

before the date of the oral proceedings
(transfer to Form 2008.1 /2310.1)

2.3 Encode ORAL(04) coded

md 28.05.18

Date Initials

2.4 Dispatch Form 2008.7 /2310.7 to division

md 28.06.18

Date Initials

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3. ☐ Arrange for the following special equipment to be provided in the conference room:

....
Date Initials

4. Return the dossier to primary examiner with Form 2041 (15 days before the oral proceedings)

....
Date Initials

21/06/18
Date

Albayrak, Tibur	Durrenberger, Anne	Trifilieff-Biolo, S	.....
Chairman	2nd examiner	1st examiner	Legal member

Enclosure(s):

Europäisches Patentamt
European Patent Office
Office européen des brevets

Wichtige Hinweise zur mündlichen Verhandlung

Das Europäische Patentamt verfügt über keine eigenen Dolmetscher. Diese müssen im Bedarfsfall von außerhalb, teilweise sogar aus anderen Ländern, beigezogen werden, was mit einem hohen Aufwand an Kosten und organisatorischen Vorbereitungen verbunden ist. Muss ein Verhandlungstermin kurzfristig abberaumt werden, können Kosten für bestellte Dolmetscher nicht mehr vermieden werden.

Es wird daher gebeten, eine Simultanübersetzung nur bei wirklichem Bedarf in Anspruch zu nehmen. Es wäre wünschenswert, wenn sich die Beteiligten (zweckmäßigerweise gleichzeitig mit der Terminabstimmung) auf die Benutzung einer Amtssprache einigen könnten. Bei Verständigungsschwierigkeiten sind die Mitglieder der Einspruchsabteilung bereit zu helfen.

Die von den Verfahrensbeteiligten bevorzugte (abgestimmte) Verhandlungssprache und ggf. eine notwendige Simultanübersetzung sind dem Amt möglichst vor der in Regel 4(1) EPÜ angegebenen Frist mitzuteilen.

☐ Verfahrenssprache ist **Deutsch**

Von der/dem/den Einsprechenden wurde

☐ Englisch

☐ Französisch benutzt.

Es wird um eilige Mitteilung - möglichst per Telefax an den zuständigen Formalprüfer - gebeten,

möglichst bis

Datum **19.11.2018**

1. welche Sprache(n) Sie in der mündlichen Verhandlung verwenden (**Sprechen**)
2. aus welcher Sprache Sie eine Simultanübersetzung benötigen (**Hören**).

Important information concerning oral proceedings

The European Patent Office has no interpreters of its own. When interpreters are needed they have to be brought in from outside, sometimes even from other countries, which is costly and involves considerable organisation. If oral proceedings have to be cancelled at short notice, the cost of interpreters already engaged still has to be borne.

Please therefore make use of simultaneous interpreting facilities only where strictly necessary. If possible the parties should agree on an official language for the proceedings, preferably at the time when they arrange a date. The members of the Opposition Division will be willing to help should any communication problems arise.

The EPO should be told if possible before the period mentioned in Rule 4 (1) EPC which language the parties prefer (agree on) and whether simultaneous interpreting facilities are required.

☒ Language of the proceedings is **English**

The language used by the opponent/s was

☐ German

☐ French.

Please inform us urgently - where possible by fax addressed to the formalities officer concerned -

if possible by

Date **19.11.2018**

1. which language(s) you intend to use during the oral proceedings (**Speaking**)
2. from which language you need simultaneous interpretation (**Listening**).

Très important Procédure orale

L'Office européen des brevets ne dispose pas de son propre service d'interprètes. Aussi faut-il appel le cas échéant à des interprètes de l'extérieur, qui viennent même parfois de l'étranger, ce qui occasionne de frais élevés et demande un grand travail d'organisation. Si la date d'une procédure orale doit être annulée au dernier moment, il n'est plus possible d'éviter les frais d'interprètes.

Les parties à une procédure sont donc priées de ne demander une traduction simultanée qu'en cas de réel besoin. Il serait souhaitable qu'elles puissent se mettre d'accord en même temps qu'elles conviennent de la date sur l'utilisation d'une langue officielle comme langue des débats. Si les parties éprouvent des difficultés de compréhension lors des débats, les membres de la division d'opposition sont disposés à leur prêter leur assistance.

L'Office doit être avisé si possible avant le début du délai mentionné dans la règle 4(1) CBE de la langue préférée par les parties pour le déroulement des débat (et sur laquelle elles se sont préalablement mises d'accord) et de la nécessité éventuelle d'une traduction simultanée.

☐ La langue de la procédure est le **français**

La langue utilisée par l'opposant/les opposants était

☐ l'allemand

☐ l'anglais.

Prière d'indiquer d'urgence à l'agent des formalités compétent si possible par téléfax

si possible jusqu'au

Date **19.11.2018**

1. quelle(s) langue(s) vous utiliserez au cours de la procédure orale (**pour parler**)
2. à partir de quelle langue vous aurez besoin d'une traduction simultanée (**pour écouter**).

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Sollten Sie Ihren Antrag auf mündliche Verhandlung zurückziehen oder zum anberaumten Verhandlungstermin nicht erscheinen wollen bzw. aus wichtigem Grund daran gehindert sein, werden Sie gebeten,

- **unverzüglich das Amt - möglichst per Telefax - davon zu benachrichtigen, wobei das Schriftstück mit einem deutlichen Vermerk "Dringend, mündliche Verhandlung am ..." oder sinngemäß gekennzeichnet sein sollte;**
- **in dringenden Fällen** (weniger als 1 Monat vor dem Verhandlungstermin) zusätzlich **auch dem/die anderen Verfahrensbeteiligten** bzw. ihre(n) Vertreter auf schnellstem Weg **direkt zu unterrichten.**

In jedem solchen Fall obliegt der Einspruchsabteilung die Entscheidung, ob die Verhandlung durchgeführt oder abberaumt wird. Es wird jedoch darauf hingewiesen, dass einem Verfahrensbeteiligten, der eine nicht rechtzeitige oder unterbliebene Benachrichtigung zu verantworten hat, die dadurch den anderen Beteiligten verursachten Kosten auferlegt werden können (Art. 104 EPÜ).

Hinweis auf Regel 4 EPÜ

Regel 4
Sprache im mündlichen Verfahren

(1) Jeder an einem mündlichen Verfahren vor dem Europäischen Patentamt Beteiligte kann sich anstelle der Verfahrenssprache einer anderen Amtssprache des Europäischen Patentamts bedienen, sofern er dies dem Europäischen Patentamt spätestens einen Monat vor dem angesetzten Termin mitgeteilt hat oder selbst für die Übersetzung in die Verfahrenssprache sorgt. Jeder Beteiligte kann sich einer Amtssprache eines Vertragsstaats bedienen, sofern er selbst für die Übersetzung in die Verfahrenssprache sorgt. Von diesen Vorschriften kann das Europäische Patentamt Ausnahmen zulassen.

Should you decide to withdraw your request for oral proceedings or not wish to attend on the date set, or if for some special reason you are unable to do so, you are requested

- **to notify the EPO immediately, where possible by fax, marking the document clearly with the words "Urgent, oral proceedings on ..." or similar;**
- **in urgent cases** (less than one month before the date set for the proceedings), additionally to **notify the other party/parties** and/or their representative(s) **direct as rapidly as possible.**

In all such cases the Opposition Division will decide whether the proceedings are to go ahead or be cancelled. You should however note that costs incurred by the other parties may be charged to a party who either fails to notify them or does not do so in good time (Article 104 EPC).

Attention is drawn to Rule 4 EPC

Rule 4
Language in oral proceedings

(1) Any party to oral proceedings before the European Patent Office may use an official language of the European Patent Office other than the language of the proceedings, if such party gives notice to the European Patent Office at least one month before the date of such oral proceedings or provides for interpretation into the language of the proceedings. Any party may use an official language of a Contracting State, if he provides for interpretation into the language of the proceedings. The European Patent Office may permit derogations from these provisions.

Si vous retirez votre requête tendant à recourir à la procédure orale ou si vous ne souhaitez pas vous présenter à la date fixée pour la procédure orale ou ne pouvez vous y présenter pour une raison sérieuse, veuillez

- **en faire avis sans retard à l'Office, si possible par téléfax, en partant sur votre communication clairement la mention "Urgent, procédure orale le ..." ou une indication similaire;**
- **dans les cas urgents** (moins d'un mois avant la date fixée pour la procédure orale) **en faire avis également directement** pas la voie la plus rapide à **l'autre/aux autres partie(s)** ou bien à son/leurs mandataire(s).

Il appartient alors à la division d'opposition de décider si la procédure orale aura lieu ou non. Il est néanmoins souligné que les frais causés aux autres parties par une partie qui est responsable de l'omission d'un tel avis ou de ce que cet avis n'a pas été fait en temps utile peuvent être mis à la charge de cette partie (art. 104 CBE).

Rappel de la Règle 4 CBE

Règle 4
Langues admissibles lors de la procédure orale

(1) Toute partie à une procédure orale devant l'Office européen des brevets peut utiliser une langue officielle de l'Office européen des brevets autre que la langue de la procédure, à condition soit d'en aviser l'Office européen des brevets un mois au moins avant la date de la procédure orale, soit d'assurer l'interprétation dans la langue de la procédure. Toute partie peut utiliser une langue officielle de l'un des Etats contractants à condition d'assurer l'interprétation dans la langue de la procédure. L'Office européen des brevets peut autoriser des dérogations aux présentes dispositions.

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(2) Die Bediensteten des Europäischen Patentamts können sich im mündlichen Verfahren anstelle der Verfahrenssprache einer anderen Amtssprache des Europäischen Patentamts bedienen.

(2) In the course of oral proceedings, employees of the European Patent Office may use an official language of the European Patent Office other than the language of the proceedings.

(2) Au cours de la procédure orale, les agents de l'Office européen des brevets peuvent utiliser une langue officielle de l'Office européen des brevets autre que la langue de la procédure.

(3) In der Beweisaufnahme können sich die zu vernehmenden Beteiligten, Zeugen oder Sachverständigen, die sich in einer Amtssprache des Europäischen Patentamts oder eines Vertragsstaats nicht hinlänglich ausdrücken können, einer anderen Sprache bedienen. Erfolgt die Beweisaufnahme auf Antrag eines Beteiligten, so werden die Beteiligten, Zeugen oder Sachverständigen mit Erklärungen, die sie in einer anderen Sprache als in einer Amtssprache des Europäischen Patentamts abgeben, nur gehört, sofern dieser Beteiligte selbst für die Übersetzung in die Verfahrenssprache sorgt. Das Europäische Patentamt kann jedoch die Übersetzung in eine seiner anderen Amtssprachen zulassen.

(3) Where evidence is taken, any party, witness or expert to be heard who is unable to express himself adequately in an official language of the European Patent Office or of a Contracting State may use another language. Where evidence is taken upon request of a party, parties, witnesses or experts expressing themselves in a language other than an official language of the European Patent Office shall be heard only if that party provides for interpretation into the language of the proceedings. The European Patent Office may, however, permit interpretation into one of its other official languages.

(3) Lors de l'instruction, les parties, témoins ou experts appelés à être entendus, qui ne possèdent pas une maîtrise suffisante d'une langue officielle de l'Office européen des brevets ou d'un Etat contractant, peuvent utiliser une autre langue. Si la mesure d'instruction est ordonnée sur requête d'une partie, les parties, témoins ou experts qui s'expriment dans une langue autre qu'une langue officielle de l'Office européen des brevets ne sont entendus que si cette partie assure l'interprétation dans la langue de la procédure. L'Office européen des brevets peut toutefois autoriser l'interprétation dans l'une de ses autres langues officielles.

(4) Mit Einverständnis aller Beteiligten und des Europäischen Patentamts kann jede Sprache verwendet werden.

(4) If the parties and the European Patent Office agree, any language may be used.

(4) Sous réserve de l'accord des parties et de l'Office européen des brevets, toute langue peut être utilisée.

(5) Das Europäische Patentamt übernimmt, soweit erforderlich, auf seine Kosten die Übersetzung in die Verfahrenssprache und gegebenenfalls in seine anderen Amtssprachen, sofern ein Beteiligter nicht selbst für die Übersetzung zu sorgen hat.

(5) The European Patent Office shall, if necessary, provide at its own expense interpretation into the language of the proceedings, or, where appropriate, into its other official languages, unless such interpretation is the responsibility of one of the parties.

(5) L'Office européen des brevets assure à ses frais, en tant que de besoin, l'interprétation dans la langue de la procédure, ou, le cas échéant, dans ses autres langues officielles, à moins que cette interprétation ne doive être assurée par l'une des parties.

(6) Erklärungen von Bediensteten des Europäischen Patentamts, Beteiligten, Zeugen und Sachverständigen, die in einer Amtssprache des Europäischen Patentamts abgegeben werden, werden in dieser Sprache in die Niederschrift aufgenommen. Erklärungen in einer anderen Sprache werden in der Amtssprache aufgenommen, in die sie übersetzt worden sind. Änderungen einer europäischen Patentanmeldung oder eines europäischen Patents werden in der Verfahrenssprache in die Niederschrift aufgenommen.

(6) Statements by employees of the European Patent Office, parties, witnesses or experts, made in an official language of the European Patent Office, shall be entered in the minutes in that language. Statements made in any other language shall be entered in the official language into which they are translated. Amendments to a European patent application or European patent shall be entered in the minutes in the language of the proceedings.

(6) Les interventions des agents de l'Office européen des brevets, des parties, témoins et experts faites dans une langue officielle de l'Office européen des brevets sont consignées au procès-verbal dans cette langue. Les interventions faites dans une autre langue sont consignées dans la langue officielle dans laquelle elles sont traduites. Les modifications apportées à une demande de brevet européen ou à un brevet européen sont consignées au procès verbal dans la langue de la procédure.

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1. European patent 2 359 834 having the title "TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS BY AN INHIBITOR OF COMPLEMENT" is based upon European patent application No. 11 001 632.6 which is a divisional application of European patent application 07 753 249.7 filed on 15.03.2007. It claims priority of US 783070 filed on 15.03.2006.

Proprietor of the patent is : Alexion Pharmaceuticals Inc., Cheshire, CT 06410, US.

2. Notices of opposition have been filed by:

Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, US (O1) on 04.08.2017 and by

Hoffmann Eitle, Patent- und Rechtsanwälte PartmbB, Arabellastrasse 30, 81925 München
DE (O2) on 09.08.2017.

The opponents request revocation of the patent in its entirety as follows:

O1 contends that the subject-matter extends beyond the content of the application as filed (A. 100(c) ; A. 76(1) and A. 123(2)) and that the invention is not sufficiently disclosed (A. 100(b); A. 83).

O1 and O2 dispute the validity of the claimed priority (A. 87) in relation to the determination of the proper prior art documents.

O1 and O2 both contend that the claimed subject-matter does not meet the requirements for inventive step (A. 100(a), A. 56).

3. The proprietor (P) requests the rejection of the oppositions and maintenance of the patent as granted.

4. All parties further request oral proceedings which are hereby summoned.

5. The present communication represents the provisional and non binding opinion of the opposition division on the above topics.

The numbering of the documents referred to hereinafter is that filed by P with his letter of 22.03.2018. It will be adhered to in the rest of the procedure.

6. A. 100(c); A 76(1), A. 123(2):

The wording of granted claim 1 concerning the expression "300 mg eculizumab single-**use** dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution" is found in the original description on p. 5, l. 8-9, p. 6, l. 22-23 and p. 22, l. 12-13. In these passages, there is no mandatory link between "a single-**use** dosage form" and "a single-**unit** dosage form". Both are cited as separate possible embodiments.

Hence it seems that the requirements of A. 76(1) would be met.

7. Validity of the priority (A. 87):

The priority document US 783070 does not disclose a single-use dosage form of a liquid and sterile composition comprising 30 ml of a 10 mg eculizumab/ml and no preservative, let alone its use to treat PNH.

Thus the actual filing date of the present patent appears to be the 15.03.2007.

8. A. 100(a), A. 83:

The claimed invention is the therapeutic use of a specific composition comprising a **known** product, namely eculizumab.

As acknowledged by O1, this product was well known and used at the filing date of the patent. The example of the patent (§74) also indicates a commercial source for eculizumab. This constatation alone would appear sufficient to consider that the skilled person would have had no difficulty in carrying out the invention as claimed which is to provide a single-use dosage form of a sterile and preservative free solution comprising 30 ml of a 10 mg eculizumab /ml and to use it to treat PNH patients.

The fact that a post-published document (D25) describes the **mature** sequence of SEQ ID NO: 4 (as mentioned in the patent) whereas the patent describes the corresponding **immature** sequence does not bear any consequence on the above reasoning.

Therefore it seems that the requirements of A. 83 would be met.

9. A. 100(a), A. 56, inventive step:

All parties consider that D1 can represent the closest prior art document.

The opposition division sees no reason to differ.

D1 (§82) describes a study wherein PNH patients are treated with a weekly 600 mg intravenous infusion of eculizumab during 4 weeks, followed by 900 mg of eculizumab 1 week later and then 900 mg on a bi-weekly basis. The study was extended for a total of 2 years for 10 out of the 11 original patients.

The effects of this treatment on different physiological parameters and symptoms involved in PNH are analyzed in D1 and show that the treatment is successful.

The differences between the disclosure of D1 and the subject-matter of granted claim 1 are: eculizumab is in a single-use dosage form of a solution comprising a total amount of 300 mg of eculizumab

the eculizumab concentration is 10 mg/ml

the volume of the dosage form is 30 ml

the solution is free of preservative

The feature concerning the sterility can be regarded as implicitly disclosed by D1 since D1 describes an intravenous infusion.

According to O1 and O2, no technical effect would result from any of these differences so that the problem with respect to D1 would be the provision of a pharmaceutical composition comprising eculizumab for use in the clinical study described in D1.

More specifically, the total amount of 300 mg of eculizumab would be obvious in view of D1 itself, the feature concerning the lack of preservative would be obvious in view of common general knowledge or in view of regulatory aspects (O1; D19) or mention of such a possibility in other prior art documents (O2; D29).

In O2's view, the claimed concentration of 10 mg/ml would be part of the common general knowledge in the field of proteins/ antibodies (D20, D29 both summarized in D31 and D32).

In O1's view the concentration of 10 mg/ml would be an arbitrary choice in the context of routine experimentation when considering the teaching of D5, D20, D21 and D22. This would be emphasized by D23 which depicts assays assessing the stability of eculizumab solutions at 2, 5, 10, 30 and 50 mg/ml and shows that they are all equally stable for 13 weeks at 4°C and 25°C.

Finally, it would follow from the obvious total amount (300 mg) and from the obvious concentration (10 mg/ml) that a volume of 300 ml would also be obvious.

According to the patent proprietor (P) the differences would lead to a special technical effect which is that the solution demonstrates the ideal balance of properties for its use to treat PNH, including enhanced maintenance of biological integrity upon prolonged storage in the absence of preservative and low-volume liquid formulation. This effect would be demonstrated in post filed D33 which compares the stabilities of a 10 mg/ml and a 30 mg/ml solution of eculizumab and shows a superior stability for the 10 mg/ml solution.

P asserts that although this effect is not mentioned in the original application, it would be intimately related to the technical problem set out in the original application which is to provide a treatment for PNH because it is mandatory for any medicament, including antibodies, to have a good stability so that the post filed data of D33 would have to be taken into account in assessing inventive step.

Hence the problem in view of D1 would lie in the provision of a eculizumab dosage form having the properties mentioned above.

The skilled person would be aware in view of D20 and D21 that the formulation of antibodies is a complex area. None of D20, D21 or any other piece of prior art such as D22 or D29 would suggest to use an eculizumab solution with a concentration of 10 mg/ml, free of preservative and comprising 300 mg eculizumab to solve the problem above.

The arguments of the parties will have to be discussed at the oral proceedings.

It will have to be examined whether the technical effect demonstrated in D33 can be taken into account for assessing inventive step. The opposition division would tend to give a positive opinion on this point.

The discussion will also have to address the issues of the claimed amount and concentration of eculizumab in relation to the prior art.

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GERMANY

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Dörries, Hans Ulrich
df-mp
Fünf Höfe
Theatinerstrasse 16
80333 München
ALLEMAGNE

Date
05.07.2018

Reference	Application No./Patent No.
AMG17203OP OPPO 01	11001632.6 - 1112 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

EPA/EPO/OEB Formblatt/Form/Formulaire : F2310

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- oder per Post / or by post / ou par courrier.

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J A Kemp
14 South Square
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ROYAUME UNI

Date: 05.07.2018

Reference	Application No./Patent No.
C411599EP PXT	11001632.6 - 1112 / 2359834

Applicant/Proprietor: Alexion Pharmaceuticals, Inc.

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Date: 05.07.2018

Reference	Application No./Patent No.
198 441 u6/ne OPPO 02	11001632.6 - 1112 / 2359834
Applicant/Proprietor: Alexion Pharmaceuticals, Inc.	

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102
06. Juli 2018

Date
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Inbox	09. Juli 2018	Data admin.
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Page 6796

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Letter accompanying subsequently filed items

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The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	11001632.6
Applicant's or representative's reference	C411599EP

	Description of document	Original file name	Assigned file name
1	Acknowledgement	EPO Acknowledgement C411599EP ALS.pdf	ADVOFDELIVRY-1.pdf

	Payment	
1	Method of payment	Not specified

Signatures

Place:	
Date:	**13 July 2018**
Signed by:	**Amanda Simons 23469**
Association:	**J A Kemp**
Representative name:	**/Amanda Simons/**
Capacity:	**(Representative)**

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J A Kemp
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ROYAUME UNI

Date
05.07.2018

Reference	Application No./Patent No.
C411599EP PXT	11001632.6 - 1112 / 2359834
Applicant/Proprietor	
Alexion Pharmaceuticals, Inc.	

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Empfangsbescheinigung über den Zugang des vorstehend bezeichneten Schriftstücks
Acknowledgement of receipt of the document specified above
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We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	6517471	
Application number	EP11001632.6	
Date of receipt	13 July 2018	
Receiving Office	European Patent Office, The Hague	
Your reference	C411599EP	
Applicant	All applicants as on file	
Documents submitted	package-data.xml epf1038.pdf (1 p.)	ep-sfd-request.xml ADVOFDELIVRY-1.pdf\EPO Acknowledgement C411599EP ALS.pdf (1 p.)
Submitted by	CN=Amanda Simons 23469	
Method of submission	Online	
Date and time receipt generated	13 July 2018, 13:48 (CEST)	
Message Digest	60:F0:48:E0:74:34:92:01:3F:D4:04:56:47:A3:6C:3A:BA:D5:D1:29	

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November 19, 2018

Opposition against European patent EP 2 359 834
(Application No. 11 001 632.6)

Title:	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor:	Alexion Pharmaceuticals, Inc.
Opponent:	Amgen Inc.
Our Ref.:	AMG17203OP

Submission in Preparation of Oral Proceedings Scheduled for January 21, 2019:

This is to confirm that I will attend the Oral Proceedings scheduled for January 21, 2018 on behalf of Opponent 1 (Amgen Inc.).

I expect to be accompanied by Dr. Simon Geiger of my office. I furthermore expect to be accompanied by Tom Wrona, Senior Patent Counsel at Amgen's Patent Department.

Finally, I expect to be accompanied by our technical expert, Dr. Michael Treuheit. Dr. Treuheit's scientific qualifications will be apparent from his declaration (**D23**, Appendix D).

It is respectfully requested that Dr. Treuheit be permitted to make oral submissions during the Oral Proceedings on specific technical aspects of the case, in particular the different aspects of chemical and physical stability of antibody formulations, the content of and the data provided in his Declaration (**D23**), and the content of, and the data provided in the declaration of Dr. Miano (**D33**). Any oral submission by Dr. Treuheit would be made under my responsibility and control, as required by **G 4/95**.

H. Ulrich Dörries [1234] Dr. sc. nat., Dipl.-Biol.
David Molnia [12347] M.S. Elec. Eng., B.S. Physics
Sandra Pohlman [568] B.S. Biology
Michael Eder [1234] Dr. sc. nat., Dipl.-Chem.
Oliver Schulz [1234] Dipl.-Phys.
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Erin Becker [1] Ph.D., B.Sc. Biology
Judith Hesse [8] LL.M.
Helena Bertelsmeier [8] LL.M.
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1 European Patent Attorney
2 European Trademark Attorney
3 European Design Attorney
4 Patentanwalt
5 Solicitor (England & Wales)
6 U.S. Patent Attorney
7 U.S. Patent Agent
8 Rechtsanwalt

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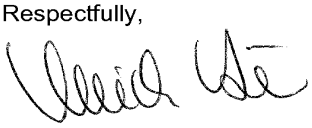
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Page 6800

dfmp

Any submissions made during Oral Proceedings on behalf of Opponent Amgen Inc., including any possible submissions by our experts, will be in English.

Assuming that the Proprietor's and the Co-Opponent's representatives will likewise make their submissions in English, no translation will be required.

Respectfully,



Dr. H. Ulrich Dörries
European Patent Attorney

UD/KS

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Page 6801

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Submission in opposition proceedings
made following summons to attend oral proceedings

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- representing the opponent(s):	Amgen Inc.
Opponent/representative's reference	AMG17203OP

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - Submission Language and attendees	AMG17203OP_Submission_attendees.pdf	OTHER-1.pdf

Signatures

Place:	**Munich, Germany**
Date:	**19 November 2018**
Signed by:	**Hans Doerries 13689**
Representative name:	**H. Ulrich Dörries**
Capacity:	**(Representative)**

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We hereby acknowledge receipt of the following submission by the opponent:

Submission number	6922420	
Application number	EP11001632.6	
Patent number	EP2359834	
Date of receipt	19 November 2018	
Your reference	AMG17203OP	
Opponent	Amgen Inc.	
Title	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement	
Documents submitted	package-data.xml ep-oppo.pdf (1 p.)	ep-opposition-data.xml OTHER-1.pdf\AMG17203OP_Submission_attendees.pdf (2 p.)
Submitted by	CN=Hans Doerries 13689	
Method of submission	Online	
Date and time receipt generated	19 November 2018, 14:39 (CET)	
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Our Ref.:	**198 441 u6/m25/ne** **Eculizumab Composition / ALEXION**
Opposition to:	**EP 2 359 834 B1 (11001632.6)**
Proprietor:	**Alexion Pharmaceuticals, Inc.** **Cheshire, CT 06410 (US)**
Opponent:	**Hoffmann Eitle** **Patent- und Rechtsanwälte PartmbB** **Arabellastr. 30** **81925 Munich (DE)**

The following observations are made in response to the preliminary opinion of the Opposition Division enclosed with their summons to oral proceedings issued on July 5, 2018.

Partnerschaftsregister Amtsgericht München | PR 1372
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1. REFORMULATION OF TECHNICAL PROBLEM AND NEW EXPERIMENTAL DATA ARE NOT ADMISSIBLE

(1) The parties have agreed that **D1** represents the closest prior art. The OD sees no reasons to differ (cf. point 9 of the preliminary opinion). Starting from **D1**, the proprietor has reformulated the technical problem which is solved by the teaching of the opposed patent to be

> *"the ideal balance of properties for use in the treatment of PNH, including the **enhanced stability** and thus maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion time and which is convenient to store."*
> (cf. submission of March 22, 2018; point 5.11.1; emphasis added)

(2) To demonstrate that the above technical problem is solved, the proprietor provides post-published experimental evidence by Dr. Miano (**D33**). In the proprietor's opinion, the experiments of **D33** would show that the eculizumab formulation at 10 mg/ml is sufficiently stable for a period of 48 months. Notably, **D33** is only concerned with long term stability of the eculizumab preparation. No data were provided to support the other alleged benefits / advantages, such as "*maintenance of biological integrity*" or an "*acceptable infusion time*".

(3) It is undisputed that the original specification contains no disclosure at all with respect to the stability of the claimed eculizumab formulation, let alone that this aspect is mentioned or merely suggested anywhere.

(4) Still, the proprietor is of the opinion that the new stability data can be used as a basis to reformulate the objective technical problem. Specifically, he points to case law (as laid down in section G-VII-11 of the Guidelines and **T184/82**), according to which it is possible to rely on new effects, provided that the skilled person would recognize

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these effects as implied by or related to the problem initially suggested. We disagree for the following reasons:

(5) The sole emphasis of the original disclosure was on the improvement of "*the quality of life of a patient*" and "*prolonging the health-adjusted life expectancy or a patient"* suffering from PNH" (cf. [0006] to [0010] of the opposed patent). Said "quality of life" and its measurement is defined in more detail in sections [0044] to [0052]. All the examples exclusively deal with clinical efficacy in the context of the improving of the patient's "*quality of life*".

(6) The proprietor now argues that the technical problem solved by the invention is to be seen in the enhancement of stability of the eculizumab formulation. We submit that this technical significance concerning the allegedly improved stability of a liquid eculizumab formulation is **not derivable** from the statements on quality of life of the patients made in the patent application. Moreover, even in the proprietor's opinion, this effect is not self-evident: he himself has indicated that the increased long-term stability was surprising.

(7) Further guidance with regard the derivability of a new technical effect or technical problem is provided by the established case law of the EPO. For example, in **T386/89** the board found that where a specific problem is identified in the description, the patentee may can put forward a

> *"**modified** version of the problem particularly if the issue of inventiveness has to be considered on an objective basis against a **new prior art** which **comes closer to the invention than that considered in the original patent application or granted patent specification**".*

(8) Reference is also made in this respect to the decision **T184/82** where the Board allowed a re-definition of the problem to such an extent that the skilled person

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"*could recognise the same as implied or related to the problem initially suggested*" (cf. point 5 of the reasons).

(9) The opposition division will appreciate that **D1** is *de facto* **no** new prior art, as it would have been required by the above case law to allow reformulation of the technical problem. As outlined in our initial opposition brief (cf. point 3.2.2), **D1** basically comprises the same disclosure as **Hillmen et al**., N Engl. J Med **2004**; 350: 552-559 **(D3)**, i.e. the latter document could likewise have served as the closest prior art. **D3** is a scientific publication corresponding to the clinical study mentioned in the Examples of **D1** and describes the details of the procedure and the results of the study in more detail.

(10) Said **Hillmen 2004** reference is cited in the description of the opposed patent (cf. **ref. 12** on page 16; 4th document in left hand column on page 31). Referring to **D3,** it is stated in [0003]:

> *"In a preliminary, 12-week, open-label clinical study in 11 PNH patients, eculizumab was shown to reduce intravascular hemolysis and transfusion requirements.[12] However, this unblinded study involved a small number of patients with no control arm and without protocol-driven transfusion standards."*

(11) Section [0003] is immediately followed by the chapter "Summary of the Invention". This makes it clear that the inventors have not only carefully considered **D1/D3**, but also acknowledged it as the closest prior art document. Accordingly, they have formulated the technical problem to overcome potential deficiencies of the prior art study, such as a small number of patients, no control arm, and no protocol-driven transfusion standards. All this concerns "***quality of life***" of the patients, but not at all stability of the eculizumab formulation. Only with unallowable hindsight one could assume that improvement of long-term stability should be the "objective" technical problem to be solved by the claimed teaching.

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(12) According to the above decisions, only if the technical the problem can be clearly deduced by the skilled person from the application as originally filed when considered in relation to the nearest prior art, reformulation of the problem would not contravene **Art. 123(2) EPC**, that is to say would not constitute added subject-matter.

(13) In this regard it is noteworthy that the whole TRIUMPH study underlying the opposed patent only lasted about **6 months** (cf. [0005], [0070], referring to a "*26 week treatment period*"). The proprietor now relies on a technical effect which – if at all – may only be visible **and** technically relevant **after two or three years** of storage of the formulation. Hence it would not have objectively been possible to identify the present technical problem of long-term stability from the present application, let alone from the closest prior art.

(14) Applying the above principles, we submit that the alleged unexpected effect, i.e. the allegedly improved long-term stability, is not deducible from the application as originally filed. It follows that it cannot be taken into account when determining the problem underlying the invention for the purpose of assessing the issue of inventive step. The post-published data of **D33** should not be admitted into the proceedings.

2. LACK OF INVENTIVE STEP (ARTICLES 100(A) AND 56 EPC)

(15) It is maintained that claim 1 of the opposed patent is obvious over **D1** (or **D3**) in combination with **D11,** or common general knowledge as reflected by **D10**.

2.1 D33 MERELY CONFIRMS COMMON KNOWLEDGE OF SKILLED PERSON BEFORE THE FILING DATE

(16) In **D33**, Dr. Miano provides data, allegedly demonstrating the improved stability of an eculizumab formulation at 10 mg/ml compared to 30 mg/ml. As outlined above, the new experiments should not be admitted into the proceedings and therefore not be

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taken into account for the assessment of inventive step. However, even if the Opposition Division considers the data relevant, they are not sufficient to support inventive step. To explain in more detail:

(17) It was common general knowledge before the filing date of the opposed patent that increasing the concentration of antibodies increases the aggregation tendency of the protein, and thus decreases its stability. In fact, there is an almost linear relationship between an increase in concentration and aggregate formation. This is reflected, e.g., by the review article **D20**:

> *"Increasing the concentration of antibodies often increases the aggregation tendency of the protein. It was demonstrated that increasing the IgG1 concentration (in 10 mM citric acid, 100 mM NaCl pH 5.5) from 2.7 mg/mL to 50 mg/mL almost linearly increased the Nephelometric Unit (NU) from 2 to 40.[52]"* (p. 9, left col., 3rd para. of **D20**)

(18) Hence, it was entirely predictable that an antibody composition formulated at 10 mg/ml shows less protein aggregation (i.e. a higher monomer content) than a composition formulated at 30 mg/ml. The data presented in **D33** merely confirm this trivial fact, which would have been obvious to the skilled person before the filing date of the opposed patent.

(19) In fact, the data submitted with **D33** illustrate the "***try-and-see***" approach the Proprietor has taken to find a concentration of 10 mg/ml. According to Dr. Miano, *"one of the stability parameters that the commercial formulation of eculizumab must meet in order to be deemed satisfactorily stable and thus suitable for release into the market is the percent of monomer antibody in the sample must be greater than or equal to 98.0%, which means that there must be less than or equal to 2.0% aggregates of the antibody […]"* (Item 3 of **D33**). In his submission, the Proprietor confirms that at least 98% monomer content represents a commercial requirement (cf. Item 5.3.4 of the Proprietor's submission dated March 22, 2018).

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(20) Having the commercial requirement of 98% monomer content in mind, and knowing that aggregate formation of antibodies increases with increasing concentration, it was a routine task for the skilled person to test a variety of antibody concentrations, and determine the storage time after which the monomer content reaches the threshold level of 98%. **D35** shows that antibody concentrations between 0.5 mg/ml and 50 mg/ml were routinely used for pharmaceutical formulations before the filing date of the opposed patent, and thus would have been included in the stability tests conducted by the skilled person.

(21) In **D33**, Dr. Miano describes the exact kind of stability studies that would have been routine to the skilled person. However, eculizumab formulations at merely two (standard) antibody concentrations were used, i.e. at 10 and 30 mg/ml. For Figure 1, these formulations were incubated at 23-27°C, in order to simulate the degradation that would occur over an extended period of time at lower temperatures. For Figure 2, the formulations were incubated at 2-8°C, which is the recommended storage temperature for eculizumab. The monomer content was assessed at various time points. However, in the experiment conducted at 2-8°C the last time point for the concentration of 10 mg/ml was taken after 24 months, whereas for the concentration of 30 mg/ml the last time point was taken after only 18 months. Any information relating to later time points is only based on extrapolation, and not on actual data, as confirmed by Dr. Miano (cf. last sentence of Item 7 of **D33**).

(22) Notably, in both of Figures 1 and 2 of **D33**, only single data points for each time point are shown for the formulation at 30 mg/ml, suggesting that only a single batch was analysed. This is contrary to the international guidelines for stability testing of biological product, which recommend providing stability information on at least 3 batches (cf. Item 4.3 of **D39**), and casts serious doubts on the reliability of any of the data for a concentration of 30 mg/ml. **D39** is filed in direct response to the Proprietor's new technical arguments regarding the stability of the claimed eculizumab composition. Thus, should the Proprietor's new technical arguments be

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admitted into the proceedings (which we object to; cf. Item 1 above), also **D39** should be admitted.

(23) Additionally, from Dr. Miano's statements in **D33** and the presented data it appears that the data for the 10 mg/ml and 30 mg/ml samples were not generated in side-by-side experiments. In Item 4 of **D33**, Dr. Miano indicates that a 30 mg/ml formulation was generated, but there is no mentioned of preparing a 10 mg/ml formulation. Consistently, according to **D33**, only the batch of 30 mg/ml (designated "ALXN5100") was subjected to the experimental procedure described in Item 5. It thus appears that only the data for the 30 mg/ml samples were generated specifically for **D33**. The source of the data for the 10 mg/ml samples remains elusive – they may have been generated for governmental approval of the 10 mg/ml eculizumab formulation. The notion that the 10 mg/ml and the 30 mg/ml samples have not been tested in side-by-side experiments could also explain the experimental differences outlined above, i.e. that only a single batch was tested for the 30 mg/ml concentration vs. several batches for the 10 mg/ml concentration, and that the samples at 10 mg/ml and 30 mg/ml were tested for different periods of time in Figure 2.

(24) If the data presented in **D33** were indeed not generated in side-by-side experiments, no reliable conclusions can be drawn from them. This is because it is known that various external (e.g., environmental) factors can significantly influence the results of biological experiments. These external factors differ from day to day. When experiments are then carried out over many months, as in the present case, the influence of these external factors multiplies, and severely distorts the experimental results. Merely to name one example, the temperatures at which the experiments of Figures 1 and 2 were performed are given as ranges, i.e. 23-27°C and 2-8°C, respectively. A high external temperature is likely to have shifted the actual temperature at which the experiments were performed close to the upper limit, and *vice versa*. Hence, it is entirely possible that the experiments for the 30 mg/ml samples were generated at a temperature close to 8°C, and the experiments for the

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10 mg/ml samples at close to 2°C. Of course, such differences would have a significant impact on degradation, which is accelerated at high temperatures. For the above reasons, no reliable conclusions can be drawn from the stability studies presented in **D33**.

(25) If the data presented in **D33** are considered despite the above-described deficiencies, Figure 2 suggests that at a temperature of 2-8°C and a concentration of 30 mg/ml, the tested eculizumab formulation reached the threshold level of 98% monomer content after about 36 months of storage. Dr. Miano concludes that *"the 30 mg/ml concentration would not meet the release standards after 36 months of storage"* (Item 5.3.7 of the Proprietor's submission dated March 22, 2018). At a concentration of 10 mg/ml, the monomer content of the eculizumab formulation after 36 months of storage was about 99.5%, and thereby above the threshold level of 98%. Accordingly, a concentration of 10 mg/ml was found acceptable.

(26) However, as mentioned above, already before the filing date of the opposed patent it was common general knowledge that aggregate formation of antibodies increases almost linearly with increasing concentration (p. 9, left col., 3rd para. of **D20**). Hence, based on Figure 2, any antibody concentration between 30 mg/ml and 10 mg/ml would have a monomer content of at least 98% after 36 months of storage, and would thus be acceptable for market release. In fact, if it was desired to obtain eculizumab compositions that are even more stable than the one formulated at 10 mg/ml, the antibody concentration could simply be reduced to below 10 mg/ml. Apparently to disguise from these trivial facts, no comparative data of eculizumab compositions formulated at any other concentrations, e.g. at 20 mg/ml or below 10 mg/ml are included in **D33**.

(27) Overall, the above demonstrates that the skilled person was in a classical "try and see" situation when finding a suitable concentration for formulating eculizumab. According to established case law, a "try and see" situation is considered to have

occurred if the skilled person, in view of the teaching in the prior art, had already clearly envisaged a group of compounds or a compound (here: standard concentrations) and then determined by routine tests whether such compound/s (here: concentrations) had the desired effect (cf. I.D.7.2 of the EPO Case Law of the Boards of Appeal).

(28) For example, in the case underlying **T1241/03**, the board considered the problem underlying the patent in suit to be the provision of an aqueous formulation of human growth hormone which remains stable during long-term storage. The Proprietor argued that that a skilled person, knowing from the prior art that the development of stable aqueous protein formulations was a risky business, would not have had a reasonable expectation of success to arrive at the claimed subject-matter upon combination of the teaching in two prior art documents. However, the board pointed out that a reasonable expectation of success does not require certainty. Thus, the board held that in spite of the understandable uncertainties which always characterise experiments using biologic compounds like proteins, the skilled person had no reason to adopt a sceptical attitude. He or she would have had either some expectations of success or, at worst, no particular expectations of any sort, but only a "try and see" attitude, which does not equate with the absence of an reasonable expectation of success (cf. Item 32 of the “Reasons for the Decision” of **T1241/03**). Accordingly, the board denied an inventive step.

(29) The present case is comparable to the case underlying **T1241/03**. However, in the present case the skilled person did not even have to combine the teachings of different prior art documents. Rather, the skilled person could simply rely and his or her common general knowledge that aggregate formation of antibodies increases with increasing concentrations. Accordingly, faced with the problem of providing a stable eculizumab formulation, he or she would have tested a number of standard concentrations and would have found that concentrations lower than 30 mg/ml, e.g. 10 mg/ml, provide for the desired stability. Just like in **T1241/03**, the skilled person

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had no reason to be sceptical that one of the tested antibody concentrations would solve the problem of providing a stable eculizumab formulation, i.e. a formulation with a monomer content of at least 98% after 36 months of storage at 2-8°C.

(30) Additionally, it is important to consider that no particular advantage of an eculizumab concentration of 10 mg/ml has been demonstrated by the Proprietor. As outlined above, based on Figure 2 any antibody concentration between 30 mg/ml and 10 mg/ml would have a monomer content of at least 98% after 36 months of storage, and would thus be acceptable for market release. Thus, the claimed antibody concentration of 10 mg/ml eculizumab represents an entirely arbitrary selection.

(31) Overall, faced with the problem of providing a stable eculizumab formulation, the skilled person was in a classic "try and see" situation. He or she would have conducted standard stability studies, such as the ones presented in **D33**. The data shown in **D33** are unreliable but, if anything, they merely confirm the skilled person's expectation that formulations are more stable at low antibody concentrations than at high concentrations. In accordance with **D33**, when performing standard stability studies the skilled person would have realized that any antibody concentration lower than 30 mg/ml would be acceptable for market release. The specific concentration of 10 mg/ml represents an arbitrary selection which is not associated with any unexpected technical effect. It is established case law of the EPO that a technical effect can only corroborate the patentability of an invention if it is unexpected or surprising. Thus, even if considered, the data of **D33** do not support an inventive step.

2.2 10 MG/ML IS A TYPICAL ANTIBODY CONCENTRATION ALSO FOR NON-IGG1 ANTIBODIES

(32) In his submission dated March 22, 2018, the Proprietor argues that based on the presented evidence, e.g. **D20**, it cannot be concluded that 10 mg/ml represents a

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typical antibody concentration in pharmaceutical formulations. To support his arguments, the Proprietor refers to **D35**, in which he presents an overview of all antibodies listed in **D20** and **D21**. Strikingly, even according to **D35**, which was prepared by the Proprietor himself, the concentration of 10 mg/ml represents the single most frequent concentration among all of the listed antibody solutions. Hence, the evidence presented by the Proprietor himself supports the view that 10 mg/ml represents a typical antibody concentration in pharmaceutical formulations, which the skilled person certainly would have considered also for eculizumab formulations.

(33) In **D35**, the Proprietor has additionally included information on the isotypes of the listed antibodies. According to **D35**, eculizumab is an IgG2/4 antibody, whereas most of the other antibodies included in the list are of the IgG1 isotype. IgG2/4 antibodies were known before the filing date of the opposed patent, as evidenced e.g. by **D40**. However, the Proprietor alleges that due to the structural differences between non-IgG1 and IgG1 antibodies, no conclusions for a suitable antibody concentration for eculizumab formulations can be drawn from prior art relating to IgG1 antibodies. Moreover, the Proprietor argues that none of the five non-IgG1 antibodies in **D35** were formulated at a concentration of 10 mg/ml. Based on these observations, the Proprietor alleges that, before the filing date of the opposed patent, a concentration of 10 mg/ml was not typical for non-IgG1 antibodies.

(34) We refute the Proprietor's allegation that a concentration of 10 mg/ml was not typical for pharmaceutical non-IgG1 antibody formulations before the filing date of the opposed patent. To support our view, we herewith submit prior art documents **D41** to **D43**, all of which demonstrate that a concentration of 10 mg/ml was also common for non-IgG1 antibodies. **D40** to **D43** are filed in direct response to the Proprietor's allegation that no conclusions for a suitable antibody concentration of non-IgG1 antibodies can be drawn from prior art relating to IgG1 antibodies. Therefore, the documents should be admitted into the proceedings.

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(35) Additionally, it should be considered that the opposed patent does not mention any significance of the antibody isotype for the stability of eculizumab formulations. The only mention of isotypes in the opposed patent is found in paragraph [0055], and relates to the recognition of IgG1 and IgG3 antibodies by phagocytic cells. The specific isotype of eculizumab (IgG2/4) is not even mentioned in the opposed patent. Hence, **D40** to **D43** are filed in response to an unpredictable technical argument by the Proprietor, and should be admitted into the proceedings also for this reason.

(36) **D41** relates to the pharmaceutical IgG4 antibody natalizumab. The particular focus of **D41** is to provide stable antibody formulations:

> *"Therefore, notwithstanding what has previously been reported in the literature, there exists a need for improved methods of formulating proteins and/or antibodies. There is also a need for stable formulations with large concentrations of antibody or protein, where the activity of the antibody or protein is retained. Also needed are stable formulations of concentrated protein which maintain a fixed volume. Applicants disclose herein stable compositions which can be further utilized to prepare antibody formulations, especially formulations with high concentrations of antibody which do not precipitate out and are stable when stored at the recommended temperatures. The highly concentrated and stable antibody formulations will greatly aid physicians in treating subjects of variable weights."* (p. 1, ll. 25-34 of **D41**)

(37) In **D41** the problem of providing a stable natalizumab composition is solved by adding phosphate buffer, polysorbate and sodium chloride. That way, pharmaceutical formulations comprising antibody concentrations between 0.1 and 200 mg/ml can be prepared (cf. p. 2, ll. 1-9 of **D41**). The concentration of 10 mg/ml is singled out on page 13, lines 2 to 5 of **D41**. Notably, Examples 11 and 12 list all ingredients of two exemplary, stable natalizumab formulations at exactly 10 mg/ml (pp. 23-24 of **D41**). Like the eculizumab composition of the opposed patent, the natalizumab compositions of **D41** are preservative-free. These data show that a

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concentration of 10 mg/ml is a typical concentration also for non-IgG1 antibody formulations, e.g. for formulations comprising IgG4 antibodies such as natalizumab.

(38) **D42** relates to the IgG4 antibody volociximab (p. 2, ll. 6-8 and p. 8, l. 22 of **D42**). The antibody targets the α5β1 integrin and inhibits the growth of cancer cells (cf. p. 1, ll. 5-7 of **D42**). On page 20, lines 19 to 23, **D42** indicates that in a preferred embodiment, the antibody is formulated as a pharmaceutical composition comprising a solution of between about 1.0 and 15.0 mg/ml antibody. **D42** further states that *"in a particularly preferred embodiment the pharmaceutical composition comprises a solution of about 10.0 mg/mL antibody, […]"* (p. 20, ll. 24-26 of **D42**). In a table listing the parameters of a phase II clinical study, the concentration of volociximab compositions for treating patients with metastatic renal cell carcinoma is indicated to be 10 mg/ml (cf. p. 41, "Dosage Form and Strength/Formulation" of **D42**). Hence, also **D42** demonstrates that a concentration of 10 mg/ml is a typical concentration for non-IgG1 antibody formulations.

(39) Finally, **D43** relates to antibodies that regulate the activity of NK cells. Although the preferred antibody is of the IgG4 isotype, reference is also made to IgG1, IgG2 and IgG3 antibodies (cf. claims 15-16 of **D43**). The preferred pharmaceutical composition comprising these antibodies has a concentration of 10 mg/ml:

> *"For example, an antibody present in a pharmaceutical composition of this invention can be supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials."* (p. 73, ll. 17-19 of **D43**)

(40) Overall, **D35** as presented by the Proprietor himself in combination with **D41**, **D42** and **D43** show that, before the filing date of the opposed patent, a concentration of 10 mg/ml was not only typical for pharmaceutical formulations of IgG1 antibodies, but also common for non-IgG1 antibodies such as IgG4. In view thereof, the skilled

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person would certainly have considered a concentration of 10 mg/ml for the IgG2/4 antibody eculizumab, e.g. when performing stability studies as outlined above.

3. CONCLUSION

(41) The data contained in **D33** should not be admitted into the proceedings. However, even if admitted, they do not support an inventive step. Moreover, before the filing date of the opposed patent, formulating antibody compositions at 10 mg/ml was common in the field, also for non-IgG1 antibodies. Thus, as laid out in our notice of opposition dated August 9, 2017, all of the features in combination as claimed in the opposed patent were obvious over **D1** (or **D3**) in combination with **D11,** or common general knowledge as reflected by **D10**.

(42) Consequently, the opposed patent does not meet the requirements of the EPC. It has therefore to be revoked *in toto*.

(43) We will use the English language in the Oral Proceedings on January 21, 2019. A translation from English into German is not required. Should any other language besides English be used by any party, translation into English is required.



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Enclosures:

D39 ICH Harmonised Tripartite Guideline on Stability Testing of Biological Products

D40 Mueller et al. (1997): Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells. Mol Immunol. 34(6): 441-452.

D41 WO 2004/071439 A2

D42 WO 2005/092073 A2

D43 WO 2006/003179 A2

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(54) Title: IMMUNOGLOBULIN FORMULATION AND METHOD OF PREPARATION THEREOF

(57) Abstract: A stable aqueous pharmaceutical formulation comprising a therapeutically effective amount of an antibody, polysorbate 80, a buffer which inhibits polysorbate oxidation is described along with methods of making the preparation. Also described are formulations with high antibody concentrations which maintain fixed volumes and which may be used on patients of variable weight.

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IMMUNOGLOBULIN FORMULATION AND METHOD OF PREPARATION THEREOF

FIELD OF THE INVENTION

The invention is directed to stable, concentrated formulations of proteins or antibodies, such as natalizumab, wherein the activity of the antibody is retained and also can be administered in a small volume and can be administered to a subject of variable weight in need thereof.

BACKGROUND OF THE INVENTION

Antibody and protein formulations are known in the art. However, preparing protein formulations, such as antibody formulations, which are chemically and biologically stable, are fraught with challenges. Preparing formulations which are also not only stable but can maintain a small volume (*i.e.,* allowing for a small volume injection) even with an increased concentration of protein, such as antibody, also is problematic. The need for such formulations exist. For example, concentrated amounts of protein in a fixed volume that is also stable would be especially beneficial to patients of variable weight. Administration of fluids to patients of variable weights may, for example, have an adverse reaction. Development of such formulations has been hindered by the proteins or the antibodies themselves, which have a high tendency to aggregate and precipitate.

SUMMARY OF THE INVENTION

Therefore, notwithstanding what has previously been reported in the literature, there exists a need for improved methods of formulating proteins and/or antibodies. There is also a need for stable formulations with large concentrations of antibody or protein, where the activity of the antibody or protein is retained. Also needed are stable formulations of concentrated protein which maintain a fixed volume. Applicants disclose herein stable compositions which can be further utilized to prepare antibody formulations, especially formulations with high concentrations of antibody which do not precipitate out and are stable when stored at the recommended temperatures. The highly concentrated and stable antibody formulations will greatly aid physicians in treating subjects of variable weights.

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One aspect of the invention provides for a stable, aqueous pharmaceutical formulation comprising an immunoglobulin (or other protein), a phosphate buffer, a polysorbate, and sodium chloride. Preferably the polysorbate is polysorbate 80, and preferably in the amount of about 0.001% to about 2.0% (w/v). Most preferably the polysorbate is present in the amount of about 0.02%. In another embodiment, the immunoglobulin or other protein is present in the formulation in an amount of about 0.1 mg/mL to about 200 mg/mL. Preferably the formulation is buffered to a pH between about 3.0 and about 7.0 and most preferably is about 6.0 ± 0.5. The formulation is preferably isotonic. The formulation may further comprise histidine. Preferably, the histidine is L-histidine.

In another aspect of the invention, the immunoglobulin of the above formulation is an anti-alpha-4 integrin antibody, such as natalizumab or another humanized antibody or monoclonal antibody. This antibody can be present in a standard amount or in a concentrated amount, *e.g.*, about 15 mg/mL or more. Preferably, the natalizumab is present in an amount from about 20 mg/mL to about 150 mg/mL. In instances wherein the formulation is present in a concentration of about 15 mg/mL or more, this formulation is maintained in a fixed volume, for example, of about 125 mL.

It is a further object of the invention to provide a method of treating a patient with variable weight for a condition with a therapeutic amount of an immunoglobulin comprising administering a formulation as described above and herein, wherein the condition is treated by administration of the formulation. It is a further aspect of the invention that the condition be one that is mediated by alpha-4 integrin, and in such conditions the immunoglobulin is one which recognizes and binds to alpha-4 integrin, such as natalizumab.

A further aspect of the invention provides for a composition comprising a sodium phosphate, a polysorbate, a protein and NaCl with a pH of 6.0±0.5, wherein the composition is stable when stored at 5°C to 8°C for a long period of time.

Another aspect of the invention provides for a method of preparing a stable protein containing formulation comprising admixing sodium phosphate, sodium chloride, a polysorbate and a protein and adjusting the pH of the mixture with phosphoric acid to about pH 6.0 ± 0.5.

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The protein may be lyophilized in the formulation of the present invention. The polysorbate is preferably polysorbate 80k, present in an amount of about 0.02% (w/v), and the protein is preferably natalizumab. The formulation may further comprise histidine.

Preferably, the the protein is lyophilized in a solution comprising 5 mM histidine, 20 mg/mL sucrose and 0.02% polysorbate 80 at a pH 6, and the protein is natalizumab at a concentration of 20 mg/mL.

It is a further object of the invention to provide for an article of manufacture comprising a container holding the stable formulation described above and herein.

Another aspect of the invention provides for a method for treating a patient with variable weight for a condition, comprising simultaneously or sequentially administering to the patient a therapeutically effective combination of a formulation described above and herein and a compound or therapy effective against the condition.

It is a further aspect of the invention to provide a use of any of the stable formulations described herein for the preparation of a medicament for the treatment of a condition wherein the medicament is effective to treat said condition. This medicament may further comprise a second compound or therapy to treat the condition.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

1. Definitions

By "protein" is meant to include but is not limited to immunoglobulins, enzymes, receptor, and fragments thereof. Although discussion of the formulation is provided mainly in reference to an antibody or immunoglobulin, other proteins are contemplated as interchangeable in the formulations disclosed.

By "immunoglobulin" is meant to include but is not limited to an antibody and antibody fragment (such as scFv, Fab, Fc, $F(ab')_2$), and other genetically engineered portions of antibodies. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM. Several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, and IgG4; IgA1 and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ), respectively. The subunit structures and

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three-dimensional configurations of different classes of immunoglobulins are well known. Preferably, the immunoglobulin recognizes and binds to alpha-4 integrin.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including agonist and antagonist antibodies), antibody compositions with polyepitopic specificity, and antibody fragments (*e.g.*, Fab, $F(ab')_2$, scFv and Fv), so long as they exhibit the desired biological activity. "Antibody" is meant to include polyclonal antibodies, monoclonal antibodies, humanized antibodies, human antibodies, primatized® antibodies and other antibodies produced via genetic engineering.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by mammalian cell expression systems or transgenic technology, uncontaminated by other immunoglobulins. For example, the monoclonal antibodies to be used in accordance with the present invention may be expressed in goats, as described by Behboodi, et al. (2002) *Transgenic cloned goats and the production of therapeutic proteins.* In *Principles of Cloning*. Elsevier Science (USA); and Meade *et al.* (1999). *Expression of recombinant proteins in the milk of transgenic animals* in *Gene expression systems: using nature for the art of expression*. J. M. Fernandez and J. P. Hoeffler ed., Academic Press. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the methods described by Shepherd *et al., Monoclonal Antibodies: A Practical Approach* (Oxford University Press, 2000).

The term "monoclonal antibodies" also includes "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or

belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity. For example, the ability to bind to alpha-4 integrin. The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described for example in Clackson *et al.*, 1991 *Nature* 352: 624-628 and Marks *et al.*, 1991 *J. Mol. Biol.*, 222: 581-597. "Humanized" forms of non-human (*e.g.*, murine, rabbit, bovine, equine, porcine, and the like) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies), which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

The expression "linear antibodies" are also included by the general term "antibody" and are a pair of tandem Fd segments (VH-CH1-VH -CH1), which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

A "variant antibody" (also included by the generic term "antibody") is a molecule which differs in amino acid sequence from a "parent" antibody's amino acid sequence by virtue of addition, deletion and/or substitution of one or more amino acid residue(s) in the parent antibody sequence. In the preferred embodiment, the variant comprises one or more amino acid substitution(s) in one or more hypervariable region(s) of the parent antibody. For

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example, the variant may comprise at least one substitution, *e.g.*, from about one to about ten, and preferably from about two to about five, in one or more hypervariable regions of the parent antibody. Ordinarily, the variant will have an amino acid sequence having at least 75% amino acid sequence identity with the parent antibody heavy or light chain variable domain sequences, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of the N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence should be construed as affecting sequence identity or homology.

To analyze such properties, one should compare a Fab form of the variant to a Fab form of the parent antibody or a full length form of the variant to a full length form of the parent antibody, for example, since it has been found that the format of the antibody impacts its activity in the biological activity assays disclosed herein. The variant antibody of particular interest is one which displays at least about 10 fold, preferably at least about 20 fold, and most preferably at least about 50 fold, enhancement in biological activity when compared to the parent antibody. The "parent" antibody is one which is encoded by an amino acid sequence used for the preparation of the variant. Preferably, the parent antibody has a human framework region and has human antibody constant region(s). For example, the parent antibody may be a humanized or a human antibody.

An "isolated antibody" is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not

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be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

"Antibody fragments" comprise a portion of an intact antibody, generally the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. "Single-chain Fv" or "sFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VH domains which enables the sFv to form the desired structure for antigen binding.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH -VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. The route of antibody administration is in accord with known methods and are well known, and may include, for example, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, or by sustained release systems. The antibody can be administered continuously by infusion or by bolus injection. Therapeutic antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. "Pharmaceutically acceptable" excipients (*e.g.*, vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed. A "stable" formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. By "stable" is also meant a formulation which exhibits little or no signs of instability, including aggregation and/or deamidation. For example, the formulations provided by the present invention may remain stable for at least two years ,when stored as indicated at a temperature of 5-8°C.

Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301 (Vincent Lee ed., New York, N.Y., 1991) and Jones, 1993 *Adv. Drug Delivery Rev.* 10: 29-90, for examples.

Stability can be measured at a selected temperature for a selected time period as exemplified by the provided examples. Storage of stable formulations if preferably for at least 6 months, more preferably 12 months, more preferably 12-18 months, and more preferably for 2 or more years.

A protein, such as an antibody or fragment thereof, "retains its physical stability" in a pharmaceutical formulation if it shows no signs of aggregation, precipitation, deamidation and/or denaturation upon visual examination of color and/or clarity, or as measured by UV light scattering or by size exclusion chromatography.

A protein "retains its chemical stability" in a pharmaceutical formulation, if the chemical stability at a given time is such that the protein is considered to still retain its biological activity. Chemical stability can be assessed by detecting and quantifying chemically altered forms of the protein. Chemical alteration may involve size modification (*e.g.*, clipping), which can be evaluated using size exclusion chromatography, SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS), for examples. Other types of chemical alteration include charge alteration (*e.g.*, occurring as a result of deamidation), which can be evaluated by ion-exchange chromatography, for example. An antibody "retains its biological activity" in a pharmaceutical formulation, if the biological activity of the antibody at a given time is within about 10% (within the errors of the assay) of the biological activity exhibited at the time the pharmaceutical formulation was prepared as determined in an antigen binding assay, for example.

By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

As used herein, "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The buffer of this invention has a pH in the range from about 3.0 to about 7.5; preferably from about pH 4.0 to about 7.0; more preferably from about pH 5.0 to about 6.5; and most preferably has a pH of about 6.0±0.5. A pH of any point in between the above ranges is also contemplated.

In a pharmacological sense, in the context of the present invention, a "therapeutically effective amount" of an antibody refers to an amount effective in the prevention or treatment

of a disorder for the treatment of which the antibody is effective. A "disorder" is any condition that would benefit from treatment with the antibody or protein. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

A "preservative" is a compound which can be included in the formulation to essentially reduce bacterial action therein, thus facilitating the production of a multi-use formulation, for example. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol.

By "patient" or "subject" is meant to include any mammal. A "mammal," for purposes of treatment, refers to any animal classified as a mammal, including but not limited to humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, and the like. Preferably, the mammal is human.

By "Antegren®" and is meant to include the antibody also known as AN100226 (antibody code number) or natalizumab (USAN name). Natalizumab is a recombinant, humanized anti-alpha-4 integrin antibody. Preferably the disease or condition being treated in the mammal is one which is modulated when a therapeutically effective dose of natalizumab is administered.

By "stable" is meant a formulation which exhibits little or no signs of instability, including aggregation and/or deamidation. In addition, "stable" may also refer to a formulation which does not exhibit any signs of instability for greater than or equal to two years ,when stored as indicated at

2. General Description

In the discussion below and the examples to follow, formulations for stable antibody formulations are disclosed. Certain stable formulations disclosed have high concentrations of

antibody but maintain a fixed volume, wherein the antibodies in these formulations are stable and the antibody does not precipitate out of solution or aggregate. Proteins other than antibodies are also contemplated for the high concentration formulations.

Antibodies are typically administered to a subject (*e.g.*, a human) at a concentration of about 0.01 mg/mL to about 200 mg/mL. More typically, antibodies range in concentration from about 0.1 mg/mL to about 150 mg/mL. However, instances exist when greater concentrations are required to be administered to a patient, *e.g.*, about 15 to about 200 mg/mL, more preferably about 15 mg/mL to 150 mg/mL, more preferably about 20 to about 50 mg/mL, and most preferably about 20 mg/mL and any integer value in between.

The antibody formulation may be administered to a mammal in need of treatment with the protein, in accordance with known methods. These methods may include, but are not limited to intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. In preferred embodiments, the antibody formulation is administered to the mammal by intravenous administration.

The appropriate dosage of the protein will depend, for example, on the condition to be treated, the severity and course of the condition, whether the protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the protein, the type of protein used, and the discretion of the attending physician. The protein is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The protein may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question. As used herein, two (or more) agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act contemporaneously.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic agents. In certain preferred embodiments, the compounds of this invention may be co-administered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. For example, the formulations of this invention can be administered in combination with other therapeutic agents or physical therapies for the treatment of rheumatoid arthritis, multiple schlerosis and Crohn's Disease.

2.1 Method of Making The Antibody Formulation

The process can be altered as would be known to the skilled artisan, but generally would follow a procedure such as the following. Obtain an ampoule from a working cell bank which contains cells which make the antibody or protein of interest. Prepare the inoculum. Culture or ferment the cells with additional feedings as is necessary. Harvest/clarify the cells by centrifugation and/or filtration. This can be done for example by concentrating the cells 10 fold by spiral wound filtration. Filter by a 0.2 μm intermediate filtration followed by purification by protein A Sepharose Fast Flow® (*i.e.*, affinity chromatography) and reverse elution. The antibody containing composition then receives a treatment at pH 3.6-3.7. The mixture then receives a viral filtration followed by a concentration/diafiltration step. The composition can then be purified by DEAE Sepharose Fast Flow® (anion exchange). This step can be performed multiple times. From this point, the composition is then further concentrated followed by a purification step using Sephacryl S300HR® (*i.e.*, gel filtration chromatography) system, wherein the running buffer used is phosphate/NaCl. The antibody containing composition can be further concentrated for the high concentration formulations (*e.g.*, 20 mg/mL or more) if so desired. The antibody containing composition is then further buffered and the concentration adjusted by adding 0.02% (w/v) polysorbate 80. This composition then receives a final filtration using a 0.2 μm filter and can be dispensed at this point into 100 mL to 10 L polypropylene bottles. The antibody or immunoglobulin so obtained can then be QC tested and QA released.

The above can be done, for example for natalizumab, as diagramed below:

200L Material (5.0 mg/mL)	2000L Material (5.0, 20 mg/mL)
Ampoule from Working Cell Bank	Ampoule from Working Cell Bank
↓	↓
Inoculum Preparation	Inoculum Preparation
↓	↓
Fermentation	Fermentation (Additional Media Feed)
↓	↓
Harvest/Clarification by filtration: Concentration 10x by spiral wound filtration	Harvest/Clarification by centrifugation & filtration: Concentration 10x by spiral would

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200L Material (5.0 mg/mL)	2000L Material (5.0, 20 mg/mL)
	filtration
↓	↓
0.2 μm Intermediate Filtration	0.2 μm Intermediate Filtration
↓	↓
Purification by Protein A Sepharose Fast Flow® (Affinity Chromatography) (Forward Elution)	Purification by Protein A Sepharose Fast Flow® (Affinity Chromatography) (Reverse Elution)
↓	↓
pH 3.7-3.8 treatment	pH 3.6-3.7 treatment
↓	↓
--	Viral filtration
↓	↓
Concentration/Diafiltration	Concentration/Diafiltration
↓	↓
Purification by DEAE Sepharose Fast Flow® (Anion Exchange Chromatography) (Single Cycle)	Purification by DEAE Sepharose Fast Flow® (Anion Exchange Chromatography) (Multiple Cycles)
↓	↓
Concentration	Concentration
↓	↓
Purification by Sephacryl S300HR® (Gel Filtration Chromatography) (Running Buffer: histidine/NaCl)	Purification by Sephacryl S300HR® (Gel Filtration Chromatography) (Running Buffer: phosphate/NaCl)
↓	↓
--	(Additional concentration for 20 mg/mL)
↓	↓
Buffer & Concentration Adjustment Phosphate/NaCl, 0.02% (w/v) polysorbate 80	Buffer & Concentration Adjustment (add 0.02% (w/v) polysorbate 80)
↓	↓
0.2 μm Final Filtration and Dispensing (100 mL- 1 L polypropylene bottles)	0.2 μm Final Filtration and Dispensing (100 mL - 10L polypropylene bottles)
↓	↓
QC Testing and QA Release of Bulk Purified AN100226	QC Testing and QA Release of Bulk Purified AN100226

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2.2 The Antibody Formulation

In one aspect of the invention, an immunoglobulin is formulated in concentrations of about 1.7, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0 or 50.0 mg/mL in 10 mM sodium phosphate, 140 mM NaCl (pH 6.0 ± 0.5) and 0.02% polysorbate 80. If necessary, the pH is adjusted to 6.0 ± 0.5 with phosphoric acid.

One example of the different formulations is displayed below.

QUANTITATIVE COMPOSITION: PHOSPHATE BUFFER FORMULATION

Component	Function	Unit Formula (per mL) 1.7 mg/mL	Unit Formula (per mL) 5.0 mg/mL	Unit Formula (per mL) 20 mg/mL
Active Ingredient:				
Anti-α4 integrin humanized monoclonal antibody	Active	1.7 mg	5.0 mg	20 mg
Other Ingredients:				
Sodium Phosphate, USP	Buffer and tonicity	1.4 mg	1.4 mg	1.4 mg
Sodium chloride, USP	Buffer and tonicity	8.2 mg	8.2 mg	8.2 mg
phosphoric acid, NF	Adjust pH to 6.0 ± 0.5	QS	QS	QS
Polysorbate 80, NF	Inhibit protein aggregation	0.2 mg	0.2 mg	0.2 mg
Water for Injection, USP	Diluent	QS to 1 mL	QS to 1 mL	QS to 1 mL

Any of the above formulations are optimally vialed in an aseptic vial. These vials can be, for example, Type I EP neutral glass vials (*e.g.*, 5.0 or 20 mL fill vials) with Helvoet Pharma V9145/FM 157/1 gray butyl rubber stoppers with aluminum seals. However other suitable aseptic vials are also contemplated. For example, the formulations can be bottled as follows:

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BOTTLING
Compounding of phosphate/sodium chloride/polysorbate 80 buffer, if needed
↓
Sterilization of vials, stoppers and manufacturing equipment
↓
Dilution with phosphate/sodium chloride/polysorbate 80 buffer antibody to 1.7 or 50 mg/mL, as required
↓
Sterile filtration
↓
Aseptic filling and stoppering of vials
Capping
↓
Release Testing

More specifically, natalizumab obtained for example by the procedures discussed above, can be bottled as follows. Natalizumab 200 L and 2000 L drug product can be filled on a fully automated filling line equipped with a vial washing, sterilization and depyrogenation tunnel. Stoppers, seals and filling equipment are washed and sterilized prior to use. This process allows for large scale tilling operations consistent with the volumes produced from the 2000 L fermentation.

When necessary, the formulation buffer, about 10 mM phosphate, about 140 mM NaCl, pH 6.0 ± 0.5, about 0.02% polysorbate 80, can be compounded in a Class 10,000 suite and used to dilute the bulk drug product to the final concentration. In-process specifications of concentration, pH and density are preferably reached prior to filtration.

The formulated natalizumab or other immunoglobulin can be sterile filtered through a 0.2 μm Millipak filter into a stainless steel surge tank inside the sterile core. Filling, stoppering and capping of natalizumab is fully automated. In-process samples for bulk sterility are collected; fill weight and headspace testing is conducted throughout the filling operation. Filled drug product is stored under refrigeration at about 2-8°C.

Filling occurs within a fully validated Class 100 sterile core. The filling line is validated to provide fill volumes within expected tolerances for the 5.0 mL and 20 mL fill volumes. Comprehensive environmental monitoring is conducted throughout the filling operation and reviewed to verify continual compliance with this standard. Media fills are conducted on a quarterly basis to support aseptic filling operations.

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Alternatively, the 200 L drug substance material produced can be bottled pursuant to the following example. Vials, filling needles, the filtration assembly and tubing are prepared and sterilized prior to use. Stoppers can be prepared by the supplier. The stoppers are then sterilized prior to filling.

Natalizumab drug product or other protein is filled on a semi-automatic filling line, with batch preparation of components, automated filling, immediate stoppering, and a subsequent capping operation. This operation is appropriate for small batch scale operations.

The final bulk solution is then sterile filtered through a 0.2 μm Millipak filter into a sterile glass receiving vessel within a Class 100 environment. Regular calibration and in-process checks ensure that the filling tolerance remains within ± 2%. Vials are stoppered and capped immediately. Filled drug product is preferably stored refrigerated at 2-8 °C.

The glass receiving vessel can be any number of vials, but can for example be a 5.0 or 20 mL neutral glass vial Type I (EP) supplied by, for example, Epsom Glass or AMILCO or a 5.0 mL or 20 mL USP Type I borosilicate glass vial, supplied by, for example Kimble or Wheaton. These vials can use any means of closure suitable. Vial closures include, but are not limited to a 13 mm Helvoet Pharma V9145/FM 157/1 grey butyl rubber stopper or a 13 mm and 20 mm Helvoet Pharma V9145/FM 157/1 grey butyl rubber stopper or a 13 mm or 20 mm West 4432/50 gray butyl rubber stopper. The rubber-stoppered bottled is then sealed most typically using an aluminum seal, such as that manufactured by West.

Although the present invention has been described in detail with reference to examples below, it is understood that various modifications can be made without departing from the spirit of the invention, and would be readily known to the skilled artisan.

EXAMPLES

Example 1

Selection of Polysorbate 80

The typical method of administering natalizumab is intravenous. Intravenous administration requires the final formulation to be isotonic. A formulation of AN100226 (natalizumab), 5 mg/mL in 50 mM L-histidine, 150 mM NaCl, pH 6.0 was initially chosen (Formulation #1). During a Phase II study, protein precipitation of the antibody was

observed during the dilution and introduction of natalizumab into the clinical dosing apparatus. Polysorbate 80 was introduced into the formulation (Formulation #2) to resolve the observed protein precipitation. Preferably, the polysorbate for use with the present invention is low in peroxide, *i.e.*, polysorbate from Sigma, Product number P6479, Lot Number 071K7283.

The two factors that have been shown to accelerate the precipitation of the AN100226 antibody are the presence of trace levels of silicone oil and denaturation at the air-liquid interface. The silicone oil was introduced into the product upon use of standard lubricated polypropylene syringes equipped with siliconized rubber stoppers. The introduction of the silicone oil is sufficient to cause discernible antibody precipitation in Formulation # 1 upon gentle agitation and room temperature storage. The aggregation, deamidation and subsequent precipitation caused by denaturation at the air-liquid interface has become more discernable problematic with the drug being shipped to more clinical sites. Both causes of protein precipitation have been resolved by the addition of polysorbate 80 at a concentration of 0.02% (w/v).

Formulation #2 shows comparable stability to the histidine/NaCl formulation (Formulation #1) in all protein characterization assays, while providing increased stability during product shipping and handling in the clinical setting.

The addition of polysorbate 80 to the formulation also overcomes the problem of precipitating or aggregating antibody when preparing formulations with higher protein content. Initial work focused on agitation-induced aggregation at high protein concentrations, including 50 mg/mL. By subjecting the material to agitation using a vortex-type mixer, aggregated species were detected by size exclusion-high performance liquid chromatography (SEC-HPLC). This model identified polysorbate 80 as an effective inhibitor of aggregation, while sucrose and other buffering components had little beneficial effect.

The effectiveness of the addition of 0.02% (w/v) polysorbate 80 in preventing agitation-induced precipitation at a protein concentration of 5 mg/mL was assessed following addition of 10 μL of a 10% polysorbate 80 solution to vials of natalizumab (Lot No. AN100226-0003). The vials were shaken on their sides along with several vials of natalizumab in Formulation #1 at 150 rotations per minute in a horizontal plane. Within 3 hours of this treatment at room temperature, the vials of Formulation #1 were laden with

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particles and appeared turbid while the vials with 0.02% (w/v) polysorbate 80 remained clear and free of particles.

The observed aggregation is presumed to be caused by the air-surface interface, as vials completely filled with AN100226 in the absence of polysorbate 80 were shaken for extended periods of time without additional particle formation being induced.

An evaluation of the ability of 0.02% (w/v) polysorbate 80 to inhibit the protein precipitation facilitated by trace levels of silicone was conducted. A vial of natalizumab (Lot No. AN100226-0003) was adjusted to 0.02% (w/v) polysorbate 80 and drawn into a commercially available, lubricated 60 mL polypropylene syringe. The material was allowed to stand for several hours at room temperature. Visual inspection confirmed that no precipitation was occurring. The material was then filtered through a 0.2 μm filter into a 5-mL vial and inspected and found to be substantially free of particles after several days, while vials treated in the same manner in the absence of polysorbate 80 (Formulation #1) were laden with particles.

Example 2

Selection of The Phosphate Buffer

During release testing of the histidine placebo (containing 0.02% w/v polysorbate 80), new trace impurities were detected. These impurities arose from degradation of polysorbate 80, apparently through an oxidation reaction involving metal ions and histidine. For active natalizumab drug product, these trace impurities have been detected only after storage at elevated temperatures (*e.g.*, 25°C and 40°C). Thus, the decision was made to modify the placebo and use phosphate to replace histidine in the buffer. The lot of histidine used in the product placebo was different than that used for the active natalizumab drug product lots AN100226-004 and AN100226-005. The impact of the source of histidine on polysorbate 80 degradation is discussed in greater detail infra.

During testing of the placebo, trace impurities were detected in the histidine/NaCl/0.02% (w/v) polysorbate 80 placebo (Formulation #2). These impurities were detected by their absorbency in the low wavelength ultraviolet region. The absorbency profiles from 200 to 400 nm for placebo stored at 5°C, 25°C, and 40°C for one month were

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determined. These data indicate that the impurities are increasing as a function of temperature.

The size exclusion HPLC method used to monitor antibody aggregation was modified to increase sensitivity for detection of the trace impurities by increasing the column load 5-fold to 100 µL, and the sample is applied undiluted rather than 10-fold diluted. In addition, the absorbency is monitored at 260 nm to reflect the absorbency maximum of the impurities. The method provides a tool to evaluate the presence of these trace impurities in both placebo and product, as the antibody emerges much earlier in the elution profile.

Placebo and natalizumab final drug product formulated in Formulations #1 and #2 were analyzed by the SEC-HPLC method described above. Analysis of the placebo in Formulation #1 spiked with polysorbate 80 to 0.02% (w/v) was performed just prior to chromatography. This shows the UV absorbency of polysorbate 80, histidine, and salt in the absence of the trace impurities. The broad peak at 16 minutes is associated with polysorbate 80 while the peaks at approximately 26-27 minutes are attributed to histidine and salt. The histidine/NaCl/0.02% (w/v) polysorbate 80 placebo (Formulation #2) stored at 5 °C for two months shows a marked increase in the late eluting peaks which implicates polysorbate 80 in the production of these impurities. In addition, the eluting at 16 minutes disappeared, indicating that the polysorbate 80 has been degraded.

AN100226 stored as bulk drug substance for approximately 5 months at 5 °C in Formulation #1 was analyzed after spiking with polysorbate 80 to 0.02% (w/v) just prior to chromatography. The antibody elutes at 16-18 minutes using these overload conditions. The remainder of the profile resembles the placebo with polysorbate 80 spike. The two-month stability samples for natalizumab lot #AN100226-0004 were also analyzed by this method. The elution profile for the 5 °C natalizumab sample indicates the absence of any additional peaks and comparable levels of the histidine/salt peaks at 26-27 minutes. The trace impurities are detected at two months for the 25 °C sample, and elevated levels are present for the two month 40 °C sample. Thus, these impurities have not been detected in the clinical supplies, which are stored at 5 °C. The appearance of these trace impurities is occurring much more rapidly in placebo than in natalizumab.

To avoid the formation of these impurities in the placebo, histidine was replaced with inorganic buffering components in the placebo formulation. The placebo product for the

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clinical trials is a sterile isotonic phosphate buffered solution with 0.02% (w/v) polysorbate 80 at pH 6.0. The substitution of histidine with phosphate was demonstrated to significantly reduce the rate of polysorbate 80 degradation. 100 μL of the phosphate/NaCl/0.02% (w/v) polysorbate 80 placebo formulation was analyzed by size exclusion HPLC monitored at 260 nm both at time zero and after 3 days at 60°C. Little change is seen in the SEC-HPLC profile as a result of this incubation. The SEC-HPLC profile for the histidine/NaCl/0.02% (w/v) polysorbate 80 formulation following only two days at 60°C shows significant levels of trace impurities related to the degradation of polysorbate 80. These data demonstrate that the polysorbate 80 degradation is significantly impeded by replacing histidine with phosphate in the placebo formulation.

Example 3

Natalizumab Formulation with Polysorbate 80 and Histidine Combined

The mechanism by which these trace impurities are produced is thought to be through a metal catalyzed oxidation of polysorbate (*see* Donbrow *et al.*, 1978 *J. Pharmaceutical Sciences*, 67(12): 28). Donbrow describes that autoxidation during storage occurs for different types of polysorbate (*e.g.*, polysorbate 20). Light, temperatures and metal ions also impact autoxidation. Donbrow *et al.* (1978). It has been confirmed that both histidine and polysorbate 80 are required for this reaction to proceed at a significant rate. Ajinomoto is the single histidine source used for formulation. However significant differences in the rate of reaction between lots supplied by the Ajinomoto have been observed.

An additional factor that plays a role in accelerating the reaction is the presence of metal. This was demonstrated by running a reaction at 60°C for five days in glass vessel with 50 mM histidine (Lot No. R016A008) and 2% (w/v) polysorbate 80. Under these conditions, minimal levels of the trace impurities are produced for this histidine lot. The reaction was then split into three vessels: (1) the first vessel remained as the control; (2) a gray butyl container closure (stopper) was added to the second vessel; and (3) a stainless steel needle was added to the third vessel. These reactions were then run for four days at 60°C and analyzed by a UV scan from 200 to 400 nm. The reaction progressed further over this time period in the presence of the needle.

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Example 4

Evaluation of Impurities — Mouse Single-Dose Limit Test

The potential toxicity of these impurities was evaluated in a mouse single-dose limit test. Histidine placebo and natalizumab samples in Formulation #2 stored at 40°C for six weeks were used because these samples provide the largest amount of the impurities. There were no signs of toxicity due to the impurities. Preliminary data are provided in the non-clinical section of the submission.

The SEC-HPLC profiles for 100 μL injections of samples used in the mouse single-dose limit test were determined. The natalizumab sample in Formulation #1 spiked with polysorbate 80 prior to analysis has little 260 nm absorbing material eluting after 20 minutes. The peak eluting at 34 minutes was found in this lot of natalizumab without polysorbate 80 addition and therefore does not indicate polysorbate 80 degradation. By contrast, the placebo in Formulation #2 stored at 40°C for six weeks, showed complete degradation, as the total area under the curve was 12.8 million μV•seconds. The natalizumab sample in Formulation #2 and stored at 40°C for six weeks had less complete degradation. The analysis confirms that the samples stored at 40°C and tested in the mouse single-dose limit test are laden with degraded polysorbate 80.

Example 5

Establishing Impurity Specifications

To assess if partially degraded polysorbate 80 is still capable of preventing antibody aggregation, Formulation #2 was heated to 60°C for 3 days in the presence of a needle to convert all the polysorbate 80 to the maximum levels of impurities. The reaction was confirmed to have degraded all the polysorbate 80 by both SEC and reverse phase HPLC. This material was diluted one-to-one with a 10 mg/mL solution of AN100226 in Formulation #2 resulting in a solution containing 0.01% (w/v) polysorbate 80 and 50% of the maximum level of degraded polysorbate 80. These antibody solutions were exposed to both shaking and siliconized syringes for several hours; aggregation of the antibody was prevented. The control samples exposed to the same conditions but without polysorbate 80 showed significant precipitation.

It is concluded from this work that as long as no more than fifty percent of the polysorbate 80 is degraded, this material can provide the appropriate environment to prevent antibody aggregation.

Although very little polysorbate degradation is occurring in the active drug product, monitoring the isoelectric focusing (IEF) pattern is done to confirm that this formulation is not compromising the antibody. IEF profiles for the six week stability time points for 5°C, 25°C and 40°C storage conditions were determined. Both the 5°C and 25°C samples are comparable to the reference standard, with no evidence of any change in overall charge of the protein. The 40°C sample showed the shift to a more acidic species typical of this product in a liquid formulation either in the presence or absence of polysorbate 80.

To establish a relationship between the extent of polysorbate 80 degradation and the peak area from the SEC-HPLC, a dilution series for six concentrations of degraded polysorbate 80 from 0 to 50% was evaluated. Formulation #2 was heated to 60°C for 3 days with a needle and confirmed to be 100% degraded by SEC and reverse phase HPLC. The reaction mixture was then diluted with AN100226 purified bulk in Formulation #1, freshly spiked with 0.02% (w/v) polysorbate 80 and analyzed by SEC-HPLC. The SEC profiles were monitored at 260 nm.

The data from this dilution series were plotted by the total peak area integrated (μV•seconds) for the absorbency profile after approximately 24 minutes as a function of the ratio of degraded polysorbate 80. This plot indicates that an area of less than 5 million μV•seconds represents about a 50% loss of polysorbate 80 to degradation products. There is a direct relationship between the amount of oxidized polysorbate 80 added and the peak area for the peaks eluting late in the chromatogram.

A preliminary limit for these trace impurities in natalizumab has been established based on the mouse single-dose limit test, the antibody solubility data with 50% degraded polysorbate 80, and estimates of the extent of the polysorbate 80 degradation. The method has been included in the ongoing stability program and a limit has been established. If additional lots are manufactured with histidine buffer, these limits will be applied at time of release as well.

Limit: total area under the peaks after approximately 24 minutes is not to exceed 4 x 106 μV•seconds.

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Example 6

1.7 mg/mL Natalizumab Formulation

1.7 mg Natalizumab
1.4 mg Sodium phosphate, USP
8.2 mg Sodium chloride, USP
0.2 mg Polysorbate 80, NF

Adjust pH to 6.0 ± 0.5 with phosphoric acid, NF. QS to 1 mL. With preferred storage at 5-8°C.

Example 7

5.0 mg/mL Natalizumab Formulation

5.0 mg Natalizumab
1.4 mg Sodium phosphate, USP
8.2 mg Sodium chloride, USP
0.2 mg Polysorbate 80, NF

Adjust pH to 6.0 ± 0.5 with phosphoric acid, NF. QS to 1 mL. With preferred storage at 5-8°C.

Example 8

20 mg/mL Natalizumab Formulation

20.0 mg Natalizumab
1.4 mg Sodium phosphate, USP
8.2 mg Sodium chloride, USP
0.2 mg Polysorbate 80, NF

Adjust pH to 6.0 ± 0.5 with phosphoric acid, NF. QS to 1 mL. With preferred storage at 5-8°C.

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Example 9

50.0 mg/mL Natalizumab Formulation

50.0 mg Natalizumab
1.4 mg Sodium phosphate, USP
8.2 mg Sodium chloride, USP
0.2 mg Polysorbate 80, NF

Adjust pH to 6.0 ± 0.5 with phosphoric acid, NF. QS to 1 mL. With preferred storage at 5-8°C.

Example 10

5.0 mg/mL Natalizumab Formulation

5.0 mg/mL natalizumab
140 mM NaCl
0.02% Polysorbate 80 (w/v)
10 mM sodium phosphate

Adjust pH to 6.0 ± 0.5 with phosphoric acid. Optimally store formulation at about 5°C to about 8°C.

Example 11

10 mg/mL Natalizumab Formulation

10.0 mg Natalizumab
1.4 mg Sodium phosphate, USP
8.2 mg Sodium chloride, USP
0.2 mg Polysorbate 80, NF

Adjust pH to 6.0 ± 0.5 with phosphoric acid, NF. QS to 1 mL. With preferred storage at 5-8°C.

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Example 12

10 mg/mL Natalizumab Formulation

10.0 mg Natalizumab
1.4 mg Sodium phosphate, USP
8.2 mg Sodium chloride, USP
0.1 mg Polysorbate 80, NF

Adjust pH to 6.0 ± 0.5 with phosphoric acid, NF. QS to 1 mL. With preferred storage at 5-8°C.

Example 13

20 mg/mL Natalizumab Formulation

20.0 mg/mL Natalizumab
140 mM NaCl
0.02% Polysorbate 80 (w/v)
10 mM sodium phosphate

Adjust pH to 6.0 ± 0.5 with phosphoric acid and bring volume to 125 mL. Optimally store formulation at about 5°C to about 8°C.

Example 15

Lyopholized Natalizumab Formulation

Additional liquid formulations of antibody at high concentration, from 20 –200 mg/mL may consist of phosphate or other suitable buffer (such as histidine, citrate, acetate or succinate) in the concentration range of 2 to 50 mM, to provide buffering in the pH range of 3.0 to 7.0. Most preferably, the pH is 6.0, +/- 0.5. The addition of polyols (such as sorbitol and mannitol), disaccharides (such as sucrose or trehalose) and amino acids (such as glycine) may be added in varying amounts with sodium chloride to maintain stability and provide an isotonic solution. The use of surfactants, such but not limited to the polysorbates, add stability when used in the range of 0.001 to 2%. To prepare a liquid formulation,

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natalizumab was concentrated to 65 mg/mL in 10 mM sodium phosphate, 140 mM sodium chloride, pH 6, with about 0.06% polysorbate 80. The resulting solution was slightly opalescent but without particulates. The sample contained greater than 99% monomer with no high molecular weight aggregate or low molecular weight species by SEC.

A stable lyophilized pharmaceutical formulation is provided. Because phosphate buffer undergoes a pH change during freezing, it is necessary to replace the phosphate with a different buffer. This buffer may be comprised of histidine, citrate or succinate,with the ability to buffer effectively in the pH range of 3.0 to 7.0, most preferably in the range of 6.0 +/- 0.5.

The use of polyols (such as mannitol) and sugars (such as sucrose) are necessary to provide cryo- and lyo-protection. These polyols may be used alone or in a combination to provide for stability and adjustment of the tonicity. Additionally, the use of amino acids (such as glycine), at levels of 10-1000 mM may used to prevent aggregation.

Surfactants, such as polysorbates or poloxamers, may be used at levels from 0.001% to 2.0% to provide for stability before lyophilization and after reconstitution and to provide for more rapid reconstitution times.

The protein, following the final purification step, may be formulated using ultrafiltration for concentration and diafiltration for buffer exchange. The protein may also be formulated using column chromatography for buffer exchange. Some combination of these techniques may also be used.

In addition, the final desired protein concentration may be obtained by filling at a protein and excipient concentration lower than desired and reconstitution at a smaller volume. For example, a 2.5 mL fill volume of a 40 mg/mL solution may be used, followed by reconstitution with 1 mL to obtain a 100 mg/mL solution.

For example, natalizumab, at a concentration of 20 mg/mL, was lyophilized in a solution containing 5 mM histidine, 20 mg/mL sucrose and 0.02% polysorbate 80, pH 6. The solution was filled at 5mL per vial into 10 mL borosilicate glass vials and fitted with gray butyl rubber lyophilization stoppers. Lyophilization was done using a Virtis Gensis model lyophilizer. The product was frozen at a shelf temperature of -60° C for 10 hours and then the shelf temperature was raised to -40°. Primary drying was performed at a shelf temperature of -10° C and a chamber pressure of 100 mTorr for 20 hours. Secondary drying

was achieved at a shelf temperature of 25°C with a chamber pressure of 100 mTorr for 10 hours. The vials were stoppered under vacuum.

The vials were then reconstituted using 1 mL of sterile WFI to give a formulation containing 100 mg/mL natalizumab. The samples were analyzed immediately after lyophilization and after 2 wks of storage in the lyophilized form at 40 deg. In both cases reconstitution times were immediate. The reconstituted solutions were clear and colorless with an absence of particulate material. The samples contained greater than 99% monomer by SEC, with no high molecular weight aggregate, or low molecular weight species. After 2 weeks storage at 40 deg, the sample showed 94% potency relative to reference (specification 80-125%).

All cited patents and publications referred to in this application are herein incorporated by reference in their entirety for all purposes.

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Claims:

We claim:

1. A stable, aqueous pharmaceutical formulation comprising an immunoglobulin, a phosphate buffer, a polysorbate, and sodium chloride.

2. The formulation of claim 1, wherein the polysorbate is polysorbate 80.

3. The formulation of claim 2, wherein the polysorbate 80 is present in an amount of about 0.001% to about 2.0% (w/v).

4. The formulation of claim 3, wherein the polysorbate 80 is present in the amount of about 0.02% (w/v).

5. The formulation of claim 1, wherein the immunoglobulin is present in the amount of about 0.1 mg/mL to about 200 mg/mL.

6. The formulation of claim 5, wherein the immunoglobulin is present in the amount of about 1.7 mg/mL.

7. The formulation of claim 5, wherein the immunoglobulin is present in the amount of about 5 mg/mL.

8. The formulation of claim 5, wherein the immunoglobulin is present in the amount of about 20 mg/mL.

9. The formulation of any one of claims 1 to 8, wherein the formulation has a pH of about 3.0 to about 7.0.

10. The formulation of claim 9, wherein the pH is about 5.5 to about 6.5.

11. The formulation of claim 10, wherein the pH is about 6.0±0.5.

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12. The formulation of claim 1, wherein the formulation is in a fixed volume and the immunoglobulin is present in the amount of about 50 mg/mL.

13. The formulation of claim 12, wherein the immunoglobulin binds alpha-4 integrin.

14. The formulation of claim 13, wherein the immunoglobulin is natalizumab.

15. The formulation of claim 1, wherein the phosphate buffer is at pH 6.0± 0.5, the polysorbate is polysorbate 80 and is present in an amount of about 0.02% (w/v), the immunoglobulin is natalizumab, and wherein the formulation is stable at a temperature of about 2°C to about 8°C for at least 6 months.

16. The formulation of claim 15, wherein natalizumab is present in an amount of about 20 mg/mL to about 150 mg/mL.

17. The formulation of claim 1, wherein the formulation is isotonic.

18. The formulation of any one of claims 1-14 or 17, wherein the immunoglobulin is a monoclonal antibody.

19. The formulation of claim 18, wherein the monoclonal antibody is natalizumab.

20. The formulation of claim 18-19, wherein the antibody is present in an amount of about 0.1 mg/mL to about 200 mg/mL.

21. The formulation of claim 19, wherein the antibody is present in an amount of about 1 mg/mL to about 150 mg/mL.

22. The formulation of claim 21, wherein the antibody is present in an amount of about 1.7 mg/mL or about 50 mg/mL.

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23. The formulation of claim 18, wherein the antibody is present in the amount of about 15 mg/mL to about 50 mg/mL.

24. The formulation of claim 18, wherein the antibody is present in the amount of about 20 mg/mL.

25. The formulation of claim 1, wherein the formulation further comprises histidine.

26. The formulation of claim 25, wherein the polysorbate is polysorbate 80.

27. A method of treating a patient with variable weight for a condition with a therapeutic amount of an immunoglobulin comprising administering a formulation of claim 1 to said patient wherein the condition is treated by administration of the formulation.

28. The method of claim 27, wherein the immunoglobulin is natalizumab.

29. A composition comprising a sodium phosphate, a polysorbate, a protein and NaCl with a pH of 6.0±0.5, wherein the composition is stable when stored at about 5°C to about 8°C for greater than 6 months.

30. The composition of claim 29, wherein the polysorbate is polysorbate 80 and is present in an amount of about 0.001% to about 2.0% (w/v).

31. The composition of claim 29, wherein the protein is an immunoglobulin which is present in an amount of about 0.01 mg/mL to about 200 mg/mL.

32. The composition of claim 29, wherein the polysorbate is polysorbate 80 and is present in the amount of about 0.02% (w/v), the NaCl is present in the amount of 150 mM, the phosphate buffer is present in the amount of 10 mM, and the immunoglobulin is natalizumab and is present in the amount of 1.7 mg/mL, 5 mg/mL, 20 mg/mL or 50 mg/mL.

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33. A method of preparing a stable protein containing formulation comprising admixing sodium phosphate, sodium chloride, a polysorbate and a protein and adjusting the pH of the mixture with phosphoric acid to about pH 6.0 ± 0.5.

34. The method of preparing a stable protein containing formulation of claim 33, wherein the sodium phosphate is present in an amount of about 10 mM, the sodium chloride is present in an amount of about 150 mM, the polysorbate is polysorbate 80 and is present in an amount of about 0.02% (w/v) and the protein is natalizumab.

35. The method of preparing a stable protein containing formulation of claim 33, wherein natalizumab is present in an amount of about 20 mg/mL to about 200 mg/mL.

36. The method of preparing a stable protein containing formulation of claim 35, wherein natalizumab is present in an amount of about 150 mg/mL.

37. The method of preparing a stable protein containing formulation of claim 33, wherein the protein is lyophilized in the formulation of claim 1.

38. The method of preparing a stable protein containing formulation of claim 37, wherein the polysorbate is polysorbate 80 and is present in an amount of about 0.02% (w/v) and the protein is natalizumab.

39. The method of preparing a stable protein containing formulation of claim 37, wherein the formulation further comprises histidine.

40. The method of preparing a stable protein containing formulation of claim 33, wherein the protein is lyophilized in a solution comprising 5 mM histidine, 20 mg/mL sucrose and 0.02% polysorbate 80 at a pH 6, and wherein the protein is natalizumab at a concentration of 20 mg/mL.

41. An article of manufacture comprising a container holding the stable formulation of any one of claims 1-29.

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42. A method for treating a patient with variable weight for a condition, comprising simultaneously or sequentially administering to the patient a therapeutically effective combination of a formulation of any one of claims 1-24 and a compound or therapy effective against the condition.

43. The use of the stable formulation of any one of claims 1-24 for the preparation of a medicament for the treatment of a condition in a patient with a variable weight condition wherein the medicament is effective to treat said condition.

44. The use of the stable formulation of any one of claims 1-24 for the preparation of a medicament to treat a condition in a patient with a variable weight condition, wherein the medicament is a combination of a formulation of any one of claims 1-24 and a compound or therapy, and wherein said medicament is effective to treat the condition.

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[Continued on next page]

(54) Title: USE OF ANTI-ALPHA5BETA1 ANTIBODIES TO INHIBIT CANCER CELL PROLIFERATION

(57) Abstract: The present invention provides methods for direct killing of cancer cells using anti-α5β1 antibodies. Generally, the method comprises contacting a cancer cell that expresses α5β1 on its surface with an anti-α5β1 antibody, and thereby inducing the death of the cancer cell. The methods of the invention may be employed at an early stage of cancer development in a patient to prevent tumor establishment. In addition, the methods may be used to treat previously formed tumors especially in cancer that have not proven susceptible to anti-angiogenesis therapy. The methods may be employed as a combination therapy of anti-α5β1 antibodies together with cancer chemotherapeutic agents or other molecular-based cancer therapeutic agents.

A. IIA1 V_H sequences
[NA, SEQ ID NO: 1; AA, SEQ ID NO: 2]

```
1   ATGGCTGTCCTGGGGCTGCTTCTCTGCCTGGTGACTTTCCCAAGCTGTGTCCTGTCCCAG
     M  A  V  L  G  L  L  L  L  C  L  V  T  F  P  S  C  V  L  S  Q
61  GTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACA
     V  Q  L  K  E  S  G  P  G  L  V  A  P  S  Q  S  L  S  I  T
121 TGCACCATCTCAGGGTTCTCATTAACCGACTATGGTGTTCACTGGGTTCGCCAGCCTCCA
     C  T  I  S  G  F  S  L  T  D  Y  G  V  H  W  V  R  Q  P  P
181 GGAAAGGGTCTGGAGTGGCTGGTAGTGATTTGGAGTGATGGAAGCTCAACCTATAATTCA
     G  K  G  L  E  W  L  V  V  I  W  S  D  G  S  S  T  Y  N  S
241 GCTCTCAAATCCAGAATGACCATCAGGAAGGACAACTCCAAGAGCCAAGTTTTCTTAATA
     A  L  K  S  R  M  T  I  R  K  D  N  S  K  S  Q  V  F  L  I
301 ATGAACAGTCTCCAAACTGATGACTCAGCCATGTACTACTGTGCCAGACATGGAACTTAC
     M  N  S  L  Q  T  D  D  S  A  M  Y  Y  C  A  R  H  G  T  Y
361 TACGGTATGACTACGACGGGGGATGCTTTGGACTACTGGGGTCAAGGAACCTCAGTCACC
     Y  G  M  T  T  T  G  D  A  L  D  Y  W  G  Q  G  T  S  V  T
421 GTCTCCTCA
     V  S  S
```

B. IIA1 V_L sequences
[NA, SEQ ID NO: 3; AA, SEQ ID NO: 4]

```
1   ATGGATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAGTCATAATGTCC
     M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S  V  I  M  S
61  AGAGGACAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAACGG
     R  G  Q  I  V  L  T  Q  S  P  A  I  M  S  A  S  L  G  E  R
121 GTCACCATGACCTGCACTGCCAGTTCAAGTGTAAGTTCCAATTACTTGCACTGGTACCAG
     V  T  M  T  C  T  A  S  S  S  V  S  S  N  Y  L  H  W  Y  Q
181 CAGAAGCCAGGATCCGCCCCCAATCTCTGGATTTATAGCACATCCAACCTGGCTTCTGGA
     Q  K  P  G  S  A  P  N  L  W  I  Y  S  T  S  N  L  A  S  G
241 GTCCCAGCTCGTTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGC
     V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I  S  S
301 ATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCTTCGTTCCCCACCGACG
     M  E  A  E  D  A  A  T  Y  Y  C  H  Q  Y  L  R  S  P  P  T
361 TTCGGTGGAGGCACCAAGCTGGAAATCAAA
     F  G  G  G  T  K  L  E  I  K
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USE OF ANTI-α5β1 ANTIBODIES TO INHIBIT CANCER CELL PROLIFERATION

FIELD OF THE INVENTION

The present invention provides antibodies that specifically recognize α5β1 integrin expressed on the surface of cancer cells, and methods for using the antibodies to inhibit proliferation of these cancer cells.

BACKGROUND OF THE INVENTION

The association of α5β1 integrin with tumor angiogenesis is well-established (see, e.g., U.S. Publ. Pat. Appl. US 2002/0172675 A1, filed May 7, 1999, which is hereby incorporated by reference herein). α5β1 is a heterodimeric integrin that specifically binds the ligand fibronectin. α5β1 is expressed on the surface of endothelial cells and mediates adhesion to and migration toward fibronectin. The binding interaction between α5β1 and fibronectin has been shown to be important for tumor angiogenesis. Angiogenesis within a tumor begins when the release of one or more pro-angiogenic growth factors (e.g., FGF, VEGF, PDGF, etc.) locally activates the endothelial cells. These activated endothelial cells then form new blood vessels by binding, via their α5β1 integrin, to the fibronectin in the extracellular matrix. Anti-α5β1 antibodies have been shown to inhibit angiogenesis in *in vivo* tumor models (see, e.g., US 2002/0172675 A1).

Anti-angiogenesis cancer therapy is based on inhibiting tumor vascularization and thereby preventing continued tumor growth and metastasis (for reviews see, e.g., Marx, "A Boost for Tumor Starvation," *Science* **301**, 452 (2003); Sato, "Molecular Diagnosis of Tumor Angiogenesis and Anti-Angiogenic Cancer Therapy," *Int. J. Clin. Oncol.* **8**, 200 (2003); Bissachi *et al.*, "Anti-Angiogenesis and Angioprevention: Mechanisms, Problems and Perspectives," *Cancer Detec. Prev.* **27**, 229 (2003)). More than 60 anti-angiogenesis based therapeutics currently are in clinical development for cancer treatment. While in some cancers, it may be possible to "starve" a tumor by preventing its vascularization, current research shows that there are cancers that do not appear to be vulnerable to anti-angiogenesis treatment (see, Sato, *supra*). For example, the anti-VEGF antibody therapeutic, AVASTIN™ (bevacizumab) succeeded in clinical trials for colon cancer but not breast cancer (see, Marx, *supra*). In addition, anti-angiogenesis based therapeutic

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methods are not well-suited to early-stage treatment when the tumor vascularization process has not yet begun.

Due to its function in angiogenesis, the α5β1 integrin has been proposed as a therapeutic target for numerous diseases mediated by angiogenic processes including cancerous tumor growth. Chimeric and humanized antibodies to α5β1 have been developed that block specific binding to fibronectin. A chimeric α5β1 antibody, M200 (also known by its generic name, volociximab) has been shown to induce apoptosis of activated endothelial cells *in vitro* regardless of the growth factor stimulus.

Thus, there remains a need for cancer therapies and early-stage treatment methods capable of directly killing cancer cells before the tumor vascularization process even begins, or for when a targeted anti-angiogenesis therapy proves ineffective.

SUMMARY OF THE INVENTION

The present invention provides methods of killing cancer cells using anti-α5β1 antibodies. In a general embodiment, the method comprises contacting a cancer cell that expresses α5β1 on its surface with an anti-α5β1 antibody.

In one preferred embodiment, the invention provides a method of inhibiting proliferation of a cancer (or tumor) cell that expresses α5β1 integrin on its surface comprising contacting the tumor cell with an antibody that binds to the α5β1 integrin expressed on the tumor cell surface. In a preferred embodiment, the tumor cell is in a patient with a refractory solid tumor. In another preferred embodiment, the tumor cell is from a cancer selected from the group consisting of: bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, renal cell carcinoma and spleen cancer.

In another embodiment, the invention provides a method of inducing death of a tumor cell that expresses α5β1 integrin on its surface comprising contacting the tumor cell with an antibody that binds to the α5β1 integrin expressed on the tumor cell surface. In a preferred embodiment, the tumor cell is in a patient with a refractory solid tumor. In another preferred embodiment, the tumor cell is from a cancer selected from the group consisting of bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, renal cell carcinoma and spleen cancer.

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In a further embodiment, the invention provides a method of inhibiting the proliferation of a cancer cell in a patient wherein the cancer cell expresses α5β1 integrin on its surface. In this embodiment, the method comprises administering to the patient a therapeutically effective amount of an antibody, wherein the antibody competitively inhibits binding of M200 to α5β1 integrin on the cancer cell surface. In another embodiment, the antibody comprises a variable region with an amino acid sequence substantially identical to SEQ ID NOs: 2, 4, 6 and 8. In a preferred embodiment of this method, the antibody administered to the patient neutralizes at least one biological activity of α5β1 integrin. In another embodiment, the antibody administered to the patient comprises a therapeutic effector moiety (e.g., an antibody-drug conjugate). In still a further embodiment of this method, the antibody is administered to the patient, serially, or together with, another chemotherapeutic agent. In another preferred embodiment of this method, the antibody is administered to a patient with a refractory solid tumor, serially, or together with, another chemotherapeutic agent.

In another embodiment, the invention provides a method for treating a subject suspected of developing a cancer that expresses α5β1 on its cell surface wherein the subject has not yet developed a tumor comprising, administering to the patient a therapeutically effective amount of a pharmaceutical composition comprising an antibody binds to the α5β1 integrin. In a preferred embodiment, the cancer is selected from the group consisting of bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, renal cell carcinoma and spleen cancer.

In another embodiment, the invention provides a method of treating a subject with a genetic predisposition for a cancer that expresses α5β1 wherein the subject has not yet developed a tumor comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an antibody binds to the α5β1 integrin. In a preferred embodiment, the cancer is selected from the group consisting of bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, renal cell carcinoma and spleen cancer.

The preferred anti-α5β1 antibodies useful with the methods of the invention include IIA1, M200, F200, and antibodies that specifically bind to the same epitope on α5β1 as IIA1, M200, and F200.

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In another embodiment, the anti-α5β1 antibodies useful with the methods of the invention include antibodies that competitively inhibit binding of IIA1 and/or M200 to α5β1 integrin expressed on the tumor cell surface.

Other antibodies useful with the method of the invention include antibodies comprising a variable region amino acid sequence substantially identical to SEQ ID NOs: 2,4,6 and 8. Also, included are antibodies comprising variable region amino acid sequences with at least about 90%, 95%, 98% or preferably, 99% or greater identity to SEQ ID NOs: 2,4,6 and 8.

In another embodiment, the present invention provides anti-α5β1 antibodies formulated as pharmaceutical compositions. These pharmaceutical compositions are useful in the various methods of the invention disclosed herein. In various embodiments, the anti-α5β1 antibody pharmaceutical compositions may be administered in a therapeutically effective amount to a subject by various routes including, but not limited to, orally, subcutaneously, topically, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, intraocularly, intraventricularly, or intrathecally. In a preferred embodiment, the pharmaceutical composition is a liquid formulation comprising between about 1.0 mg/mL and 15 mg/mL of an anti-α5β1 antibody, about 22-27 mM citrate, about 145-165 mM sodium chloride, about 0.04% to 0.06% polysorbate (TWEEN®) 80, at a pH of about 5.5 to 7.5. In another preferred embodiment, the pharmaceutical composition is a liquid formulation comprising about 10 mg/mL anti-α5β1 antibody, about 25 mM citrate, about 150 mM sodium chloride, about 0.05% polysorbate (TWEEN®) 80, at a pH of about 6.5. In a particularly preferred embodiment, the pharmaceutical composition is a liquid formulation comprising about 10 mg/mL M200, about 25 mM citrate, about 150 mM sodium chloride, about 0.05% polysorbate (TWEEN®) 80, with a pH of about 6.5. In other preferred embodiments, each of the pharmaceutical compositions described herein may further comprise a chemotherapeutic agent. In another embodiment, the pharmaceutical composition comprising an anti-α5β1 antibody may be administered to a patient together with a pharmaceutically effective amount of another chemotherapeutic agent.

The pharmaceutical compositions described above may be used in a method of treating a patient diagnosed with or suspected of having a cancer selected from the group consisting of: bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, renal cell

carcinoma and spleen cancer, with the method comprising: administering intravenously to the patient a therapeutically effective dose of the liquid formulation comprising between about 1.0 mg/mL and 15 mg/mL of an anti-α5β1 antibody, about 22-27 mM citrate, about 145-165 mM sodium chloride, about 0.04% to 0.06% polysorbate (TWEEN®) 80, at a pH of about 5.5 to 7.5. In one embodiment of the treatment methods, the therapeutically effective dose administered is about 10 mg/kg. In a preferred embodiment, the patient treated with the pharmaceutical composition has been diagnosed with or is suspected of having renal cell carcinoma or metastatic melanoma, and the therapeutically effective dose is about 10 mg/kg.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts: (A) IIA1 V_H nucleic acid sequence (SEQ ID NO: 1) and amino acid sequence (SEQ ID NO: 2); (B) IIA1 V_L nucleic acid sequence (SEQ ID NO: 3) and amino acid sequence (SEQ ID NO: 4).

Figure 2 depicts: (A) M200 V_H nucleic acid sequence (SEQ ID NO: 5) and amino acid sequence (SEQ ID NO: 6); (B) M200 V_L nucleic acid sequence (SEQ ID NO: 7) and amino acid sequence (SEQ ID NO: 8).

DETAILED DESCRIPTION OF THE INVENTION

For clarity and understanding, the following disclosure describes the invention in detail by way of illustrative embodiments and exemplifications. The embodiments and examples included in this disclosure are not intended to limit the scope of the invention. It will be readily apparent to one of ordinary skill in the art that equivalent materials and/or methods may be employed, and/or obvious changes, variations or modifications may be made in any of the disclosed embodiments and examples without departing from the scope of the appended claims.

All publications and patent applications cited in this disclosure are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Unless defined otherwise, all terms used herein have the common ordinary meaning ascribed to them by a person of ordinary skill in the art to which this invention belongs.

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Overview

The present invention is based on the surprising discovery that α5β1 integrin is expressed on the surface of tumor epithelial cells for many types of cancer. Further, it has been found that targeted antibody binding to this surface α5β1 results in direct killing of these cancer cells. Because this method of attacking and killing cancer cells is direct, it is amenable to very early-stage treatment (i.e., prior to substantial tumor formation). Furthermore, the direct cancer cell killing method of the present invention may be particularly useful for treating cancers that express α5β1 on the cell surface but have not proven susceptible to anti-angiogenic approaches. Cancers in this category include, but are not limited to, bladder cancer, breast cancer, renal cancer, pancreatic cancer, lung cancer, prostate cancer, ovarian cancer, and metastatic melanoma.

The present invention provides methods of killing or otherwise preventing the proliferation of cancer cells using anti-α5β1 antibodies. In its most general embodiment, the method comprises contacting a cancer cell that expresses α5β1 on its surface with an anti-α5β1 antibody, and thereby inducing the death (e.g., via apoptosis) of the cancer cell.

The methods of the invention may be employed to kill cancer cells *in vivo* (e.g., in a patient) and thereby prevent or attenuate tumor formation and growth. In addition, the method may be used to treat previously formed tumors, and may be employed together with other cancer therapies (e.g., chemotherapeutic agents or other molecular-based cancer therapeutic agents). For example, a patient suffering from the growth of a cancerous tumor may be treated with a formulation of the antibody M200 in tandem with a chemotherapeutic compound such as doxorubicin. Because M200 is a chimeric antibody with relatively low toxicity in humans, this combination treatment may provide comparable cancer cell killing power without the toxic side effects associated with a higher dosage of the chemotherapeutic alone.

The present invention provides a method in which anti-α5β1 antibodies kill and/or inhibit the proliferation of cancer cells directly, even in the absence of any tumor vasculature that may be susceptible to the anti-angiogenic effect of these antibodies. Therefore, the method is particularly well-suited for the prophylaxis or therapeutic treatment of cancers that express α5β1 but do not form heavily vascularized tumors, and/or are not otherwise susceptible to anti-angiogenesis therapeutics e.g., pancreatic cancer, renal cancer, metastatic melanoma, lung cancer and breast cancer.

Furthermore, because of the direct cancer cell killing ability of M200 (and other anti-α5β1 antibodies disclosed herein) it is possible to use these antibodies in early stage cancer therapy prior to the formation of vascularized tumors. An early-stage treatment method is particularly significant in view of the advent of new, more sensitive diagnostic tests for cancers that have been developed with the genetic marker information mined from the human genome sequence. It is likely that many common cancers will be detected and diagnosed at a very early stage, i.e., a pretumor stage wherein cancer cells may be present in tissue and/or circulating but have not established a tumor structure detectable by less sensitive non-genetic diagnostics. In such an early stage diagnosis scenario, anti-angiogenesis therapies may have little or no effect when a tumor vasculature has not yet been established and common chemotherapeutics may create too many toxic side-effects to warrant their use. Because the method of the present invention results in antibody-targeted direct killing of cancer cells that express α5β1 integrin on their surface it is particularly well-suited for early-stage preventative treatment.

Those subjects most likely to benefit from the early-stage method of treatment would include, but are not limited to: 1) a subject who has had pretumor tests indicating a high probability of the development and/or presence of tumors (or microtumors); 2) a subject exposed to a very potent carcinogenic environment whose probability of tumor progression is high; and, 3) a subject whose has a high genetic predisposition to develop a cancer wherein the cancer cells express α5β1 on their surface.

Anti-α5β1 Antibodies

The methods of the present invention employ anti-α5β1 antibodies as direct cancer cell killing agents. As used herein, the term "antibody" refers to an immunoglobulin molecule that specifically binds to, or is immunologically reactive with a particular antigen, and includes polyclonal, monoclonal, genetically engineered and otherwise modified forms of antibodies, including but not limited to chimeric antibodies, humanized antibodies, heteroconjugate antibodies (*e.g.*, bispecific antibodies, diabodies, triabodies, and tetrabodies), and antigen binding fragments of antibodies, including e.g., Fab', $F(ab')_2$, Fab, Fv, rIgG, and scFv fragments. The term "scFv" refers to a single chain Fv antibody in which the variable domains of the heavy chain and the light chain from a traditional antibody have been joined to form one chain. In addition, the term "antibody," as used in the context of the invention disclosed herein encompasses mixtures of more

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than one antibody reactive with a specific antigen (e.g., a cocktail of different types of monoclonal antibodies reactive with α5β1 integrin).

Preferably, the anti-α5β1 antibodies used in the methods of the present invention are monoclonal antibodies. Monoclonal antibodies useful with the methods present invention may be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow and Lane, "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory Press, New York (1988); Hammerling et al., in: "Monoclonal Antibodies and T-Cell Hybridomas," Elsevier, New York (1981), pp. 563-681 (both of which are incorporated herein by reference in their entireties). Production of antibodies by selection of libraries of recombinant antibodies in phage or similar vectors, *see, e.g.,* Huse *et al., Science* 246:1275-1281 (1989); Ward *et al., Nature* 341:544-546 (1989); and Vaughan *et al., Nature Biotech.* 14:309-314 (1996), or by immunizing an animal with the antigen or with DNA encoding the antigen.

In preferred embodiments, the direct cancer cell killing methods of the present invention may be carried out using the previously characterized anti-α5β1 antibodies, IIA1, M200, or F200. IIA1 is the parent mouse IgG1 class antibody that has been shown to inhibit α5β1 integrin binding to fibronectin (see, e.g., U.S. Publ. Pat. Appl. US 2002/0172675 A1, filed May 7, 1999, which is hereby incorporated by reference herein). M200 is a chimeric IgG4 antibody derived from IIA1. F200 is a Fab fragment derived from M200. These antibodies have been generated, functionally characterized and their specific amino acid sequences disclosed in U.S. Pat. Appl. Ser. Nos. 10/724,274, filed Nov. 26, 2003, and 10/830,956, filed April 23, 2004, each of which is hereby incorporated by reference herein. Both M200 and F200 have been shown to exhibit *in vivo* anti-angiogenic efficacy in monkey eye and rabbit eye models (see, U.S. Pat. Appl. Ser. Nos. 10/724,274, filed Nov. 26, 2003, and 10/830,956, filed April 23, 2004).

Antibodies useful with the method of the present invention also include those that specifically bind to the same epitope on α5β1 as IIA1, M200, and F200. An "epitope" (or "antigenic determinant") refers to a site on an antigen to which an antibody binds. Epitopes may be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. For example, the epitope on α5β1 integrin

may comprise amino acids on each of the α and β polypeptide chains that make up the heterodimer structure. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 6-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed (1996).

Two antibodies are said to bind to the same epitope of a protein if amino acid mutations in the protein that reduce or eliminate binding of one antibody also reduce or eliminate binding of the other antibody. Also, it may be concluded that two antibodies bind the same epitope if the two antibodies compete for binding to the protein, i.e., binding of one antibody to the protein competitively inhibits, reduces or eliminates binding of the other antibody. Consequently, the methods of the present invention may be carried out with an antibody that has been determined to competitively inhibit the binding of IIA1, M200 (volociximab), or F200 to α5β1 integrin expressed on the cancer cell surface.

The anti-α5β1 antibodies useful with the methods of the present invention are not limited to IIA1, M200 and F200, but may include antibodies comprising a variable region, framework region, or CDR amino acid sequence substantially identical to those of IIA1, M200 and F200. The extent of the variable regions, framework regions and CDRs are well-known to those of ordinary skill in the art (see e.g., Kabat, et al., "Sequences of Proteins of Immunological Interest", 5th ed., National Institutes of Health, Bethesda, MD (1991)). As used herein, the variable region of the heavy chain ("V_H" or "VH") or light chain ("V_L" or a "VL") of an antibody, includes the heavy or light chains of an antigen binding fragment, e.g., Fv, scFv, dsFv or Fab. Antibody light and heavy chain variable regions also contain four "framework" regions interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs." The CDRs are primarily responsible for binding to an epitope of an antigen.

A "substantially identical" variable, constant, framework region, or CDR refers to an antibody region wherein at least about 85-90%, and preferably at least 95% of the amino acid sequence is identical to a natural or unaltered antibody variable or constant

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region. The terms "identical" or percent "identity," in the context of two or more amino acid or nucleotide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (see, e.g., description of BLAST at the National Center for Biotechnology Information (NCBI) web site located at www.ncbi.nlm.nih.gov).

Identical or substantially identical sequences include sequences having deletions and/or additions, as well as those that have substitutions, as well as naturally occurring, e.g., polymorphic or allelic variants, and man-made variants such as conservatively modified variants. The well-known algorithms for measuring sequence identity can account for gaps and the like. Preferably, sequence identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

The amino acid sequences of the anti-$\alpha 5\beta 1$ antibodies useful with the methods of the present invention are not confined to the sequences found in natural antibodies; antibodies can be redesigned to obtain desired characteristics using well-known recombinant DNA techniques. Such "genetically altered antibodies" include those where the amino acid sequence has been varied from that of a parent (i.e., unaltered) antibody. The possible variations range from the changing of just one or a few amino acids to the complete redesign of, for example, the variable or constant region. Changes, by site-directed mutation, in the constant region may be made in order to improve or alter the functional characteristics of a therapeutic antibody such as immunogenicity, pharmacokinetic characteristics (e.g., serum half-life), complement fixation, interaction with membranes and other effector functions. Generally, changes to the antibody variable region may be made in order to improve the antigen binding characteristics.

In one preferred embodiment, the chimeric antibody, M200, may be employed in the direct cancer cell killing methods of the present invention. The term "chimeric antibody" refers to an immunoglobulin molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable

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region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. Methods for producing chimeric antibodies are well-known to those of ordinary skill in the art. See e.g., Morrison *et al.*, Science 229:1202-1207 (1985); Oi *et al.*, BioTechniques 4:214-221 (1986); Gillies et al., J. Immunol. Methods 125:191-202 (1989); and U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, each of which is hereby incorporated herein by reference in its entirety.

In another preferred embodiment, humanized anti-$\alpha 5\beta 1$ antibodies may be employed in the direct cancer cell killing methods of the present invention. The additional human sequences in humanized antibodies further decrease the possible immunogenicity of the antibody when it is used as a human therapeutic. Humanized versions of IIA1 are disclosed in U.S. Pat. Appl. Ser. Nos. 10/724,274, filed Nov. 26, 2003, and 10/830,956, filed April 23, 2004, each of which is hereby incorporated by reference herein. The term "humanized antibody" refers to an immunoglobulin comprising a human framework, at least one and preferably all CDRs from a non-human antibody, and in which any constant region present is substantially identical to a human immunoglobulin constant region, i.e., at least about 85-90%, and preferably at least 95% identical. Hence, all parts of a humanized immunoglobulin, except the CDRs, are substantially identical to corresponding parts of one or more native human immunoglobulin sequences. Accordingly, such humanized antibodies are chimeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions may be identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, e.g., Queen et al., U.S. Patent Nos: 5,530,101; 5,585,089; 5,693,761; 5,693,762; 6,180,370 (each of which is incorporated by reference in its entirety). Antibodies may be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT

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publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Mol. Immunol., 28:489-498 (1991); Studnicka et al., Prot. Eng. 7:805-814 (1994); Roguska et al., Proc. Natl. Acad. Sci. 91:969-973 (1994), and chain shuffling (U.S. Patent No. 5,565,332), all of which are hereby incorporated by reference in their entireties.

In another embodiment, human antibodies (i.e., antibodies comprising both a human variable and constant region) to $\alpha 5\beta 1$ may be employed for therapeutic treatment of human patients according to the methods of the present invention. Human antibodies can be made or obtained by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each of which is incorporated herein by reference in its entirety. Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entireties. Completely human antibodies that recognize a selected epitope also can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers et al., Biotechnology 12:899-903 (1988).

In an alternative embodiment, primatized antibodies (i.e., an antibody comprising monkey variable regions and human constant regions) may be employed for therapeutic treatment according to the methods of the present invention. Methods for producing primatized antibodies are known in the art. See e.g., U.S. Patent Nos. 5,658,570; 5,681,722; and 5,693,780, which are incorporated herein by reference in their entireties.

Antibody Function

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The methods of the present invention employ functional antibodies that exhibit specific binding to α5β1 integrin. Avidity testing of antibodies for specific binding to antigen allows one skilled in the art to identify antibodies specifically recognizing one or more epitopes of α5β1 integrin. Antibodies are defined to be specifically binding if: 1) they exhibit a threshold level of binding activity; and/or 2) they do not significantly cross-react with related polypeptide molecules.

First, anti-α5β1 antibodies useful for the methods disclosed herein specifically bind (or "specifically react" or "specifically immunoreact") if they bind to a α5β1 integrin polypeptide, peptide or epitope with a binding affinity (K_a) of 10^6 mol^{-1} or greater, preferably 10^7 mol^{-1} or greater, more preferably 10^8 mol^{-1} or greater, and most preferably 10^9 mol^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, Ann. NY Acad. Sci. 51: 660-72, 1949), or by surface plasmon resonance using BIAcore. A variety of antibody binding assays useful for characterizing anti-α5β1 antibodies are disclosed in U.S. Pat. Appl. Ser. Nos. 10/724,274, filed Nov. 26, 2003, and 10/830,956, filed April 23, 2004, each of which is hereby incorporated by reference herein.

Second, antibodies specifically bind if they do not significantly cross-react with related polypeptides. Antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect α5β1 integrin polypeptide but not known related polypeptides using a standard Western blot analysis (see, e.g., "Current Protocols in Molecular Biology," eds. Ausubel et al., 1995). Examples of known related polypeptides include orthologs, proteins from the same species that are members of the integrin family of proteins, or mutant α5β1 integrin polypeptides where the mutation alters the anti-α5β1 epitope. Moreover, antibodies may be "screened against" known related polypeptides to isolate a population that specifically binds to the α5β1 integrin. For example, antibodies raised to human α5β1 integrin polypeptides are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to human α5β1 integrin polypeptides will flow through the matrix under the proper buffer conditions. Such screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to closely related polypeptides (Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and isolation

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of specific antibodies is well known in the art (see, Fundamental Immunology, Paul (eds.), Raven Press, 1993; Getzoff et al., Adv. in Immunol. 43: 1-98, 1988; Monoclonal Antibodies: Principles and Practice, Goding, J. W. (eds.), Academic Press Ltd., 1996; Benjamin et al., Ann. Rev. Immunol. 2: 67-101, 1984). Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmuno-assay, radioimmuno-precipitation, enzyme-linked immuno-sorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. For a review of immunological and immunoassay procedures, see *Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991).

Specific binding anti-α5β1 antibodies useful with the methods of the present invention may be prepared through a process of selection for α5β1 binding. Generally, polyclonal antibodies raised to specifically bind to a particular protein, or its polymorphic variants, alleles, orthologs, conservatively modified variants, splice variants, may be selected to obtain only those antibodies that are specifically immunoreactive with the selected protein (e.g., α5β1 integrin) and not with other proteins. Specific binding selection is achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically reactive with the α5β1 integrin polypeptide. For example, solid-phase ELISA immunoassays may be used to select antibodies specifically reactive with protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

Antibody Drug Conjugates

In some embodiments, the method of killing cancer cells may employ an anti-α5β1 antibody conjugated to an effector moiety. The effector moiety may be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or preferably may be a therapeutic moiety. The effector moiety (or "effector component") may be bound (or linked, or conjugated), to the anti-5β1 antibody either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds.

In one aspect the therapeutic moiety is a small molecule that modulates the activity of the α5β1 integrin. In another aspect, the therapeutic moiety affects the activity of molecules or cells associated with or in close proximity to the α5β1 integrin. For

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example, the therapeutic moiety may be a cytotoxic agent. The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof. Suitable toxins and their corresponding fragments include diphtheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin, auristatins (e.g., auristatin E, or auristatin F), and the like. Targeting the therapeutic moiety to the $\alpha5\beta1$ integrin expressed on the surface of a cancer cell not only serves to increase the local concentration of therapeutic moiety in the cancer afflicted area, but also serves to reduce deleterious side effects that may be associated with the therapeutic moiety.

Examples of chemotherapeutic agents that may used as therapeutic moieties with the anti-$\alpha5\beta1$ antibodies of the present invention include, but are not limited to, adriamycin, doxorubicin, doxil, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, e.g., paclitaxel (TAXOL™, Bristol-Myers Squibb Oncology, Princeton, N.J.), and doxetaxel (TAXOTERE™, Rhone-Poulenc Rorer), toxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), 5-FU, 6-thioguanine, 6-mercaptopurine, actinomycin D, VP-16, chlorambucil, melphalan, and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone.

Alternatively, the method of the invention may be carried out wherein the chemotherapeutic agents disclosed above are administered in a formulation together with the anti-$\alpha5\beta1$ antibody although not as a conjugate.

Cancer Indications

The present invention is directed to methods of killing cancer cells, inhibiting cancer cell proliferation and/or metastasis, by targeting $\alpha5\beta1$ integrin on the cancer cell's surface with an antibody. Cancer is a physiological condition typically characterized by cells undergoing unregulated growth. Cancer includes all malignant neoplasms including but not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More

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particular examples of cancers that express α5β1 on the cell surface that may be targeted using the methods disclosed herein include, but are not limited to, bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, renal cell carcinoma and spleen cancer.

A tumor is a proliferating mass of cells lacking normal growth controls. A tumor may benign or malignant, and may include pre-cancerous, or cancerous, cells and tissues. Cancer cells typically form tumors as a cancer progresses. Tumor growth is accompanied by an increase in vascular density. This tumor neovasculature provides required nourishment that allows tumors to grow. The anti-angiogenic antibody therapeutics anti-α5β1 and anti-VEGF (VEGF = vascular endothelial growth factor) have demonstrated efficacy in impeding tumor growth in a variety of cancer models (see, e.g., Kim *et al.*, *J Clin Invest.*, 110(7):933-41 (2002); Ferrara, *Nat Rev Cancer*, 2(10):795-803 (2002)). The anti-α5β1 antibody, M200, has been shown to inhibit angiogenesis *in* vitro and *in vivo* in rabbit and monkey models of angiogenic ocular diseases (e.g., advance macular degeneration; see, U.S. Pat. Appl. Ser. Nos. 10/724,274, filed Nov. 26, 2003, and 10/830,956, filed April 23, 2004, each of which is hereby incorporated by reference herein).

The present invention is based on the surprising discovery that for many types of cancer α5β1 integrin is expressed on the surface of tumor epithelial cells (in addition to the endothelial cells of the tumor vasculature), and that targeted binding to this surface α5β1 with an antibody results in direct killing of these cancer cells. Consequently, cancers characterized by proliferating epithelial cells expressing α5β1 may be treated and/or targeted with anti-α5β1 antibodies even absent any tumor vasculature formation. Because this method of attacking and killing cancer cells is direct, it is amenable to very early stage treatment (i.e., prior to substantial tumor formation).

Patients most likely to benefit from the early-stage method of treatment would include, but are not limited to: 1) a patient who has had a pretumor diagnostic tests indicating a high probability of the development and/or presence of tumors (or microtumors); 2) a patient exposed to a very potent carcinogenic environment whose probability of tumor progression is high; and, 3) a patient whose genetic predisposition (e.g., as evidenced by a genetic marker for a cancer) makes the likelihood of cancer development high wherein the cancer cells express α5β1 on their surface. For example, a patient with a genetic predisposition for breast cancer (e.g., BRCA gene positive), or in

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whom some pretumor cancer marker has been detected (e.g., micro-metastases detected by PCR), will be particularly well-suited for the early-stage preventative direct cancer-cell killing method wherein a pharmaceutical composition of an anti-α5β1 antibody is administered.

Genes indicating an increased cancer predisposition, and tumor markers indicating a likelihood of developing a tumor may be found in the cancer biomedical literature and databases. In addition, lists of carcinogens and their exposure levels that greatly increase cancer risk are available in the biomedical literature well-known to those of ordinary skill in the art. One of ordinary skill may use these literature resources together with the well-known methods for detecting α5β1 integrin expression on cancer cells, disclosed below, to determine whether a subject may be amenable to the early-stage cancer treatment methods of the present invention. As described in Example 2 below, flow cytometry analysis reveals α5β1 integrin is expressed on the surface of cell lines originating from at least the following cancers: bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, and renal cell carcinoma.

Furthermore, the direct cancer cell killing method of the present invention may be particularly useful for treating cancers that express α5β1 but have not proven susceptible to anti-angiogenic approaches. Cancers in this category include, but are not limited to, bladder cancer, breast cancer, fibrosarcoma, renal cancer, pancreatic cancer, prostate cancer, ovarian cancer, lung cancer, and metastatic melanoma. In a particularly preferred embodiment, the methods of the present invention may be used to treat any of the above listed cancers wherein the patient exhibits refractory solid tumors. The anti-α5β1 antibody, M200 (volociximab) has demonstrated some efficacy for treating human patients with a variety of cancers exhibiting refractory solid tumors, including: colorectal cancer (CRC), melanoma (MEL), renal cell carcinoma (RCC), hepatocellular cancer (HCC), lung cancer (NSCLC), pancreatic cancer (PC), parotid cancer (PARO) and breast cancer (BC).

One of ordinary skill may determine those cancers that express α5β1 on the surface of tumor epithelial cells by using an anti-α5β1 antibody (e.g., IIA1 or M200) to probe tumor biopsy samples according to standard methods of immunohistochemistry. As described in Example 1 below, immunohistochemical (IHC) analysis of tumor biopsy samples taken from melanoma, lung, renal, pancreatic and breast cancer patients all

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showed α5β1 integrin expression on tumor epithelial cells. It is reasonable to expect that there other cancers will be found to express α5β1 on the surface of tumor epithelial cells using IHC (or other methods), and it is recognized that these cancers will be susceptible to the cancer cell kill method of the present invention.

In addition to IHC of tumor samples, cancer cell lines may be screened for α5β1 cell surface expression using an anti-α5β1 antibody and standard flow cytometry techniques well-known to those of ordinary skill in the art. As detailed in Example 2, below, flow cytometry screening revealed α5β1 expression on the surface of 21 well-known cancer cell lines. Based on this result, these cell lines are susceptible to direct cell killing using anti-α5β1 antibodies according to the methods of the invention. Furthermore, to the extent a cancer cell line is determined to express α5β1 on its surface, and the cell-line corresponds to, originated from, or is derived from, cancer cells present in a cancer patient, the methods of the present invention may be used to treat such a patient. For example, the NW231 cell line, which was found in Example 2 to express α5β1 integrin on its surface, originated from breast cancer tumors. Consequently, one of ordinary skill would immediately recognize that an anti-α5β1 antibody may be used to treat a breast cancer patient in accordance with the methods taught herein.

Compositions, Formulations and Administration of Antibodies

The anti-α5β1 antibodies useful in the methods of the present invention may be used in an isolated and purified form and directly contacted with cancers cells or tumors. Methods of purifying anti-α5β1 antibodies (e.g., IIA1, M200, and F200) are disclosed in U.S. Pat. Appl. Ser. Nos. 10/724,274, filed Nov. 26, 2003, and 10/830,956, filed April 23, 2004, each of which is hereby incorporated by reference herein. F200 may also be prepared as a Fab'-NAC fragment according to the methods disclosed in U.S. Pat. Appl. 60/583,127, filed June 25, 2004, which is hereby incorporated by reference herein. The F200-Fab'-NAC exhibits increased stability in liquid and lyophilized formulations of the antibody. Purity and homogeneity may be determined using standard analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. An antibody that is the predominant species present in a preparation is considered to be substantially purified. For example, antibody solution that exhibits essentially one band in an electrophoretic gel is substantially purified.

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Preferably, the antibody used in the pharmaceutical compositions of the invention is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

In preferred embodiments, the direct cancer cell killing method is carried out wherein the purified anti-α5β1 antibodies are formulated into a pharmaceutical composition that is administered to a subject in a therapeutically effective amount. As used herein, "therapeutically effective amount" refers to the amount of a pharmaceutical formulation or composition that is sufficient to cure, alleviate, attenuate or at least partially arrest the cancer and/or its symptoms, and/or complications. Clinical methods for determining the therapeutically effective amount of an anti-α5β1 antibody for treatment of cancer are well-known to those of ordinary skill in the art and may be determined empirically using routine experimentation. For example, in the context of cancer treatment, a "therapeutically effective amount" is an amount capable of invoking one or more of the following effects: (1) inhibition, to some extent, of tumor growth, including, slowing down and complete growth arrest; (2) reduction in the number of cancer cells; (3) reduction in tumor size; (4) inhibition (i.e., reduction, slowing down or complete stopping) of cancer cell infiltration into peripheral organs; (5) inhibition (i.e., reduction, slowing down or complete stopping) of cancer cell metastasis; (6) enhancement of anti-cancer immune response, which may, but does not have to, result in the regression or rejection of a tumor; and/or (7) relief, to some extent, of one or more symptoms associated with the disorder.

The pharmaceutical compositions for administration will commonly comprise an anti-α5β1 antibody dissolved in a pharmaceutically acceptable carrier or excipient, preferably an aqueous carrier. Acceptable carriers, excipients, or stabilizers, for the pharmaceutical composition are those which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids, antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming, counter-ions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and

PLURONICS™. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions should sterile and generally free of undesirable matter. The pharmaceutical compositions may be sterilized by conventional, well known sterilization techniques.

The pharmaceutical compositions also may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and other pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol. The concentration of antibody in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs (e.g., "Remington's Pharmaceutical Science" (15th ed., 1980) and Goodman & Gillman, "The Pharmacologial Basis of Therapeutics" (Hardman et al., eds., 1996)).

In a preferred embodiment of the methods of the present invention, the anti-α5β1 antibody is formulated as a pharmaceutical composition comprising a solution of between about 1.0 mg/mL and 15.0 mg/mL antibody, about 22 mM to 28 mM citrate, about 135 mM to 165 mM Sodium Chloride, 0.04%-0.06% Polysorbate (TWEEN®) 80, at a pH of 5.5 to 7.5. Preferably, the pH range of the liquid formulation is between about pH 6.0 and pH 7.0, and most preferably between about pH 6.3 and pH 6.7. In a particularly preferred embodiment the pharmaceutical composition comprises a solution of about 10.0 mg/mL antibody, about 25 mM citrate, about 150 mM sodium chloride, about 0.05% polysorbate (TWEEN®) 80, at a pH of about 6.5. In other embodiments, the above anti-α5β1 antibody pharmaceutical composition may further comprise a chemotherapeutic agent, or alternatively, may be administered to a patient together with a pharmaceutically effective amount of another chemotherapeutic agent.

Preferably the liquid formulation of the pharmaceutical composition is a stable, colorless, or clear to slightly opalescent solution exhibiting no more than 10%, and preferably 5% or less of degaded antibody monomer as measured by SEC-HPLC.

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Preferably, no more than 10%, and preferably 5% or less of hydrolysis clipping is observed, and no more than 10%, and preferably 5% or less of antibody aggregation is formed. Preferably, the concentration, pH and osmolality of the formulation have no more than ± 10% change. Potency is within 70-130%, preferably 80-120% of the control.

The administration of the pharmaceutical compositions comprising anti-α5β1 antibodies to a subject may be carried in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, topically, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, intraocularly, intraventricularly, or intrathecally. It is well recognized that antibodies when administered orally, should be protected from digestion. This is typically accomplished either by complexing the molecules with a composition to render them resistant to acidic and enzymatic hydrolysis, or by packaging the molecules in an appropriately resistant carrier, such as a liposome or a protection barrier. Means of protecting agents from digestion are well known in the art.

The pharmaceutical compositions may be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules and lozenges.

The exact dosage to be used in a particular embodiment of the method of the invention will depend on the purpose of the treatment, and may be ascertained by one skilled in the art using well-known techniques (e.g., Ansel et al., "Pharmaceutical Dosage Forms and Drug Delivery;" Lieberman, "Pharmaceutical Dosage Forms" (vols. 1-3, 1992), Dekker, ISBN 0824770846, 082476918X, 0824712692, 0824716981; Lloyd, "The Art, Science and Technology of Pharmaceutical Compounding" (1999); and Pickar, "Dosage Calculations" (1999)). As is known in the art, adjustments for cancer degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

In one embodiment of the methods of the present invention, a pharmaceutical compositions comprising an anti-α5β1 antibody is administered to a patient based on the weight of antibody (in mg) per patient body weight (in kg). Thus, preferred dose levels include at least about 0.5 mg/kg, 1.0 mg/kg, 2.5 mg/kg, 5.0 mg/kg, 10.0 mg/kg, and 15

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mg/kg. Preferably, the dose is administered to the patient as an intravenous infusion over 1 hour. Additional doses may be administered over an extended time period such that a steady state serum concentration is established in the patient. For example, an infusion of 10 mg/kg may be administered once a week over the course of a year.

In one preferred embodiment, the anti-α5β1 antibody dosing level and schedule are selected to ensure that the dose produces a maximum serum concentration below the safe mean peak serum concentrations seen in pharmacokinetic studies carried out in monkeys (e.g., cynomolgus). For example, in cynomolgus monkeys, the mean peak level of M200 at a dose of 50 mg/kg after 4 weekly doses was 1862 μg/mL (range: 1000-2606 μg/mL). The monkeys showed no toxicity at these serum concentrations. In addition, or alternatively, a dose may be selected so that the trough serum level is > 1 μg/mL, the concentration that produces 80% inhibition of binding of α5β1 to fibronectin in an *in vitro* activity assay.

In accordance with one embodiment of the methods of therapeutic cancer cell killing of the present invention, a pharmaceutical composition comprising an anti-α5β1 antibody is administered to a patient intravenously at a fixed dosage, typically about 0.1 to 10 mg per patient per day. In embodiments where the pharmaceutical composition is administered to a secluded site, such as into a body cavity or into a lumen of an organ, and not into the blood stream, fixed dosages from 0.1 mg up to about 100 mg per patient per day may be used. Substantially higher dosages are possible for embodiments where topical administration is desired. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art, e.g., "Remington's Pharmaceutical Science," and Goodman and Gillman, "The Pharmacologial Basis of Therapeutics," *supra*.

The pharmaceutical compositions employed in the cancer cell killing method of the invention may be administered as part of a therapeutic or prophylactic treatment. In a therapeutic method, the pharmaceutical composition is administered to a patient already suffering from a cancer in an amount sufficient to cure, or at least partially arrest the progress of the disease and its complications. Generally, in a therapeutic treatment context, the progress of the therapy may be measured as decrease in tumor size or a decrease in the rate of tumor growth. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the cancer and the general state of the patient's health. Single or multiple

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administrations of the pharmaceutical compositions may be employed depending on the dosage and frequency tolerated by the patient.

A early-stage treatment method is directed to preventing or slowing the development of cancer in a subject that is suspected of developing the disease, or in the very early stage of the disease. The particular dose required for an early-stage treatment will depend upon the medical condition and history of the patient, the particular cancer being prevented, as well as other factors such as age, weight, gender, administration route, efficiency, etc. An early-stage treatment method also may be used prophylactically, e.g., in a patient who has previously had cancer to prevent a recurrence of the cancer, or in a patient who is suspected of having a significant likelihood of developing cancer. For example, a patient with a genetic predisposition for breast cancer, in whom some pre-tumorous cancer marker has been detected (e.g., micro-metastases detected by PCR), will be particularly well-suited for the early-stage treatment method.

In an alternative embodiment of the present invention, the direct cancer cell killing method may be carried out wherein a chemotherapeutic agent is administered in addition to the anti-$\alpha5\beta1$ antibody. Typical chemotherapeutic agents useful in this embodiment are disclosed *supra.* This combination therapy method may be particularly preferred in an early-stage, or prophylactic treatment context where the patient lacks fully developed disease symptoms. At this early stage, or in a preventative context, many patients may not agree to undergo the toxic side effects that accompany the use of a standard chemotherapy agent alone. By administering a lower dosage of the standard chemotherapeutic together with a relatively non-toxic anti-$\alpha5\beta1$ antibody, the cancer may be treated prophylactically, or at a very early-stage, with a regimen that is effective yet much more tolerable for the patient.

The following examples are intended to illustrate but not limit the invention disclosed herein.

Examples

Example 1: $\alpha5\beta1$ expression on tumor samples detected by IHC

Tumor biopsy samples taken from melanoma, lung, renal, pancreatic and breast cancer patients were surveyed for $\alpha5\beta1$ integrin expression by immunohistochemistry (IHC).

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Materials and Methods

Frozen tissue samples (obtained from the Mayo Clinic or Cleveland Clinic), were frozen in optimal cutting temperature (OCT) compound and stored at –70°C. Cryostat tissue sections (7 μm) were fixed in 75% acetone/25% ethanol for 1 minute. Samples were incubated with either the anti-α5β1 mouse antibody IIA1 (5 μg/ml) or control mouse IgG1 (anti-trinitrophenyl, ATCC hybridoma clone 1B7.11) for 30 minutes. Antibody binding was detected using the biotinylated secondary antibody, goat-anti-mouse IgG (3 μg/ml, 30 minutes; Jackson ImmunoResearch), and developed using the Vectastain Elite ABC Kit (Vector Laboratories) and stable DAB (diaminobenzidine and H_2O_2; Research Genetics). Staining was performed using the DAKO Autostainer at room temperature.

Results

As shown in Table 1, nearly all of the tumor sections analyzed stained positive for α5β1 on the tumor vasculature. Surprisingly, a significant proportion of tumor samples also exhibited positive staining for α5β1 integrin expression on the tumor epithelium itself. These results indicate that an anti-α5β1 antibody will directly target cancer cells in addition to the invading neovasculature.

Table 1: IHC results for tumor samples stained with anti-α5β1 antibody IIA1

	Tumor Vasculature					**Tumor Epithelium**				
Tumor type (# samples)	**Neg**	**1+**	**2+**	**3+**	**4+**	**Neg**	**1+**	**2+**	**3+**	**4+**
Renal (n=39)	2	2	13	18	4	27	8	4	0	0
Pancreatic (n=32)	0	1	17	12	2	15	2	7	7	1
Lung (n=39)	0	1	0	28	1	19	14	3	2	1
Colon (n=21)	2	2	3	8	6	19	0	1	1	0
Melanoma (n=19)	3	0	2	13	1	13	5	1	0	0
Ovarian omental mets (n=4)	0	0	1	3	0	2	2	0	0	0
Bladder (n=8)	0	0	4	3	1	5	2	1	0	0

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Example 2: α5β1 expression on cancer cell lines detected by flow cytometry

A panel of 24 cancer cell lines was surveyed by flow cytometry for α5β1 integrin expression on the cell surface. As shown in Table 2, these 24 cell lines originated in a variety of different cancers including: bladder, breast, colon, fibrosarcoma, lung, melanoma, pancreas, prostate, ovarian, renal, and spleen.

Materials and Methods

Cells were removed with 5 mM EDTA in Tris-HCl (pH 8.0) and blocked by centrifugation in Hank's balanced salt solution containing 3% heat inactivated FBS, 1% normal goat serum (Sigma) and 1% BSA at 4°C for 5 minutes. Cells were incubated for 30 – 60 minutes at 4°C with the mouse anti-α5β1 antibody, IIA1 (10 μg/ml) in FACS buffer (PBS containing 0.1% BSA). Excess monoclonal antibody was removed by centrifugation and cells were washed two times with FACS buffer prior to resuspending in PE-anti-mouse IgG (H+L) antibody (Southern Biotech, 1:400 dilution) for 30 – 60 minutes at 4°C. After washing cells were re-suspended in FACS buffer containing propidium iodide (1 μg/ml). Mean fluorescence intensity (MFI) was measured on a FACSCalibur (Becton Dickinson). Background MFI was ~5.

Results

As shown in Table 2, significant detectable surface expression of α5β1 integrin was observed for 21 of the 24 cell lines assessed. Three cancer cell lines CHL-1, COLO 357 and C32 exhibited MFI values that were very close to background indicating little or no surface expression of α5β1 integrin. The 21 cell lines expressing α5β1 integrin on their surface should be vulnerable to direct cell killing by an anti-α5β1 antibody. In addition, the cancer from which these cell lines were originated may be susceptible to treatment with an anti-α5β1 antibody therapeutic.

Table 2: Results for FACS analysis of various cancer cell lines with IIA1, and *in vitro* proliferation assay with M200.

Cell Line	Cancer of Origin	α5β1 Surface Expression (MFI)	% Growth Inhibition	
			- Serum	+ Serum
A549	lung	71.41	40	0
H460	lung	87.5	40	0
SW839	renal	65.8	30	0
MIA PACA-2	pancreas	61.9	20	0

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HCT-116	colon	42.5	15	0
786-0	renal	144.4	10	0
EKVX	lung	36.7	10	0
SW1990	spleen	nd[1]	10	0
SN12C	renal	129.1	5	0
A498	renal	67.1	5	0
A375	melanoma	156.1	5	0
A2058	melanoma	36.2	0	0
CHL-1	melanoma	6.8	0	0
TK-10	renal	36.6	0	0
COLO 357	pancreas	7.8	0	0
HT144	melanoma	102.1	0	0
C8161	melanoma	122	0	0
HT1376	bladder	111.9	0	0
DU145	prostate	87.5	0	0
UACC-62	melanoma	88.5	0	0
HT1080	fibrosarcoma	421.7	0	0
ES-2	ovarian	132.8	0	0
CAPAN-1	pancreas	nd[1]	0	0
CAPAN-2	pancreas	nd[1]	0	0
ASPC-1	pancreas	nd[1]	0	0
C32	melanoma	5.7	0	0
LOX	melanoma	261.6	40[2]	35
NW231	breast	132.2	10[2]	35

[1] nd = not determined
[2] Values for % growth inhibition were determined in a 2-day rather than 4-day assay.

Example 3: M200 inhibition of *in vitro* cancer cell proliferation

A panel of 28 cancer cell lines was assessed for sensitivity to the chimeric anti-α5β1 antibody M200 in a cell proliferation assay in the presence or absence of serum.

Materials and Methods

Cancer cell lines were plated at a density of 2500 cells/well in 96-well plates in IMDM with supplements in the presence or absence of 10% FBS. At the time of plating, cells were challenged with various concentrations M200 or a non-function blocking anti-α5 antibody, VC5. Four days later, cell viability was assessed by the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega) according to the manufacturer's instructions. All growth studies were done at least 3 times in triplicate.

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Results

As shown in Table 2, M200 inhibited growth of thirteen cancer cell lines in the absence of serum and two cell lines in the presence of serum. Two of the cell lines, LOX and NW231 were found to be sensitive to M200, under both conditions. Based on these results, the cancers from which these cell lines originate (melanoma and breast) are likely to respond to treatment with M200.

Example 4: *In vivo* inhibition of tumor cell proliferation in NW231 and LOX xenograft models

NW231 and LOX cells were grown as orthotopic xenografts in SCID mice and challenged with the anti-$\alpha5\beta1$ antibodies M200 and IIA1 by intraperitoneal injection.

Materials and Methods

Immune compromised mice CB-17 SCID (strain C.B-Igh1/IcrTac-Prkdc) were obtained from Taconic Farms (Germantown, NY). Studies were initiated using female mice between the ages of 6-10 weeks (~20 grams in weight). Animals were pre-dosed with M200, IIA1 or control IgG intraperitoneally at a dose of 10 mg/kg 1 hour prior to NW231 inoculation ($1x10^7$ cells in IMDM) into the mammary fat pad. Dosing continued for 3 weeks at a frequency of 3 times/week at 10 mg/kg. Tumor volume was measured twice weekly by caliper and calculated by $\pi/6$ x length x width x height. Clinical and mortality observations were performed daily according to IACUC regulations.

Results

IIA1, was found to reproducibly inhibit both NW231 and LOX xenograft tumor growth in this model. M200 was not found to reproducibly inhibit tumor growth in these models. Since IIA1 does not recognize mouse $\alpha5\beta1$ in the xenograft tumor vasculature, the entire inhibitory effect of IIA1 on tumor growth may be attributed to a direct anti-proliferative effect on the cancer cells of the tumor.

Example 5: IIA1 in combination with DOXIL® prevents tumor establishment in an *in vivo* NW231 xenograft model

The following experiment was conducted in order to determine the efficacy of the anti-$\alpha5\beta1$ integrin antibody, IIA1 in combination with the chemotherapeutic agent, DOXIL®, for preventing establishment of human NW231 tumors *in vivo*. DOXIL® is a liposome encapsulated formulation of doxorubicin HCl, a cytotoxic anthracycline

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antibiotic isolated from *Streptomyces peucetius*. The NW231 cell line originated from breast cancer and is considered a good model for studying treatments for breast cancer.

Materials and Methods

Four to six week old female SCID mice obtained from Taconic Farms and maintained in micro-isolator cages were inoculated in the mammary fat pads with $1x10^7$ NW231 cells. Animals received either TIB control (n=20) or M200 (n=20) at 5 mg/kg for the first injection at the time of tumor cell inoculation. Subsequent treatments were at 0.7 mg/kg per injection. DOXIL® treatment was commenced 5 days post tumor cell inoculation. Chemotherapeutic doses were at 4 mg/kg for the first injection and at 2 mg/kg for subsequent injections. Reagents were delivered by intraperitoneal injection for four doses. Tumor volume was measured twice weekly and clinical and mortality observations were performed daily according to IACUC regulations.

Results

IIA1 treatment exhibited a significant effect in slowing the establishment of the NW231 tumors in mice. At 24 days post-implant of NW-231 tumors, the mean tumor volume of control TIB treated xenografts had increased in an exponential fashion to ~425 mm^3 whereas the IIA1 treated xenografts had increased to a mean volume of ~175 mm^3.

DOXIL® treatment by itself also exhibited a significant effect in preventing tumor establishment. For the DOXIL® alone treated xenografts, mean tumor volume initially increased to only ~25 mm^3 during the first 45 days post-implant, and then increased gradually up to ~275 mm^3 by day 74.

Treatment of mice with the combination therapy of IIA1 and doxil had an even greater inhibitory effect on the rate of tumor establishment compared to either treatment alone. For the IIA1 plus DOXIL® treated xenografts, mean tumor volume remained near zero out to day 54, then increased gradually but only up to ~125 mm^3 by day 74. These results indicate increased efficacy (i.e., a combined tumor inhibitory effect) *in vivo* for the combined treatment of an anti-$\alpha5\beta1$ antibody, IIA1 and a chemotherapeutic agent, DOXIL®.

Example 6: M200 efficacy measured in an VX2 rabbit tumor model

Although M200 does not cross react with mouse or rat $\alpha5\beta1$ integrin, it does recognize $\alpha5\beta1$ integrin found in rabbit. Thus, the rabbit VX2 carcinoma may represent a good model for determining the *in vivo* direct cancer cell killing efficacy of M200. VX2

is a widely accepted rabbit model for studies on treatment of primary tumors of various locations (see e.g., Chen JG, et al, Lab Anim. 2004 Jan;38(1):79-84; Purdie, TG et al, Phys Med Biol. 2001 Dec;46(12):3161-75); Geschwind, JH, et al, Cancer Res. 2002 Jul 62(1): 3909-3913).

A. M200 VX2 Pilot Study

An initial Pilot Study was carried out to determine the general parameters for conducting the M200 efficacy study in the rabbit VX2 tumor model.

Tumor inoculation

Rabbits were inoculated on Day 0 with a cell suspension (100 µl) subcutaneously (left hind limb), and intramuscularly at a depth of about 3 mm (right hind limb). Intramuscular inoculation was carried out as follows. With the rabbit under ketamine/isoflurane anesthesia, a ~2 cm incision was made parallel to the right femur with a scalpel on the anterior, lateral aspect of the femoral axis, ~1/3 of the total femur length distally from the femoral-pelvic (hip) joint. Muscle groups were separated to create a ~0.5 cm deep cavity. One VX2 tumor fragment from a donor animal was placed into the cavity. Skin was securely closed with sterile surgical staples or sutures and a topical antibiotic was applied to the wound site.

Tumor measurements

Starting on Day 5, tumor dimensions (Length, Width and Height) were measured in millimeters via electronic vernier calipers connected to a laptop computer at a minimum frequency of twice weekly. For consistency, the same trained technician performed the tumor measurements throughout the course of the study. Tumor volume was calculated using the formula: Length x Width x Height x 0.52. Upon animal termination, tumors were carefully excised, trimmed and weighed. In addition, animals were weighed a minimum of once weekly. Representative samples of each tumor were preserved in sample cassette in formalin or OCT and flash frozen in liquid nitrogen.

In vivo passaging of VX2 tumors

The VX2 tumors used in both the Pilot and M200 efficacy study were maintained by *in vivo* passaging on a monthly basis. Either a cell suspension (100 µl) or pieces of tumor (~5-10 mm^3) were used for intramuscular inoculation in each hind limb. The animals and tumors were visually monitored and tumors removed before reaching 2 cm in diameter for *in vivo* passaging. *In vivo* passage was carried out by sacrificing the animal,

removing the tumor, and processing the tumor into 5-10 mm^3 pieces. The pieces were then re-implanted into the next group of 2 rabbits.

IHC analysis of VX2 tumors from M200 treated rabbits

M200 was administered intravenously at 10 mg/kg dosage to a tumor-bearing rabbit and the tumor was excised 1 hour later. Tumor sections were stained with either: (i) an anti-human secondary antibody specific for tumor bound M200; (ii) IIA1 followed by an anti-mouse secondary to detect total $\alpha5\beta1$; or (iii) a control IgG and anti-mouse secondary.

Results

Both the subcutaneous tumors and the intramuscular tumors were found to be accessible to M200 injected intravenously into the animals, as assessed by IHC. IHC analysis of the stained sections revealed high levels of $\alpha5\beta1$ expression both in VX2 tumor cells and rabbit tumor vasculature.

B. M200 VX2 Efficacy Study

Based on the pilot study IHC results, the VX2 rabbit model was used to assess M200 efficacy *in vivo*.

Methods

Rabbits (30 total) were inoculated, either subcutaneously or intramuscularly with either a VX2 cell suspension (100 µl) or pieces of tumor (~5-10 mm^3). The test group of 20 was treated with M200 at 10 mg/kg intravenously, twice weekly for 3 weeks. The control group of 10 animals was treated with a control IgG delivered by the same method as the M200. Tumor measurements, termination, weighing, tumor preservation and study duration were carried out according to the methods described above for the Pilot Study. In addition, one milliliter of blood was taken from an ear vessel on a weekly basis for serum analysis.

Results

A strong correlation was observed between inhibition of tumor growth and circulating levels of M200 in the animals. In general, when the levels of M200 were maintained at or above 50 µg/mL, then tumor growth was inhibited. Since M200 is immunogenic in rabbits, some of the test group rabbits generated an immune response to M200 as early as two weeks following M200 administration, and eventually all of the animals were found to seroconvert. In the test group animals that generated an anti-

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idiotype response resulting in the clearance of M200 (i.e. M200 < 50 μg/mL at day 14), larger tumor growth was observed. Thus, the results observed with the VX2 carcinoma model indicate that M200 is able to inhibit tumor growth in a robust *in vivo* oncology model.

Example 7: M200 and IIA1 efficacy in a SCID mouse VX2 xenograft model

As described in Example 6, M200 exhibited efficacy in a rabbit VX2 carcinoma model. A further measure of the direct cancer cell killing ability of M200 and IIA1 was carried out in a SCID mouse VX2 xenograft model. Because M200 and IIA1 do not recognize mouse α5β1 integrin present on the VX2 xenograft tumor vasculature, any inhibitory effect on VX2 xenograft tumor growth may be attributed to a direct anti-proliferative effect that M200 and IIA1 has on VX2 cancer cells.

Materials & Methods

Immune compromised mice CB-17 SCID (strain C.B-Igh1/IcrTac-Prkdc) were obtained from Taconic Farms (Germantown, NY). Studies were initiated using female mice between the ages of 6-10 weeks (~20 grams in weight). Animals were pre-dosed with M200, IIA1, or control IgG intraperitoneally at a dose of 10 mg/kg one hour prior to VX2 inoculation ($1x10^7$ cells in Iscove's Modified Dulbecco's Medium) into the mammary fat pad. Dosing was continued for 3 weeks at a frequency of 3 times/week at 10 mg/kg. Tumor volume was measured twice weekly by caliper and calculated by π/6 x length x width x height. Clinical and mortality observations were performed daily according to IACUC regulations.

Results

Dosing with M200 or IIA1 did not correlate with a decreased rate of VX2 tumor growth or an overall decreased tumor size versus the mice dosed with control IgG. These results suggest that neither M200 and IIA1 are able to directly kill VX2 cells in the mouse xenograft model.

Example 8: Phase I Dose-Escalation Study of M200 in Human Patients with Refractory Solid Tumors

A. Overview

A two-part Phase I open-label study was carried to determine the effects of treatment with up to 6 dose levels, from 0.5 mg/kg to 15.0 mg/kg of M200 (volociximab)

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in 21 human patients with a variety of refractory solid tumor types. The total duration of treatment for the first part was 6 weeks with physical assessments through 45 days after the last dose. The second part was an extension study wherein 6 of the 11 patients who exhibited a stable disease response in the first part received continued M200 dosing for up to 52 weeks.

B. Study Parameters and Protocols

1. Patient selection

Patients selected for the study had to meet the following inclusion/exclusion criteria:

a. At least one measurable lesion on routine computed tomography (CT) imaging or magnetic resonance imaging (MRI).
b. An estimated survival of $\geq$ 3 months; Eastern Collaborative Oncology Group (ECOG) performance status $\leq$ 2;
c. No central nervous system (CNS) tumors or metastasis (documented at screening by head imaging);
d. No major surgery within 4 weeks prior to entry;
e. No minor surgery within one week prior to entry; no chemotherapy, immunotherapy, or radiotherapy within 4 weeks prior to entry;
f. No active bleeding disorder or thromboembolic events;
g. No other clinically significant or unstable medical condition.
h. Patients who had previously received murine or chimeric mAb therapy were required to test negative in screening for anti-M200 antibodies (i.e., cross-reactive human-anti-murine-antibody [HAMA] or human-anti-chimeric antibody [HACA]).

A total of 22 patients were enrolled in the study. Tumor types of the 22 patients included: colorectal (4 patients; CRC), melanoma (4 patients; MEL), renal cell carcinoma (3 patients; RCC), esophageal (2 patients; EC), hepatocellular (2 patients; HCC), lung (1 patient; NSCLC), prostate (1 patient; PRO), thyroid (1 patient; THY), pancreatic (2 patients; PC), parotid (1 patient; PARO) and breast (1 patient; BC). Twenty-one of the 22 patients were treated, and 20 of the 21 treated patients completed all 5 doses plus follow-up through day 45. The 21 treated patients (12 male, 9 female) had a median age of 59 years (range 29-81), and a mean Eastern Collaborative Oncology Group (ECOG) score of 1 (range 0-2).

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2. Selection of M200 Dosing Levels and Schedule

Dosing levels and schedule were chosen to ensure that the highest dose (15.0 mg/kg) would produce maximum serum concentrations well below the safe mean peak serum concentrations seen in cynolmolgus monkeys, and so that the trough serum levels for doses of ≥ 1.0 mg/kg would produce serum concentrations > 1 μg/mL, the concentration that produces 80% inhibition of binding of α5β1 to fibronectin in an *in vitro* activity assay.

The dosing levels and schedule used in the study were as follows. M200 was administered to the 21 patients on days 1, 15, 22, 29, and 36. The dosing levels and numbers of patients per level were: 0.5 mg/kg (1 patient), 1.0 mg/kg (2 patient), 2.5 mg/kg (3 patients), 5.0 mg/kg (3 patients), 10.0 mg/kg (6 patients) and 15 mg/kg (6 patients). The dose was administered as an intravenous infusion over 1 hour. The first and second doses were separated by a two week period to allow sampling for single-dose pharmacokinetic data. The real-time PK measurements performed following the first dose were used to predict each patient's peak serum concentration following the fifth dose. If the predicted peak serum concentration following the fifth dose was <750 μg/mL, the patient received all 5 doses of M200. The remaining 3 doses were given weekly and followed by a 45-day evaluation period.

Human serum concentrations of M200 were predicted based on the above dosing scheme and applying the same range of variabilty that was observed in the cynomolgus monkeys (i.e., 54%-140% of the mean). At the highest dose in humans (15.0 mg/kg), the mean peak after 5 doses was predicted to be 592 μg/mL with the range of variability in humans predicted as 320 to 829 μg/mL. Table 3, was compiled using PK data from the monkey studies to predict the peak and tough serum concentrations (Cmax and Cmin, respectively) for each dose at each dose level in humans.

TABLE 3: Estimated Peak and Trough Serum Levels of M200 in Humans

Level	Dose (mg/kg)	PK Value[a]	Dose Number				
			1	2	3	4	5
1	0.5	Cmax	11	11	12	13	13
		Cmin[b]	1.5	1.6	2	2.1	2.2
2	1.0	Cmax	22	22	25	26	26
		Cmin	3	3	4	5	5
3	2.5	Cmax	54	57	64	67	68
		Cmin	9	10	13	14	15
4	5.0	Cmax	108	114	132	141	147

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		Cmin	20	24	33	39	43
5	10.0	Cmax	216	236	282	317	346
		Cmin	51	66	101	131	156
6	15.0	Cmax	324	366	450	524	592
		Cmin	91	126	200	268	332
		Cmax range (54-140%)	175-453	197-512	243-629	283-733	320-829

[a]Values expressed in μg/mL.
[b]Calculated for values expected 1 week after first dose.

Based on the above table, the terminal half-life in humans at 15.0 mg/kg was predicted to be about 13 days, and less for lower doses. M200 accumulation in serum was predicted to be substantive at dose levels ≥ 5.0 mg/kg, with a steady state concentration reached within 4 weeks for all doses < 10.0 mg/kg and within 5 weeks for doses ≥10.0 mg/kg.

3. M200 Formulation and Administration

M200 bulk biologic was manufactured in accordance with current Good Manufacturing Practices (cGMP). The composition of the M200 formulation used in the present study was 10 mg/mL M200, 25 mM Citrate, 150 mM Sodium Chloride, 0.05% Polysorbate (TWEEN®) 80, with a pH of 6.5. This formulation is a sterile, colorless, clear-to-slightly opalescent, preservative-free liquid for I.V. use. Each 20-mL single-use vial was filled to deliver 15 mL of M200 at 10.0 mg/mL. Each 10-mL single-use vial was filled to deliver 10 mL of M200 at 10.0 mg/mL. Intact vials were stored in a refrigerator at 2°C to 8°C (36°F-46°F) and maintained without freezing or shaking.

Once prepared, the M200 was administered within 6 hours if stored at room temperature (25°C) or 48 hours if refrigerated (between 2-8°C). After that time, the prepared solution was discarded.

The appropriate dose of M200 to be administered to the patient was calculated by multiplying the patient's weight (kg) by the appropriate dose level (mg/kg) for the patient. The patient's pre-dose weight on Study Day 1 was used to calculate the dose throughout the study. For patients enrolled in the 0.5 mg/kg through the 10.0 mg/kg dose cohorts and for patients weighing ≤80 kg enrolled in the 15.0 mg/kg dose cohort, the dose was administered in a fixed total volume of 120 mL over one hour.

Although 120 mL of the diluted study drug was administered to the patient, the infusion bag was prepared to contain a total of 150 mL. The additional 30 mL was used to prime the infusion line and was not be administered to the patient. Thus, the total dose of

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study drug placed into the infusion bag was the patient's dose (i.e., the patient's weight (kg) × dose level [mg/kg] multiplied by 1.25). The total volume in the infusion bag was brought up to 150 mL by adding Sodium Chloride for Injection, USP (0.9%).

Patients weighing >80 kg enrolled in the 15.0 mg/kg dose cohort had their dose administered in a fixed total volume of 180 mL over one hour. Although 180 mL of the diluted study drug was administered to the patient, the infusion bag was prepared to contain a total of 210 mL. The additional 30 mL was used to prime the infusion line and was not administered to the patient. Thus, the total dose of study drug placed into the infusion bag was the patient's dose (i.e., the patient's weight (kg) x dose level [mg/kg] multiplied by 1.167). The total volume in the infusion bag was brought up to 210 mL by adding Sodium Chloride for Injection, USP (0.9%).

The pre-filled infusion line was attached directly to the patient's IV access (e.g., heparin lock). The infusate was administered at a rate of 2 mL/min (or 3 mL/min for 15.0 mg/kg cohort patients weighing >80 kg) over one hour using an infusion pump.

4. Primary Endpoints and Adverse Events

The study endpoints included maximum tolerated dose, dose-limiting toxicity, safety profile, immunogenicity, pharmacokinetics (PK) , monocyte saturation (monocytes express the $\alpha 5\beta 1$ receptor), and tumor response based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria (see, Therasse P, *et al.,* "New guidelines to evaluate the response to treatment in solid tumors," *Journal of the National Cancer Institute,* 92(3):205-216 (2000), which is hereby incorporated by reference herein in its entirety).

Adverse events were graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Dose-limiting toxicity was defined as any Grade-3 or Grade-4 adverse event, excluding scheduled hospitalizations or elective surgeries. Grade-3 or Grade-4 adverse events thought to be unrelated to M200 were excluded where appropriate following a review with the medical monitor and regulatory agencies.

Patient evaluations during the study included the following:

a. Screening (within 14 days of study entry) of: medical history and physical examination, ECOG performance status, routine chemistry panel, complete blood count (CBC) with differential and platelets, sensitive C-reactive protein (CRP), urinalysis with microscopic analysis (UA/micro), electrocardiogram (ECG), chest radiograph (CXR), disease-directed body CT or MRI, urine

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pregnancy test within 48 hours prior to dosing, and coagulation studies (prothrombin [PT] and partial thromboplastin time [PTT]), anti-M200 antibodies (i.e., to detect cross-reactive HAMA or HACA) for patients who previously received murine or chimeric antibodies, head CT for patients with tumors that commonly metastasize to brain or CNS, and, in some patients, a biopsy to evaluate tumor vessel vascularity.

b. On-study laboratory evaluations: routine chemistry panel, urate, serum amylase, CBC with differential and platelets, UA/micro.

c. Routine disease-directed radiographic imaging (e.g., CT scanning) before and after treatment with M200 was used to evaluate tumor response using uni-dimensional, RECIST criteria. Target lesions were selected on the basis of their size (lesions with the longest diameter (LD)) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the LD for all target lesions was calculated and reported as the baseline sum LD. The baseline sum LD was used as reference by which to characterize the objective tumor response. The following response criteria for the target lesions was used to assess the best overall response.

- Complete Response (CR): Disappearance of all target lesions confirmed by repeat imaging 25 to 28 days after CR first documented.
- Partial Response (PR): At least a 30% decrease in the sum of LD of target lesions, taking as reference the baseline sum LD, confirmed by repeat imaging 25 to 28 days after PR first documented.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as references the smallest sum LD.
- Progressive Disease (PD): At least a 20% increase in the sum of LD of target lesions, taking as reference the smallest sum LD recorded since treatment started, or the appearance of one or more new lesions.

d. Blood drawn for serum PK measurements, immunogenicity assays (anti-M200 antibodies), vascular growth factors, and measurement of bound and unbound $\alpha 5\beta 1$ on peripheral blood monocytes.

e. A 3-mm punch biopsy of superficial tumor metastasis was obtained and frozen after the last treatment with M200 from patients who consented (not required for enrollment) to the procedure. This biopsy specimen could be used evaluate tumor vessel vascularity following treatment with M200.

5. Pharmacokinetic Measurements

The first human PK data at low doses was used to predict the human serum levels at higher doses (described above). The serum for measuring M200 concentrations was obtained immediately before and after the first dose, and at 4, 24, 48, and 168 hours after completion of the first dose. Serum samples for measuring M200 concentrations were also taken immediately before, at the end, and 4 hours after the completion of each subsequent infusion. These samples were split and aliquots assayed before the next dose and again at the end of the study: end of 1st dose, 168 hours after 1st dose, immediately pre-dose (trough) and immediately post-dose (peak) for the 2nd, 3rd, 4th and 5th doses. The serum levels of M200 were measured by ELISA.

The plasma concentration-time data of M200 from each patient was subjected to a PK analysis. The following parameters were included in the calculations: peak (Cmax) and trough (Cmin) levels, terminal phase half-life ($T_{1/2\,\beta}$), area under the plasma-concentration time curve (AUC), elimination clearance (CL), and volume of distribution (V). Summary parameters were computed across patients from each treatment group. Changes in the PK parameters as a function of dose and time were also evaluated.

6. Immunogenicity

Immunogenicity of M200 was determined by a double-antigen bridging ELISA assay. Serum samples for anti-M200 antibodies were obtained from patients before treatment, prior to the second dose, and at study exit, 45 days after the last dose of M200. In addition, serum for anti-M200 antibodies was drawn if the interval between doses was $\geq$ 2 weeks. Samples were stored and evaluated at the end of the study. Samples that tested positive for antibodies to M200 were re-assayed for neutralizing antibodies to M200. Patients with known exposure to murine or chimeric monoclonal antibodies were screened for the presence of anti-M200 antibodies prior to receiving the first dose of M200.

7. Flow Cytometry Analysis

The integrin α5β1 is also expressed on monocytes in the peripheral blood. To determine the dose at which the α5β1 sites on these circulating cells is saturated by M200, samples of peripheral blood were taken before the first dose on Study Day 1, Study Days 2 and 8, just before each subsequent dose, and on Study Day 43. The saturation of α5β1 sites on monocytes was determined by flow cytometry. The monocytes were identified by using antibodies to CD14. Bound M200 on the monocyte surface was detected by adding labeled anti-human IgG4. Unoccupied (free) α5β1 on the cells was detected by adding labeled IIA1. The percentages of monocytes and lymphocytes expressing α5β1 was also assessed.

C. Results – Dose Escalation Study

M200 was well tolerated by patients at doses up to 15 mg/kg and no dose-limiting M200 toxicities were observed. The overall response outcome for the study was: stable disease (SD) in 11 patients and progressive disease (PD) in 10 patients. Table 4 shows a breakdown of the response outcome by dosage and patient tumor type.

TABLE 4: Response Outcomes by Dosage and Tumor Type

0.5 mg/kg	1.0 mg/kg	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	15.0 mg/kg
CRC (PD)	HCC (SD)	CRC (PD)	HCC (SD)	CRC (SD)	EC (PD)
	PC (SD)	NSCLC (SD)	EC (PD)	CRC (SD)	RCC (SD)
		MEL Ocular (PD)	THY (PD)	PARO (SD)	PRO (PD)
				BC (SD)	MEL (PD)
				RCC (SD)	MEL (SD)
				MEL (PD)	RCC (PD)

Abbreviations: CRC = colorectal; MEL = melanoma; RCC = renal; EC = esophogeal; HCC = hepatocellular; NSCLC = lung; PRO = prostate; THY = thyroid; PC = pancreatic; PARO = parotid; BC = breast; SD = Stable Disease; PD = Progressive Disease

Adverse events were generally mild to moderate in intensity and included fatigue, nausea, constipation, headache and anorexia. There were no severe or serious adverse events that were dose limiting or considered by investigators to be related to M200. Three patients at the 0.5 mg/kg and 1.0 mg/kg dose levels tested positive for anti-M200 antibodies, but there were no apparent associated adverse events. No patients in the

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higher dose cohorts tested positive for anti-M200. The one patient who received 0.5 mg/kg had fever after the first dose, which was recorded as an infusion reaction. The patient, however, did complete all 5 doses of M200 without subsequent episodes of fever or other signs of infusion reaction.

The study data indicated that M200 exhibits non-linear pharmacokinetics. Slower-clearance was observed at the higher concentrations with the $T_{1/2}$ = 15.7 days at the 10 mg/kg dosage level. In addition, The 10 mg/kg dosage resulted in monocyte saturation, and had a mean trough level of 82 μg/mL two weeks after the 1st dose, which is above the minimum effective *in vitro* concentration of 2-3 μg/mL.

D. Results – Extension Study

Six of the 11 patients exhibiting a stable disease response or better entered an extension study and continued dosing. As shown in Table 5, below, 5 of the 6 patients exhibited stable disease (SD) or better response. One of these patients with renal cell cancer (RCC) in the 15.0 mg/kg cohort achieved a partial response.

Table 5: Extension Study Outcomes

Dose Cohort (# patients; tumor type)	Best Response Outcome	Time to Progression* (Days)
2.5 mg/kg (1; NSCLC)	SD	129
5 mg/kg (1; HCC)	SD	122
10 mg/kg (1; CRC)	PD	70
10 mg/kg (1; PARO)	SD	143
15 mg/kg (1; RCC)	PR	214
15 mg/kg (1; MEL)	SD	172+ (on Study)

* Includes 43 days on dose escalation study plus days on extension study.
SD defined as stabilization for ≥16 weeks (112 days)

Example 9: Phase II Open-Label Study of M200 in Human Patients with Metastatic Renal Cell Carcinoma

A. Overview

Based on the efficacy of M200 demonstrated by the Phase I study of Example 8, a Phase II, open-label, multi-center, single-arm, 2-stage study of the efficacy of M200 for

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treating human patients has been undertaken. The primary objective of the study is to evaluate the efficacy (tumor response) of M200 in patients with metastatic renal cell carcinoma (RCC), as defined using Response Criteria for Solid Tumors (see RECIST, Example 8 above). The study also has as secondary objectives evaluation of other efficacy measures (ie, time to disease progression and duration of response), and further evaluation of the safety, immunogenicity and PK parameters of M200 that were initially evaluated in the Phase I study of Example 8. An additional exploratory objective is to measure detectable biomarkers in serum and plasma. The study will enroll up to 40 patients at up to eight investigational sites. Twenty patients will be enrolled into stage one of the study. If one confirmed complete response (CR) or partial response (PR) is observed by 4 months (16 weeks) or if one stable disease (SD) is observed at 4 months, an additional 20 patients will be enrolled (stage 2). All patients will receive M200 (10 mg/kg) as an intravenous infusion over 30 minutes once every other week for up to 52 weeks or until disease progression, whichever occurs first. The study exit visit will occur 45 days after the last dose or at the time of early termination if the patient is unable to return. A follow-up visit will occur at 3 months after the last dose. If a visit is not feasible, follow-up will be conducted by telephone. Long-term follow-up will be conducted by telephone at 6 months after the last dose.

B. Study Parameters and Protocols

The Phase II study is being conducted according to the parameters and protocols described in Table 6 below.

Table 6: Parameters of Phase II Study in Patients with Metastatic Renal Cell Carcinoma

Study Population:	Males and females of at least 18 years of age with metastatic RCC of clear cell histology.
Key Patient Inclusion/Exclusion Criteria:	**Inclusion:** Males and females of at least 18 years of age with metastatic RCC of predominantly clear cell histology who have received 0 to 2 prior regimens for metastatic disease; measurable disease according to Response Criteria for Solid Tumors (RECIST); Eastern Cooperative Oncology Group (ECOG) performance status ≤1; negative pregnancy test (women of childbearing potential only); pretreatment laboratory levels that meet specific criteria; and signed informed consent, including permission to use protected health information (PHI). **Exclusion:** Any of the following histologies of RCC: papillary, chromophobe, collecting duct, or unclassified; known sensitivity

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	to murine proteins or chimeric antibodies or other components of the product; use of any investigational drug within 4 weeks prior to screening or 5 half-lives of the prior investigational drug (whichever is longer); systemic chemotherapy, immunotherapy, radiation therapy, or monoclonal antibody therapy within 4 weeks of M200 administration; documented CNS tumor or CNS metastasis; history of thromboembolic events and bleeding disorders within the past year; and medical conditions that may be exacerbated by bleeding.
Sample Size:	Up to 40 patients may be enrolled. Twenty patients will be enrolled into stage one. If one confirmed complete response (CR) or partial response (PR) is observed by 4 months (16 weeks) or if one stable disease (SD) is observed at 4 months, an additional 20 patients will be enrolled (stage 2).
Dosage Form and Strength/Formulation:	M200 is a sterile, colorless, clear-to-slightly opalescent, preservative-free liquid for IV use. Each 10-mL single-use vial is filled to deliver 10 mL of M200 at 10 mg/mL. The composition of each vial is 10 mg/mL M200, 25 mM citrate, 150 mM sodium chloride, and 0.05% Polysorbate (Tween®) 80, with a pH of 6.5.
Storage and Filtration:	Intact vials will be stored in a refrigerator at 2°C to 8°C (36°F to 46°F). Do not freeze or shake. M200 must be administered within 6 hours if stored at room temperature (25°C) or 48 hours if refrigerated (between 2°C to 8°C). After that time, the prepared solution must be discarded.
Dosing Regimen/Route of Administration:	All eligible patients will receive 10 mg/kg M200 by intravenous (IV) infusion over 30 minutes every other week for up to 52 weeks or until disease progression, whichever occurs first. The dose of M200 to be administered to the patient will be calculated by multiplying the patient's weight (kg) by the dose level (10 mg/kg). The patient's predose weight on Study Day 0 will be used to calculate the dose throughout the study, provided the weight does not vary by more than 10%. The appropriate volume of the 10 mg/mL M200 formulation will be removed and diluted with 0.9% sodium chloride to a final volume of 120 mL for IV infusion into the patient over the 30 minute period.
Duration of Treatment and Follow-up:	All patients will receive M200 (10 mg/kg) intravenously once every other week for up to 52 weeks or until disease progression, whichever occurs first. The study exit visit will occur 45 days after the last dose or at the time of early termination if the patient is unable to return. A follow-up visit will occur at 3 months after the last dose. If a visit is not feasible, follow-up will be conducted by telephone. Long-term follow-up will be conducted by telephone at 6 months after the last dose.

Endpoints:	The primary endpoint of this study is the proportion of patients with a confirmed tumor response at any time during the study. The secondary endpoints are as follows: (1) time to disease progression; (2) duration of tumor response; (3) PK of M200; and (4) immunogenicity. The exploratory endpoint is the measurement of detectable biomarkers in serum and plasma.
Efficacy Measurements:	Disease-directed radiographic imaging every 8 weeks (2 months) to evaluate tumor response using uni-dimensional RECIST. At screen, CT or MRI of the head, chest, abdomen, and pelvis will be obtained. Every 8 weeks, a complete physical examination and imaging scans of all lesions present at screen plus all disease-directed anatomical lesions will be performed. For any PR or CR, confirmatory radiographic imaging will be repeated one month (between 28 to 35 days) post-PR or -CR. All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and will be recorded and measured at baseline. Measurable lesions are defined as follows: 2.0 cm in one dimension using conventional CT/MRI and 1.0 cm in one dimension using spiral CT. A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as the reference by which objective tumor response will be characterized.
Safety Measurements:	The following safety measurements will be monitored: adverse events (AE); serious adverse events (SAE); physical examination findings; vital signs; and abnormal laboratory values.
Pharmacokinetic Measurements:	Pharmacokinetic (PK) measurements will be taken in all patients at predose (within 15 minutes prior to dosing) and postdose (one hour post-end of infusion): Day 0, Weeks 2, 4, and 6, once every other month (Weeks 8, 16, 24, 32, 40, 48), Week 52, study exit visit, and 3-month follow-up (if possible). Samples obtained for PK may be used for anti-Ab analyses, if appropriate.
Immunogenicity:	Anti-Ab measurements will be taken in all patients within 15 minutes prior to dosing on Day 0, Week 8, study exit visit, and 3-month follow-up (if possible). Samples obtained for PK may be used for anti-Ab analyses, if appropriate.
Other Measurements:	An exploratory assay will evaluate the presence of biomarkers in serum and plasma. The following cancer markers are included in the assay: CEA, CA 19-9, Syndecan, IGFBP-2, and LFL2. The following cytokines/growth factors are included: MIA, IL-6, TNF-alpha, PGF, VEGF, EGF, and bFGF.
Statistical Methods:	This study design will yield a power of at least 79.5% for detecting an overall response rate of ≥20% with up to 20% of the responders displaying stable disease (SD). Summary statistics and 95% confidence intervals will be provided for dichotomous

	endpoints. Kaplan-Meier methods will be used to summarize temporal variables.

It is understood that the examples described above in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All publications, sequences of accession numbers, and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

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CLAIMS

We claim:

1. A method of inhibiting the proliferation of a cancer cell in a patient wherein the cancer cell expresses α5β1 integrin on its surface, the method comprising administering to the patient a therapeutically effective amount of a pharmaceutical composition comprising a liquid formulation comprising:
   about 1.0 mg/mL to 15 mg/mL anti-α5β1 antibody;
   about 22 mM to 27 mM citrate;
   about 145 mM to 165 mM sodium chloride;
   about 0.04% to 0.06% polysorbate (TWEEN®) 80;
   and a pH of about 5.5 to 7.5.

2. The method of Claim 1, wherein the antibody neutralizes at least one biological activity of α5β1 integrin.

3. The method of claim 1, wherein the anti-α5β1 antibody binds to the same epitope of α5β1 integrin as the M200 antibody.

4. The method of claim 1, wherein the anti-α5β1 antibody competitively inhibits binding of M200 to α5β1 integrin expressed on the cell surface.

5. The method of claim 1, wherein the anti-α5β1 antibody is selected from the group consisting of M200, F200 and IIA1.

6. The method of Claim 1, wherein the antibody competitively inhibits binding of a second antibody to α5β1 integrin on the cancer cell surface wherein the second antibody comprises an amino acid sequence selected from SEQ ID NOs: 2, 4, 6 and 8.

7. The method of claim 1, wherein the therapeutically effective dose is about 10 mg/kg.

8. The method of Claim 1, wherein the cancer cell is selected from the group consisting of breast cancer cell, lung cancer cell, metastatic melanoma cell, pancreatic cancer cell, and renal cell carcinoma cell.

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9. The method of claim 1, wherein the cancer is renal cell carcinoma or metastatic melanoma.

10. The method of Claim 1, further comprising administering a chemotherapeutic agent to the patient either serially or in combination with the antibody.

11. A method of treating a subject for a cancer that expresses $\alpha5\beta1$ wherein the subject has not yet developed a tumor comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a liquid formulation comprising:

about 1.0 mg/mL to 15 mg/mL anti-$\alpha5\beta1$ antibody;
about 22 mM to 27 mM citrate;
about 145 mM to 165 mM sodium chloride;
about 0.04% to 0.06% polysorbate (TWEEN®) 80;
and a pH of about 5.5 to 7.5.

12. The method of Claim 11, wherein the antibody binds to the same epitope of $\alpha5\beta1$ integrin as M200.

13. The method of Claim 11, wherein the antibody competitively inhibits binding of M200 to $\alpha5\beta1$ integrin expressed on the cancer cell surface.

14. The method of Claim 11, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, colon cancer, fibrosarcoma, lung cancer, metastatic melanoma, pancreatic cancer, prostate cancer, ovarian cancer, renal cell carcinoma and spleen cancer.

15. The method of claim 11, wherein the cancer is renal cell carcinoma or metastatic melanoma.

16. The method of claim 11, wherein the therapeutically effective dose is about 10 mg/kg.

17. The method of Claim 11, further comprising administering a chemotherapeutic agent to the patient either serially or in combination with the antibody.

18. A pharmaceutical composition comprising a liquid formulation comprising:

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about 1.0 mg/mL to 15 mg/mL anti-α5β1 antibody;
about 22 mM to 27 mM citrate;
about 145 mM to 165 mM sodium chloride;
about 0.04% to 0.06% polysorbate (TWEEN®) 80;
and a pH of about 5.5 to 7.5.

19. The pharmaceutical composition of claim 18, wherein the concentration of anti-α5β1 antibody is about 10 mg/mL.

20. The pharmaceutical composition of claim 18, wherein the anti-α5β1 antibody is M200.

21. The pharmaceutical composition of claim 18, wherein the anti-α5β1 antibody is M200.

22. The pharmaceutical composition of claim 18, wherein the composition further comprises a chemotherapeutic agent.

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A. IIA1 V_H sequences
[NA, SEQ ID NO: 1; AA, SEQ ID NO: 2]

```
1   ATGGCTGTCCTGGGGCTGCTTCTCTGCCTGGTGACTTTCCCAAGCTGTGTCCTGTCCCAG
     M  A  V  L  G  L  L  L  C  L  V  T  F  P  S  C  V  L  S  Q
61  GTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACA
     V  Q  L  K  E  S  G  P  G  L  V  A  P  S  Q  S  L  S  I  T
121 TGCACCATCTCAGGGTTCTCATTAACCGACTATGGTGTTCACTGGGTTCGCCAGCCTCCA
     C  T  I  S  G  F  S  L  T  D  Y  G  V  H  W  V  R  Q  P  P
181 GGAAAGGGTCTGGAGTGGCTGGTAGTGATTTGGAGTGATGGAAGCTCAACCTATAATTCA
     G  K  G  L  E  W  L  V  V  I  W  S  D  G  S  S  T  Y  N  S
241 GCTCTCAAATCCAGAATGACCATCAGGAAGGACAACTCCAAGAGCCAAGTTTTCTTAATA
     A  L  K  S  R  M  T  I  R  K  D  N  S  K  S  Q  V  F  L  I
301 ATGAACAGTCTCCAAACTGATGACTCAGCCATGTACTACTGTGCCAGACATGGAACTTAC
     M  N  S  L  Q  T  D  D  S  A  M  Y  Y  C  A  R  H  G  T  Y
361 TACGGTATGACTACGACGGGGGATGCTTTGGACTACTGGGGTCAAGGAACCTCAGTCACC
     Y  G  M  T  T  T  G  D  A  L  D  Y  W  G  Q  G  T  S  V  T
421 GTCTCCTCA
     V  S  S
```

B. IIA1 V_L sequences
[NA, SEQ ID NO: 3; AA, SEQ ID NO: 4]

```
1   ATGGATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAGTCATAATGTCC
     M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S  V  I  M  S
61  AGAGGACAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCTCTAGGGGAACGG
     R  G  Q  I  V  L  T  Q  S  P  A  I  M  S  A  S  L  G  E  R
121 GTCACCATGACCTGCACTGCCAGTTCAAGTGTAAGTTCCAATTACTTGCACTGGTACCAG
     V  T  M  T  C  T  A  S  S  S  V  S  S  N  Y  L  H  W  Y  Q
181 CAGAAGCCAGGATCCGCCCCCAATCTCTGGATTTATAGCACATCCAACCTGGCTTCTGGA
     Q  K  P  G  S  A  P  N  L  W  I  Y  S  T  S  N  L  A  S  G
241 GTCCCAGCTCGTTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGC
     V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I  S  S
301 ATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCTTCGTTCCCCACCGACG
     M  E  A  E  D  A  A  T  Y  Y  C  H  Q  Y  L  R  S  P  P  T
361 TTCGGTGGAGGCACCAAGCTGGAAATCAAA
     F  G  G  G  T  K  L  E  I  K
```

FIGURE 1

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A. M200 V_H sequences
[NA, SEQ ID NO: 5; AA, SEQ ID NO: 6]

```
1    TCTAGACCACCATGGCTGTCCTGGGGCTGCTTCTCTGCCTGGTGACTTTCCCAAGCTGTG
                M  A  V  L  G  L  L  L  C  L  V  T  F  P  S  C
61   TCCTGTCCCAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCC
     V  L  S  Q  V  Q  L  K  E  S  G  P  G  L  V  A  P  S  Q  S
121  TGTCCATCACATGCACCATCTCAGGGTTCTCATTAACCGACTATGGTGTTCACTGGGTTC
     L  S  I  T  C  T  I  S  G  F  S  L  T  D  Y  G  V  H  W  V
181  GCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGTAGTGATTTGGAGTGATGGAAGCTCAA
     R  Q  P  P  G  K  G  L  E  W  L  V  V  I  W  S  D  G  S  S
241  CCTATAATTCAGCTCTCAAATCCAGAATGACCATCAGGAAGGACAACTCCAAGAGCCAAG
     T  Y  N  S  A  L  K  S  R  M  T  I  R  K  D  N  S  K  S  Q
301  TTTTCTTAATAATGAACAGTCTCCAAACTGATGACTCAGCCATGTACTACTGTGCCAGAC
     V  F  L  I  M  N  S  L  Q  T  D  D  S  A  M  Y  Y  C  A  R
361  ATGGAACTTACTACGGAATGACTACGACGGGGGATGCTTTGGACTACTGGGGTCAAGGAA
     H  G  T  Y  Y  G  M  T  T  T  G  D  A  L  D  Y  W  G  Q  G
421  CCTCAGTCACCGTCTCCTCAG^GTAAGAATGGCCTCTAGA
     T  S  V  T  V  S  S
```

B. M200 V_L sequences
[NA, SEQ ID NO: 7; AA, SEQ ID NO: 8]

```
1    ACGCGTCCACCATGGATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAG
                M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S
61   TCATAATGTCCAGAGGACAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCTC
     V  I  M  S  R  G  Q  I  V  L  T  Q  S  P  A  I  M  S  A  S
121  TAGGGGAACGGGTCACCATGACCTGCACTGCCAGTTCAAGTGTCAGTTCCAATTACTTGC
     L  G  E  R  V  T  M  T  C  T  A  S  S  S  V  S  S  N  Y  L
181  ACTGGTACCAGCAGAAGCCAGGATCCGCCCCCAATCTCTGGATTTATAGCACATCCAACC
     H  W  Y  Q  Q  K  P  G  S  A  P  N  L  W  I  Y  S  T  S  N
241  TGGCTTCTGGAGTCCCAGCTCGTTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCA
     L  A  S  G  V  P  A  R  F  S  G  S  G  S  G  T  S  Y  S  L
301  CAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCTTCGTT
     T  I  S  S  M  E  A  E  D  A  A  T  Y  Y  C  H  Q  Y  L  R
361  CCCCACCGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAAC^GTAAGTAGAATCCAAAGT
     S  P  P  T  F  G  G  G  T  K  L  E  I  K
421  CTAGA
```

FIGURE 2

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(54) Title: HUMAN ANTI-KIR ANTIBODIES

(57) Abstract: Compositions and methods for regulating an immune response in a subject are described. More particularly, described are human antibodies that regulate the activity of NK cells and allow a potentiation of NK cell cytotoxicity in mammalian subjects, and antibodies having antigen-binding properties similar to those of human monoclonal antibody 1-7F9 or 14F1. Described also are also fragments and derivatives of such antibodies, as well as pharmaceutical compositions comprising the same and their uses, particularly for use in therapy, to increase NK cell activity or cytotoxicity in subjects.

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HUMAN ANTI-KIR ANTIBODIES

FIELD OF THE INVENTION

The present invention relates to human antibodies, as well as fragments and derivatives thereof, which cross-react with and/or block two or more inhibitory KIR receptors present on the surface of NK cells, and potentiate NK cell cytotoxicity in mammalian subjects or in a biological sample. The invention also relates to methods of making such antibodies, fragments, variants, and derivatives; pharmaceutical compositions comprising the same; and the use of such molecules and compositions, particularly in therapy, to increase NK cell activity or cytotoxicity in subjects.

BACKGROUND OF THE INVENTION

Natural killer (NK) cells are a sub-population of lymphocytes, involved in immunity and in the host immune surveillance system.

NK cells are mononuclear cell that develop in the bone marrow from lymphoid progenitors, and morphological features and biological properties typically include the expression of the cluster determinants (CDs) CD16, CD56, and/or CD57; the absence of the alpha/beta or gamma/delta TCR complex on the cell surface; the ability to bind to and kill target cells that fail to express "self" major histocompatibility complex (MHC)/human leukocyte antigen (HLA) proteins; and the ability to kill tumor cells or other diseased cells that express ligands for activating NK receptors. NK cells are characterized by their ability to bind and kill several types of tumor cell lines without the need for prior immunization or activation. NK cells can also release soluble proteins and cytokines that exert a regulatory effect on the immune system; and can undergo multiple rounds of cell division and produce daughter cells with similar biologic properties as the parent cell. Upon activation by interferons and/or cytokines, NK cells mediate the lysis of tumor cells and of cells infected with intracellular pathogens by mechanisms that require direct, physical contacts between the NK cell and the target cell. Lysis of target cells involves the release of cytotoxic granules from the NK cell onto the surface of the bound target, and effector proteins such as perforin and granzyme B that penetrate the target plasma membrane and induce apoptosis or programmed cell death. Normal, healthy cells are protected from lysis by NK cells.

Based on their biological properties, various therapeutic and vaccine strategies have been proposed in the art that rely on a modulation of NK cells. However, NK cell activity is regulated by a complex mechanism that involves both stimulating and inhibitory signals.

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Briefly, the lytic activity of NK cells is regulated by various cell surface receptors that transduce either positive or negative intracellular signals upon interaction with ligands on the target cell. The balance between positive and negative signals transmitted via these receptors determines whether or not a target cell is lysed (killed) by a NK cell. NK cell stimulatory signals can be mediated by Natural Cytotoxicity Receptors (NCR) such as NKp30, NKp44, and NKp46; as well as NKG2C receptors, NKG2D receptors, certain activating Killer Ig-like Receptors (KIRs), and other activating NK receptors (Lanier, Annual Review of Immunology 2005;23:225-74). NK cell inhibitory signals can be mediated by receptors like Ly49, CD94/NKG2A, as well as certain inhibitory KIRs, which recognize major histocompatibility complex (MHC) class I-molecules (Kärre et al., Nature 1986;319:675-8; Öhlén et al, Science 1989;246:666-8). These inhibitory receptors bind to polymorphic determinants of MHC class I molecules (including HLA class I) present on other cells and inhibit NK cell-mediated lysis.

KIRs, sometimes also referred to as Killer Inhibitory Receptors, have been characterized in humans and non-human primates, and are polymorphic type 1 trans-membrane molecules present on certain subsets of lymphocytes, including NK cells and some T cells. KIRs interact with determinants in the alpha 1 and 2 domains of the MHC class I molecules and, as described above, distinct KIRs are either stimulatory or inhibitory for NK cells.

The nomenclature for KIRs is based upon the number of extracellular domains (KIR2D and KIR3D having two and three extracellular Ig-domains, respectively) and whether the cytoplasmic tail is long (KIR2DL or KIR3DL) or short (KIR2DS or KIR3DS). The presence or absence of a given KIR is variable from one NK cell to another within the NK population present in a single individual. Among humans, there is also a relatively high level of polymorphism of KIR genes, with certain KIR genes being present in some, but not all individuals. The expression of KIR alleles on NK cells is stochastically regulated, meaning that, in a given individual, a given lymphocyte may express one, two, or more different KIRs, depending on the genoptype of the individual. The NK cells of a single individual typically express different combinations of KIRs, providing a repertoire of NK cells with different specificities for MHC class I molecules.

Certain KIR gene products cause stimulation of lymphocyte activity when bound to an appropriate ligand. The activating KIRs all have a short cytoplasmic tail with a charged transmembrane residue that associates with an adapter molecule having an Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) which transduce stimulatory signals to the NK cell. By contrast, inhibitory KIRs have a long cytoplasmic tail containing Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM), which transduce inhibitory signals to the NK cell upon engagement of their MHC class I ligands. The known inhibitory KIRs include members of the

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KIR2DL and KIR3DL subfamilies. Inhibitory KIRs having two Ig domains (KIR2DL) recognize HLA-C allotypes: KIR2DL2 (formerly designated p58.2) and the closely related, allelic gene product KIR2DL3 both recognize "group 1" HLA-C allotypes (including HLA-Cw1, -3, -7, and -8), whereas KIR2DL1 (p58.1) recognizes "group 2" HLA-C allotypes (such as HLA-Cw2, -4, -5, and -6). The recognition by KIR2DL1 is dictated by the presence of a Lys residue at position 80 of HLA-C alleles. KIR2DL2 and KIR2DL3 recognition is dictated by the presence of an Asn residue at position 80 in HLA-C. Importantly, the great majority of HLA-C alleles have either an Asn or a Lys residue at position 80. Therefore, KIR2DL1, -2, and -3 collectively recognize essentially all HLA-C allotypes found in humans. One KIR with three Ig domains, KIR3DL1 (p70), recognizes an epitope shared by HLA-Bw4 alleles. Finally, KIR3DL2 (p140), a homodimer of molecules with three Ig domains, recognizes HLA-A3 and -A11.

Although multiple inhibitory KIRs and/or other MHC class I-specific inhibitory receptors (Moretta et al, Eur J Immunogenet. 1997;24(6):455-68; Valiante et al, Immunol Rev 1997;155:155-64; Lanier, Annu Rev Immunol 1998;16:359-93) may be co-expressed by NK cells, in any given individual's NK repertoire there are cells that express only a single KIR, and thus are inhibited only by specific MHC class I alleles (or alleles belonging to the same group of MHC class I allotypes). Human MHC class I molecules often are referred to as Human Histocompatibility Antigen (HLA) class I.

NK cell populations or clones that are KIR-ligand mismatched with respect to their targets, *i.e.*, that express KIRs which do not recognize any HLA molecule of a host, have been shown to mediate potent, life-saving anti-tumor responses after allogeneic bone-marrow transplantation in leukemia patients (Ruggeri et al., Science 2002,295:2097-2100). The underlying mechanism is believed to be that HLA mismatched hematopoietic transplantation leads to the expansion of donor-derived NK cells expressing KIR which do not recognize any HLA ligands in the recipient, and thus are not inhibited via KIR. These allogeneic NK clones exert potent anti-tumor activity. This response is very strong in patients diagnosed with acute myeloid leukaemia (AML), and treated with KIR-MHC mismatched haploidentical transplants. One way of reproducing this effect by pharmacological treatment of a patient would be to administer reagents that block the KIR/HLA interaction to activate the patient's endogenous NK cells.

Certain monoclonal antibodies specific for KIR2DL1 have been shown to block the interaction of KIR2DL1 with "group 2" HLA-C allotypes, such as HLA-Cw4 (Moretta et al., J Exp Med 1993;178:597-604), and to promote NK-mediated lysis of target cells that express those HLA-C allotypes. Monoclonal antibodies against KIR2DL2/3 that block the interaction of KIR2DL2/3 with HLA-Cw3 or similar allotypes have also been described (Moretta et al., J

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Exp Med 1993;178:597-604). Such antibodies are not ideal for use in clinical situations, as the development of two therapeutic monoclonal antibodies (mAbs) and administration of both of such antibodies or a selection of one of such antibodies (after appropriate diagnosis) would be required to treat all potential patients, depending on whether any given patient was expressing group 1 or group 2 HLA-C allotypes.

Watzl et al. (Tissue Antigens 2000;56:240-247) produced cross-reacting murine antibodies recognizing multiple isotypes of KIRs, but those antibodies did not potentiate the lytic activity of NK cells. Further, Spaggiari et al. (Blood 2002;99:1706-1714 and Blood 2002;100:4098-4107) carried out experiments utilizing numerous murine monoclonal antibodies against various KIRs. One of those antibodies, NKVSF1 (also known as Pan2D), was reported to recognize a common epitope of KIR2DL1 (CD158a), KIR2DL2 (CD158b) and KIR2DS4 (p50.3). Shin et al. (Hybridoma 1999;18:521-7) also reported the production of two monoclonal antibodies, denoted A210 and A803g, capable of binding to all of KIR2DL1, KIR2DL3, and KIR2DS4. However, for therapeutic use of an antibody in blocking the inhibitory KIRs of a patient's NK cells, the fewer activating KIR molecules an antibody cross-reacts with, the better, since blockade of activating receptors could impair the stimulation of NK cells. Thus, an antibody having the antigen-binding characteristics of NKVSF1, A210, or A803g would not be optimal in a clinical setting. Additionally, the use of murine monoclonal antibodies in the treatment of a human patient may result in a host immune-response against the antibodies, thus compromising the efficacy of the treatment.

Accordingly, practical and effective approaches for the therapeutic modulation of inhibitory KIRs have not been made available so far in the art.

SUMMARY OF THE INVENTION

This invention provides novel and useful human antibodies that specifically bind to KIR2DL1 and at least one of KIR2DL2 and KIR2DL3, or to all three of these KIRs, and/or human antibodies which potentiate NK-cell lytic activity by blocking the interactions between one or more such KIRs and HLA-C. Fragments and derivatives of such antibodies are also provided. The invention also pertains to novel and useful antibodies, antibody fragments, and derivatives of antibodies, which comprise VH and VL sequences substantially identical to those of human antibodies 1-7F9 and 1-4F1, as described herein. The invention also provides nucleic acids comprising nucleotide sequences encoding such antibodies; vectors comprising such nucleic acids; host cells and organisms comprising such nucleic acids and/or vectors; and compositions, such as pharmaceutically acceptable compositions and kits, comprising such proteins, nucleic acids, vectors, and/or cells and typically one or more

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additional ingredients that can be active ingredients or inactive ingredients that promote formulation, delivery, stability, or other characteristics of the composition (*e.g.*, various carriers). The invention further provides various new and useful methods making and using such antibodies, nucleic acids, vectors, cells, organisms, and/or compositions, such as in the modulation of KIR-mediated biological activities, for example in the treatment of diseases related thereto.

In one aspect, the invention provides a human or humanized antibody that binds to each one of KIR2DL1, KIR2DL2, and KIR2DL3, but which does not bind to KIR2DS4. In one embodiment, the antibody further does not bind to KIR2DS3. In another embodiment, the human or humanized antibody blocks the binding of at least one of KIR2DL1, KIR2DL2, and KIR2DL3 to an HLA-C class I molecule. In a further embodiment, the antibody may block the binding of an HLA-Cw4 molecule to KIR2DL1, and the binding of an HLA-Cw3 molecule to at least one of KIR2DL2 or KIR2DL3. For example, the antibody may block the binding of an HLA-Cw4 molecule to residues M44, F45 and D72 of the extracellular portion of KIR2DL1 (SEQ ID NO:23). In yet another embodiment, the antibody potentiates the lytic activity of an NK cell against a human target cell expressing an HLA-C class I molecule.

In another aspect, the invention provides a human or humanized antibody that competes with an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17, in the binding to at least one of KIR2DL1, KIR2DL2, and KIR2DL3. In one embodiment, the antibody competes with an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17, in the binding to each of KIR2DL1, KIR2DL2, and KIR2DL3. In another embodiment, the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:15. In yet another embodiment, the antibody comprises (a) a heavy chain CDR1 amino acid sequence corresponding to residues 31-35 of SEQ ID NO:17; (b) a heavy chain CDR2 amino acid sequence corresponding to residues 50-65 of SEQ ID NO:17; and (c) a heavy chain CDR3 amino acid sequence corresponding to residues 99-112 of SEQ ID NO:17. For example, the antibody may comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17.

In another aspect, the invention provides an isolated human or humanized antibody that binds to a KIR2DL1 epitope substantially comprising the amino acid residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88.

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In another aspect, the invention provides an isolated human or humanized antibody that has a dissociation constant (K_d) for KIR2DL1 of no more than about 0.45 nM and/or a K_d for KIR2DL3 of no more than about 0.025 nM.

The invention also provides a human or humanized antibody or antibody fragment, or a derivative thereof, which has any of the foregoing properties, alone or in any suitable combination. In one embodiment, the antibody is a monoclonal antibody. In another embodiment, the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. For example, the antibody may be an IgG4 antibody.

The invention also provides a nucleic acid encoding the human or humanized antibody or antibody fragment having any of the foregoing properties, a vector comprising such a nucleic acid, a cell comprising such a vector, and a method of producing a human anti-KIR antibody, comprising culturing such a cell under conditions suitable for expression of the anti-KIR antibody.

The invention also provides a pharmaceutical composition comprising a human or humanized antibody or antibody fragment having one or more of the foregoing properties or produced by any method, in an amount effective to detectably potentiate NK cell cytotoxicity in a patient, together with a pharmaceutically acceptable carrier or excipient. The composition may, for example, comprise Polysorbate 80, sucrose, or both Polysorbate 80 and sucrose.

The invention also provides a method of potentiating NK cell activity in a subject in need thereof, which method comprises administering to the subject an effective amount of any of the foregoing compositions. In one embodiment, the subject is a patient suffering from cancer. For example, the patient may be suffering from a cancer selected from acute myeloid leukaemia, chronic myeloid leukaemia, multiple myeloma, and non-Hodgkin's lymphoma. Alternatively, the patient may be suffering from a cancer selected from colorectal cancer, renal cancer, ovarian cancer, lung cancer, breast cancer, and malignant melanoma. In another embodiment, the subject is a patient suffering from an infectious disease. In yet another embodiment, the method further comprises administering a therapeutic agent selected from an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody that binds to an inhibitory KIR, an anti-infective agent, a targeting agent, and an adjunct compound.

It should be understood that description of antibodies "provided" by the invention or antibodies to which the invention "relates", etc., also, by implication, refers to antibodies that may be useful in practice of the inventive methods described herein, unless otherwise stated or clearly contradicted by context.

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These and other aspects and features of the invention are described in further detail herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the murine monoclonal antibody DF200 binding to a common determinant of various human KIR2DL receptors. (A) Clone CP11, KIR2DL1+, DF200. (B) Clone CP502, KIR2DL3+. (C) Clone CP11, KIR2DL1+, Anti-KIR2DL1. (D) Clone CP502, KIR2DL3+, Anti-KIR2DL2/3.

Figure 2 depicts the murine monoclonal antibody DF200 neutralizing the KIR2DL1-mediated inhibition of NK cell cytotoxicity against Cw4-expressing target cells, showing the reconstitution of lysis with the anti-KIR mAb on C1R-Cw4 target at effector/target (E/T) ratios of 4/1.

Figure 3 depicts the murine monoclonal antibody DF200, a Fab fragment of DF200, and conventional murine antibodies specific for either KIR2DL1 (mAbs EB6 and XA-141) or KIR2DL2/3 (mAbs GL183 and Y249) neutralizing the KIR2DL1-mediated inhibition of cytotoxicity against Cw4-positive target cells, and the KIR2DL2/3-mediated inhibition of NK cell cytotoxicity against Cw3-positive target cells. (A) and (C) KIR2DL1+, target cells 721.221-Cw4+. (B) KIR2DL2+, target cells 721.221-Cw3+. (D) KIR2DL3+, target cells 721.221-Cw3+.

Figure 4 depicts reconstitution of cell lysis by NK cells that express KIR2DL1 against HLA-Cw4 positive target cells in the presence of F(ab')2 fragments of the DF200 and EB6 antibodies. (A) Target cells 721.221-Cw4; E/T ratio = 1. (B) Target cells B-EBV cells expressing Cw4, E/T ratio = 2.

Figure 5 depicts induction of NK-mediated killing by cross-reactive murine (DF200 and NKVSF1 (Pan2D)) and human (1-7F9, 1-4F1, 1-6F5 and 1-6F1) mAbs, and a murine conventional KIR2DL1-specific mAb (EB6). The mAbs induce (reconstitute) killing by KIR2DL1-expressing NK cells (YTS-KIR2DL1) of 721.221 target cells transfected with HLA-Cw4. E/T ratio = 1. (A) 30 µg/ml mAb. (B) 10 µg/ml mAb.

Figure 6 depicts induction of NK-mediated killing by the same antibodies as in Figure 5. The mAbs induce (reconstitute) killing by KIR2DL1-expressing NK cells (YTS-KIR2DL1) of B-EBV cells expressing endogenous HLA-Cw4. E/T ratio = 2. (A) 30 µg/ml mAb. (B) 10 µg/ml mAb.

Figure 7 depicts an epitope map showing results of competitive binding experiments obtained by surface plasmon resonance (BIAcore®) analysis with anti-KIR antibodies to KIR2DL1, where overlapping circles designate overlap among the mAbs in binding to

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KIR2DL1. Results show that 1-7F9 is competitive with EB6 and 1-4F1, but not with NKVSF1 (Pan2D) and DF200, on KIR2DL1. Antibody 1-4F1 in turn is competitive with EB6, DF200, NKVSF1 (Pan2D), and 1-7F9. Antibody NKVSF1 (Pan2D) competes with DF200, 1-4F1, and EB6, but not 1-7F9, on KIR2DL1. DF200 competes with NKVSF1 (Pan2D), 1-4F1, and EB6, but not 1-7F9, on KIR2DL1.

Figure 8 depicts an epitope map showing results of competitive binding experiments obtained by BIAcore® analysis with anti-KIR antibodies to KIR2DL3, where overlapping circles designate overlap in binding to KIR2DL3. Results show that 1-4F1 is competitive with NKVSF1 (Pan2D), DF200, gl183, and 1-7F9 on KIR2DL3. 1-7F9 is competitive with DF200, gl183, and 1-4F1, but not with NKVSF1 (Pan2D), on KIR2DL3. NKVSF1 (Pan2D) competes with DF200, 1-4F1, and GL183, but not 1-7F9, on KIR2DL3. DF200 competes with NKVSF1 (Pan2D), 1-4F1, and 1-7F9, but not with GL183, on KIR2DL3.

Figure 9 depicts an epitope map showing results of competitive binding experiments obtained by BIAcore® analysis with anti-KIR antibodies to KIR2DS1, where overlapping circles designate overlap in binding to KIR2DS1. Results show that antibody 1-4F1 is competitive with NKVSF1 (Pan2D), DF200, and 1-7F9 on KIR2DS1. Antibody 1-7F9 is competitive with 1-4F1, but not competitive with DF200 and NKVSF1 (Pan2D) on KIR2DS1. NKVSF1 competes with DF200 and 1-4F1, but not with 1-7F9, on KIR2DS1. DF200 competes with NKVSF1 and 1-4F1, but not with 1-7F9, on KIR2DS1.

Figure 10 depicts Pan2D (NKVSF1) mAb titration demonstrating binding of the mAb to cynomolgus NK cells. Cynomolgus NK cells (NK bulk day 16) were incubated with different amount of NKVSF1 (Pan2D) mAb followed by PE-conjugated goat F(ab')2 fragments anti-mouse IgG (H+L) antibodies. The percentage of positive cells was determined with an isotypic control (purified mouse IgG1). Samples were done in duplicate. Mean fluorescence intensity = MFI.

Figure 11 shows the binding of soluble KIR2DL1 and KIR2DL1(R131W) mutant to cells. (A) Binding of increasing concentrations of soluble KIR2DL1-Fc, and a KIR2DL1(R131W)-hFc mutant, to cells expressing HLA-Cw3 or –Cw4. The bound KIR-Fc proteins were detected using a secondary fluorochrome-conjugated antibody, and revealed by flow-cytometry. Mean fluorescence is shown on the y-axis. (B) Binding of the indicated anti-KIR mAbs (GL183, EB6, DF200, and Pan2D (NKVSF1)) to KIR2DL1-Fc and the 2DL1(R131W) mutant protein, using human Ig as control. The figure shows that DF200 and Pan2D (NKVSF1) bind less well to the mutant than the wild-type KIR2DL1-Fc, indicating that both mAbs are affected by the R131W mutation. Therefore, R131 is one of the residues constituting the epitope for DF200 and Pan2D on KIR2DL1.

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Figure 12 provides a comparative alignment of the amino acid sequences of the light chain variable regions, and light chain CDRs of antibodies DF200 and Pan2D (NKVSF1). (A) Alignment of anti-KIR variable light (VL) regions of DF200 (SEQ ID NO:1) and Pan-2D (SEQ ID NO:2). Numbers above amino acid sequences indicate position respective to initiation of translation Met (+1) in the immature (non-secreted) immunoglobulin. (B) Alignment of CDR-L1 sequences. Residue before: Normally Cys. Residues after: Trp. Typically Trp-Tyr-Leu.Length: 10-17 aa. (C) Alignment of CDR-L2 sequences. Residues before: Generally Ile-Tyr. Length: 7 aa. Start: approximately 16 aa after the end of CDR-L1. Start: approximately 24 aa from the beginning of secreted protein. (D) Alignment of CDR-L3 sequences. Residues before: Cys. Residues after: Phe-Gly-XXX-Gly. Length: 7-11 aa. Start: approximately 33 aa after the end of CDR-L2.

Figure 13 provides the heavy chain variable region, and the heavy-chain CDRs of antibody DF200. (A) DF-200 VH region, immature protein. The secreted, mature VH starts at position 20: residue Q. The VH region ends with residue S and thereafter the constant region (not shown) continues. (B) CDR-H1. Residues before: Cys-XXX-XXX-XXX. Residues after: Trp. Generally Trp-Val or Trp-Ile. Length: 10-14 aa. Start: Approximately 22-26 aa from the beginning of the secreted protein. (C) CDR-H2. Residues before: Leu-Glu-Trp-Ile-Gly but other variations possible. Residues after: Lys or Arg / Leu or Ile or Val or Phe or Thr or Ala / Thr or Ser or Ile or Ala. Length: 16-20 aa. Start: Approximately 15 aa after the end of CDR-H1. (D) CDR-H3. Residues before: Cys-XXX-XXX (Typically Cys-Ala-Arg). Residues after: Trp-Gly-XXX-Gly. Length: 3-25 aa. Start: Approximately 33 after the end of CDR-H2.

Figure 14 depicts the nucleotide and amino acid sequences of the VH and VL sequence of human antibody 1-7F9. (A) Translation of HuKIR 1-7F9 mature variable light chain. (B) Nucleotide sequence encoding HuKIR 1-7F9 mature variable light chain. (C) Translation of HuKIR 1-7F9 mature variable heavy chain. (D) Nucleotide sequence encoding HuKIR 1-7F9 mature heavy chain.

Figure 15 shows the amino acid sequences of the VH and VL sequences of monoclonal antibodies 1-7F9, DF200 (VH sequence: SEQ ID NO:19; VL sequence: SEQ ID NO:21), and Pan2D (NKVSF1; VH sequence: SEQ ID NO:20; VL sequence: SEQ ID NO:22). The CDRs are boxed.

Figure 16 depicts flow cytometry data, showing binding of the murine antibody, Pan2D (NKVSF1) and the human mAbs 1-7F9 and 1-4F1 to cells transfected with KIR2DL1, KIR2DL3, KIR2DS1, KIR2DS2, KIR2DS3 or KIR2DS4, as indicated (purified antibody: 1 µg/ml). (A) - (R) NKVSF1 (pan2D), 1-7F9, and 1-4F1 all bind to KIR2DL1, KIR2DL3,

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KIR2DS1, and KIR2DS2. Neither antibody binds to KIR2DS3. NKVSF1, but not 1-7F9 or 1-4F1, binds to KIR2DS4.

Figure 17 depicts FACS analysis results. (A) Antibody-mediated neutralization of binding of soluble KIR-Fc to HLA-Cw4 on cells, detected by flow cytometry (FACS). FACS-measurement of KIR2DL1-mFc binding to LCL721.221-Cw4 cells, after pre-incubation with various concentrations of the cross-reactive mAb DF200, or the KIR2DL2/3-specific mAb GL183. DF200, but not GL183, reduces binding of KIR2DL1 to HLA-Cw4. Measurements are plotted as a percentage of KIR2DL1-hFc-binding in the absence of inhibitory antibodies. (B) FACS-measurement of KIR2DL1-mFc binding to LCL721.221-Cw4 cells, after pre-incubation with various concentrations of 1-1F4 and 1-7F9. Measurements are plotted as a percentage of KIR2DL1-mFc-binding in the absence of inhibitory antibodies.

Figure 18 depicts results of ^{51}Cr-release cytotoxicity assays. (A) LCL 721.221-Cw3 cells are efficiently killed by both YTS cells (diamonds) and YTS-2DL1 cells (triangles) at various E:T ratios. In contrast, LCL 721.221-Cw4 cells are efficiently killed by YTS cells (squares), but can not be killed by YTS-2DL1 cells (crosses) due to KIR-restrictions. (B) In the absence of antibody, YTS-2DL1 cells cannot kill LCL-721.221-Cw4 (E:T ratio 12:1). 1-7F9 induces killing of LCL 721.221-Cw4 cells by YTS-2DL1 cells in a dose-dependent fashion.

Figure 19 depicts a superposition of two crystal complex structures; the KIR2DL1/1-7F9 Fab' and the KIR2DL1/MHC class I, PDB-code 1IM9 (Fan et al., Nat. Immunol. 2001;2:452-460), using the C_α-atoms of the common KIR molecule (labeled 'KIR') as template. KIR2DL1/1-7F9 Fab' is indicated in grey line ribbon style while KIR2DL1/MHC class I in a dark tube style. 1-7F9 Fab' is labeled '1-7F9' while MHC class I is labeled 'MHC'. An overlap between the MHC and the Fab' is seen in the superposition, demonstrating the ability of 1-7F9 Fab' to obstruct MHC class I binding to the KIR2DL1 receptor. See Example 11.

Figure 20 shows the binding epitope of 1-7F9 on KIR2DL1, as indicated in the KIR2DL1 sequence. Amino acids within 4.0 Å distance from 1-7F9 are highlighted in grey and black background. Amino acids highlighted by a black background are involved in hydrogen-bonding to 1-7F9.

DEFINITIONS

For convenience, several terms are defined here. However, the list of defined terms provided here is not exclusive. Other terms may be defined throughout the description of the invention.

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Within the context of this invention "active" NK cells designate biologically active NK cells, including NK cells having the capacity of lysing target cells or enhancing the immune function of other cells. For instance, an "active" NK cell can be able to kill cells that express a ligand for an activating NK receptor and/or fail to express MHC/HLA antigens recognized by a KIR on the NK cell. NK cells can be obtained by various techniques known in the art, such as isolation from blood samples, cytapheresis, tissue or cell collections, etc. Useful protocols for assays involving NK cells can be found in Natural Killer Cells Protocols (edited by Campbell KS and Colonna M). Human Press. pp. 219-238 (2000).

As used herein, a "Killer Ig-like Receptor", "Killer Inhibitory Receptor", or "KIR", refers to a protein or polypeptide encoded by a gene that is a member of the KIR gene family or by a cDNA prepared from such a gene. A detailed review of the KIR gene family, including the nomenclature of KIR genes and KIR gene products, and Genbank accession numbers for exemplary KIRs, is "The KIR Gene Cluster" by M. Carrington and P. Norman, available at the NCBI web-site called "Bookshelf" (accessible via the World-Wide Web (WWW) address ncbi.nlm.nih.gov/books). The sequences of human KIR genes and cDNAs, as well as their protein products, are available in public databases, including GenBank. Non-limiting exemplary GenBank entries of human KIRs have the following accession numbers: KIR2DL1: Genbank accession number U24076, NM_014218, , AAR16197, or L41267; KIR2DL2: Genbank accession number U24075 or L76669; KIR2DL3: Genbank accession number U24074 or L41268; KIR2DL4: Genbank accession number X97229; KIR2DS1: Genbank accession number X89892; KIR2DS2: Genbank accession number L76667; KIR2DS3: Genbank accession number NM_012312 or L76670 (splice variant); KIR3DL1: Genbank accession number L41269; and KIR2DS4: Genbank accession number AAR26325. A KIR may comprise from 1 to 3 extracellular domains, and may have a long (*i.e.*, more than 40 amino acids) or short (*i.e.*, less than 40 amino acids) cytoplasmic tail. As previously described herein, these features determine the nomenclature of a KIR. Exemplary KIR2DL1, KIR2DL2, KIR2DL3, and KIR2DS4 molecules comprise the following respective amino acid sequences:

KIR2DL1 extracellular domain:

HEGVHRKPSLLAHPGXLVKSEETVILQCWSDVMFEHFLLHREGMFNDTLRLIGEHH DGVSKANFSISRMTQDLAGTYRCYGSVTHSPYQVSAPSDPLDIVIIGLYEKPSLSAQXGPTVL AGENVTLSCSSRSSYDMYHLSREGEAHERRLPAGPKVNGTFQADFPLGPATHGGTYRCFG SFHDSPYEWSKSSDPLLVSVTGNPSNSWPSPTEPSSKTGNPRHLH (SEQ ID NO:23), where "X" at position 16 is P or R, and where "X" at position 114 is P or L, representing allelic variants.

KIR2DL2 extracellular domain:

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HEGVHRKPSLLAHPGRLVKSEETVILQCWSDVRFEHFLLHREGKFKDTLHLIGEHH DGVSKANFSIGPMMQDLAGTYRCYGSVTHSPYQLSAPSDPLDIVITGLYEKPSLSAQPGPTV LAGESVTLSCSSRSSYDMYHLSREGEAHECRFSAGPKVNGTFQADFPLGPATHGGTYRCF GSFRDSPYEWSNSSDPLLVSVIGNPSNSWPSPTEPSSKTGNPRHLH (SEQ ID NO:24)

KIR2DL3 extracellular domain:

HEGVHRKPSLLAHPGPLVKSEETVILQCWSDVRFQHFLLHREGKFKDTLHLIGEHH DGVSKANFSIGPMMQDLAGTYRCYGSVTHSPYQLSAPSDPLDIVITGLYEKPSLSAQPGPTV LAGESVTLSCSSRSSYDMYHLSREGEAHERRFSAGPKVNGTFQADFPLGPATHGGTYRCF GSFRDSPYEWSNSSDPLLVSVTGNPSNSWPSPTEPSSETGNPRHLH (SEQ ID NO:25).

KIR2DS4 extracellular domain:

QEGVHRKPSFLALPGHLVKSEETVILQCWSDVMFEHFLLHREGKFNNTLHLIGEHH DGVSKANFSIGPMMPVLAGTYRCYGSVPHSPYQLSAPSDPLDMV (SEQ ID NO:38).

The term "KIR2DL2/3" refers to either or both of the KIR2DL2 and KIR2DL3 receptors. These two receptors have a very high homology, they are allelic forms of the same gene, and are considered by the art to be functionally similar.

Unless otherwise specified, term "MHC" encompasses MHC molecules in all mammals, whereas an "HLA" molecule refers to a human MHC molecule.

Within the context of this invention, stating that an antibody "binds" a determinant (*i.e.*, the word "bind" in the context of antibody:determinant interaction) designates that the antibody binds the determinant with specificity and/or affinity. For example, GL183 is a conventional monoclonal antibody that binds to KIR2DL2/3. EB6 is a conventional monoclonal antibody that binds to KIR2DL1. EB6 and GL183 are both commercially available (Beckman Coulter Inc., Fullerton, CA).

A "cross-reactive" anti-KIR antibody is an antibody that binds more than one KIR molecule with specificity and/or affinity. For example, DF200 and 1-7F9 are monoclonal antibodies cross-reactive with KIR2DL1, -2, and -3. The hybridoma producing antibody DF200 has been deposited at the CNCM culture collection, under identification no. "DF200", registration no. CNCM I-3224, registered 10 June 2004, Collection Nationale de Cultures de Microorganismes, Institut Pasteur, 25, Rue du Docteur Roux, F-75724 Paris Cedex 15, France. NKVSF1, also referred to as "Pan2D" herein, is cross-reactive with KIR2DL1, -2, and -3 and KIR2DS4. This antibody is commercially available from Serotec (Cergy Sainte-Christophe, France), Catalog ref no. MCA2243.

"Specific binding" or "specificity" refers to the ability of an antibody or other agent to detectably bind an epitope presented on an antigen, such as a KIR, while having relatively little detectable reactivity with other proteins or structures (such as other proteins presented

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on NK cells, or on other cell types). Specificity can be relatively determined by binding or competitive binding assays, using, *e.g.*, Biacore instruments, as described elsewhere herein. Specificity can be exhibited by, *e.g.*, an about 10:1, about 20:1, about 50:1, about 100:1, 10.000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules (in this case the specific antigen is a KIR). A KIR-binding antibody, specific for a KIR presented on the NK cells of a particular organism, such as a human, can sometimes exhibit binding to similar KIRs of other species (*i.e.*, a KIR-binding antibody, or other KIR-binding agent, may cross-react with KIRs of various species).

"Selectivity" refers to the preferential binding of a protein to a particular region, target, or peptide as opposed to one or more other biological molecules, structures, cells, tissues, etc. A KIR-binding antibody can also be selective for a KIR produced in a particular organism (*e.g.*, in a human as opposed to in a primate), and/or for a particular type of KIR (*e.g.*, a KIR with a long cytoplasmic tail), particularly where the antibody cross-reacts with more than one type of KIR produced in a particular organism, and/or a particular portion of a KIR (such as a particular epitope or antigenic determinant region). For example, selectivity can be determined by competitive ELISA or Biacore assays. The difference in affinity/avidity that marks selectivity can be any detectable preference (*e.g.*, a ratio of more than 1:1.1, or more than about 1:5, if detectable, would be suitable, including 1:10, 1:100, 1:1000 or more). Unless otherwise specified, any quantitative data on "affinity" presented herein refers to measurements of bivalent (as opposed to monovalent) binding.

An "epitope" or "binding site" is an area or region on an antigen to which an antigen-binding peptide (such as an antibody) specifically binds. A protein epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide (in other words, the amino acid residue is within the "footprint" of the specifically antigen binding peptide). The term epitope herein includes both types of amino acid binding sites in any particular region of a KIR that specifically binds to an anti-KIR antibody, or another KIR-specific agent according to the invention, unless otherwise stated (*e.g.*, in some contexts the invention relates to antibodies that bind directly to particular amino acid residues) . KIRs may comprise a number of different epitopes, which may include, without limitation, (1) linear peptide antigenic determinants, (2) conformational antigenic determinants which consist of one or more non-contiguous amino acids located near each other in a mature KIR conformation; and (3) post-translational antigenic determinants which consist, either

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in whole or part, of molecular structures covalently attached to a KIR, such as carbohydrate groups.

The phrase that a first antibody binds "substantially" or "at least partially" the same epitope as a second antibody means that the epitope binding site for the first antibody comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the amino acid residues on the antigen that constitutes the epitope binding site of the second antibody. Also, that a first antibody binds substantially or partially the same epitope as a second antibody means that the first and second antibodies compete in binding to the antigen, as described above. Thus, the term "binds to substantially the same epitope or determinant as" the monoclonal antibody 1-7F9 means that an antibody "competes" with 1-7F9. Generally, an antibody that "binds to substantially the same epitope or determinant as" the monoclonal antibody of interest (*e.g.* DF200, NKVSF1, 1-7F9) means that the antibody "competes" with said antibody of interest for binding to one or more KIR molecules, preferably a KIR molecule selected from the group consisting of KIR2DL1 and KIR2DL2/3. In other examples, an antibody that binds to substantially the same epitope or determinant on a KIR2DL1 molecule as the antibody of interest "competes" with the antibody of interest for binding to KIR2DL1. An antibody that binds to substantially the same epitope or determinant on a KIR2DL2/3 molecule as the antibody of interest "competes" with the antibody of interest for binding to KIR2DL2/3.

The phrase "binds to essentially the same epitope or determinant as" an antibody of interest means that an antibody "competes" with said antibody of interest for at least one, or for any and all KIR molecules to which said antibody of interest specifically binds. The phrase "binds to essentially the same epitope or determinant as" the monoclonal antibody 1-7F9 means that an antibody "competes" with 1-7F9 for at least one, preferably any and all KIR molecules, to which 1-7F9 specifically binds. For example, an antibody that binds to essentially the same epitope or determinant as the monoclonal antibodies 1-7F9 or NKVSF1 can "compete" with said 1-7F9 or NKVSF1 respectively for binding to KIR2DL1, KIR2DL2/3, KIR2DS1 and KIR2DS2.

The ability of an anti-KIR antibody to "block" the binding of a KIR molecule and an HLA molecule means that the antibody, in an assay using soluble or cell-surface associated KIR and HLA molecules, can detectably reduce the binding of a KIR-molecule to an HLA molecule in a dose-dependent fashion, where the KIR molecule detectably binds to the HLA molecule in the absence of the antibody. An exemplary assay for determining whether an anti-KIR antibody is capable of such blocking is provided in Example 8.

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The ability of an anti-KIR antibody to "reduce the inhibitory activity of a KIR", "facilitate NK cell activity," "facilitate NK cell cytotoxicity," "facilitate NK cells," "potentiate NK cell activity," "potentiate NK cell cytotoxicity," or "potentiate NK cells" means that an NK cell expressing a KIR, when contacted with the antibody, is capable of lysing target cells that express on their surface a particular MHC or HLA class I which is a ligand for said KIR. For example, the term "potentiation in NK cytotoxicity" means any substantial potentiation, or at least 5%, 10%, 20%, 30% or greater potentiation in NK cytotoxicity, *e.g.* at least about 50% potentiation of NK cytotoxicity (*e.g.*, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% as compared to a control. This includes, for example, about 55-100%, about 65-100%, about 75-100%, about 80-100%, or about 90-100% potentiation of NK cell cytotoxicity, as compared to a control. It also includes more than about 100%, more than about 500%, more than about 1000%, more than about 2000%, or higher increase in the cytotoxicity. This can be measured in, *e.g.*, an assay, such as a standard cytotoxicity assay, where a higher number, amounting to a higher percentage, of target cells are lysed by NK cells in the presence of an NK potentiator (such as an anti-KIR mAb) than in the absence of the potentiator (*i.e.*, the control).

The term "neutralize KIR-mediated inhibition of NK cell cytotoxicity" or "neutralize the inhibitory activity of a KIR," as used herein means the ability to increase specific lysis to more than about 20%, preferably with at least about 30%, at least about 40%, at least about 50%, at least about 100%, or more of the specific lysis obtained at the same effector:target cell ratio with NK cells or NK cell lines that are not blocked by their KIR, as measured by a classical chromium release test of cytotoxicity. "Neutralize KIR mediated inhibition" can also mean that in a chromium-release assay, or other cytotoxicity assay, using an NK cell clone or transfectant expressing one or several inhibitory KIRs and a target cell expressing at least one HLA class I allele that is recognized by one of the KIRs on the NK cell, the specific lysis obtained with the antibody is more than about 100%, preferably at least about 101%, at least about 150%, at least about 200%, or more (*e.g.*, about 101-150%, about 120-150%, about 120-200%, about 150-200%, or about 200-1000%), or more of the specific lysis obtained with the same concentration of NKVSF1 (commercially available from Serotec), using the same NK cells and target cells, at the same effector:target cell ratio.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions identical to, essentially identical to, or derived from human germline immunoglobulin sequences. Such human antibodies can include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). How-

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ever, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

In the context of the present invention, unless otherwise stated, "treatment" or "treating" refers to preventing, alleviating, managing, curing or reducing one or more symptoms or clinically relevant manifestations of a disease or disorder, unless contradicted by context. For example, "treatment" of a patient in whom no symptoms or clinically relevant manifestations of a disease or disorder have been identified is preventive therapy, whereas "treatment" of a patient in whom symptoms or clinically relevant manifestations of a disease or disorder have been identified generally does not constitute preventive therapy. Nonetheless, it should be understood that the various therapeutic and prophylactic method and use facets of the invention are distinct from one in many respects (*e.g.*, dosage of compound(s) to be delivered to a subject, timing of application, impetus for application, etc.) and may each be considered a unique facet of the invention.

In the context of the present invention "cancer" refers to any neoplastic disorder, including, but not limited to, such cellular disorders as sarcoma, carcinoma, melanoma, leukaemia, and lymphoma, which may include cancers in the breast, head and neck, ovaries, bladder, lung, pharynx, larynx, oesophagus, stomach, small intestines, liver, pancreas, colon, female reproductive tract, male reproductive tract, prostate, kidneys and central nervous system.

The term "biological sample" as used herein includes but is not limited to a biological fluid (for example serum, lymph, and blood), cell sample or tissue sample (for example bone marrow or tumor tissue) derived from a human or non-human mammal.

The term "substantially identical" in the context of two amino acid sequences means that the sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 95, at least about 98, or at least about 99 percent sequence identity. In one embodiment, residue positions that are not identical differ by conservative amino acid substitutions (further described elsewhere herein). Sequence identity is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, the publicly available GCG software contains programs such as "Gap" and "BestFit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species

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of organisms or between a wild-type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences can also be compared using FASTA, applying default or recommended parameters. A program in GCG Version 6.1., FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, Methods Enzymol. 1990;183:63-98; Pearson, Methods Mol. Biol. 2000;132:185-219). Another preferred algorithm when comparing a sequence to a database containing a large number of sequences from various organisms is the computer program BLAST, especially blastp, using default parameters. See, *e.g.*, Altschul et al., J. Mol. Biol. 1990;215:403-410; Altschul et al., Nucleic Acids Res. 1997;25:3389-402 (1997); each herein incorporated by reference. "Corresponding" amino acid positions in two substantially identical amino acid sequences are those aligned by any of the protein analysis software mentioned herein, using default parameters.

A "1-7F9-like" or "1-4F1-like" antibody as used herein means (1) an antibody that binds substantially the same epitope as an antibody comprising the VH and VL sequences of 1-7F9 or 1-4F1, respectively, such as, *e.g.*, monoclonal antibody 1-7F9 or 1-4F1, respectively, and/or (2) an antibody comprising VH and VL sequences identical or substantially identical to the VH and VL sequences of 1-7F9 or 1-4F1, respectively.

A "conservative" amino acid substitution is one in which an amino acid residue is substituted by another amino acid residue having a side chain ("R-group") with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where amino acid sequences differ from each other by conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, *e.g.*, Pearson, Methods Mol. Biol. 1994;243:307-31. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Exemplary conservative amino acids substitution groups include: valine-leucine-isoleucine, phenylalanine- tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

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DESCRIPTION OF THE INVENTION

This invention is based on the generation of novel cross-reactive and neutralizing antibodies binding to inhibitory KIRs, the antibodies allowing effective activation of NK cells in most or all individuals in human populations.

Described herein are, for example, antibodies that bind to all of KIR2DL1, KIR2DL2, and KIR2DL3 and potentiate NK-cell lytic activity by blocking the interactions between these KIRs and HLA-C. Such antibodies are referred to herein as "cross-reactive and neutralizing anti-KIR mAbs".

In a particular aspect, the present invention relates to novel cross-reactive, neutralizing, or both crossreacting and neutralizing, fully human anti-KIR antibodies, as well as compositions comprising such antibodies and methods of using such antibodies or compositions. These antibodies include human antibodies 1-7F9 and 1-4F1, described in PCT/DK2004/000470, filed on July 1, 2004, which is hereby incorporated by reference in its entirety,

Antibodies, compositions, and methods described herein may, among other things, overcome current limitations associated with therapeutic modulation of KIR, and with therapeutic NK cell activation, and provide additional advantageous features and benefits. For example, the antibodies can cross-react with multiple inhibitory KIRs and reduce or neutralize their inhibitory signals, resulting in potentiation of NK cell cytotoxicity by NK cells expressing such inhibitory KIR receptors. The ability to cross-react with multiple KIR gene products can allow the antibodies of the invention to be effectively used to increase NK cell activity in most or all human subjects, without the burden or expense of pre-determining the KIR or HLA type of the subject. In one embodiment, the antibodies do not bind KIR2DS4, thus avoiding the reduction in stimulatory potential that would be associated with neutralization of the activating KIR2DS4 receptor. Additionally or alternatively, the antibodies do not bind KIR2DS3.

From such antibodies, various antibody fragments and derivatives can also be generated and used for the same or similar purposes as are described herein with respect to the antibodies of the invention, and aspects of the invention described with respect to antibodies apply equally to antibody fragments and derivatives, unless otherwise stated or clearly contradicted by context. In other words, a feature of the invention described herein with respect to an antibody should, unless otherwise indicated, also implicitly be understood to describe a similar feature with respect to an antibody fragment or derivative having similar functionality in terms of specificity. It should be recognized, however, that "full-length" antibodies and an-

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tibody "fragments" can be characterized as distinct aspects of the invention, inasmuch as their biological and physiochemical properties may differ significantly.

Some antibodies of the invention can be characterized as "human", which are typically associated with a lower risk for an immune response against the antibodies by a human subject to whom they are administered. In one exemplary aspect, an isolated antibody that facilitates the activation of human NK cells in virtually all humans is described. In an exemplary embodiment, the antibody is human antibody 1-7F9 or a human 1-7F9-like antibody.

Antibodies, fragments, or derivatives of either thereof can cross-react with at least two inhibitory KIR receptors at the surface of NK cells, reduce or neutralize the inhibitory signals of the NK cells, and potentiate the activity of the NK cells. For example, an antibody, antibody fragment, or derivative may bind a common determinant of human KIR2DL receptors, so that the antibody, antibody fragment, or derivative binds at least KIR2DL1, KIR2DL2, and KIR2DL3 receptors. For the purposes of this invention, the term "KIR2DL2/3" refers to either or both of the KIR2DL2 and KIR2DL3 receptors.

As described herein, the anti-KIR mAbs 1-7F9 and 1-4F1 have several advantages over previously produced anti-KIR antibodies. For example, 1-7F9 and 1-4F1 are fully human, thus reducing or minimizing any immune response against the antibody once administered to a subject. Furthermore, both 1-7F9 and 1-4F1 are of suitable isotypes for therapeutic anti-KIR antibodies (IgG4 and IgG2, respectively), as described below. 1-7F9 is also more more effective at inducing killing by NK cells that express either KIR2DL1, -2, and/or -3 than murine mAbs EB6, GL183, DF200, and NKVSF1 (Pan2D). For example, as shown in Figures 5 and 6, 1-7F9 induced higher levels of specific lysis by KIR2DL1-expressing NK cells of target cells that expressed HLA-Cw4 than did EB6, DF200 or NKVSF1 (Pan2D). 1-7F9 further has a higher affinity for KIR compared to previously known anti-KIR mAbs. For example, 1-7F9 binds to KIR2DL1 and KIR2DL3 with dissociation constants (K_d's) of 0,43 nM and 0.025 nM, respectively, representing a higher affinity for both antigens than, for example, DF200 (see Examples 3 and 8). As opposed to the murine antibodies NKVSF1 (Pan2D), A210, and A208g, none of 1-7F9 and 1-4F1 binds to KIR2DS4, making them better suited for therapeutic purposes. Like NKVSF1 (Pan2D) and DF200, 1-7F9 and 1-4F1 also bind to KIR2DS1 and KIR2DS2, but KIR2DS1 and KIR2DS2 are not believed to be important in anti-leukemia efficacy. Particular antibodies according to the invention therefore have the same or similar antigen-specificities as 1-7F9 and/or 1-4F1. For example, antibodies comprising the same or similar VH and VL regions as 1-7F9 can have the same or similar antigen-binding and/or NK-stimulatory properties as 1-7F9; and antibodies comprising the same or

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similar VH and VL regions as 1-4F1 can have the same or similar antigen-binding properties as 1-4F1

As shown in Figure 14, the amino acid sequences of the VL and VH regions of 1-7F9 have been determined:

1-7F9 VL region (SEQ ID NO:15):

EIVLTQSPVTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWMYTFGQGTKLEIKRT

1-7F9 VH region (SEQ ID NO:17):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSFYAISWVRQAPGQGLEWMGGFIPIF GAANYAQKFQGRVTITADESTSTAYMELSSLRSDDTAVYYCARIPSGSYYYDYDMDVWGQ GTTVTVSS

The amino acid sequences of the 1-4F1 VL and VH regions are provided in SEQ ID NOS: 39 and 41, respectively, and the nucleotide sequences encoding the 1-4F1 VL and VH regions are provided in SEQ ID NOS:40 and 42, respectively. In a particular embodiment, residues 3, 4, 9, 24, 32, 41, 47, 50, 55, 71, and 74 of SEQ ID NO:39 are Q, L, S, R, A, G, L, D, E, F, and A, respectively. In another particular embodiment, residues 3, 4, 9, 24, 32, 41, 47, 50, 55, 71, and 74 of SEQ ID NO:39 are R, M, F, W, Y, A, F, Y, Q, Y, and T, respectively.

As shown in Figure 15, the amino acid sequences of the 1-7F9 CDRs have been identified as follows: the light chain CDR1 amino acid sequence corresponds to residues 24-34 of SEQ ID NO:15; the light chain CDR2 amino acid sequence corresponds to residues 50-56 of SEQ ID NO:15; the light chain CDR3 amino acid sequence corresponds to residues 89-97 of SEQ ID NO:15; the heavy chain CDR1 amino acid sequence corresponds to residues 31-35 of SEQ ID NO:17; the heavy chain CDR2 amino acid sequence corresponds to residues 50-65 of SEQ ID NO:17; and the heavy chain CDR3 amino acid sequence corresponds to residues 99-112 of SEQ ID NO:17. The amino acid sequences of the 1-4F1 CDRs have been identified as follows: the light chain CDR1 amino acid sequence corresponds to residues 24-34 of SEQ ID NO:39; the light chain CDR2 amino acid sequence corresponds to residues 50-56 of SEQ ID NO:39; the light chain CDR3 amino acid sequence corresponds to residues 89-97 of SEQ ID NO:39; the heavy chain CDR1 amino acid sequence corresponds to residues 31-35 of SEQ ID NO:41; the heavy chain CDR2 amino acid sequence corresponds to residues 50-66 of SEQ ID NO:41; and the heavy chain CDR3 amino acid sequence corresponds to residues 99-113 of SEQ ID NO:41.

Amino acid sequences for the entire 1-7F9 light and heavy chains are provided in SEQ ID NOS:36 and 37, respectively.

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Accordingly, additional antibodies of, for example, various human antibody subclasses; antibody fragments, antibody derivatives, and other KIR-binding peptides, can be readily produced by, *e.g.*, recombinant techniques, based upon this information. For example, in one aspect, the invention provides an antibody having a VL and a VH sequence consisting essentially of SEQ ID NO:15 and SEQ ID NO:17, respectively, and/or an antibody having a VL and a VH sequence consisting essentially of SEQ ID NO:39 and SEQ ID NO:41, respectively. In another aspect, the invention provides an antibody comprising CDR regions consisting essentially of the 1-7F9 or 1-4F1 VH CDR1-3 and VL CDR1-3 described above. In another aspect, the invention provides an antibody comprising CDR regions as follows: a light chain CDR1 amino acid sequence corresponding to about residues 24-34 of SEQ ID NO:15; the light chain CDR2 amino acid sequence corresponding to about residues 50-56 of SEQ ID NO:15; the light chain CDR3 amino acid sequence corresponding to about residues 89-97 of SEQ ID NO:15; the heavy chain CDR1 amino acid sequence corresponding to about residues 31-35 of SEQ ID NO:17; the heavy chain CDR2 amino acid sequence corresponding to about to residues 50-65 of SEQ ID NO:17; and the heavy chain CDR3 amino acid sequence corresponding to about residues 99-112 of SEQ ID NO:17. In another aspect, the invention provides an antibody comprising CDR regions as follows: a light chain CDR1 amino acid sequence corresponding to about residues 24-34 of SEQ ID NO:39; a light chain CDR2 amino acid sequence corresponding to about residues 50-56 of SEQ ID NO:39; a light chain CDR3 amino acid sequence corresponding to about residues 89-97 of SEQ ID NO:39; a heavy chain CDR1 amino acid sequence corresponding to about residues 31-35 of SEQ ID NO:41; a heavy chain CDR2 amino acid sequence corresponding to about residues 50-66 of SEQ ID NO:41; and a heavy chain CDR3 amino acid sequence corresponding to about residues 99-113 of SEQ ID NO:41. In another aspect, the invention provides an antibody comprising a light chain CDR1 amino acid sequence consisting essentially of residues 24-34 of SEQ ID NO:15; a light chain CDR2 amino acid sequence consisting essentially of residues 50-56 of SEQ ID NO:15; a light chain CDR3 amino acid sequence consisting essentially of residues 89-97 of SEQ ID NO:15; a heavy chain CDR1 amino acid sequence consisting essentially of residues 31-35 of SEQ ID NO:17; a heavy chain CDR2 amino acid sequence consisting essentially of residues 50-65 of SEQ ID NO:17; and a heavy chain CDR3 amino acid sequence consisting essentially of residues 99-112 of SEQ ID NO:17. In another aspect, the invention provides an antibody comprising CDR regions as follows: a light chain CDR1 amino acid sequence consisting essentially of residues 24-34 of SEQ ID NO:39; a light chain CDR2 amino acid sequence consisting essentially of residues 50-56 of SEQ ID NO:39; a light chain CDR3 amino acid sequence consisting essentially of residues 89-97 of

SEQ ID NO:39; a heavy chain CDR1 amino acid sequence consisting essentially of residues 31-35 of SEQ ID NO:41; a heavy chain CDR2 amino acid sequence consisting essentially of residues 50-66 of SEQ ID NO:41; and a heavy chain CDR3 amino acid sequence consisting essentially of residues 99-113 of SEQ ID NO:41.

The invention also encompasses an anti-KIR antibody, antibody fragment, or antibody derivative, or a KIR-binding polypeptide, comprising at least one variant amino acid sequence substantially identical to the 1-7F9 or 1-4F1 VH or VL sequence, or to a CDR-region therein. A variant amino acid sequence can comprise or consist essentially of an amino acid sequence that is at least about 50, 80, 90, 95, 98, or 99 (*e.g.*, about 50-99, about 65-99, about 75-99, or about 85-99) percent identical to a 1-7F9 or 1-4F1 CDR, VH, or VL region. A variant amino acid sequence can also or alternatively comprise 1, 2, or 3 CDRs that comprise or consist of amino acid sequences that are at least about 80%, at least about 90%, or at least about 95% identical to 1-7F9 or 1-4F1 CDRs. Thus, in one aspect, the invention provides a human antibody comprising a light chain CDR1 amino acid sequence at least about 80%, at least about 90%, or at least about 95% identical to residues 24-34 of SEQ ID NO:15 or SEQ ID NO:39; a light chain CDR2 amino acid sequence at least about 80%, at least about 90%, or at least about 95% identical to residues 50-56 of SEQ ID NO:15 or SEQ ID NO:39; a light chain CDR3 amino acid sequence at least about 80%, at least about 90%, or at least about 95% identical to residues 89-97 of SEQ ID NO:15 or SEQ ID NO:39; a heavy chain CDR1 amino acid sequence at least about 80%, at least about 90%, or at least about 95% identical to residues 31-35 of SEQ ID NO:17 or SEQ ID NO:41; a heavy chain CDR2 amino acid sequence at least about 80%, at least about 90%, or at least about 95% identical to residues 50-65 of SEQ ID NO:17 or to residues 50 to 66 of SEQ ID NO:41; and a heavy chain CDR3 amino acid sequence at least about 80%, at least about 90%, or at least about 95% identical to residues 99-112 of SEQ ID NO:17 or to residues 99 to 113 of SEQ ID NO:41. The basic properties of 1-7F9- or 1-4F1-derived KIR-binding amino acid sequences that are retained in such variant amino acid sequences desirably include the specificity and/or avidity of the 1-7F9 or 1-4F1 sequence for one or more KIRs, and may also or alternatively include the capability of 1-7F9 in blocking KIR/HLA-C interaction and potentiating the lytic activity of NK cells.

In another aspect, the invention provides an anti-KIR antibody, antibody fragment, or antibody derivative, or a KIR-binding polypeptide, that comprises a KIR-binding amino acid sequence that differs from a 1-7F9 or 1-4F1 KIR-binding sequence in one or more amino acid residues (*e.g.*, at least 2, 3, 5, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 50, or

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more amino acid residues) by way of one or more residue insertions, deletions, and/or substitutions. In one embodiment, such a variant KIR-binding sequence confers greater affinity; greater or different specificity; less immunogenicity (in terms of host response to the sequence); greater *in vivo* stability; and/or other beneficial properties to the variant sequence over an essentially identical amino acid sequence comprising the native 1-7F9 or 1-4F1 sequence. Suitable sequence variations are further described elsewhere herein. A KIR-binding portion of an anti-KIR antibody, antibody fragment, or antibody derivative, or a KIR-binding polypeptide, can also comprise any suitable number of non-amino acid components or substituents, such as non-amino acid organic moieties, that facilitate KIR binding and/or provide other advantageous physiochemical or immunological properties.

Some antibodies of the invention can also or alternatively be characterized by their binding affinity to one or more KIRs. For example, as shown in Examples 3 and 8, in terms of bivalent binding, DF200 has a K_d for KIR2DL1 of about 11 nM, and a K_d for KIR2DL3 of about 2.0 nM, and 1-7F9 has a K_d for KIR2DL1 of about 0.43 nM, and a K_d for KIR2DL3 of about 0,025 nM. Accordingly, in one aspect, the invention provides human or non-human (*e.g.*, murine, chimeric, or humanized) antibodies having a K_d in bivalent binding to KIR2DL1 of no more than about 20 nM, no more than about 11 nM, no more than about 5 nM, no more than about 1 nM, no more than about 0.5 nM, or no more than about 0.43 nM. Additionally or alternatively, human or non-human (*e.g.*, murine, chimeric, or humanized) antibodies of the invention may have a K_d for KIR2DL3 of no more than about 20 nM, no more than about 2 nM, no more than about 1 nM, no more than about 0.1 nM, or no more than about 0.05 nM, or no more than about 0.025 nM. In a particular aspect, the antibodies have about the same K_d values for bivalent binding to KIR2DL1 and KIR2DL3 as 1-7F9. As shown in Example 13, in terms of monovalent binding, 1-7F9 and 1-4F1 have K_d-values for KIR2DL3 of about 3.5 and 7 nM, respectively. Accordingly, in one aspect, the invention provides human or non-human (*e.g.*, murine, chimeric, or humanized) antibodies having a K_d in monovalent binding to KIR2DL3 of no more than about 20 nM, no more than about 10 nM, no more than about 7 nM, or no more than about 3.5 nM.

An anti-KIR antibody, antibody fragment, or antibody derivative, or a KIR-binding polypeptide selectively and/or specifically (typically specifically) binds at least one KIR, and more particularly an antigenic determinant region or epitope of at least one KIR, under appropriate conditions (*e.g.*, with respect to temperature, pH, etc., which typically will reflect human physiological conditions in a normal or NK cell-associated disease state, and in the context of a suitable protein comprising the sequence or combination). For example, in one aspect, the invention relates to antibodies that can be characterized in (among other things)

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the ability to compete with 1-7F9, 1-4F1 or a 1-7F9- or 1-4F1-like antibody, and various methods involving the same. Other antibodies of the invention (and/or that are useful in the practice of inventive methods described herein) also or alternatively can be characterized on having the ability to compete with one or more of antibody DF200, antibody NKVSF1, antibody EB6, and antibody GL183.

The cross-reactive and neutralizing anti-KIR antibodies, antibody fragments, or derivatives of the invention reduce or neutralize the inhibitory activity of KIR by specifically inhibiting binding of MHC and/or HLA molecules to at least two inhibitory KIR receptors and facilitating NK cell activity, meaning that such antibodies, fragments or derivatives permit NK cells expressing an inhibitory KIR receptor on their surface to be capable of lysing cells that express a corresponding HLA ligand for that particular inhibitory KIR receptor (*e.g.*, a particular HLA antigen). In one aspect, the invention provides antibodies that specifically inhibit the binding of HLA-C molecules to KIR2DL1 and KIR2DL2/3 receptors. In another aspect, the invention provides antibodies that inhibit the binding of KIR2DL1 and/or KIR2DL2/3 to HLA-C. In yet another aspect, the invention provides antibodies that facilitate NK cell activity *in vivo*, and/or *in vitro*.

At least one of KIR2DL1 or KID2DL2/3 is present in at least about 90% or more of the human population, and the more preferred antibodies of this invention are capable of potentiating the activity of NK cells expressing either or both of these KIR. Therefore, compositions of this invention may be used to effectively activate or potentiate NK cells in most human individuals, typically in about 90% of human individuals or more. Accordingly, a single antibody composition according to the invention may be used to treat most human subjects, and there is seldom need to determine KIR- or HLA-allelic groups or to use mixtures or cocktails of two or more anti-KIR mAbs.

In one aspect, the antibody specifically binds both KIR2DL1 and KIR2DL2/3 human receptors and reverses inhibition of NK cell cytotoxicity mediated by these KIRs. The antibody may also be human and compete with monoclonal antibody 1-7F9 and/or 1-4F1. The term "competes with" when referring to a particular pair of antibodies (*e.g.*, one or more antibodies selected from DF200, NKVSF1 (Pan2D), 1-7F9, EB6, and GL183), means that a first antibody detectably competes with a second antibody (or other molecule) in a binding assay using either recombinant KIR molecules or cell-surface expressed KIR molecules. For example, in one aspect, where the percentages of inhibition for a certain antibody pair are above about 20%, above about 30%, above about 40%, above about 50%, regardless of which antibody is used as first antibody, the antibodies compete. Alternatively, if the percentages of inhibition for a certain antibody pair average at least about 29%, at least about

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30%, at least about 40%, or at least about 50%, the antibodies compete. The percentage of inhibition of a second antibody binding to KIR2D protein by a first antibody can be calculated as: 100*(1-(detected binding second antibody)/(detected binding of first antibody)). The same applies to antibody fragments and antibody derivatives. Unless otherwise specified, an antibody that "competes" with 1-7F9, 1-4F1 or a 1-7F9- or 1-4F1-like antibody may compete with 1-7F9, 1-4F1 or the 1-7F9- or 1-4F1-like antibody for binding to human KIR2DL1, human KIR2DL2/3, or both human KIR2DL1 and KIR2DL2/3. For example, the antibody DF200 competes with 1-7F9 and 1-4F1 for binding to KIR2DL3.

Optionally, an antibody which competes with 1-7F9 or 1-4F1 is not 1-7F9 or 1-4F1 themselves, respectively (*i.e.*, the invention provides antibodies other than 1-7F9 and 1-4F1 that are characterized by, among other things, the ability to compete with 1-7F9 and/or 1-4F1 in binding to one or both of these KIRs).

In another aspect, the antibody binds both KIR2DL1 and KIR2DL2/3 human receptors, reduces or neutralizes or reverses inhibition of NK cell cytotoxicity mediated by these KIRs, and competes with 1-7F9 for binding to the KIR2DL1 human receptor, the KIR2DL2/3 human receptor, or both KIR2DL1 and KIR2DL2/3 human receptors. Optionally, said antibody is a chimeric, human, or humanized antibody.

In another aspect, the antibody binds both KIR2DL1 and KIR2DL2/3 human receptors, reduces, neutralizes or reverses inhibition of NK cell cytotoxicity mediated by these KIRs, and competes with EB6 for binding to the KIR2DL1 human receptor, or competes with GL183 and/or DF200 for binding to the KIR2DL2/3 human receptor; or it competes with both EB6 for binding to the KIR2DL1 human receptor and GL183 and/or DF200 for binding to the KIR2DL2/3 human receptor. In one embodiment, the antibody is not NKVSF1 (Pan2D), not A210, not A803g, and/or not DF200. The antibody can be, *e.g.*, a murine, chimeric, human, or humanized antibody.

In another aspect, the antibody comprises VH, VL, or both VH and VL regions that are at least substantially identical to the VH and/or VL regions of 1-7F9 or 1-4F1. The antibody can be of any subclass, including IgG1, IgG2, IgG3, and IgG4. In a particular aspect, the antibody is a human IgG4 antibody. In another particular aspect, the antibody is a human IgG2 antibody. The antibody can be a chimeric, human, or humanized antibody.

In another aspect, the antibody is human, competes with 1-7F9 or 1-4F1, and recognizes, binds to, or has immunospecificity for at least partially the same, or the same, epitope or "epitopic site" on a KIR molecule as the monoclonal antibody 1-7F9 or 1-4F1. Preferably, said KIR molecule is a human KIR2DL1 or KIR2DL2/3 receptor.

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In another aspect, the antibody binds a common determinant present in both KIR2DL1 and KIR2DL2/3 human receptors and reduces, neutralizes or reverses inhibition of NK cell cytotoxicity mediated by these KIRs. The antibody more specifically can bind at least partially the same, substantially the same, or the same epitope on KIR as monoclonal antibody 1-7F9.

In a particular aspect, the antibody is a monoclonal antibody that exhibits one or more of the above-described characteristics.

In another aspect, functional fragments and derivatives of the antibodies described herein can be prepared which have substantially similar antigen binding, specificity and/or activity, including, without limitation, Fab fragments, Fab'2 fragments, immunoadhesins, diabodies, camelized antibodies, Janusins, minibodies, CDRs, and ScFv fragments.

Unless otherwise specified, antibodies or bivalent fragments or derivatives thereof are monospecific, *i.e.*, both "arms" of the antibodies, fragments, or derivatives bind the same antigen(s).

In yet another aspect, antibody derivatives comprising an antibody of the invention conjugated or covalently bound to a toxin, a radionuclide, a detectable moiety (*e.g.*, a fluor), or a solid support, can be prepared.

The invention also encompasses pharmaceutical compositions comprising an antibody as disclosed above, a fragment thereof, or a derivative of either thereof. Accordingly, the invention also relates to use of an antibody as disclosed herein in a method for the manufacture of a medicament. In preferred embodiments, the medicament or pharmaceutical composition is for the treatment of a cancer or other proliferative disorder, an infection, or for use in transplantation.

In one aspect, the invention relates to a composition (*e.g.*, a composition formulated for pharmaceutical administration, a kit for preparing such a composition, an assay kit or media, purification media, etc.) that comprises an antibody that binds at least two different human inhibitory KIR receptor gene products and is capable of neutralizing KIR-mediated inhibition cytotoxicity by NK cells expressing at least one of said two different human inhibitory KIR receptors, wherein the antibody is incorporated into a liposome. The liposome may also comprise an additional substance such as, *e.g.*, a nucleic acid molecule for the delivery of genes for gene therapy; a nucleic acid molecule for the delivery of antisense RNA, RNAi, or siRNA for suppressing a gene in an NK cell; or a toxin or a drug for the targeted killing of NK cells.

Described herein are also methods of regulating human NK cell activity *in vitro*, *ex vivo*, or *in vivo*, comprising contacting human NK cells with an effective amount of an anti-

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body of the invention, a fragment of such an antibody, a derivative of either thereof, or a pharmaceutical composition comprising at least one of any thereof. Preferred methods comprise administration of an effective amount of a pharmaceutical compositions of this invention and are directed at increasing the cytotoxic activity of human NK cells, most preferably *ex vivo* or *in vivo*, in a subject having a cancer, an infectious disease, or an immune disease. For example, administration can be effected by intravenous infusion of the antibody in a suitable buffer to a patient with cancer or an infectious disease (such as a viral disease).

The invention also provides a composition comprising an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein the antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity in NK cells expressing at least one of the two different human inhibitory KIR receptors, the antibody being present in an amount effective to detectably potentiate NK cell cytotoxicity in a patient or in a biological sample comprising NK cells; and a pharmaceutically acceptable carrier or excipient. Preferably the antibody binds a common determinant present on KIR2DL1 and KIR2DL2/3. Compositions comprising an antibody of the invention may optionally further comprise a second therapeutic agent (an agent that induces, promotes, and/or enhances a therapeutic effect in a host that is related to a condition or disorder for which the antibody is administered – *e.g.*, a condition related to a cancer, transplantation, an infectious disease, a viral infection, etc.). In one aspect, a second therapeutic agent that can be co-administered with an antibody of the invention in such a combination composition may be selected from, for example, an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody specific for a non-KIR antigen, optionally a second antibody that binds to and inhibits and neutralizes an inhibitory KIR, or reduces the signalling from an inhibitory KIR, an anti-infective agent, a targeting agent, or an adjunct compound. Advantageous immunomodulatory agents may be selected from IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-21, TGF-beta, GM-CSF, M-CSF, G-CSF, TNF-alpha, TNF-beta, LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-alpha, IFN-beta, or IFN-gamma. Examples of the chemotherapeutic agents include alkylating agents, antimetabolites, cytotoxic antibiotics, adriamycin, dactinomycin, mitomycin, carminomycin, daunomycin, doxorubicin, tamoxifen, taxol, taxotere, vincristine, vinblastine, vinorelbine, etoposide (VP-16), 5-fluorouracil (5FU), cytosine arabinoside, cyclophosphamide, thiotepa, methotrexate, camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP), aminopterin, combretastatin(s), other vinca alkyloids and derivatives or prodrugs thereof. Examples of hormonal agents include leuprorelin, goserelin, triptorelin, buserelin, tamoxifen, toremifene, flutamide, nilutamide, cyproterone bicalutamid anastrozole, exeme-

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stane, letrozole, fadrozole medroxy, chlormadinone, megestrol, other LHRH agonists, other anti-estrogens, other anti-androgens, other aromatase inhibitors, and other progestagens. Preferably, the second antibody that binds to and inhibits an inhibitory KIR receptor is an antibody or a derivative or fragment thereof that binds to an epitope of an inhibitory KIR receptor that differs from the epitope bound by the antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products. In another aspect, a second antibody may be directed to a target associated with the disease state that is at least partially treatable by administration of the antibody of the invention (*e.g.*, a cancer-associated antigen, a viral infection-associated antigen, etc.).

The invention further provides a method of detectably potentiating NK cell activity in a patient in need thereof, comprising the step of administering to the patient a composition according to the invention. A patient in need of NK cell activity potentiation can be any patient diagnosed as having a disease or disorder wherein such potentiation may promote, enhance, and/or induce a therapeutic effect (or promotes, enhances, and/or induces such an effect in at least a substantial proportion of patients with the disease or disorder and substantially similar characteristics as the patient – as may determined by, *e.g.*, clinical trials). A patient in need of such treatment may be suffering from, *e.g.*, cancer, another proliferative disorder, an infectious disease or an immune disorder. Preferably, the method comprises the additional step of administering to the patient an appropriate additional therapeutic agent selected from an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody specific for an antigen distinct from KIR, optionally a second antibody that binds to and inhibits and neutralizes an inhibitory KIR receptor, or reduces the signalling from an inhibitory KIR, an anti-infective agent, a targeting agent or an adjunct compound wherein said additional therapeutic agent is administered to said patient as a single dosage form together with said antibody, or as separate dosage form. The dosage of the antibody (or antibody fragment/derivative) and the dosage of the additional therapeutic agent collectively are sufficient to detectably induce, promote, and/or enhance a therapeutic response in the patient which comprises the potentiation of NK cell activity. Where administered separately, the antibody, fragment, or derivative and the additional therapeutic agent are desirably administered under conditions (*e.g.*, with respect to timing, number of doses, etc.) that result in a detectable combined therapeutic benefit to the patient.

Further encompassed by the present invention are antibodies which are capable of specifically binding NK cells and/or KIR receptors in a non-human primate, preferably a monkey. Also encompassed are methods for evaluating the toxicity, dosage and/or activity or

efficacy of antibodies of the invention which are candidate medicaments. In one aspect, the invention encompasses a method for determining a dose of an antibody that is toxic to an animal or target tissue by administering an antibody of the invention to an non-human primate recipient animal having NK cells, and assessing any toxic or deleterious or adverse effects of the agent on the animal, or preferably on a target tissue. In another aspect, the invention is a method for identifying an antibody that is toxic to an animal or target tissue by administering an antibody of the invention to an non-human primate recipient animal having NK cells, and assessing any toxic or deleterious or adverse effects of the agent on the animal, or preferably on a target tissue. In another aspect, the invention is a method for identifying an antibody that is efficacious as treatment of an infection, disease or tumor by administering an antibody of the invention to a non-human primate model of infection, disease or cancer, and identifying the antibody that ameliorates the infection, disease or cancer, or a symptom thereof. In one embodiment, said antibody of the invention is an antibody which (a) cross reacts with at least two inhibitory human KIR receptors at the surface of human NK cells, and (b) cross-reacts with NK cells or a KIR receptor of the non-human primate.

Further encompassed by the present invention is a method of detecting the presence of NK cells bearing an inhibitory KIR on their cell surface in a biological sample or a living organism, said method comprising the steps of:

a) contacting said biological sample or living organism with an antibody of the invention, wherein said antibody is conjugated or covalently bound to a detectable moiety; and

b) detecting the presence of said antibody in said biological sample or living organism.

The invention also provides a method of purifying from a sample NK cells bearing an inhibitory KIR on their cell surface comprising the steps of:

a) contacting said sample with an antibody of the invention under conditions that allow said NK cells bearing an inhibitory KIR on their cell surface to bind to said antibody, wherein said antibody is conjugated or covalently bound to a solid support (*e.g.*, a bead, a matrix, etc.); and

b) eluting said bound NK cells from said antibody conjugated or covalently bound to a solid support.

It has been found that antibody NKVSF1 (Pan2D) also binds to NK cells from cynomolgus monkeys, see Figure 10.

The invention therefore provides an antibody, as well as fragments and derivatives thereof, wherein said antibody, fragment or derivative cross-reacts with at least two inhibitory human KIR receptors at the surface of human NK cells, and which furthermore binds to NK

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cells from cynomolgus monkeys. In one embodiment hereof, the antibody is not antibody NKVSF1, not A210, and/or not A802g. The invention also provides a method of testing the toxicity of an antibody, as well as fragments and derivatives thereof, wherein said antibody, fragment or derivative cross-reacts with at least two inhibitory human KIR receptors at the surface of human NK cells, wherein the method comprises testing the antibody in a cynomolgus monkey.

In a further aspect, the invention provides an antibody, antibody fragment, or derivative of either thereof, that comprises the light variable region or one or more light variable region CDRs of antibody 1-7F9 or 1-4F1. In still another aspect, the invention provides an antibody, antibody fragment, or derivative of either thereof that comprises a sequence that is highly similar to all or essentially all of the light chain variable region sequence of 1-7F9 or 1-4F1.

In a further aspect, the invention provides an antibody, antibody fragment, or derivative of either thereof, which comprises the heavy chain variable region or one or more heavy chain variable region CDRs of antibody 1-7F9 or 1-4F1. In still another aspect, the invention provides an antibody, antibody fragment, or derivative of either thereof that comprises a sequence that is highly similar to all or essentially all of the heavy chain variable region sequence of 1-7F9 or 1-4F1.

Antibodies

The present invention provides novel antibodies and fragments or derivatives thereof that bind common determinants conserved among human inhibitory KIR receptors, preferably including a determinant present on at least two different KIR2DL gene products, but not on KIR2DS4, and which cause potentiation of NK cells expressing at least one of those KIR receptors. The invention discloses, for the first time, that such cross-reacting and neutralizing antibodies can be produced, and effectively used in modulation of NK cell activity, which represents an unexpected result and opens an avenue towards novel and effective NK-based therapies, particularly in human subjects.

Within the context of this invention, a "common determinant" designates a determinant or epitope that is shared by several gene products of the human inhibitory KIR receptors. Preferably, the common determinant is shared by at least two members of the KIR2DL receptor group. More preferably, the determinant is shared by at least KIR2DL1 and KIR2DL2/3. Certain antibodies of this invention may, in addition to recognizing multiple gene products of the KIR2DL type, also recognize determinants present on other inhibitory KIRs, such as gene product of the KIR3DL receptor group; *e.g.*, KIR3DL1 and/or KIR3DL2. The

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determinant or epitope may represent a peptide fragment or a conformational epitope shared by said members. In a more specific embodiment, the antibody of this invention specifically binds to substantially the same epitope recognized by monoclonal antibody DF200. This determinant is present on both KIR2DL1 and KIR2DL2/3. In another preferred embodiment, the antibody of this invention specifically binds to substantially the same epitope recognized by human mAb 1-7F9, or the epitope recognized by human mAb 1-4F1, said epitopes being present on KIR2DL1, -2 and -3, but not on KIR2DS4.

The term "antibodies," as used herein, refers to polyclonal and monoclonal antibodies, as well as to fragments and derivatives of said polyclonal and monoclonal antibodies unless otherwise stated or clearly contradicted by context. Depending on the type of constant domain in the heavy chains, full length antibodies typically are assigned to one of five major classes: IgA, IgD, IgE, IgG, and IgM. Several of these are further divided into subclasses or isotypes, such as IgG1, IgG2, IgG3, IgG4, and the like. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are termed "alpha," "delta," "epsilon," "gamma" and "mu," respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. IgG and/or IgM are the preferred classes of antibodies employed in this invention because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting. Typically, an "antibody" in the context of this invention refers to a monoclonal antibody, more preferably a "human" monoclonal antibody.

One embodiment of the invention provides a method of blocking the interaction of an inhibitory KIR and its corresponding HLA ligand *in vivo*, for therapeutic purposes that involve activation of endogenous NK cells. In such a setting, it may be advantageous to avoid depleting the NK cells, since, if depleted, the NK cells could not exert their therapeutically beneficial effects. Therefore, antibody isotypes such as IgG4 and IgG2 which exhibit little binding to Fc-receptors, and which do not activate the complement system, are typically preferred. The isotype of 1-7F9 is IgG4. IgG2 antibodies are also generally considered to be non-depleting, and may also be more stable molecules than IgG4 antibodies, thereby conferring a longer half-life *in vivo*. 1-4F1 is of the IgG2 isotype.

Antibody Production

The antibodies of this invention may be produced by a variety of techniques known in the art. Typically, they are produced by immunization of a non-human animal, preferably a mouse, with an immunogen comprising an inhibitory KIR polypeptide, preferably a KIR2DL polypeptide, more preferably a human KIR2DL polypeptide. The inhibitory KIR polypeptide

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may comprise the full length sequence of a human inhibitory KIR polypeptide, or a fragment or derivative thereof, typically an immunogenic fragment, *i.e.*, a portion of the polypeptide comprising an epitope exposed on the surface of the cell expressing an inhibitory KIR receptor. Such fragments typically contain at least about 7 consecutive amino acids of the mature polypeptide sequence, even more preferably at least about 10 consecutive amino acids thereof. Fragments typically are essentially derived from the extra-cellular domain of the receptor. Even more preferred is a human KIR2DL polypeptide which includes at least one, more preferably both, extracellular Ig domains, of the full length KIRDL polypeptide and is capable of mimicking at least one conformational epitope present in a KIR2DL receptor. In other embodiments, said polypeptide comprises at least about 8 consecutive amino acids of an extracellular Ig domain of amino acid positions 1-224 of the KIR2DL1 polypeptide (amino acid numbering of according to Wagtmann et al., Immunity 1995;2:439-449, which is incorporated by reference herein in its entirety, or according to the PROW internet website found at the World Wide Web (www) address ncbi.nlm.nih.gov/prow/guide/1326018082.htm).

In one embodiment, the immunogen comprises a wild-type human KIR2DL polypeptide in a lipid membrane, typically at the surface of a cell. In a specific embodiment, the immunogen comprises intact NK cells, particularly intact human NK cells. In another embodiment, the cells are lysed or otherwise treated so as to not be intact. The immunogen can be, for example, suspended or dissolved in a buffer, optionally with an adjuvant, such as complete Freund's adjuvant, prior to administration to a non-human mammal, such as a mouse, rabbit, goat, horse, dog, sheep, guinea pig, rat, hamster, etc.

The step of immunizing a non-human mammal with an antigen may be carried out in any manner well known in the art for stimulating the production of antibodies in a mouse (see, for example, E. Harlow and D. Lane, Antibodies: A Laboratory Manual., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988)). The immunogen is then suspended or dissolved in a buffer, optionally with an adjuvant, such as complete Freund's adjuvant. Methods for determining the amount of immunogen, types of buffers and amounts of adjuvant are well known to those of skill in the art and are not limiting in any way on the present invention. These parameters may be different for different immunogens, but are easily elucidated.

Similarly, principles relevant to selection of the location and frequency of immunization sufficient to stimulate the production of antibodies are also well known in the art. In an exemplary immunization protocol, the non-human animals are injected intraperitoneally with antigen on day 1 and again about a week later. This is followed by recall injections of the antigen around day 20, optionally with adjuvant such as incomplete Freund's adjuvant. The

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recall injections are performed intravenously and may be repeated for several consecutive days. This is followed by a booster injection at day 40, either intravenously or intraperitoneally, typically without adjuvant. This protocol results in the production of antigen-specific antibody-producing B cells after about 40 days. Other protocols may also be utilized as long as they result in the production of B cells expressing an antibody directed to the antigen used in immunization.

For polyclonal antibody preparation, serum is obtained from an immunized non-human animal and the antibodies present therein isolated by well-known techniques. The serum may be affinity purified using any of the immunogens set forth above linked to a solid support so as to obtain antibodies that react with inhibitory KIR receptors.

In an alternate embodiment, lymphocytes from an unimmunized non-human mammal are isolated, grown *in vitro*, and then exposed to the immunogen in cell culture. The lymphocytes are then harvested and the fusion step described below is carried out.

For monoclonal antibodies, the next step is the isolation of splenocytes from the immunized non-human mammal and the subsequent fusion of those splenocytes with an immortalized cell in order to form an antibody-producing hybridoma. The isolation of splenocytes from a non-human mammal is well-known in the art and typically involves removing the spleen from an anesthetized non-human mammal, cutting it into small pieces and squeezing the splenocytes from the splenic capsule and through a nylon mesh of a cell strainer into an appropriate buffer so as to produce a single cell suspension. The cells are washed, centrifuged and resuspended in a buffer that lyses any red blood cells. The solution is again centrifuged and remaining lymphocytes in the pellet are finally resuspended in fresh buffer.

Once isolated and present in single cell suspension, the lymphocytes can be fused to an immortal cell line. This is typically a mouse myeloma cell line, although many other immortal cell lines useful for creating hybridomas are known in the art. Preferred murine myeloma lines include, but are not limited to, those derived from MOPC-21 and MPC-11 mouse tumors (available from the Salk Institute Cell Distribution Center, San Diego, Calif. U.S.A.), or the X63 Ag8653 and SP-2 cell lines (available from the American Type Culture Collection, Rockville, Maryland U.S.A.). The cell fusion is effected using polyethylene glycol or the like. The resulting hybridomas are then grown in selective media that contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

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Hybridomas are typically grown on a feeder layer of macrophages. The macrophages are preferably from littermates of the non-human mammal used to isolate splenocytes and are typically primed with incomplete Freund's adjuvant or the like several days before plating the hybridomas. Fusion methods are described in Goding, "Monoclonal Antibodies: Principles and Practice," pp. 59-103 (Academic Press, 1986), the disclosure of which is herein incorporated by reference.

The cells are allowed to grow in the selection media for sufficient time for colony formation and antibody production. This is usually between about 7 and about 14 days. The hybridoma colonies are then assayed for the production of antibodies that cross-react with multiple inhibitory KIR receptor gene products. The assay is typically a colorimetric ELISA-type assay, although several other types of assays may be employed, including immunoprecipitation and radioimmunoassay, or FACS, Biacore, Scintillation-proximity assays (SPA), or other types of assays well known in the art. The wells containing antibodies of the desired specificity are examined to determine if one or more distinct hybridoma cell colonies are present. If more than one colony is present, the cells may be re-cloned and grown to ensure that only a single cell has given rise to the colony producing the desired antibody. Positive wells with a single apparent colony are typically re-cloned and re-assayed to insure that only one monoclonal antibody is being detected and produced.

In a preferred embodiment, the non-human animal used to produce antibodies according to applicable methods of the invention is a mammal, such as a rodent (*e.g.*, mouse, rat, etc.), bovine, porcine, horse, rabbit, goat, sheep, etc. Also, the non-human mammal may be genetically modified or engineered to produce "human" antibodies, such as the Xenomouse™ (Abgenix) or HuMAb-Mouse™ (Medarex), as described below.

Antibodies also may be produced transgenically through the generation of a chordate (such as a mammal or bird) or a plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom (see, *e.g.*, Ma et al., Nature Rev. Genetics 4:794-805 (2003); Nolke et al., Expert Opin Biol Ther. 2003 Oct;3(7):1153-62; Schillberg et al., Cell Mol Life Sci. 2003 Mar;60(3):433-45; Tekoah et al., Arch Biochem Biophys. 2004 Jun 15;426(2):266-78; Fischer et al., Eur. J. of Biochem., 262(3):810 (1999); and US Patent Application 20030084482 regarding production of antibodies and antibody-like proteins in plants). In connection with the transgenic production in mammals, antibodies and other proteins can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, *e.g.*, US Patents 5,827,690, 5,756,687, 5,750,172, and 5,741,957. Antibodies also may be produced in the

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eggs of birds and recovered therefrom. See, *e.g.*, Tini et al., Comp Biochem Physiol A Mol Integr Physiol. 2002 Mar;131(3):569-74 and US Patent 4,550,019.

Antibodies may also be produced by selection of combinatorial libraries of immunoglobulins, as disclosed for instance in Ward et al., Nature, 341 (1989) p. 544.

According to another embodiment, the invention provides a hybridoma derived from a B cell from a non-human host, wherein said B-cell produces an antibody that binds a determinant present on at least two different human inhibitory KIR receptor gene products and said antibody is capable of neutralizing the inhibitory activity of said receptors. More preferably, the hybridoma of this aspect of the invention is not a hybridoma that produces the monoclonal antibody NKVSF1, not A210, and/or not A802g. The hybridoma according to this aspect of the invention can be created as described above by the fusion of splenocytes from the immunized non-human mammal with an immortal cell line. Hybridomas produced by this fusion can be screened for the presence of such a cross-reacting antibody as described elsewhere herein. Preferably, the hybridoma produces an antibody that recognizes a determinant present on at least two different KIR2DL gene products, and cause potentiation of NK cells expressing at least one of those KIR receptors. Even more preferably, the hybridoma produces an antibody that binds to substantially the same epitope or determinant as 1-7F9 and which potentiates NK cell activity, or which binds substantially the saem epitope as 1-4F1. Most preferably, the hybridoma is hybridoma 1-7F9 which produces monoclonal antibody 1-7F9, or hybridoma 1-4F1 which produces monoclonal antibody 1-4F1.

Hybridomas that are confirmed to produce a monoclonal antibody of this invention can be grown up in larger amounts in an appropriate medium, such as DMEM or RPMI-1640. Alternatively, the hybridoma cells can be grown *in vivo* as ascites tumors in an animal.

After sufficient growth to produce the desired monoclonal antibody, the growth media containing monoclonal antibody (or the ascites fluid) can be separated away from the cells, and the monoclonal antibody is purified. Purification is typically achieved by chromatography using protein A or protein G-Sepharose, or an anti-mouse Ig linked to a solid support such as agarose or Sepharose beads (all described, for example, in the Antibody Purification Handbook, Amersham Biosciences, publication No. 18-1037-46, Edition AC, the disclosure of which is hereby incorporated by reference), or by other known techniques such as electrophoresis or dialysis. The bound antibody is typically eluted from protein A/protein G columns by using low pH buffers (glycine or acetate buffers of pH 3.0 or less) with immediate neutralization of antibody-containing fractions. These fractions can be pooled, dialyzed, and concentrated as needed.

Human Antibodies

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In one aspect, the invention provides human anti-KIR antibodies. "Human" antibodies are distinguishable from "humanized" antibodies (which are described separately below). Such "human" antibodies may include amino acid residues not encoded by human germ line immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs, such as in CDR3. However, the term "human antibody", as used herein, is intended to not include humanized antibodies or human/mouse chimera antibodies in which CDR sequences derived from the germ line of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

Transgenic animals can and have been developed that harbour human Ig-genes and, upon immunization, produce a full repertoire of human antibodies in the absence of mouse immunoglobulin production. Such human Ig-transgenic mice can be employed to produce human antibodies. Such human antibodies can be generated in human Ig-transgenic animals (*e.g.*, mice, rats, sheep, pigs, goats, cattle, horses, etc.) comprising human immunoglobulin loci and native immunoglobulin gene deletions, such as in a XenoMouse™ (Abgenix – Fremont, CA, USA) (see, *e.g.*, Green et al. Nature Genetics 7:13-21 (1994); Mendez et al. Nature Genetics 15:146-156 (1997); Green and Jakobovits J. Exp. Med. 188:483-495 (1998); European Patent No., EP 0 463 151 B1; International Patent Application Nos. WO 94/02602, WO 96/34096; WO 98/24893, WO 99/45031, WO 99/53049, and WO 00/037504; and US Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 5,994,619, 6,075,181, 6,091,001, 6,114,598 and 6,130,364) or transgenic animals comprising a minilocus of human Ig-encoding genes such as the HuMab-mouse™ (Medarex – Princeton, NJ, USA) (see, *e.g.*, EP 0546073, EP0546073; U.S. Pat. Nos. 5,545,807, 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, 5,643,763; and International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884). Splenocytes from such transgenic mice can be used to produce hybridomas that secrete human monoclonal antibodies according to well known techniques, as described herein. Similar techniques and principles are described in, *e.g.*, Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551-255 (1993); Jakobovits et al., Nature, 362:255-258 (1993); and Bruggemann et al., Year in Immuno., 7:33 (1993)).

Further, human antibodies or antibodies from other species can be generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other related techniques, using methods well known in the art, and the resulting molecules can be subjected to additional maturation methods, such as af-

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finity maturation, as such techniques also are well known (see, *e.g.*, (Hoogenboom et al ., J. Mol. Biol. 227: 381 (1991) (phage display); Vaughan, et al., Nature Biotech 14:309 (1996) (phage display); Hanes and Plucthau PNAS USA 94:4937-4942 (1997) (ribosomal display), Parmley and Smith Gene 73:305-318 (1988) (phage display), Marks et al., J. Mol. Biol., 222:581 (1991), Scott TIBS 17:241-245 (1992), Cwirla et al. PNAS USA 87:6378-6382 (1990), Russel et al. Nucl. Acids Research 21:1081-1085 (1993), Hoganboom et al. Immunol. Reviews 130:43-68 (1992), Chiswell and McCafferty TIBTECH 10:80-84 (1992), and US Patent 5,733,743). If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized, *e.g.*, as described elsewhere herein.

Accordingly, as described herein, anti-KIR mAbs are promising agents for the treatment of cancer and viral infections and other diseases and disorders. Anti-KIR mAbs may be generated by various approaches, such as humanization of murine mAbs or by fusion of splenocytes from human Ig-transgenic mice (XenoMouse, or HuMab mice), by phage-display, or by immortalization of human Ab-producing B cells, or by other methods. In either case, the antibody can be produced by cell lines and purified in quantities suitable for formulation, packaging and injection into patients in need thereof.

Recombinant Production

Anti-KIR antibodies can also be prepared by recombinant expression in single cell organisms, such as yeast; or in bacterial cell cultures (such as in *E. coli*); or in eukaryotic cell culture (*e.g.*, in a culture of a mammalian cells) using standard techniques.

Thus, according to an alternate embodiment, the DNA encoding heavy and light chains of a cross-reactive and neutralizing anti-KIR antibody, that binds a determinant present on at least two different human inhibitory KIRs, is isolated from the hybridoma of this invention and placed in an appropriate expression vector for transfection into an appropriate host. The host is then used for the recombinant production of the antibody, or variants thereof, such as a humanized version of that monoclonal antibody, active fragments of the antibody, or chimeric antibodies comprising the antigen recognition portion of the antibody. Preferably, the DNA used in this embodiment encodes an antibody that recognizes a determinant present on at least two different KIR2DL gene products, but not on KIR2DS3 or -4, and that cause potentiation of NK cells expressing at least one of those KIR2DL receptors. Even more preferably, the DNA encodes an antibody that binds to substantially the same epitope or determinant as 1-7F9 and which potentiates NK cell activity. Most preferably, that DNA encodes monoclonal antibody 1-7F9.

DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capa-

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ble of binding specifically to genes encoding the heavy and light chains of murine or human antibodies). Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant expression in bacteria of DNA encoding fragments of the antibody is well known in the art (see, for example, Skerra et al., Curr. Opinion in Immunol., 5, pp. 256 (1993); and Pluckthun, Immunol. Revs. 130, pp. 151 (1992).

Additionally, recombinant production of antibodies from known variable heavy (VH) and variable light (VL) chains, and human constant regions has been described by, for example, Ruker et al. (Annals of the New York Academy of Sciences. 1991;646:212-219), who reports the expression of a human monoclonal anti-HIV-1 antibody in CHO cells; Bianchi et al. (Biotechnology and Bioengineering. 2003;84:439-444), who describes high-level expression of full-Length antibodies using trans-complementing expression vectors, No Soo Kim et al. (Biotechnol. Prog. 2001;17:69-75), who describes key determinants in the occurrence of clonal variation in humanized antibody expression of CHO cells during dihydrofolate reductase mediated gene amplification; King et al. (Biochemical Journal. 1992;281:317-323), who reports expression, purification and characterization of a mouse-human chimeric antibody and chimeric Fab' fragment; WO 2003064606 which describes isolated human monoclonal antibodies comprising a human heavy and a human light chain variable regions, both comprising FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4 sequences; and WO 2003040170 which describes chimeric or human monoclonal antibodies and antigen-binding portions that specifically binds to and activates human CD40.

The entire cDNA sequences encoding the constant regions of human IgG can be found in the following GenBank entries, each of which incorporated by reference in its entirety, accessed on January 6, 2005:

Human IgG1 constant heavy chain region: GenBank accession #: J00228
Human IgG2 constant heavy chain region: GenBank accession #: J00230
Human IgG3 constant heavy chain region: GenBank accession #: X04646
Human IgG4 constant heavy chain region: GenBank accession #: K01316
Human kappa light chain constant region: GenBank accession #: J00241.

In an exemplary embodiment, to produce recombinant mAb production from 1-7F9 or 1-4F1 VH and VL sequences, the following protocol can be applied. Steps 1-3 describe retrieval of the VH and VL regions from a hybridoma or other cell producing 1-7F9 or 1-4F1. However, the cDNA encoding the 1-7F9 or 1-4F1 VH and VL sequences (or mutants or de-

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rivatives thereof), to be used in step 4, can also be prepared from the sequence information provided in Figures 14 or 15, using well-established techniques for synthesizing cDNA fragments. Alternatively, the VH and VL fragments of 1-7F9 or 1-4F1, or mutants or derivatives thereof, may be cloned into any one of a number of expression vectors described in the scientific literature or commercially available expression vectors, containing a constant region of the desired Ig subclass, in order to express a full-length antibody. Additionally, VH and VL fragments of 1-7F9 or 1-4F1, or mutants or derivatives thereof can be cloned into vectors encoding truncated constant regions in order to express antibody fragments (*e.g.*, Fab fragments). One example of a commercially available vector is pASK84, available from the ATCC (American Type Culture Collection, catalog number 87094).

(1) Isolation of total RNA from hybridoma cells:

$4x10^6$ hybridoma cells (such as 1-7F9 or 1-4F1) secreting antibodies against human KIR are used for isolation of total RNA using RNeasy Mini Kit from Qiagen, according to manufacturers instructions, and briefly outlined here: The cells are pelleted by centrifugation for 5 min at 1000 rpm and disrupted by addition of 350 µl RLT buffer containing 10 µl/ml β-mercaptoethanol. The lysate is transferred onto a QIAshredder column from Qiagen and centrifuged for 2 min at maximum speed. The flow-through is mixed with an equal volume of 70% ethanol. Up to 700 µl sample is applied per RNeasy spin column (Qiagen) and centrifuged at 14000 rpm, and the flow-through discarded. 700µl RW1 buffer is applied per column which is centrifuged at 14000 rpm for 15s to wash the column. The column is washed twice with 500µl RPE buffer and centrifuged for 14000rpm for 15s. To dry the column it is centrifuged for additionally 2 min at 14000rpm. The column is transferred to a new collection tube and the RNA is eluted with 50µl of nuclease-free water and centrifuged for 1min at 14000rpm. The RNA concentration is measured by absorbance at OD=260nm. The RNA is stored at -80ºC until needed.

(2) cDNA synthesis:

1 µg RNA is used for first-strand cDNA synthesis using SMART RACE cDNA Amplification Kit from Clontech. For preparation of 5'-RACE-Ready cDNA, a reaction mixture is prepared containing RNA isolated as described above, the reverse-primer 5'-CDS primer back, and SMART II A oligo, and this mixture is incubated at 72ºC for about 2 min., and subsequently cooled on ice for about 2 min. before adding 1xFirst-Strand buffer, DTT (20mM), dNTP (10mM) and PowerScript Reverse Transcriptase. The reaction mixture is incubated at 42ºC for 1.5 hour and Tricine-EDTA buffer is added and incubated at 72ºC for 7 min. At this point samples can be stored at -20ºC.

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(3) PCR amplification and cloning of human variable light (VL) and human variable heavy (VH) chains:

A PCR (Polymerase Chain Reaction) reaction mixture containing 1xAdvantage HF 2 PCR buffer, dNTP (10mM) and 1xAdvantage HF 2 polymerase mix is established for separate amplification of variable regions of both VL and VH from cDNA made as above.

For amplification of VL the following primers are used:

UPM (Universal Primer Mix):

5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3' (SEQ ID NO:26)

5'-CTAATACGACTCACTATAGGG-3' (SEQ ID NO:27)

VK RACE2:

5'-GCAGGCACACAACAGAGGCAGTTCCAGATTTC-3' (SEQ ID NO:28)

For amplification of VH the following primers are used:

UPM (Universal Primer Mix):

5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3' (SEQ ID NO:29)

5'-CTAATACGACTCACTATAGGG-3' (SEQ ID NO:30)

AB90RACE:

5'-GTGCCAGGGGGAAGACCGATGGG-3' (SEQ ID NO:31)

Three rounds of PCR are conducted. Round 1: PCR is run for 5 cycles at 94ºC for 5s and 72ºC for 3 min. Round 2: PCR is run for 5 cycles at 94ºC for 5s, 70ºC for 10s, and 72ºC for 1 min. Round 3: PCR is run for 28 cycles at 94ºC for 5s, 68ºC for 10s, and 72ºC for 1 min.

The PCR products are analyzed by electrophoresis on a 1% agarose gel and the DNA purified from the gel using QIAEX11 agarose gel extraction kit from Qiagen.

The purified PCR products are introduced into PCR4-TOPO vector using TOPO TA Cloning kit from Invitrogen and used for transformation of TOP10 competent cells.

A suitable amount of colonies are analyzed by colony PCR using Taq polymerase, 1xTaq polymerase buffer, dNTP (10mM) and the following primers and PCR program:

M13forward primer: 5'-GTAAAACGACGGCCAG-3' (SEQ ID NO:32)

M13reverse primer: 5'-CAGGAAACAGCTATGAC-3' (SEQ ID NO:33)

PCR Program:

25 cycles are run at 94ºC for 30s, 55ºC for 30s, and 72ºC for 1 min.

Plasmid DNA from clones comprising VL and VH inserts, respectively, is extracted and sequenced using primer M13forward and M13reverse listed above. In the case of the

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human anti-KIR mAb 1-7F9, the sequences encoding the heavy and light chain variable regions are shown in Fig 15.

(4) Subcloning of antibody genes into mammalian expression vectors

Based on the sequence data for cDNAs encoding the heavy and light chain variable regions of the mAb, primers are designed for the amplification of the variable light (VL) and variable heavy (VH) chain genes, respectively. The variable regions are formatted by PCR to include a Kozak sequence, leader sequence and unique restriction enzyme sites. For the VL, this is achieved by designing 5' PCR primers to introduce a HindIII site, the Kozak sequence and to be homologous to the 5' end of the leader sequence of the variable light chain region. The 3' primer is homologous to the 3' end of the variable region and introduced a BsiWI site at the 3' boundary of the variable region. The VH region is generated in a similar fashion except that a NotI and a NheI site are introduced in the 5' and 3' end instead of HindIII and BsiWI, respectively.

The amplified gene products are each cloned into a eukaryotic expression vector containing the light and heavy chain constant regions, using standard techniques. The VL DNA fragments is digested with HindIII and BsiWI and ligated into a eukaryotic expression vector containing the beta-lactamase gene encoding resistance to ampicillin and an E. coli replication origin (pUC); the resulting plasmid is designated VLCL. The VH DNA fragments, is digested with NotI and NheI and introduced into the VLCL vector resulting from the introduction of VL fragment as described above. The resulting plasmid contains functional expression cassettes encoding both the heavy and light chains of the antibody on the same plasmid. The ligated plasmid is used to transform *E. coli*. Plasmid DNA is prepared from these ampicillin resistant bacterial populations and used for transfection into Chinese hamster Ovary cells, or other mammalian cell lines. Transfection and cell culture is done be standard methods, as described for example in "Molecular Cloning", Sambrook et al. The result is transfected cell lines that stably express and secrete the antibody molecule of interest, such as the 1-7F9 or 1-4F1 human anti-KIR mAb or a mAb comprising the VH and VL regions of 1-7F9 or 1-4F1, or another human anti-KIR mAb.

Variants of the antibody can easily be generated. For example, an antibody with the exact same specificity as 1-7F9 or 1-4F1 but of a different isotype than IgG4 or IgG2, respectively, can be obtained by sub-cloning the cDNA encoding VL and VH of 1-7F9 or 1-4F1 into plasmids containing cDNA encoding the kappa light chain constant regions and a heavy constant chain region selected from IgG1 or IgG2 or IgG3 or IgG4 constant heavy chain regions. Thus, an antibody as generated can possess any isotype and the antibody can then be isotype switched using conventional techniques in the art. Such techniques include the use of

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direct recombinant techniques (see, *e.g.*, US Patent 4,816,397), cell-cell fusion techniques (see *e.g.*, US Patent 5,916,771), and other suitable techniques known in the art. Accordingly, the effector function of antibodies provided by the invention may be "changed" with respect to the isotype of a parent antibody by isotype switching to, *e.g.*, an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody for various uses, including therapeutic ones.

Thus, in further aspects, the invention provides a hybridoma comprising: (a) a B cell from a mammalian host (typically a non-human mammalian host) that has been immunized with an antigen that comprises an epitope present on an inhibitory KIR polypeptide, fused to (b) an immortalized cell (*e.g.*, a myeloma cell), wherein said hybridoma produces a monoclonal antibody that binds at least two different human inhibitory KIR receptors and is capable of at least substantially neutralizing KIR-mediated inhibition of NK cell cytotoxicity in a population of NK cells expressing said at least two different human inhibitory KIR receptors. In one embodiment, the mammalian host is a transgenic animal capable of producing human antibodies. Optionally, said hybridoma does not produce monoclonal antibody NKVSF1, not A210, and/or not A802g. In various embodiments, the antibody binds KIR2DL1 and KIR2DL2/3 receptors or a common determinant present on both KIR2DL1 and KIR2DL2/3. The hybridoma may produce an antibody that inhibits the binding of a HLA-C allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors. For example, the hybridoma may produce an antibody that binds to substantially the same epitope as monoclonal antibody 1-7F9 or 1-4F1 on either KIR2DL1 or KIR2DL2/3 or both KIR2DL1 and KIR2DL2/3.

The invention also provides methods of producing an antibody which cross-reacts with multiple KIR2DL gene products and which reduces or neutralizes the inhibitory activity of such KIRs, said method comprising the steps of:

(a) immunizing a non-human mammal with an immunogen comprising a KIR2DL polypeptide;

(b) preparing antibodies from said immunized mammal, wherein said antibodies bind said KIR2DL polypeptide,

(c) selecting antibodies of (b) that cross-react with at least two different KIR2DL gene products, and

(d) selecting antibodies of (c) that potentiate NK cells. In one embodiment, said non-human mammal is a transgenic animal engineered to express a human antibody repertoire (*e.g.*, a non-human mammal comprising human immunoglobulin loci and native immunoglobulin gene deletions, such as a Xenomouse™ (Abgenix – Fremont, CA, USA) or non-

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human mammal comprising a minilocus of human Ig-encoding genes, such as the HuMab-mouse™ (Medarex – Princeton, NJ, USA)). Optionally, step a and b may be replaced by alternative methods for obtaining anti-KIR mAbs, including, without limitation, the use of phage-display or viral transduction of human B cells, or other methods known in the art. Optionally, the method further comprises selecting an antibody that binds to KIR in a primate, preferably a cynomolgus monkey, NK cell or KIR polypeptide. Optionally, the invention further comprises a method of evaluating an anti-KIR antibody, wherein an antibody produced according to the above method is administered to a primate, preferably a cynomolgus monkey, preferably wherein the monkey is observed for the presence or absence of an indication of toxicity of the antibody.

The invention also provides a method of producing an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein the antibody is capable of neutralizing KIR-mediated inhibition of cytotoxicity (or potentiating NK cytotoxicity) by a population of NK cells expressing the at least two different human inhibitory KIR receptor gene products, the method comprising the steps of:

a) immunizing a non-human mammal with an immunogen comprising an inhibitory KIR polypeptide;

b) preparing antibodies from the immunized animal, wherein the antibodies bind the KIR polypeptide,

c) selecting antibodies of (b) that cross-react with at least two different human inhibitory KIR receptor gene products, and

selecting antibodies of (c) that capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing the at least two different human inhibitory KIR receptor gene products, wherein the order of steps (c) and (d) is optionally reversed and any number of the steps are optionally repeated 1 or more times. The inhibitory KIR polypeptide used for immunization can, in one embodiment, be one or more KIR2DL polypeptide(s) and the antibodies selected in step (c) cross-react with at least KIR2DL1 and KIR2DL2/3. Preferably the antibody recognizes a common determinant present on at least two different KIR receptor gene products; most preferably the KIRs are KIR2DL1 and KIR2DL2/3. In one embodiment, step (c) comprises selecting antibodies which react with at least one KIR2DL and one KIR3DL receptor gene products. For example, the selected antibody could react with KIR2DL1 and KIR2DL2/3 as well as KIR3DL1 and/or KIR3DL2. Optionally, the method further comprises selecting an antibody that binds a primate, preferably a cynomolgus monkey, NK cell or KIR polypeptide. Optionally, the invention further comprises a method of evaluating an antibody, wherein an antibody produced according to the

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above method is administered to a primate, preferably a cynomolgus monkey, preferably wherein the monkey is observed for the presence or absence of an indication of toxicity of the antibody.

Optionally, in the above-described methods, the antibody selected in step c) or d) is not NKVSF1, not A210, and/or not A802g. Preferably, the antibody prepared in step (b) in the above methods is a monoclonal antibody. Preferably the antibody selected in step (c) in the above methods inhibits the binding of one or more HLA-C allotypes having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of one or more HLA-C allotypes having an Asn residue at position 80 to human KIR2DL2/3 receptors. Preferably, the antibodies selected in step (d) in the above methods cause a potentiation in NK cytotoxicity, or a neutralization of KIR-mediated inhibition of NK cytotoxicity. Preferably, the antibody binds to substantially the same epitope as monoclonal antibody 1-7F9 on KIR2DL1 and/or KIR2DL2/3. Optionally the methods also or alternatively comprise the additional step of making fragments of the selected monoclonal antibodies, making derivatives of the selected monoclonal antibodies (*e.g.*, by conjugation with a radionuclide, cytotoxic agent, reporter molecule, or the like), or making derivatives of antibody fragments produced from, or that comprise sequences that correspond to, the sequences of such monoclonal antibodies. In another aspect, a variant of such an antibody can be produced by preparing an antibody that comprises a variant amino acid sequence from the antibody obtained by the above-described methods by use of known genetic engineering methods (*e.g.*, a sequence that is modified so as to change the binding properties of the antibody, increase or decrease the stability of the antibody, enhance purification of the antibody, enhance detection of the antibody, and/or change the immunological properties of the antibody with respect to a host to which it is to be administered).

The invention further provides a method of producing an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein the antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on (or potentiating NK cytotoxicity by) a population of NK cells expressing at least one of the different human inhibitory KIR receptor gene products, the method comprising the steps of:

(a) selecting, from a library or repertoire, a monoclonal antibody or an antibody fragment that cross-reacts with at least two different human inhibitory KIR2DL receptor gene products, and

(b) selecting an antibody of (a) that is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity in a population of NK cells expressing the at least two different human inhibitory KIR2DL receptor gene products. Preferably the antibody binds a common determi-

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nant present on KIR2DL1 and KIR2DL2/3. Optionally, the antibody selected in step (b) is not NKVSF1. Preferably, the antibody selected in step (b) inhibits the binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2/3 receptors. Preferably, the antibody selected in step (b) causes a potentiation in NK cytotoxicity. Preferably, the antibody binds to substantially the same epitope as monoclonal antibody 1-7F9 on KIR2DL1 and/or KIR2DL2/3. Alternatively, the antibody binds substantially the same epitope as monoclonal antibody 1-4F1. Optionally the method comprises the additional step of making fragments of the selected monoclonal antibodies, making derivatives of the selected monoclonal antibodies, or making derivatives of selected monoclonal antibody fragments.

Additionally, the invention provides a method of producing an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein the antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity in a population of NK cells expressing at least one of the two different human inhibitory KIR receptor gene products, the method comprising the steps of:

a) culturing a hybridoma of the invention under conditions permissive for the production of the monoclonal antibody; and

b) separating the monoclonal antibody from the hybridoma cells. Optionally the method comprises the additional step of making fragments of the the monoclonal antibody, making derivatives of the monoclonal antibody, or making derivatives of such monoclonal antibody fragments. Preferably the antibody binds a common determinant present on KIR2DL1 and KIR2DL2/3.

Also provided by the present invention is a method of producing an antibody that binds at least two different human inhibitory KIR receptor gene products, wherein the antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity in a population of NK cells expressing at least one of the two different human inhibitory KIR receptor gene products, the method comprising the steps of:

a) isolating from a hybridoma of the invention a nucleic acid encoding the monoclonal antibody;

b) optionally modifying the nucleic acid so as to obtain a modified nucleic acid that comprises a sequence that encodes a modified or derivatized antibody comprising an amino acid sequence that corresponds to a functional sequence of the monoclonal antibody or is substantially similar thereto (*e.g.*, is at least about 65%, at least about 75%, at least about 85%, at least about 90%, at least about 95% (such as about 70-99%) identical to such a se-

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quence) selected from a humanized antibody, a chimeric antibody, a single chain antibody, an immunoreactive fragment of an antibody, or a fusion protein comprising such an immunoreactive fragment;

c) inserting the nucleic acid or modified nucleic acid (or related nucleic acid coding for the same amino acid sequence) into an expression vector, wherein the encoded antibody or antibody fragment is capable of being expressed when the expression vector is present in a host cell grown under appropriate conditions;

d) transfecting a host cell with the expression vector, wherein the host cell does not otherwise produce immunoglobulin protein;

e) culturing the transfected host cell under conditions which cause the expression of the antibody or antibody fragment; and

f) isolating the antibody or antibody fragment produced by the transfected host cell. Preferably the antibody binds a common determinant present on KIR2DL1 and KIR2DL2/3.

Antibody Screening and Selection

Particular antibodies of this invention are able to reduce or neutralize the KIR-mediated inhibition of NK cell cytotoxicity; particularly inhibition mediated by KIR2DL receptors and more particularly at least both KIR2DL1- and KIR2DL2/3-mediated inhibition. These antibodies are thus "neutralizing", "blocking", or "inhibitory" antibodies, in the sense that they reduce, neutralize and/or block, at least partially and detectably, the inhibitory signaling pathway mediated by KIR receptors when these interact with MHC class I molecules. More importantly, this neutralization activity is displayed with respect to several types of inhibitory KIR receptors, preferably several KIR2DL receptor gene products, and more preferably at least both KIR2DL1 and KIR2DL2/3 so that these antibodies may be used in most or all human subjects with high efficacy. Neutralization of KIR-mediated inhibition of NK cell cytotoxicity can be assessed by various assays or tests, such as standard *in vitro* cellular cytotoxicity assays, as described herein.

Once an antibody that cross-reacts with multiple inhibitor KIR receptors is identified it can be tested for its ability to reduce or neutralize the inhibitory effect of those KIR receptors in intact NK cells. In a specific variant, the neutralizing activity can be illustrated by the capacity of said antibody to reconstitute lysis by KIR2DL-expressing NK cells of targets expressing HLA-C. In another specific embodiment, the neutralizing activity of the antibody is defined by the ability of the antibody to block or reduce the binding of HLA-C molecules to KIR2DL1 and KIR2DL3 (or the closely related KIR2DL2) receptors, further preferably as it is the capacity of the antibody to alter the binding of KIR2DL2/3 to a HLA-C molecule selected

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from Cw1, Cw3, Cw7, and Cw8 (or of another HLA-C molecule having an Asn residue at position 80), and/or the binding of KIR2DL1 to a HLA-C molecule selected from Cw2, Cw4, Cw5 and Cw6 (or of another HLA-C molecule having a Lys residue at position 80).

In another variant, the neutralizing activity of an antibody of this invention can be assessed in a cell-based cytotoxicity assay, as disclosed in the Examples provided herein.

In another variant, the neutralizing activity of an antibody of this invention can be assessed in a cytokine-release assay, wherein NK cells are incubated with the test antibody and a target cell line expressing one HLA-C allele recognized by a KIR molecule of the NK population, to stimulate NK cell cytokine production (for example IFN-γ and/or GM-CSF production). In an exemplary protocol, IFN-γ production from PBMC is assessed by cell surface and intracytoplasmic staining and analysis by flow cytometry after about 4 days in culture. Briefly, Brefeldin A (Sigma Aldrich) can be added at a final concentration of about 5 µg/ml for the least about 4 hours of culture. The cells can then incubated with anti-CD3 and anti-CD56 mAb prior to permeabilization (IntraPrep™; Beckman Coulter) and staining with PE-anti-IFN-γ or PE-IgG1 (Pharmingen). GM-CSF and IFN- production from polyclonal activated NK cells can be measured in supernatants using ELISA (GM-CSF: DuoSet Elisa, R&D Systems, Minneapolis, MN; IFN-γ: OptE1A set, Pharmingen).

Antibodies of this invention may partially (*i.e.*, reduce) or fully neutralize the KIR-mediated inhibition of NK cell cytotoxicity. For example, preferred antibodies of this invention are able to induce or augment the lysis of HLA-matched, or HLA-compatible, or autologous target cell populations, *i.e.*, cell populations that would not be effectively lysed by NK cells in the absence of said antibody. Accordingly, the antibodies of this invention may also be defined as facilitating NK cell activity *in vivo*, and/or *in vitro*.

In a specific embodiment, the antibody binds substantially the same epitope as monoclonal antibody DF200 (produced by hybridoma DF200), 1-7F9, or 1-4F1. Such antibodies can be referred to herein as "DF200 like antibodies", "1-7F9-like antibodies", and "1-4F1-like antibodies", respectively. In a further preferred embodiment, the antibody is a monoclonal antibody. More preferred "DF200 like antibodies" of this invention are antibodies other than the monoclonal antibody NKVSF1. Most preferred is monoclonal antibody 1-7F9 or 1-4F1, and antibodies comprising the VH and/or VL regions of 1-7F9 or 1-4F1.

The identification of one or more antibodies that bind(s) to substantially or essentially the same epitope as the monoclonal antibodies described herein can be readily determined using any one of variety of immunological screening assays in which antibody competition can be assessed. A number of such assays are routinely practiced and well known in the art (see, *e.g.*, U.S. Pat. No. 5,660,827, issued Aug. 26, 1997, which is specifically incor-

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porated herein by reference). It will be understood that actually determining the epitope to which an antibody described herein binds is not in any way required to identify an antibody that binds to the same or substantially the same epitope as the monoclonal antibody described herein.

For example, where the test antibodies to be examined are obtained from different source animals, or are even of a different Ig isotype, a simple competition assay may be employed in which the control antibody (DF200, for example) is mixed with the test antibody and then applied to a sample containing either or both KIR2DL1 and/or KIR2DL2/3, each of which is known to be bound by DF200. Protocols based upon ELISAs, radioimmunoassays, Western blotting, and the use of BIACORE analysis (as described, for example, in the Examples section herein) are suitable for use in such simple competition studies.

In certain embodiments, one would pre-mix the control antibody (1-7F9 or 1-4F1, for example) with varying amounts of the test antibody (*e.g.*, in ratios of about 1:1, 1:2, 1:10 or about 1:100) for a period of time prior to applying to the KIR antigen sample. In other embodiments, the control and varying amounts of test antibody can simply be added separately and admixed during exposure to the KIR antigen sample. As long as one can distinguish bound from free antibodies (*e.g.*, by using separation or washing techniques to eliminate unbound antibodies) and control antibody from the test antibody (*e.g.*, by using species specific or isotype specific secondary antibodies or by specifically labelling the control antibody with a detectable label) one will be able to determine if the test antibody reduce the binding of the control antibody to the different KIR2DL antigens, indicating that the test antibody recognizes substantially the same epitope as 1-7F9. The binding of the (labeled) control antibody in the presence of a completely irrelevant antibody (that does not bind KIR) can serve as the control high value. The control low value can be obtained by incubating the labeled control antibody with the same but unlabelled control antibody, where competition would occur and reduce binding of the labeled antibody. In a test assay, a significant reduction in labeled antibody reactivity in the presence of a test antibody is indicative of a test antibody that recognizes substantially the same epitope, *i.e.*, one that competes with the labeled control antibody. For example, any test antibody that reduces the binding of 1-7F9 or 1-4F1 to both of KIR2DL1 and KIR2DL2/3 antigens by at least about 50%, such as at least about 60%, or more preferably at least about 70% (*e.g.*, about 65-100%), at any ratio of 1-7F9:test antibody or 1-4F1:test antibody between about 1:1 or 1:10 and about 1:100 is considered to be an antibody that binds to substantially the same epitope or determinant as 1-7F9 or 1-4F1, respectively. Preferably, such test antibody will reduce the binding of 1-7F9 to at least one other, preferably each of the KIR2DL antigens preferably at least about 50%, at least about 60%, at

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least about 80% or at least about 90% (*e.g.*, about 95%) of the binding of 1-7F9 or 1-4F1 observed in the absence of the test antibody. Of course, such methods can be adapted to identify and/or evaluate antibodies that compete with other antibodies and/or KIR antigens.

Competition can also or alternatively be assessed by, for example, a flow cytometry test. In such a test, cells bearing a given KIR can be incubated first with a control antibody (1-7F9 or 1-4F1, for example), and then with the test antibody labeled with a fluorochrome or biotin. The antibody is said to compete with control antibody if the binding obtained upon preincubation with saturating amount of control antibody is about 80%, preferably about 50%, about 40% or less (*e.g.*, about 30%) of the binding (as measured by mean of fluorescence) obtained by the test antibody without preincubation with control antibody. Alternatively, an antibody is said to compete with the control antibody if the binding obtained with a labeled control antibody (by a fluorochrome or biotin) on cells preincubated with saturating amount of test antibody is about 80%, preferably about 50%, about 40%, or less (*e.g.*, about 30%) of the binding obtained without preincubation with the test antibody.

A simple competition assay in which a test antibody is pre-adsorbed and applied at saturating concentration to a surface onto which either KIR2DL1 or KIR2DL2/3, or both, are immobilized also may be advantageously employed. The surface in the simple competition assay is preferably a BIACORE chip (or other media suitable for surface plasmon resonance analysis). The binding of a control antibody (*e.g.*, 1-7F9 or 1-4F1) to the KIR-coated surface is measured. This binding to the KIR-containing surface of the control antibody alone is compared with the binding of the control antibody in the presence of a test antibody. A significant reduction in binding to the KIR2DL1 and KIR2DL2/3-containing surface by the control antibody in the presence of a test antibody indicates that the test antibody recognizes substantially the same epitope as the control antibody such that the test antibody "competes" with the control antibody. Any test antibody that reduces the binding of control antibody (such as 1-7F9 or 1-4F1) to both of KIR2DL1 and KIR2DL2/3 antigens by at least about 20% or more, at least about 40%, at least about 50%, at least about 70%, or more, can be considered to be an antibody that binds to substantially the same epitope or determinant as the control antibody (*e.g.*, 1-7F9 or 1-4F1). Preferably, such test antibody will reduce the binding of the control antibody (*e.g.*, 1-7F9 or 1-4F1) to each of the KIR2DL antigens by at least about 50% (*e.g.*, at least about 60%, at least about 70%, or more). It will be appreciated that the order of control and test antibodies can be reversed; *i.e.* the control antibody can be first bound to the surface and then the test antibody is brought into contact with the surface thereafter in a competition assay. Preferably, the antibody having higher affinity for KIR2DL1 and KIR2DL2/3 antigens is bound to the KIR2DL1 and KIR2DL2/3-containing surface first, as

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it will be expected that the decrease in binding seen for the second antibody (assuming the antibodies are competing) will be of greater magnitude. Further examples of such assays are provided in the Examples herein, and in *e.g.*, Saunal and Regenmortel, (1995) J. Immunol. Methods 183: 33-41, the disclosure of which is incorporated herein by reference.

While described in the context of 1-7F9 or 1-4F1 for the purposes of exemplification, it will be appreciated that the above-described screening assays can also be used to identify antibodies that compete with one or more of NKVSF1, DF200, EB6, GL183, and other antibodies, antibody fragments, and antibody derivatives according to the invention.

Upon immunization and production of antibodies in a vertebrate or cell, particular selection steps may be performed to isolate antibodies as claimed. In this regard, in a specific embodiment, the invention also relates to methods of producing such antibodies, comprising:

(a) immunizing a non-human mammal with an immunogen comprising an inhibitory KIR polypeptide;

(b) preparing antibodies from said immunized animal, wherein said antibodies bind said KIR polypeptide,

(c) selecting antibodies of (b) that cross-react with at least two different inhibitory KIR gene products, and

(d) selecting antibodies of (c) that are capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing at least one of said two different human inhibitory KIR receptor gene products.

In certain embodiments, an additional step is performed between steps (c) and (d) above, to select anti-KIR mAbs that do not react with KIR2DS4.

The selection of an antibody that cross-reacts with at least two different inhibitory KIR gene products may be achieved by screening the antibody against two or more different inhibitory KIR antigens. In a more preferred embodiment, the antibodies prepared in step (b) are monoclonal antibodies. Thus, the term "preparing antibodies from said immunized animal," as used herein, includes obtaining B-cells from an immunized animal and using those B cells to produce a hybridoma that expresses antibodies, as well as obtaining antibodies directly from the serum of an immunized animal. In another preferred embodiment, the antibodies selected in step (c) are those that cross-react with at least KIR2DL1 and KIR2DL2/3.

In yet another preferred embodiment, the antibodies selected in step (d) cause at least about 10% more specific lysis mediated by NK cells displaying at least one KIR recognized by the antibody, and preferably at least about 40% more specific lysis, at least about 50% higher specific lysis, or more preferably at least about 70% increased specific lysis (*e.g.*, about 60-100% increased specific lysis), as measured in a standard chromium release assay

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towards a target cell expressing cognate HLA class I molecule, compared to the specific lysis in the absence of an anti-KIR mAb.. Alternatively, the antibodies selected in step (d) when used in a chromium-release assay employing an NK cell clone expressing one or several inhibitory KIRs and a target cells expressing at least one HLA allele that is recognized by one of the KIRs on the NK clone, the level of cytotoxicity obtained with the antibody should be at least about 20%, preferably at least about 30 %, or more of the specific cytotoxicity obtained with the same concentration of DF200 or with a blocking anti-MHC class I antibody, at the same effector:target cell ratio.

The order of steps (c) and (d) of the immediately above-described method can be changed. Optionally, the method also or alternatively may further comprise additional steps of making fragments of the monoclonal antibody or derivatives of the monoclonal antibody or such fragments, *e.g.*, as described elsewhere herein.

In another variant, the invention provides a method for obtaining an antibody that comprises:

(a) selecting, from a library or repertoire, a monoclonal antibody, a fragment of a monoclonal antibody, or a derivative of either thereof that cross-reacts with at least two different human inhibitory KIR2DL receptor gene products, and

(b) selecting an antibody, fragment, or derivative of (a) that is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on a population of NK cells expressing said at least two different human inhibitory KIR2DL receptor gene products.

The repertoire may be any (recombinant) repertoire of antibodies or fragments thereof, optionally displayed by any suitable structure (*e.g.*, phage, bacteria, synthetic complex, etc.). Selection of inhibitory antibodies may be performed as disclosed above and further illustrated in the examples.

Computer modelling of the extra-cellular domains of KIR2DL1, -2 and -3 (KIR2DL1-3), based on their published crystal-structures (Maenaka et al. Structure with Folding and design 1999;7:391-398; Fan et al., Nature immunology 2001;2:452-460; Boyington et al. Nature, 2000;405:537-543), revealed the involvement of certain regions or KIR2DL1, -2 and -3 in the interaction between these KIR and the anti-KIR2DL cross-reactive murine monoclonal antibodies DF200 and NKVSF1. Thus, in one embodiment, the present invention provides antibodies that exclusively bind to KIR2DL1 within a region defined by the amino acid residues (105, 106, 107, 108, 109, 110, 111, 127, 129, 130, 131, 132, 133, 134, 135, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 181, and 192). In another embodiment the invention provides antibodies that bind to KIR2DL1 and KIR 2DL2/3 without interacting with amino acid residues outside the region defined by the residues (105, 106, 107, 108, 109,

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110, 111, 127, 129, 130, 131, 132, 133, 134, 135, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 181, and 192).

In another embodiment, the invention provides antibodies that bind to KIR2DL1 and which does not bind to a mutant of KIR2DL1 in which R131 (*i.e.*, the Arg residue at position 131 of the KIR2DL1 mutant) is (*i.e.*, is substituted with) an Ala residue.

In another embodiment, the invention provides antibodies that bind to KIR2DL1 and which does not bind to a mutant of KIR2DL1 in which R157 is Ala.

In another embodiment, the invention provides antibodies that bind to KIR2DL1 and which does not bind to a mutant of KIR2DL1 in which R158 is Ala.

In another embodiment, the invention provides antibodies that bind to KIR2DL1 residues 131, 157, and 158.

In another embodiment, the invention provides antibodies that bind to KIR2DS3(R131W), but not to wild type KIR2DS3.

In a particular embodiment, the antibodies exclusively bind to KIR2DL1 within a region defined by the amino acid residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88 (in which the letters designate amino acids in single-letter code, and the number is the position of that residue in KIR2DL1 (SEQ ID NO:23), using the numbering system described in Wagtmann et al., Immunity 1995;2:439-449). These residues have been identified as the 1-7F9 KIR2DL1 epitope (see Example 11).

In another embodiment, the antibodies bind to an antigenic epitope in a KIR2DL1 sequence consisting of the fragment L38 to Y88 of SEQ ID NO:23. In yet another embodiment, the antibodies bind to an antigenic epitope in a KIR2DL1 sequence comprising the fragment L38 to Y88 of SEQ ID NO:23.

In another embodiment, the invention provides antibodies that bind to KIR2DL1 and KIR 2DL2/3 without interacting with amino acid residues outside the region defined by the residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88.

In another embodiment, the antibodies bind to KIR2DL1 within a region comprising at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the amino acid residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88. In a particular embodiment, the KIR2DL1 epitope for the antibodies comprises the amino acid residues M44 and F45 (see Examples 9 and 11).

In another embodiment, the antibodies bind to KIR2DL1 within a region comprising at least 1, 2, 4, 6, 8, 10, or 12 or all of the amino acid residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88. In a particular embodiment, the region comprises amino acid residues M44 and F45, and optionally at least 1, 2, 4, 6, 8, 10, or

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all of L38, R41, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88 (see Examples 9 and 11). In another particular embodiment, the region comprises at least amino acid residues M44, F45, and D72, which are in the KIR2DL1 region where the 1-7F9 epitope and the HLA-C binding sites overlap.

In another embodiment, the invention provides antibodies that bind to KIR2DL1, KIR2DL2/3, and KIR2DS4.

In another embodiment, the invention provides antibodies that bind to both KIR2DL1 and KIR2DL2/3, but not to KIR2DS4.

In another embodiment, the invention provides antibodies that bind to both KIR2DL1 and KIR2DL2/3, but not to KIR2DS3 or to KIR2DS4.

Determination of whether an antibody, antibody fragment, or antibody derivative binds within one of the epitope regions defined above can be carried out in ways known to the person skilled in the art. See, for example, Examples 9 and 11. In another example of such mapping/characterization methods, an epitope region for an anti-KIR antibody may be determined by epitope "foot-printing" using chemical modification of the exposed amines/carboxyls in the KIR2DL1 or KIR2DL2/3 protein. One specific example of such a foot-printing technique is the use of HXMS (hydrogen-deuterium exchange detected by mass spectrometry) wherein a hydrogen/deuterium exchange of receptor and ligand protein amide protons, binding, and back exchange occurs, wherein the backbone amide groups participating in protein binding are protected from back exchange and therefore will remain deuterated. Relevant regions can be identified at this point by peptic proteolysis, fast microbore high-performance liquid chromatography separation, and/or electrospray ionization mass spectrometry. See, *e.g.*, Ehring H, Analytical Biochemistry, Vol. 267 (2) pp. 252-259 (1999) and/or Engen, J.R. and Smith, D.L. (2001) Anal. Chem. 73, 256A-265A. Another example of a suitable epitope identification technique is nuclear magnetic resonance epitope mapping (NMR), where typically the position of the signals in two-dimensional NMR spectres of the free antigen and the antigen complexed with the antigen binding peptide, such as an antibody, are compared. The antigen typically is selectively isotopically labeled with 15N so that only signals corresponding to the antigen and no signals from the antigen binding peptide are seen in the NMR-spectrum. Antigen signals originating from amino acids involved in the interaction with the antigen binding peptide typically will shift position in the spectres of the complex compared to the spectres of the free antigen, and the amino acids involved in the binding can be identified that way. See, *e.g.*, Ernst Schering Res Found Workshop. 2004;(44):149-67; Huang et al, Journal of Molecular Biology, Vol. 281 (1) pp. 61-67 (1998); and Saito and Patterson, Methods. 1996 Jun;9(3):516-24.

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Epitope mapping/characterization also can be performed using mass spectrometry methods. See, *e.g.*, Downward, J Mass Spectrom. 2000 Apr;35(4):493-503 and Kiselar and Downard, Anal Chem. 1999 May 1;71(9):1792-801.

Protease digestion techniques also can be useful in the context of epitope mapping and identification. Antigenic determinant-relevant regions/sequences can be determined by protease digestion, *e.g.* by using trypsin in a ratio of about 1:50 to KIR2DL1 or KIR2DL2/3 o/n digestion at 37 °C and pH 7-8, followed by mass spectrometry (MS) analysis for peptide identification. The peptides protected from trypsin cleavage by the anti-KIR antibody can subsequently be identified by comparison of samples subjected to trypsin digestion and samples incubated with antibody and then subjected to digestion by *e.g.* trypsin (thereby revealing a foot print for the antibody). Other enzymes like chymotrypsin, pepsin, etc., also or alternatively can be used in a similar epitope characterization methods. Moreover, enzymatic digestion can provide a quick method for analyzing whether a potential antigenic determinant sequence is within a region of the KIR2DL1 in the context of a KIR-binding polypeptide. If the polypeptide is not surface exposed, it is most likely not relevant in terms of immunogenicity/antigenicity. See, *e.g.*, Manca, Ann Ist Super Sanita. 1991;27(1):15-9 for a discussion of similar techniques.

Site-directed mutagenesis is another technique useful for elucidation of a binding epitope. For example, in "alanine-scanning", each residue within a protein segment is replaced with an alanine residue, and the consequences for binding affinity measured. If the mutation leads to a significant resuction in binding affinity, it is most likely involved in binding. Monoclonal antibodies specific for structural epitopes (*i.e.*, antibodies which do not bind the unfolded protein) can be used to verify that the alanine-replacement does not influence overall fold of the protein. See, *e.g.*, Clackson and Wells, Science 1995;267:383–386; and Wells, Proc Natl Acad Sci USA 1996;93:1–6.

Electron microscopy can also be used for epitope "foot-printing". For example, Wang et al., Nature 1992;355:275-278 used coordinated application of cryoelectron microscopy, three-dimensional image reconstruction, and X-ray crystallography to determine the physical footprint of a Fab-fragment on the capsid surface of native cowpea mosaic virus.

Other forms of "label-free" assay for epitope evaluation include surface plasmon resonance (SPR, BIACORE) and reflectometric interference spectroscopy (RifS). See, *e.g.*, Fägerstam et al., Journal Of Molecular Recognition 1990;3:208-14; Nice et al., J. Chromatogr. 1993;646:159–168; Leipert et al., Angew. Chem. Int. Ed. 1998;37:3308–3311; Kröger et al., Biosensors and Bioelectronics 2002;17:937-944.

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Antibody Fragments and Derivatives

Fragments and derivatives of antibodies of this invention (which are encompassed by the terms "antibody" and "antibodies" as used in this application, unless otherwise stated or clearly contradicted by context), preferably a 1-7F9- or 1-4F1-like antibody, can be produced by techniques that are known in the art. "Immunoreactive fragments" comprise a portion of the intact antibody, generally the antigen binding site or variable region. Examples of antibody fragments include "kappa bodies" (see, *e.g.*, Ill et al., Protein Eng 10: 949-57 (1997)) and "janusins" (described further elsewhere herein).Fab, Fab', Fab'-SH, F(ab)2, F(ab')2, dAb (Ward et al., (1989) Nature 341:544-546); consisting essentially of a VH domain), Fd (consisting essentially of the VH and CH1 domains), and Fv (consisting essentially of the VL and VH domains) fragments; diabodies; kappa bodies; and janusins. For example, antibody fragments include a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (1) single-chain Fv (scFv) molecules (2) single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety and (3) single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multispecific antibodies formed from antibody fragments. For instance, Fab or F(ab')2 fragments may be produced by protease digestion of the isolated antibodies, according to conventional techniques.

It will be appreciated that immunoreactive fragments can be modified using known methods, for example to slow clearance *in vivo* and obtain a more desirable pharmacokinetic profile. For example, the fragment may be modified with polyethylene glycol (PEG) or polyoxyethylated polyols. Methods for coupling and site-specifically conjugating PEG to a Fab' fragment are described in, for example, Leong et al, Cytokine 16(3):106-119 (2001) and Delgado et al, Br. J. Cancer 73(2):175-182 (1996), the disclosures of which are incorporated herein by reference.

In a particular aspect, the invention provides antibodies, antibody fragments, and antibody derivatives comprising the light and/or heavy chain variable region sequence of 1-7F9 or 1-4F1 as set forth in Figure 14. In another aspect, the invention provides antibodies, antibody fragments, and derivatives thereof that comprise one or more of the light and/or heavy chain variable region CDRs of 1-7F9 or 1-4F1 as set forth in Fig. 15 or described above. Functional variants/analogs of such sequences can be generated by making suitable substitutions, additions, and/or deletions in these disclosed amino acid sequences using

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standard techniques, which may be aided by the comparison of the sequences. From superimposition of crystal structures of the 1-7F9/KIR2DL1 complex and the and the KIR2DL1/MHC class I complex (Fan et al., Nat. Immunol. 2001; 2: 452-460), PDB-code 1IM9, it can be concluded that a scFv derivative of 1-7F9 will be able to block MHC class I binding to KIR2DL1. Also the single L-chain of 1-7F9 or the single VL domain of 1-7F9 alone, when bound to KIR2DL1, would block MHC class I binding.

Functional variants/analogs of such sequences can be generated by making suitable substitutions, additions, and/or deletions in these disclosed amino acid sequences using standard techniques, which may be aided by the comparison of the sequences. In another aspect, positions where a residue is present in a sequence of one of these antibodies, but not another, may be suitable for deletions, substitutions, and/or insertions.

Alternatively, the DNA of a encoding an antibody of this invention, such as a 1-7F9- or 1-4F1-like antibody, may be modified so as to encode for a fragment of this invention. The modified DNA is then inserted into an expression vector and used to transform or transfect an appropriate cell, which then expresses the desired fragment, as described elsewhere herein.

In an alternate embodiment, the DNA of a hybridoma producing a murine antibody of this invention, such as a DF200-like antibody, can be modified prior to insertion into an expression vector, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous non-human sequences (as described by *e.g.*, Morrison et al., Proc. Natl. Acad. Sci. U.S.A., 81, pp. 6851 (1984)). In another embodiment, the variable regions encoding the antibody of this invention may be joined, by recombinant DNA engineering, to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of the original antibody. Typically, such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention.

Thus, according to another embodiment, the antibody of this invention, preferably a DF-200-like antibody is humanized. "Humanized" forms of antibodies according to this invention are specific chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab') 2, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from the murine immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of the original antibody (donor antibody) while maintaining the desired specificity, affin-

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ity, and capacity of the original antibody. In some instances, Fv framework residues of the human immunoglobulin may be replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in either the recipient antibody or in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of the original antibody and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones et al., Nature, 321, pp. 522 (1986); Reichmann et al., Nature, 332, pp. 323 (1988); and Presta, Curr. Op. Struct. Biol., 2, pp. 593 (1992).

Methods for humanizing the antibodies of this invention are well known in the art. Generally, a humanized antibody according to the present invention has one or more amino acid residues introduced into it from the original antibody. These murine or other non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321, pp. 522 (1986); Riechmann et al., Nature, 332, pp. 323 (1988); Verhoeyen et al., Science, 239, pp. 1534 (1988)). Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly et al., U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from the original murine antibody. In practice, humanized antibodies according to this invention are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in the original murine antibody.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of an antibody of this invention is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the mouse is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol., 151, pp. 2296 (1993); Chothia and Lesk, J. Mol. Biol., 196, pp. 901 (1987)). Another method uses a particular framework from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework can be used for several different humanized antibodies (Carter

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et al., Proc. Natl. Acad. Sci. U.S.A., 89, pp. 4285 (1992); Presta et al., J. Immunol., 51, pp. 1993)).

It is further important that antibodies be humanized with retention of high affinity for multiple inhibitory KIR receptors and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

As described above, a method of making human monoclonal antibodies is to use a XenoMouse® (Abgenix, Fremont, CA) as the mouse used for immunization. A XenoMouse is a murine host according to this invention that has had its immunoglobulin genes replaced by functional human immunoglobulin genes. Thus, antibodies produced by this mouse or in hybridomas made from the B cells of this mouse, are already humanized. The XenoMouse is described in United States Patent No. 6,162,963, which is herein incorporated in its entirety by reference. An analogous method can be achieved using a HuMAb-Mouse™ (Medarex).

Human antibodies may also be produced according to various other techniques, such as by using, for immunization, other transgenic animals that have been engineered to express a human antibody repertoire (Jakobovitz et al., Nature 362 (1993) 255), or by selection of antibody repertoires using phage display methods. Such techniques are known to the skilled person and can be implemented starting from monoclonal antibodies as disclosed in the present application.

The antibodies of the present invention, preferably a 1-7F9- or 1-4F1-like antibody, may also be derivatized to "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in the original antibody, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another

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antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly et al., supra; Morrison et al., Proc. Natl. Acad. Sci. U.S.A., 81, pp. 6851 (1984)).

Other derivatives within the scope of this invention include functionalized antibodies, *i.e.*, antibodies that are conjugated or covalently bound to a toxin, such as ricin, diphtheria toxin, abrin and *Pseudomonas exotoxin*, or a therapeutic radionuclide such as, *e.g.*, ^{90}Y or ^{131}I; to a detectable moiety, such as a fluorescent moiety, a diagnostic radioisotope or an imaging agent; or to a solid support, such as agarose beads or the like. Methods for conjugation or covalent bonding of these other agents to antibodies are well known in the art.

Conjugation to a toxin is useful for targeted killing of NK cells displaying one of the cross-reacting KIR receptors on its cell surface. Once the antibody of the invention binds to the cell surface of such cells, it is internalized and the toxin is released inside of the cell, selectively killing that cell. Such use is an alternate embodiment of the present invention.

Conjugation to a detectable moiety is useful when the antibody of this invention is used for diagnostic purposes. Such purposes include, but are not limited to, assaying biological samples for the presence of the NK cells bearing the cross-reacting KIR on their cell surface and detecting the presence of NK cells bearing the cross-reacting KIR in a living organism. Such assay and detection methods are also alternate embodiments of the present invention.

Conjugation of an antibody of this invention to a solid support is useful as a tool for affinity purification of NK cells bearing the cross-reacting KIR on their cell surface from a source, such as a biological fluid. This method of purification is another alternate embodiment of the present invention, as is the resulting purified population of NK cells.

PHARMACEUTICAL COMPOSITIONS AND APPLICATIONS

The invention also provides pharmaceutical compositions that comprise a human or humanized antibody, or fragments and derivatives thereof, wherein said antibody, fragment or derivative cross-reacts with at least two inhibitory KIR receptors at the surface of NK cells, reduces or neutralizes their inhibitory signals and potentiates the activity of those cells, in any suitable vehicle in an amount effective to detectably potentiate NK cell cytotoxicity in a patient or in a biological sample comprising NK cells. The pharmaceutical compositions comprising a human or humanized anti-KIR mAb, optionally together with another active agent, for use according to the present invention may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington: The Science and Prac-

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tice of Pharmacy, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 1995. The compositions may appear in conventional forms, for example, capsules, tablets, aerosols, solutions or suspensions, or as freeze-dried powder.

Accordingly, one object of the present invention is to provide a pharmaceutical formulation comprising a human or humanized antibody, or fragments or derivatives thereof, which is present in a concentration from 1 mg/ml to 500 mg/ml, and wherein said formulation has a pH from 2.0 to 10.0. The formulation may further comprise a buffer system, preservative(s), tonicity agent(s), chelating agent(s), stabilizers and surfactants. In one embodiment of the invention the pharmaceutical formulation is an aqueous formulation, *i.e.*, a formulation comprising water. Such formulation is typically a solution or a suspension. In a further embodiment of the invention the pharmaceutical formulation is an aqueous solution. As used herein, the term "aqueous formulation" is a formulation comprising at least 50 %w/w water. Likewise, the term "aqueous solution" is a solution comprising at least 50 %w/w water, and the term "aqueous suspension" a suspension comprising at least 50 %w/w water.

In another embodiment, the pharmaceutical formulation is a freeze-dried formulation, whereto the physician or the patient adds solvents and/or diluents prior to use.

In another embodiment, the pharmaceutical formulation is a dried formulation (*e.g.*, freeze-dried or spray-dried) ready for use without any prior dissolution.

In a further aspect, the invention relates to a pharmaceutical formulation comprising an aqueous solution of an antibody as described herein, and a buffer, wherein said the antibody is present in a concentration from 1 mg/ml or above, and wherein said formulation has a pH from about 2.0 to about 10.0.

In another embodiment, the pH of the formulation is selected from the list consisting of 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, and 10.0.

In a further embodiment, the buffer is selected from the group consisting of sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine, lysine, arginine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, bicine, tricine, malic acid, succinate, maleic acid, fumaric acid, tartaric acid, aspartic acid or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment of the invention.

In a further embodiment, the formulation also comprises a pharmaceutically acceptable preservative. In another embodiment, the preservative is selected from the group con-

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sisting of phenol, o-cresol, m-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thiomerosal, bronopol, benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, benzethonium chloride, chlorphenesine (3p-chlorphenoxypropane-1,2-diol) or mixtures thereof. In one embodiment, the preservative is present in a concentration from 0.1 mg/ml to 20 mg/ml. In another embodiment, the preservative is present in a concentration from 0.1 mg/ml to 5 mg/ml. In yet another embodiment, the preservative is present in a concentration from 5 mg/ml to 10 mg/ml. In still another embodiment, the preservative is present in a concentration from 10 mg/ml to 20 mg/ml. Each one of the specific preservatives listed constitutes an alternative embodiment of the invention. While the use of a preservative in pharmaceutical compositions is well-known to the skilled person; for convenience, reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

In a further embodiment, the formulation also comprises an isotonic agent. In one embodiment, the isotonic agent is selected from the group consisting of a salt (*e.g.*, sodium chloride), a sugar, a sugar alcohol, an amino sugar, an amino acid (*e.g.*, L-glycine, L-histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine), an alditol (*e.g.*, glycerol (glycerine), 1,2-propanediol (propyleneglycol), 1,3-propanediol, 1,3-butanediol), and polyethyleneglycol (*e.g.*, PEG400), or mixtures thereof. Any suitable sugar can be used, including, but not limited to mono-, di-, or polysaccharides; and water-soluble glucans, such as, for example, fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch and carboxymethylcellulose-Na. In one embodiment, the sugar additive is sucrose. Sugar alcohol is defined as a C4-C8 hydrocarbon having at least one --OH group and includes, for example, mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. In one embodiment the sugar alcohol additive is mannitol. The sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used, as long as the sugar or sugar alcohol is soluble in the liquid preparation and does not adversely effect the stabilizing effects achieved using the methods of the invention. In one embodiment, the sugar or sugar alcohol concentration is between about 1 mg/ml and about 150 mg/ml. In another embodiment, the isotonic agent is present in a concentration from 1 mg/ml to 50 mg/ml. In another embodiment, the isotonic agent is present in a concentration from 1 mg/ml to 7 mg/ml. In another embodiment, the isotonic agent is present in a concentration from 8 mg/ml to 24 mg/ml. In another embodiment, the isotonic agent is present in a concentration from 25 mg/ml to 50 mg/ml. Each one the specific isotonic agents listed above constitutes an alterna-

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tive embodiment of the invention. While the use of an isotonic agent in pharmaceutical compositions is well-known to the skilled person; for convenience, reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

In a further embodiment, the formulation also comprises a chelating agent. In one embodiment, the chelating agent is selected from salts of ethylenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. In another embodiment, the chelating agent is present in a concentration from 0.1mg/ml to 5mg/ml. In another embodiment, the chelating agent is present in a concentration from 0.1 mg/ml to 2 mg/ml. In a further embodiment of the invention the chelating agent is present in a concentration from 2 mg/ml to 5 mg/ml. Each one of these specific chelating agents constitutes an alternative embodiment of the invention. The use of a chelating agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

In a further embodiment, the formulation also comprises a stabilizer. While the use of a stabilizer in pharmaceutical compositions is well-known to the skilled person; for convenience, reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

Compositions of the invention may, for example, be stabilized liquid pharmaceutical compositions whose therapeutically active components include a polypeptide that possibly exhibits aggregate formation during storage in liquid pharmaceutical formulations. By "aggregate formation" is intended a physical interaction between the polypeptide molecules that results in formation of oligomers, which may remain soluble, or large visible aggregates that precipitate from the solution. By "during storage" is intended a liquid pharmaceutical composition or formulation once prepared, is not immediately administered to a subject. Rather, following preparation, it is packaged for storage, either in a liquid form, in a frozen state, or in a dried form for later reconstitution into a liquid form or other form suitable for administration to a subject. By "dried form" is intended the liquid pharmaceutical composition or formulation is dried either by freeze drying (*i.e.*, lyophilization; see, for example, Williams and Polli (1984) J. Parenteral Sci. Technol. 38:48-59), spray drying (see Masters (1991) in Spray-Drying Handbook (5th ed; Longman Scientific and Technical, Essez, U.K.), pp. 491-676; Broadhead et al. (1992) Drug Devel. Ind. Pharm. 18:1169-1206; and Mumenthaler et al. (1994) Pharm. Res. 11:12-20), or air drying (Carpenter and Crowe (1988) Cryobiology 25:459-470; and Roser (1991) Biopharm. 4:47-53). Aggregate formation by a polypeptide during storage of a liquid pharmaceutical composition can adversely affect biological activity of that polypeptide, resulting in loss of therapeutic efficacy of the pharmaceutical composition. Furthermore, aggregate

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formation may cause other problems such as blockage of tubing, membranes, or pumps when the polypeptide-containing pharmaceutical composition is administered using an infusion system.

The pharmaceutical compositions of the invention may also comprise an amount of an amino acid base sufficient to decrease aggregate formation by the polypeptide during storage of the composition. By "amino acid base" is intended an amino acid or a combination of amino acids, where any given amino acid is present either in its free base form or in its salt form. Where a combination of amino acids is used, all of the amino acids may be present in their free base forms, all may be present in their salt forms, or some may be present in their free base forms while others are present in their salt forms. In one embodiment, amino acids to use in preparing the compositions of the invention are those carrying a charged side chain, such as arginine, lysine, aspartic acid, and glutamic acid. Any stereoisomer (*i.e.*, L, D, or a mixture thereof) of a particular amino acid (*e.g.* methionine, histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine and mixtures thereof) or combinations of these stereoisomers, may be present in the pharmaceutical compositions of the invention so long as the particular amino acid is present either in its free base form or its salt form. In one embodiment the L-stereoisomer is used. Compositions of the invention may also be formulated with analogues of these amino acids. By "amino acid analogue" is intended a derivative of the naturally occurring amino acid that brings about the desired effect of decreasing aggregate formation by the polypeptide during storage of the liquid pharmaceutical compositions of the invention. Suitable arginine analogues include, for example, aminoguanidine, ornithine and N-monoethyl L-arginine, suitable methionine analogues include ethionine and buthionine and suitable cysteine analogues include S-methyl-L cysteine. As with the other amino acids, the amino acid analogues are incorporated into the compositions in either their free base form or their salt form. In a further embodiment of the invention the amino acids or amino acid analogues are used in a concentration, which is sufficient to prevent or delay aggregation of the protein.

In a further embodiment, methionine (or other sulphuric amino acids or amino acid analogous) may be added to inhibit oxidation of methionine residues to methionine sulfoxide when the polypeptide acting as the therapeutic agent is a polypeptide comprising at least one methionine residue susceptible to such oxidation. By "inhibit" is intended minimal accumulation of methionine oxidized species over time. Inhibiting methionine oxidation results in greater retention of the polypeptide in its proper molecular form. Any stereoisomer of methionine (L or D) or combinations thereof can be used. The amount to be added should be an amount sufficient to inhibit oxidation of the methionine residues such that the amount of me-

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thionine sulfoxide is acceptable to regulatory agencies. Typically, this means that the composition contains no more than about 10% to about 30% methionine sulfoxide. Generally, this can be achieved by adding methionine such that the ratio of methionine added to methionine residues ranges from about 1:1 to about 1000:1, such as 10:1 to about 100:1.

In a further embodiment, the formulation also comprises a stabilizer selected from the group of high molecular weight polymers or low molecular compounds. In one embodiment, the stabilizer is selected from polyethylene glycol (*e.g.* PEG 3350), polyvinyl alcohol (PVA), polyvinylpyrrolidone, carboxy-/hydroxycellulose or derivates thereof (*e.g.*, HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, sulphur-containing substances as monothioglycerol, thioglycolic acid and 2-methylthioethanol, and different salts (*e.g.* sodium chloride). Each one of these specific stabilizers constitutes an alternative embodiment of the invention.

The pharmaceutical compositions may also comprise additional stabilizing agents, which further enhance stability of a therapeutically active polypeptide therein. Stabilizing agents of particular interest to the present invention include, but are not limited to, methionine and EDTA, which protect the polypeptide against methionine oxidation, and a nonionic surfactant, which protects the polypeptide against aggregation associated with freeze-thawing or mechanical shearing.

In a further embodiment, the formulation also comprises a surfactant. In one embodiment, the surfactant is selected from a detergent, ethoxylated castor oil, polyglycolyzed glycerides, acetylated monoglycerides, sorbitan fatty acid esters, polyoxypropylene-polyoxyethylene block polymers (eg. poloxamers such as Pluronic® F68, poloxamer 188 and 407, Triton X-100), polyoxyethylene sorbitan fatty acid esters, polyoxyethylene and polyethylene derivatives such as alkylated and alkoxylated derivatives (tweens, *e.g.* Tween-20, Tween-40, Tween-80 and Brij-35), monoglycerides or ethoxylated derivatives thereof, diglycerides or polyoxyethylene derivatives thereof, alcohols, glycerol, lectins and phospholipids (eg. phosphatidyl serine, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, diphosphatidyl glycerol and sphingomyelin), derivates of phospholipids (eg. dipalmitoyl phosphatidic acid) and lysophospholipids (eg. palmitoyl lysophosphatidyl-L-serine and 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine) and alkyl, alkoxyl (alkyl ester), alkoxy (alkyl ether)- derivatives of lysophosphatidyl and phosphatidylcholines, *e.g.* lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, that is cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, and the positively charged DODAC, DOTMA, DCP, BISHOP, lysophosphatidylserine and lysophosphatidylthreonine, and glycerophospholipids (eg. cephalins), glyceroglycolipids (eg. galactopyransoide), sphingoglycol-

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ipids (eg. ceramides, gangliosides), dodecylphosphocholine, hen egg lysolecithin, fusidic acid derivatives- (*e.g.* sodium tauro-dihydrofusidate etc.), long-chain fatty acids and salts thereof C6-C12 (eg. oleic acid and caprylic acid), acylcarnitines and derivatives, N^{α}-acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, N^{α}-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, N^{α}-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, DSS (docusate sodium, CAS registry no [577-11-7]), docusate calcium, CAS registry no [128-49-4]), docusate potassium, CAS registry no [7491-09-0]), SDS (sodium dodecyl sulphate or sodium lauryl sulphate), sodium caprylate, cholic acid or derivatives thereof, bile acids and salts thereof and glycine or taurine conjugates, ursodeoxycholic acid, sodium cholate, sodium deoxycholate, sodium taurocholate, sodium glycocholate, N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulphonates) monovalent surfactants, zwitterionic surfactants (*e.g.* N-alkyl-N,N-dimethylammonio-1-propanesulfonates, 3-cholamido-1-propyldimethylammonio-1-propanesulfonate, cationic surfactants (quaternary ammonium bases) (*e.g.* cetyl-trimethylammonium bromide, cetylpyridinium chloride), non-ionic surfactants (*e.g.* dodecyl β-D-glucopyranoside), poloxamines (*e.g.*, Tetronic's), which are tetrafunctional block copolymers derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, or the surfactant may be selected from the group of imidazoline derivatives, or mixtures thereof. Each one of these specific surfactants constitutes an alternative embodiment of the invention.

While the use of a surfactant in pharmaceutical compositions is well-known to the skilled person; for convenience, reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

In a further embodiment, the formulation also comprises protease inhibitors such as EDTA (ethylenediamine tetraacetic acid) and benzamidineHCl, although other commercially available and suitable protease inhibitors may also be used. The use of a protease inhibitor is particular useful in pharmaceutical compositions comprising zymogens of proteases in order to inhibit autocatalysis.

Other ingredients may also be present in the pharmaceutical formulation of the present invention. Such additional ingredients may include wetting agents, emulsifiers, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (*e.g.*, human serum albumin, gelatine or proteins) and a zwitterion (*e.g.*, an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Such additional ingredi-

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ents preferably do not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

Pharmaceutical compositions containing an antibody according to the present invention may be administered to a patient in need of such treatment at several sites, for example, at topical sites, for example, skin and mucosal sites, at sites which bypass absorption, for example, administration in an artery, in a vein, in the heart, and at sites which involve absorption, for example, administration in the skin, under the skin, in a muscle or in the abdomen.

Administration of pharmaceutical compositions according to the invention may be through several routes of administration, for example, lingual, sublingual, buccal, in the mouth, oral, in the stomach and intestine, nasal, pulmonary, for example, through the bronchioles and alveoli or a combination thereof, epidermal, dermal, transdermal, vaginal, rectal, ocular, for examples through the conjunctiva, uretal, and parenteral to patients in need of such a treatment.

Compositions of the current invention may be administered in several dosage forms, for example, as solutions, suspensions, emulsions, microemulsions, multiple emulsion, foams, salves, pastes, plasters, ointments, tablets, coated tablets, rinses, capsules, for example, hard gelatine capsules and soft gelatine capsules, suppositories, rectal capsules, drops, gels, sprays, powder, aerosols, inhalants, eye drops, ophthalmic ointments, ophthalmic rinses, vaginal pessaries, vaginal rings, vaginal ointments, injection solution, in situ transforming solutions, for example in situ gelling, in situ setting, in situ precipitating, in situ crystallization, infusion solution, and implants.

Compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the antibody, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polylactic and polyglycolic acid and block copolymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticulates, liquid crystals and dispersions thereof, L2 phase and dispersions there of, well known to those skilled in the art

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of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

Compositions of the current invention are useful in the formulation of solids, semisolids, powder and solutions for pulmonary administration of the antibody, using, for example a metered dose inhaler, dry powder inhaler and a nebulizer, all being devices well known to those skilled in the art.

Compositions of the current invention are specifically useful in the formulation of controlled, sustained, protracting, retarded, and slow release drug delivery systems. More specifically, but not limited to, compositions are useful in formulation of parenteral controlled release and sustained release systems (both systems leading to a many-fold reduction in number of administrations), well known to those skilled in the art. Even more preferably, are controlled release and sustained release systems administered subcutaneous. Without limiting the scope of the invention, examples of useful controlled release system and compositions are hydrogels, oleaginous gels, liquid crystals, polymeric micelles, microspheres, nanoparticles,

Methods to produce controlled release systems useful for compositions of the current invention include, but are not limited to, crystallization, condensation, co-crystallization, precipitation, co-precipitation, emulsification, dispersion, high pressure homogenisation, encapsulation, spray drying, microencapsulating, coacervation, phase separation, solvent evaporation to produce microspheres, extrusion and supercritical fluid processes. General reference is made to Handbook of Pharmaceutical Controlled Release (Wise, D.L., ed. Marcel Dekker, New York, 2000) and Drug and the Pharmaceutical Sciences vol. 99: Protein Formulation and Delivery (MacNally, E.J., ed. Marcel Dekker, New York, 2000).

Parenteral administration may be performed by subcutaneous, intramuscular, intraperitoneal or intravenous injection by means of a syringe, optionally a pen-like syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a solution or suspension for the administration of the antibody in the form of a nasal or pulmonal spray. As another option, the pharmaceutical compositions containing the antibody of the invention can also be adapted to transdermal administration, *e.g.* by needle-free injection or from a patch, optionally an iontophoretic patch, or transmucosal, *e.g.* buccal, administration.

The antibody can be administered via the pulmonary route in a vehicle, as a solution, suspension or dry powder using any of known types of devices suitable for pulmonary drug delivery. Examples of these comprise of, but are not limited to, the three general types of aerosol-generating for pulmonary drug delivery, and may include jet or ultrasonic nebuliz-

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ers, metered-dose inhalers, or dry powder inhalers (Cf. Yu J, Chien YW. Pulmonary drug delivery: Physiologic and mechanistic aspects. Crit Rev Ther Drug Carr Sys 14(4) (1997) 395-453).

Based on standardised testing methodology, the aerodynamic diameter (d_a) of a particle is defined as the geometric equivalent diameter of a reference standard spherical particle of unit density (1 g/cm3). In the simplest case, for spherical particles, d_a is related to a reference diameter (d) as a function of the square root of the density ratio as described by:

$$d_a = \sqrt{\frac{\rho}{\rho_a}} d$$

Modifications to this relationship occur for non-spherical particles (cf. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). The terms "MMAD" and "MMEAD" are well-described and known to the art (cf. Edwards DA, Ben-Jebria A, Langer R and represents a measure of the median value of an aerodynamic particle size distribution. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). Mass median aerodynamic diameter (MMAD) and mass median effective aerodynamic diameter (MMEAD) are used inter-changeably, are statistical parameters, and empirically describe the size of aerosol particles in relation to their potential to deposit in the lungs, independent of actual shape, size, or density (cf. Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385). MMAD is normally calculated from the measurement made with impactors, an instrument that measures the particle inertial behaviour in air.

In a further embodiment, the formulation could be aerosolized by any known aerosolisation technology, such as nebulisation, to achieve a MMAD of aerosol particles less than 10 µm, more preferably between 1-5 µm, and most preferably between 1-3 µm. The preferred particle size is based on the most effective size for delivery of drug to the deep lung, where protein is optimally absorbed (cf. Edwards DA, Ben-Jebria A, Langer A, Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 84(2) (1998) 379-385).

Deep lung deposition of the pulmonal formulations comprising the antibody may optional be further optimized by using modifications of the inhalation techniques, for example, but not limited to: slow inhalation flow (eg. 30 L/min), breath holding and timing of actuation.

The term "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability.

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The term "physical stability" of the protein formulation as used herein refers to the tendency of the protein to form biologically inactive and/or insoluble aggregates of the protein as a result of exposure of the protein to thermo-mechanical stresses and/or interaction with interfaces and surfaces that are destabilizing, such as hydrophobic surfaces and interfaces. Physical stability of the aqueous protein formulations is evaluated by means of visual inspection and/or turbidity measurements after exposing the formulation filled in suitable containers (*e.g.* cartridges or vials) to mechanical/physical stress (*e.g.* agitation) at different temperatures for various time periods. Visual inspection of the formulations is performed in a sharp focused light with a dark background. The turbidity of the formulation is characterized by a visual score ranking the degree of turbidity for instance on a scale from 0 to 3 (a formulation showing no turbidity corresponds to a visual score 0, and a formulation showing visual turbidity in daylight corresponds to visual score 3). A formulation is classified physical unstable with respect to protein aggregation, when it shows visual turbidity in daylight. Alternatively, the turbidity of the formulation can be evaluated by simple turbidity measurements well-known to the skilled person. Physical stability of the aqueous protein formulations can also be evaluated by using a spectroscopic agent or probe of the conformational status of the protein. The probe is preferably a small molecule that preferentially binds to a non-native conformer of the protein. One example of a small molecular spectroscopic probe of protein structure is Thioflavin T. Thioflavin T is a fluorescent dye that has been widely used for the detection of amyloid fibrils. In the presence of fibrils, and perhaps other protein configurations as well, Thioflavin T gives rise to a new excitation maximum at about 450 nm and enhanced emission at about 482 nm when bound to a fibril protein form. Unbound Thioflavin T is essentially non-fluorescent at the wavelengths.

Other small molecules can be used as probes of the changes in protein structure from native to non-native states. For instance the "hydrophobic patch" probes that bind preferentially to exposed hydrophobic patches of a protein. The hydrophobic patches are generally buried within the tertiary structure of a protein in its native state, but become exposed as a protein begins to unfold or denature. Examples of these small molecular, spectroscopic probes are aromatic, hydrophobic dyes, such as antrhacene, acridine, phenanthroline or the like. Other spectroscopic probes are metal-amino acid complexes, such as cobalt metal complexes of hydrophobic amino acids, such as phenylalanine, leucine, isoleucine, methionine, and valine, or the like.

The term "chemical stability" of the protein formulation as used herein refers to chemical covalent changes in the protein structure leading to formation of chemical degradation products with potential less biological potency and/or potential increased immunogenic

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properties compared to the native protein structure. Various chemical degradation products can be formed depending on the type and nature of the native protein and the environment to which the protein is exposed. Elimination of chemical degradation can most probably not be completely avoided and increasing amounts of chemical degradation products is often seen during storage and use of the protein formulation as well-known by the person skilled in the art. Most proteins are prone to deamidation, a process in which the side chain amide group in glutaminyl or asparaginyl residues is hydrolysed to form a free carboxylic acid. Other degradations pathways involves formation of high molecular weight transformation products where two or more protein molecules are covalently bound to each other through transamidation and/or disulfide interactions leading to formation of covalently bound dimer, oligomer and polymer degradation products (Stability of Protein Pharmaceuticals, Ahern. T.J. & Manning M.C., Plenum Press, New York 1992). Oxidation (of for instance methionine residues) can be mentioned as another variant of chemical degradation. The chemical stability of the protein formulation can be evaluated by measuring the amount of the chemical degradation products at various time-points after exposure to different environmental conditions (the formation of degradation products can often be accelerated by for instance increasing temperature). The amount of each individual degradation product is often determined by separation of the degradation products depending on molecule size and/or charge using various chromatography techniques (*e.g.* SEC-HPLC and/or RP-HPLC).

Hence, as outlined above, a "stabilized formulation" refers to a formulation with increased physical stability, increased chemical stability or increased physical and chemical stability. In general, a formulation must be stable during use and storage (in compliance with recommended use and storage conditions) until the expiration date is reached.

In one embodiment of the invention the pharmaceutical formulation comprising the antibody is stable for more than 6 weeks of usage and for more than 3 years of storage.

In another embodiment of the invention the pharmaceutical formulation comprising the antibody is stable for more than 4 weeks of usage and for more than 3 years of storage.

In a further embodiment of the invention the pharmaceutical formulation comprising the antibody is stable for more than 4 weeks of usage and for more than two years of storage.

In an even further embodiment of the invention the pharmaceutical formulation comprising the antibody is stable for more than 2 weeks of usage and for more than two years of storage.

Thus, as described above, pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers; alumina; aluminium

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stearate; lecithin; serum proteins such as human serum albumin; buffer substances such as phosphates and glycine; sorbic acid; potassium sorbate; partial glyceride mixtures of saturated vegetable fatty acids; water; salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, and zinc salts; colloidal silica; magnesium trisilicate; polyvinyl pyrrolidone; cellulose-based substances; polyethylene glycol; sodium carboxymethylcellulose; polyacrylates; waxes; polyethylene-polyoxypropylene-block polymers; polyethylene glycol; and wool fat.

The compositions of this invention may be employed in a method of potentiating the activity of NK cells in a patient or a biological sample. This method comprises the step of contacting said composition with said patient or biological sample. Such method will be useful for both diagnostic and therapeutic purposes.

For use in conjunction with a biological sample, the antibody composition can be administered by simply mixing with or applying directly to the sample, depending upon the nature of the sample (fluid or solid). The biological sample may be contacted directly with the antibody in any suitable device (plate, pouch, flask, etc.). For use in conjunction with a patient, the composition must be formulated for administration to the patient.

As described above, the compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously.

Sterile injectable forms of the compositions of this invention may be aqueous or an oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly

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used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the compositions may be formulated in an ointment such as petrolatum.

The compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

Several monoclonal antibodies have been shown to be efficient in clinical situations, such as Rituxan (Rituximab), Herceptin (Trastuzumab) or Xolair (Omalizumab), and similar administration regimens (*i.e.*, formulations and/or doses and/or administration protocols) may be used with the antibodies of this invention. Schedules and dosages for administration of the antibody in the pharmaceutical compositions of the present invention can be determined in accordance with known methods for these products, for example using the manufacturers' instructions. For example, an antibody present in a pharmaceutical composition of this invention can be supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for IV administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5. An exemplary suitable dosage range for an antibody in a pharmaceutical composition of this invention may between about 10 mg/m2 and 500 mg/m2. However, it will be appreciated that these schedules are exemplary and that an optimal schedule and regimen can be adapted taking into account the affinity and tolerability of the particular antibody in the pharmaceutical composition that must be determined in clinical trials. Quantities and schedule of injection of an antibody in a pharmaceutical composition of this invention that saturate NK cells for 24 hours, 48 hours 72 hours or a week or a month will be determined considering the affinity of the antibody and its pharmacokinetic parameters in humans and non-human mammals.

According to another embodiment, the antibody compositions of this invention may further comprise another therapeutic agent, including agents normally utilized for the particular therapeutic purpose for which the antibody is being administered. The additional therapeutic agent will normally be present in the composition in amounts typically used for that agent in a monotherapy for the particular disease or condition being treated. Such therapeutic agents include, but are not limited to, therapeutic agents used in the treatment of cancers,

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therapeutic agents used to treat infectious disease, therapeutic agents used in other immunotherapies, cytokines (such as IL-2 or IL-15), other antibodies and fragments of other antibodies.

In an alternate embodiment, an antibody that binds a common determinant present on at least two different human inhibitory KIR receptor gene products, wherein said antibody is capable of neutralizing KIR-mediated inhibition of NK cell cytotoxicity on NK cells expressing at least one of said two different human inhibitory KIR receptors of this invention, may be incorporated into liposomes ("immunoliposomes"), alone or together with another substance for targeted delivery to a tumor, or the site of an infection, in a human or other non-human mammal. Such other substances include nucleic acids for the delivery of genes for gene therapy or for the delivery of antisense RNA, RNAi or siRNA for suppressing a gene in an NK cell, or toxins or drugs for the targeted killing of NK cells.

For example, a number of therapeutic agents are available for the treatment of cancers. The antibody compositions and methods of the present invention may be combined with any other methods generally employed in the treatment of the particular disease, particularly a tumor, cancer disease, or other disease or disorder that the patient exhibits. So long as a particular therapeutic approach is not known to be detrimental to the patient's condition in itself, and does not significantly counteract the activity of the antibody in a pharmaceutical composition of this invention, its combination with the present invention is contemplated.

In connection with solid tumor treatment, the pharmaceutical compositions of the present invention may be used in combination with classical approaches, such as surgery, radiotherapy, chemotherapy, and the like. The invention therefore provides combined therapies in which a pharmaceutical composition of this invention is used simultaneously with, before, or after surgery or radiation treatment; or is administered to patients with, before, or after conventional chemotherapeutic, radiotherapeutic or anti-angiogenic agents, or targeted immunotoxins or coaguligands.

When one or more agents are used in combination with an antibody-containing composition of this invention in a therapeutic regimen, there is no requirement for the combined results to be additive of the effects observed when each treatment is conducted separately. Although at least additive effects are generally desirable, any increased anti-cancer effect above one of the single therapies would be of benefit. Also, there is no particular requirement for the combined treatment to exhibit synergistic effects, although this is possible and advantageous.

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To practice combined anti-cancer therapy, one would simply administer to a patient an antibody composition of this invention in combination with another anti-cancer agent in a manner effective to result in their combined anti-cancer actions within the animal. The agents would therefore be provided in amounts effective and for periods of time effective to result in their combined presence within the tumor vasculature and their combined actions in the tumor environment. To achieve this goal, an antibody composition of this invention and anti-cancer agents may be administered to the patient simultaneously, either in a single combined composition, or as two distinct compositions using the same or different administration routes.

Alternatively, the administration of an antibody composition of this invention may precede, or follow, the anti-cancer agent treatment by, *e.g.*, intervals ranging from minutes to weeks and months. One would ensure that the anti-cancer agent and an antibody in the antibody composition of this invention exert an advantageously combined effect on the cancer. As an example, mAbs of the present invention may be administered to patients with Non-Hodgkin's Lymphoma (NHL). Such patients are typically treated with a combination of Rituximab and a combination of chemotherapy agents known as CHOP. Accordingly, anti-KIR antibodies of this invention may be used to treat NHL patients who are undergoing treatment with Rituximab and CHOP, by combining the administration of all the agents in a treatment schedule where the agents are given on the same day, or on different days, with a longer treatment-period.

Other anti-cancer agents may be given prior to, at the same time as, or following, administration of an anti-KIR antibody composition of this invention. However, when immunoconjugates of an antibody are used in the antibody composition of this invention, various anti-cancer agents may be simultaneously or subsequently administered.

In some situations, it may even be desirable to extend the time period for treatment significantly, where several days (2, 3, 4, 5, 6 or 7), several weeks (1, 2, 3, 4, 5, 6, 7 or 8) or even several months (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administration of the anti-cancer agent or anti-cancer treatment and the administration of an antibody composition of this invention. This might be advantageous in circumstances where the anti-cancer treatment was intended to substantially destroy the tumor, such as surgery or chemotherapy, and administration of an antibody composition of this invention was intended to prevent micrometastasis or tumor re-growth.

It also is envisioned that more than one administration of either an anti-KIR antibody-based composition of this invention or the anti-cancer agent will be utilized. These agents may be administered interchangeably, on alternate days or weeks; or a cycle of

treatment with an anti-KIR antibody composition of this invention, followed by a cycle of anti-cancer agent therapy. In any event, to achieve tumor regression using a combined therapy, all that is required is to deliver both agents in a combined amount effective to exert an anti-tumor effect, irrespective of the times for administration.

In terms of surgery, any surgical intervention may be practiced in combination with the present invention. In connection with radiotherapy, any mechanism for inducing DNA damage locally within cancer cells is contemplated, such as gamma-irradiation, X-rays, UV-irradiation, microwaves and even electronic emissions and the like. The directed delivery of radioisotopes to cancer cells is also contemplated, and this may be used in connection with a targeting antibody or other targeting means.

In other aspects, immunomodulatory compounds or regimens may be administered in combination with or as part of the antibody compositions of the present invention. Preferred examples of immunomodulatory compounds include cytokines. Various cytokines may be employed in such combined approaches. Examples of cytokines useful in the combinations contemplated by this invention include IL-1alpha IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-21, TGF-beta, GM-CSF, M-CSF, G-CSF, TNF-alpha, TNF-beta, LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-alpha, IFN-beta, IFN-gamma. Cytokines used in the combination treatment or compositions of this invention are administered according to standard regimens, consistent with clinical indications such as the condition of the patient and relative toxicity of the cytokine. Other immunomodulatory compounds that may be administered in combination with, or as part of, the antibody compositions of the present invention include antibodies that bind specifically to other inhibitory receptors on lymphocytes, including without limitation antibodies such as anti-CTLA4 antibodies, or anti-CD94/NKG2A antibodies (see, for example, U.S. published patent application 20030095965). Variants and derivatives of these molecules that are known in the art also or alternatively can be used in such methods, and incorporated into compositions of the invention, as appropriate.

In certain embodiments, the cross-reacting, blocking, and/or inhibitory anti-KIR antibody-comprising therapeutic compositions of the present invention may be administered in combination with or may further comprise a chemotherapeutic or hormonal therapy agent. A variety of hormonal therapy and chemotherapeutic agents may be used in the combined treatment methods disclosed herein. Chemotherapeutic agents contemplated as exemplary include, but are not limited to, alkylating agents, antimetabolites, cytotoxic antibiotics, vinca alkaloids, for example adriamycin, dactinomycin, mitomycin, carminomycin, daunomycin, doxorubicin, tamoxifen, taxol, taxotere, vincristine, vinblastine, vinorelbine, etoposide (VP-

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16), 5-fluorouracil (5FU), cytosine arabinoside, cyclophosphamide, thiotepa, methotrexate, camptothecin, actinomycin-D, mitomycin C, cisplatin (CDDP), aminopterin, combretastatin(s) and derivatives and prodrugs thereof.

Hormonal agents include, but are not limited to, for example LHRH agonists such as leuprorelin, goserelin, triptorelin, and buserelin; anti-estrogens such as tamoxifen and toremifene; anti-androgens such as flutamide, nilutamide, cyproterone and bicalutamide; aromatase inhibitors such as anastrozole, exemestane, letrozole and fadrozole; and progestagens such as medroxy, chlormadinone and megestrol.

As will be understood by those of ordinary skill in the art, the appropriate doses of chemotherapeutic agents will approximate those already employed in clinical therapies wherein the chemotherapeutics are administered alone or in combination with other chemotherapeutics. By way of example only, agents such as cisplatin, and other DNA alkylating may be used. Cisplatin has been widely used to treat cancer, with efficacious doses used in clinical applications of 20 mg/m2 for 5 days every three weeks for a total of three courses. Cisplatin is not absorbed orally and must therefore be delivered via injection intravenously, subcutaneously, intratumorally or intraperitoneally.

Further useful chemotherapeutic agents include compounds that interfere with DNA replication, mitosis and chromosomal segregation, and agents that disrupt the synthesis and fidelity of polynucleotide precursors. A number of exemplary chemotherapeutic agents for combined therapy are listed in Table C of U.S. Patent No. 6,524,583, the disclosure of which agents and indications are specifically incorporated herein by reference. Each of the agents listed are exemplary and not limiting. The skilled artisan is directed to "Remington's Pharmaceutical Sciences" 15th Edition, chapter 33, in particular pages 624-652. Variation in dosage will likely occur depending on the condition being treated. The physician administering treatment will be able to determine the appropriate dose for the individual subject.

The present cross-reacting, blocking, and/or inhibitory anti-KIR antibody compositions of this invention may be used in combination with any one or more anti-angiogenic therapies or may further comprise anti-angiogenic agents. Examples of such agents include neutralizing antibodies, antisense RNA, siRNA, RNAi, RNA aptamers and ribozymes each directed against VEGF or VEGF receptors (U.S. Patent No. 6,524,583, the disclosure of which is incorporated herein by reference). Variants of VEGF with antagonistic properties may also be employed, as described in WO 98/16551, specifically incorporated herein by reference. Further exemplary anti-angiogenic agents that are useful in connection with combined therapy are listed in Table D of U.S. Patent No. 6,524,583, the disclosure of which agents and indications are specifically incorporated herein by reference.

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The anti-KIR antibody compositions of this invention may also be advantageously used in combination with methods to induce apoptosis or may comprise apoptotic agents. For example, a number of oncogenes have been identified that inhibit apoptosis, or programmed cell death. Exemplary oncogenes in this category include, but are not limited to, bcr-abl, bcl-2 (distinct from bcl-1, cyclin D1; GenBank accession numbers M14745, X06487; U.S. Pat. Nos. 5,650,491; and 5,539,094; each incorporated herein by reference) and family members including Bcl-x1, Mcl-1, Bak, A1, and A20. Overexpression of bcl-2 was first discovered in T cell lymphomas. The oncogene bcl-2 functions by binding and inactivating Bax, a protein in the apoptotic pathway. Inhibition of bcl-2 function prevents inactivation of Bax, and allows the apoptotic pathway to proceed. Inhibition of this class of oncogenes, *e.g.*, using antisense nucleotide sequences, RNAi, siRNA or small molecule chemical compounds, is contemplated for use in the present invention to give enhancement of apoptosis (U.S. Pat. Nos. 5,650,491; 5,539,094; and 5,583,034; each incorporated herein by reference).

The anti-KIR antibody compositions of this invention may also comprise or be used in combination with molecules that comprise a targeting portion, *e.g.*, antibody, ligand, or conjugate thereof, directed to a specific marker on a target cell ("targeting agent"), for example a target tumor cell. Generally speaking, targeting agents for use in these additional aspects of the invention will preferably recognize accessible tumor antigens that are preferentially, or specifically, expressed in the tumor site. The targeting agents will generally bind to a surface-expressed, surface-accessible or surface-localized component of a tumor cell. The targeting agents will also preferably exhibit properties of high affinity; and will not exert significant *in vivo* side effects against life-sustaining normal tissues, such as one or more tissues selected from heart, kidney, brain, liver, bone marrow, colon, breast, prostate, thyroid, gall bladder, lung, adrenals, muscle, nerve fibers, pancreas, skin, or other life-sustaining organ or tissue in the human body. The term "not exert significant side effects," as used herein, refers to the fact that a targeting agent, when administered *in vivo*, will produce only negligible or clinically manageable side effects, such as those normally encountered during chemotherapy.

In the treatment of tumors, an antibody composition of this invention may additionally comprise or may be used in combination with adjunct compounds. Adjunct compounds may include by way of example anti-emetics such as serotonin antagonists and therapies such as phenothiazines, substituted benzamides, antihistamines, butyrophenones, corticosteroids, benzodiazepines and cannabinoids; bisphosphonates such as zoledronic acid and pamidronic acid; and hematopoietic growth factors such as erythropoietin and G-CSF, for example filgrastim, lenograstim and darbepoietin.

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In another embodiment, two or more antibodies of this invention recognizing different epitopes or determinants, including NKVSF1, DF200, 1-4F1 and/or 1-7F9, may be combined in a single composition so as to reduce or neutralize the inhibitory effects of as many KIR gene products as possible. Compositions comprising combinations of cross-reactive inhibitory KIR antibodies of this invention, or fragments or derivatives thereof, will allow even wider utility because there likely exists a small percentage of the human population that may lack each of the inhibitory KIR gene products recognized by a single cross-reacting antibody. Similarly, an antibody composition of this invention may further comprise one or more antibodies that recognize single inhibitory KIR subtypes. Such combinations would again provide wider utility in a therapeutic setting. Accordingly, an antibody of this invention can be combined with another anti-KIR antibody binding to one or more of, *e.g.*, KIR2DL1, KIR2DLK2, KIR2DL3, KIR3DL1, KIR3DL2, and KIR3DL3.

The invention also provides a method of potentiating NK cell activity in a patient in need thereof, comprising the step of administering a composition according to this invention to said patient. The method is more specifically directed at increasing NK cell activity in patients having a disease in which increased NK cell activity is beneficial, which involves, affects or is caused by cells susceptible to lysis by NK cells, or which is caused or characterized by insufficient NK cell activity, such as a cancer, another proliferative disorder, an infectious disease or an immune disorder. More specifically, the methods of the present invention are utilized for the treatment of a variety of cancers and other proliferative diseases including, but not limited to, carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and Burketts lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, teratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma.

Other preferred disorders that can be treated according to the invention include hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but

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not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; Sezary syndrome (SS); Adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL).

Other proliferative disorders can also be treated according to the invention, including for example hyperplasias, fibrosis (especially pulmonary, but also other types of fibrosis, such as renal fibrosis), angiogenesis, psoriasis, atherosclerosis and smooth muscle proliferation in the blood vessels, such as stenosis or restenosis following angioplasty. The cross-reacting inhibitory KIR antibody of this invention can be used to treat or prevent infectious diseases, including preferably any infections caused by viruses, bacteria, protozoa, molds or fungi. Such viral infectious organisms include, but are not limited to, hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-1), herpes simplex type 2 (HSV-2), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papilloma virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, Ebola-virus, and human immunodeficiency virus type I or type 2 (HIV-1, HIV-2).

Bacterial infections that can be treated according to this invention include, but are not limited to, infections caused by the following: *Staphylococcus; Streptococcus,* including *S. pyogenes; Enterococcl; Bacillus,* including *Bacillus anthracis,* and *Lactobacillus; Listeria; Corynebacterium diphtheriae; Gardnerella* including *G. vaginalis; Nocardia; Streptomyces; Thermoactinomyces vulgaris; Treponerna; Camplyobacter, Pseudomonas* including *Raeruginosa; Legionella; Neisseria including N.gonorrhoeae* and *N.meningitides; Flavobacterium* including *F. meningosepticum and F. odoraturn; Brucella; Bordetella* including *B. pertussis and B. bronchiseptica; Escherichia* including *E. coli, Klebsiella; Enterobacter, Serratia* including *S. marcescens* and *S. liquefaciens; Edwardsiella; Proteus* including *P. mirabilis* and *P. vulgaris; Streptobacillus; Rickettsiaceae* including *R. fickettsfi, Chlamydia* including *C. psittaci* and *C. trachornatis; Mycobacterium* including *M. tuberculosis, M. intracellulare, M. folluiturn, M. laprae, M. avium, M. bovis, M. africanum, M. kansasii, M. intracellulare,* and *M. lepraernurium;* and *Nocardia.*

Protozoa infections that may be treated according to this invention include, but are not limited to, infections caused by *leishmania*, *kokzidioa*, and *trypanosoma*. A complete list of infectious diseases can be found on the website of the National Center for Infectious Dis-

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ease (NCID) at the Center for Disease Control (CDC) (World Wide Web (www) at cdc.gov/ncidod/diseases/), which list is incorporated herein by reference. All of said diseases are candidates for treatment using the cross-reacting inhibitory KIR antibodies of the invention.

Such methods of treating various infectious diseases may employ the antibody composition of this invention, either alone or in combination with other treatments and/or therapeutic agents known for treating such diseases, including anti-viral agents, anti-fungal agents, antibacterial agents, antibiotics, anti-parasitic agents and anti-protozoal agents. When these methods involve additional treatments with additional therapeutic agents, those agents may be administered together with the antibodies of this invention as either a single dosage form or as separate, multiple dosage forms. When administered as a separate dosage form, the additional agent may be administered prior to, simultaneously with, of following administration of the antibody of this invention.

Further aspects and advantages of this invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of this application.

EXAMPLES

Example 1: Purification of PBLs and generation of polyclonal or clonal NK cell lines

PBLs were derived from healthy donors by Ficoll-Hypaque gradients and depletion of plastic adherent cells. To obtain enriched NK cells, PBLs were incubated with anti-CD3, anti-CD4 and anti-HLA-DR mAbs for 30 minutes at 4°C, followed by incubation with goat anti-mouse magnetic beads (Dynal) (30 minutes at 4°C) and immunomagnetic selection by methods known in the art (Pende et al., J Exp Med 1999;190:1505-1516). $CD3^-$, $CD4^-$, DR^- cells were cultivated on irradiated feeder cells and 100 U/ml Interleukin 2 (Proleukin, Chiron Corporation) and 1.5 ng/ml Phytohemagglutinin A (Gibco BRL) to obtain polyclonal NK cell populations. NK cells were cloned by limiting dilution and clones of NK cells were characterized by flow cytometry for expression of cell surface receptors.

The mAbs used were JT3A (IgG2a, anti-CD3), EB6 and GL183 (IgG1, anti-KIR2DL1 and KIR2DL3, respectively), XA-141 (IgM, anti-KIR2DL1 with the same specificity as EB6), anti-CD4 (HP2.6), and anti-DR (D1.12, IgG2a). Instead of JT3A, HP2.6, and DR1.12, other commercially available mAbs of the same specificities can be used. EB6 and GL183 are commercially available (Beckman Coulter Inc., Fullerton, CA). XA-141 is not commercially available, but mAb EB6 can equally well be used for control reconstitution of lysis, as shown

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in the examples and Figures herein, as described by Moretta et al., J Exp Med 1993;178:597-604.

Cells were stained with the appropriate antibodies (30mns at 4°C) followed by PE- or FITC-conjugated polyclonal anti-mouse antibodies (Southern Biotechnology Associates Inc). Samples were analyzed by cytofluorometric analysis (flow cytometry) on a FACScan apparatus (Becton Dickinson, Mountain View, CA).

The following NK cell clones were used in this study. CP11, CN5 and CN505 are KIR2DL1 positive clones and are stained by EB6 (IgG1, anti-KIR2DL1) or XA-141 (IgM, anti KIR2DL1 with same specificity as compared to EB6 antibodies). CN12 and CP502 are KIR2DL3-expressing NK clones and are stained by GL183 antibody (IgG1, anti-KIR2DL2/3).

The cytolytic activity of NK clones was assessed by a standard 4-hour 51Cr-release assay in which effector NK cells were tested for their ability to kill target cells expressing HLA-Cw3 or -Cw4, HLA positive cell lines known for their sensitivity to NK cell lysis. All the targets were used at 5000 cells per well in microtitration 96-well plates and the effector:target ratio is indicated in the Figures (usually 4 effectors per target cells). The cytolytic assay was performed with or without hybridoma supernatant of the indicated monoclonal antibodies at a ½ (1:1) dilution. The procedure was essentially the same as described in Moretta et al., J Exp Med 1993;178:597-604.

Example 2: Generation of new mAbs

mAbs were generated by immunizing 5 week old Balb/C mice with activated poly-clonal or monoclonal human NK cell lines as described in (Moretta et al., J Exp Med 1990;172:1589-1598). After different cell fusions, the mAbs were first selected for their ability to cross-react with EB6- and GL183-positive NK cell lines and clones. Positive monoclonal antibodies were further screened for their ability to reconstitute lysis by EB6- positive or GL183-positive NK clones of Cw4 or Cw3 positive targets, respectively.

Cell staining was carried out as follows. Cells were stained with a panel of antibodies (1µg/ml or 50µl supernatant, 30mns at 4°C) followed by PE-conjugated goat F(ab')2 fragments anti-mouse IgG (H+L) or PE-conjugated goat F(ab')2 fragment anti-human IgG (Fc gamma) antibodies (Beckman Coulter). Cytofluorometric analysis was performed on an Epics XL.MCL apparatus (Beckman Coulter).

One of the monoclonal antibodies, the DF200 mAb, was found to react with various members of the KIR family including KIR2DL1, and KIR2DL2/3. Both KIR2DL1-positive and KIR2DL2/3-positive NK cells were stained brightly with DF200mAb (Figure 1).

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NK clones expressing one or another (or even both) of these HLA class I-specific inhibitory receptors were used as effectors cells against target cells expressing one or more HLA-C alleles. Cytotoxicity assays were carried out as follows. The cytolytic activity of YTS-KIR2DL1 or YTS-Eco (KIR-negative) cell lines was assessed by a standard 4 hours Cr-51 release assay. The target cells were HLA-Cw4-positive or -negative B-EBV cell lines, or HLA-Cw4-transfected 721.221 cells. All targets were used at 3000 cells per well in microtitration plate. The effector/target ratio is indicated in the figures. The cytolytic assay was performed with or without the indicated full length or F(ab')2 fragments of monoclonal mouse or human antibodies. As expected, KIR2DL1-positive ($KIR2DL1^+$) NK clones displayed little if any cytolytic activity against target cells expressing HLA-Cw4 and KIR2DL3+ NK clones displayed little or no activity on Cw3 positive targets. However, in the presence of DF200mAb (used to mask their KIR2DL receptors) NK clones became unable to recognize their HLA-C ligands and displayed strong cytolytic activity against Cw3- or Cw4-expressing targets (examples of results are shown in Figures 2-6) .

For example, the C1R cell line (CW4+ EBV cell line, ATCC No. CRL-1993), which expresses HLA-Cw4 (but no group 1 HLA-C allotypes) was not killed by KIR2DL1-positive NK clones (CN5/CN505), but the inhibition could be efficiently reversed by the use of either DF200 or a conventional anti KIR2DL1 mAb. On the other hand, NK clones expressing the $KIR2DL2/3^+$ $KIR2DL1^-$ phenotype (CN12) efficiently killed C1R cells and this killing was unaffected by the DF200mAb (Figure 2). Similar results are obtained with KIR2DL2- or KIR2DL3-positive NK clones on Cw3-positive targets.

Similarly, the Cw4+ 221 EBV cell line was not killed by KIR2DL1+transfected NK cells, but the inhibition could be efficiently reversed by the use of the DF200 mAb, a Fab fragment of DF200, or the a conventional anti-KIR2DL1 mAbs EB6 or XA141. Also, a Cw3-positive 721.221-transfectant was not killed by $KIR2DL2^+$ NK cells, but this inhibition could be reversed by the use of either DF200 or a DF200 Fab fragment. Finally, the Cw3+ 721.221-transfectant was not killed by $KIR2DL3^+$ NK cells, but this inhibition could be reversed by the use of either a DF200 Fab fragment or the conventional anti-KIR2DL3 mAb GL183 or Y249. The results are shown in Figure 3.

F(ab')2 fragments were also tested for their ability to reconstite lysis of Cw4 positive targets. F(ab')2 fragments of the DF200 and EB6 Abs were both able to reverse inhibition of lysis by KIR2DL1-transfected NK cells of the Cw4 transfected 221 cell line and the Cw4+ TUBO EBV cell line. Results are shown in Figure 4.

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Example 3: Biacore analysis of DF200 mAb/KIR2DL1 and DF200 mAb/KIR2DL3 interactions

Production and purification of recombinant proteins. The KIR2DL1 and KIR2DL3 recombinant proteins were produced in *E. coli*. cDNA encoding the entire extracellular domain of KIR2DL1 (SEQ ID NO:23) and KIR2DL3 (SEQ ID NO:25) were amplified by PCR from pCDM8 clone 47.11 vector (Biassoni et al, Eur J Immunol. 1993;23:1083-7) and RSV.5(gpt)183 clone 6 vector (Wagtmann et al, Immunity 1995;2:439-49 and 1995;3:801-809) respectively, using the following primers:

Sense: 5'-GGAATTCCAGGAGGAATTTAAAATGCATGAGGGAGTCCACAG-3' (SEQ ID NO:13)

Anti-sense: 5'- CGGGATCCCAGGTGTCTGGGGTTACC -3' (SEQ ID NO:14)

They were cloned into the pML1 expression vector in frame with a sequence encoding a biotinylation signal (Saulquin et al, J Exp Med. 2003;197:933-8).

Protein expression was performed in the BL21(DE3) bacterial strain (Invitrogen). Transfected bacteria were grown to OD600=0.6 at 37°C in medium supplemented with ampicillin (100 µg/ml) and expression was induced with 1 mM IPTG.

Proteins were recovered from inclusion bodies under denaturing conditions (8 M urea). Refolding of the recombinant proteins was performed in 20 mM Tris, pH 7.8, NaCl 150 mM buffer containing L-arginine (400 mM, Sigma) and β-mercaptoethanol (1 mM), at room temperature, by decreasing the urea concentration in a six step dialysis (4, 3, 2, 1 0.5 and 0 M urea, respectively). Reduced and oxidized glutathione (5 mM and 0.5 mM respectively, Sigma) were added during the 0.5 and 0 M urea dialysis steps. Finally, the proteins were dialyzed extensively against 10 mM Tris, pH 7.5, NaCl 150 mM buffer. Soluble, refolded proteins were concentrated and then purified on a Superdex 200 size-exclusion column (Pharmacia; ÄKTA system).

Surface plasmon resonance measurements were performed on a Biacore apparatus (Biacore). In all Biacore experiments HBS buffer supplemented with 0.05% surfactant P20 served as running buffer.

Protein immobilisation. Recombinant KIR2DL1 and KIR2DL3 proteins produced as described above were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5 (Biacore). The sensor chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimidehydrochloride and N-hydroxysuccinimide, Biacore). Proteins, in coupling buffer (10 mM acetate, pH 4.5) were injected. Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore).

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Affinity measurements. For kinetic measurements, various concentrations of the soluble antibody (1 x 10-7 to 4 x 10-10 M) were applied onto the immobilized sample. Measurements were performed at a 20 µl/min continuous flow rate. For each cycle, the surface of the sensor chip was regenerated by 5 µl injection of 10 mM NaOH pH 11. The BIAlogue Kinetics Evaluation program (BIAevaluation 3.1, Biacore) was used for data analysis. The soluble analyte (40 µl at various concentrations) was injected at a flow rate of 20 µl/min in HBS buffer, on dextran layers containing 500 or 540 reflectance units (RU), and 1000 or 700 RU of KIR2DL1 and KIR2DL3, respectively. Data are representative of 6 independent experiments. The results are shown in Table 1, below.

Table 1

BIAcore analysis of DF200 mAb binding to immobilized KIR2DL1 and KIR2DL3. K_D: Dissociation constant.

Protein	K_D (10^{-9} M)
KIR2DL1	10.9 +/- 3.8
KIR2DL3	2.0 +/- 1.9

Example 4: Generation of new human anti-KIR mAbs

Human monoclonal anti-KIR mAbs were generated by immunizing transgenic mice, engineered to express a human antibody repertoire, with recombinant, soluble KIR proteins. After different cell fusions to establish hybridomas, mAbs were first selected for their ability to cross-react with immobilized KIR2DL1 and KIR2DL3 protein (produced as described in Example 3, above). Several human monoclonal antibodies, including 1-7F9, 1-4F1, 1-6F5 and 1-6F1, were found to be cross-reactive with KIR2DL1 and KIR2DL2/3.

Positive monoclonal antibodies were further screened for their ability to reconstitute lysis by KIR2DL1-positive NK transfectants against Cw4-positive target cells. The NK cells expressing the HLA class I-specific inhibitory receptors were used as effectors cells against target cells expressing one or more HLA-C alleles (Figures 5 and 6). Cytotoxicity assays were carried out as described above. The effector/target ratio is indicated in the figure legends, and antibodies were used at either 10 µg/ml or 30 µg/ml.

As expected, KIR2DL1+ NK cells displayed little if any cytolytic activity against target cells expressing HLA-Cw4. However, in the presence of 1-7F9 mAb, NK cells became unable to recognize their HLA-C (*i.e.*, were no longer inhibited by HLA-C molecules), and now displayed strong cytolytic activity against the Cw4-positive targets. For example, the two cell lines tested (the HLA-Cw4 transfected 721.221 and the CW4+ EBV cell lines) were

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not killed by KIR2DL1+ NK cells, but the inhibition could be efficiently reversed by the use of either mAb 1-7F9 or the conventional anti-KIR2DL1 mAb EB6. The murine mAbs DF200 and Pan2D (NKVSF1) were compared to the human mAb 1-7F9 and in all experiments 1-7F9 was more potent than any of the other mAbs tested in terms of inducing cytotoxicity by the NK cells. Antibodies 1-4F1, 1-6F5 and 1-6F1 on the other hand were not able to reconstitute cell lysis by NK cells against Cw4-positive targets.

Example 5: Biacore competitive binding analysis of murine and human anti-KIR antibodies

Epitope mapping analysis was performed according to a previously described method (Gauthier et al., Journal of Immunology 1999;162:41-50; Saunal and van Regenmortel, Journal of Immunology 1995;183:33-41) on immobilized KIR2DL1 (900 RU), KIR 2DL3 (2000 RU) and KIR2DS1 (1000 RU) with mouse anti-KIR 2D antibodies DF200, NKVSF1 (Pan2D), gl183 and EB6, and human anti-KIR2D antibodies 1-4F1, 1-6F1, 1-6F5 and 1-7F9.

All experiments were done at a flow rate of 5 µl/min in HBS buffer with 2 min injection of the different antibodies at 15 µg/ml. For each pair of antibodies, competitive binding analysis was performed in two steps. In the first step the first monoclonal antibody (mAb) was injected on KIR2D target protein followed by the second mAb (without removing the first mAb) and second mAb RU value (RU2) was monitored. In the second step the second mAb was injected first, directly on nude KIR2D protein, and mAb RU value (RU1) was monitored. Percent inhibition of second mAb binding to KIR2D protein by first mab was calculated by: 100*(1-RU2/RU1).

Results are shown in Tables 2, 3 and 4, where the antibodies designated "first antibody" are listed on vertical column and the 'second antibody' are listed on the horizontal column. For each antibody combination tested, the values for direct binding level (RU) of the antibodies to the chip are listed in the table, where direct binding of the second antibody to the KIR2D chip is listed in the upper portion of the field and the value for binding of the second antibody to the KIR2D chip when the first antibody is present is listed in the lower portion of the field. Listed in the right of each field is the percentage inhibition of second antibody binding. Table 2 shows binding on a KIR2DL1 chip, Table 3 shows binding of antibodies to a KIR2DL3 chip, and Table 4 shows binding of antibodies to a KIR2DS1 chip.

Competitive binding of murine antibodies DF200, NKVSF1 and EB6, and human antibodies 1-4F1, 1-7F9 and 1-6F1 to immobilized KIR2DL1, KIR2DL2/3 and KIR2DS1 was assessed. Epitope mapping (Figure 7) from experiments with anti-KIR antibodies' binding to KIR2DL1 showed that (a) antibody 1-7F9 is competitive with EB6 and 1-4F1, but not with

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NKVSF1 and DF200; (b) antibody 1-4F1 in turn is competitive with EB6, DF200, NKVSF1 and 1-7 F9; (c) NKVSF1 competes with DF200, 1-4F1 and EB6, but not 1-7F9; and (d) DF200 competes with NKVSF1, 1-4F1 and EB6, but not 1-7F9. Epitope mapping (Figure 8) from experiments with anti-KIR antibodies' binding to KIR2DL3 showed that (a) 1-4F1 is competitive with NKVSF1, DF200, gl183 and 1-7F9; (b) 1-7F9 is competitive with DF200, gl183 and 1-4F1, but not with NKVSF1; (c) NKVSF1 competes with DF200, 1-4F1 and GL183, but not 1-7F9; and (d) DF200 competes with NKVSF1, 1-4F1 and 1-7F9, but not with GL183. Epitope mapping (Figure 9) from experiments with anti-KIR antibodies' binding to KIR2DS1 showed that (a) 1-4F1 is competitive with NKVSF1, DF200 and 1-7F9; (b) 1-7F9 is competitive with 1-4F1 but not competitive with DF200 and NKVSF1; (c) NKVSF1 competes with DF200 and 1-4F1, but not 1-7F9; and (d) DF200 competes with NKVSF1 and 1-4F1, but not with 1-7F9.

Table 2

KIR2DL1 epitope mapping using competitive binding between first and second antibodies (ND = not determined).

First Ab (below)	Second Ab						
	DF200	Pan2D	EB6	1-4F1	1-7F9	1-6F1	1-6F5
DF200		80%	90%	490 92% 40	480 27% 350	540 15% 460	400 15% 340
Pan2D	90%		90%	900 95% 50	860 2% 840	750 12% 660	600 13% 520
EB6	60%	40%	460 57% 200	370 48% 190	490 65% 170	260 23% 200	Nd
1-4F1							
1-7F9	600 10% 545	545 2% 534	460 60% 180	360 95% 16		330 9% 300	Nd
1-6F1	350 11% 310	475 7% 440	260 18% 320	360 23% 275	490 10% 440		Nd
1-6F5	350 17% 290	475 7% 440	Nd	360 17% 300	Nd	290 40% 170	

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Table 3

KIR2DL3 epitope mapping using competitive binding between first and second antibodies (ND = not determined)

First Ab (below)	Second Ab						
	DF200	Pan2D	Gl183	1-4F1	1-7F9	1-6F1	1-6F5
DF200		75%	20%	1270 75% 320	520 62% 200	550 16% 460	440 4% 420
Pan2D	95%		85%	2250 68% 730	880 15% 750	840 8% 770	560 18% 460
gl183	8%	40%		1300 75% 330	670 76% 160	530 18% 430	Nd
1-4F1	1140 82% 210	2400 63% 890	1240 73% 330		1050 87% 140		
1-7F9	770 42% 450	870 5% 830	800 75% 200	1000 63% 270			
1-6F1	790 4% 760	990 0% 1090	620 8% 570				
1-6F5	800 5% 760	990 4% 950	Nd				

Table 4

KIR2DS1 epitope mapping using competitive binding between first and second antibodies (ND = not determined)

First Ab (below)	Second Ab			
	DF200	Pan2D	1-4F1	1-7F9
DF200		70%	660 87% 80	975 15% 825
Pan2D	100%		650 100% -8	920 45% 500
1-7F9	900 17% 1090	1350 11% 1200	660 96% 23	

Example 6: Anti-KIR mAb titration with cynomolgus NK cells

Anti-KIR antibody NKVSF1 was tested for its ability to bind to NK cells from cynomolgus monkeys.

Purification of monkey PBMC and generation of polyclonal NK cell bulk. Cynomolgus Macaque PBMC were prepared from Sodium citrate CPT tube (Becton Dickinson). NK

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cells purification was performed by negative depletion (Macaque NK cell enrichment kit, Stem Cell Technology). NK cells were cultivated on irradiated human feeder cells with 300 U/ml Interleukin 2 (Proleukin, Chiron Corporation) and 1ng/ml Phytohemagglutinin A (Invitrogen, Gibco) to obtain polyclonal NK cell populations.

Pan2D mAb titration with cynomolgus NK cells. Cynomolgus NK cells (NK bulk day 16) were incubated with different amount of Pan2D mAb followed by PE-conjugated goat F(ab')2 fragments anti-mouse IgG (H+L) antibodies. The percentage of positive cells was determined with an isotypic control (purified mouse IgG1). Samples were done in duplicate. Mean fluorescence intensity = MFI.

Binding of the antibody to monkey NK cells is shown in Figure 10.

Example 7: Screening of human anti-KIR mAbs

Human monoclonal anti-KIR Abs were generated by immunizing transgenic mice engineered to express a human antibody repertoire with recombinant KIR protein, as described in Example 4. To generate cross-reactive anti-KIR antibodies, animals were sequentially immunized with different KIRs, either in soluble form as described in Example 3, or expressed at the surface of cells.

Next, after different cell fusions, the mAbs were first selected for their ability to cross-react with immobilized KIR2DL1 and KIR2DL3 protein, by ELISA, using standard methods. Several human monoclonal antibodies, including 1-7F9, 1-4F1, 1-6F5 and 1-6F1, were found to react with all of KIR2DL1, KIR2DL2 and KIR2DL3, by ELISA. The mAbs that reacted with both KIR2DL1 and KIR2DL2/3 were designated "KIR2DL-crossreactive mAbs."

The KIR2DL cross-reactive mAbs were then tested for their ability to react with KIR at the surface of cells by flow cytometry. Briefly, the binding of the human anti-KIR mAbs was tested by incubating them with various cell lines stably over-expressing KIRs (*e.g.*, YTS-2DL1, BWZ-KIR2DL2 and BWZ-KIR2DS4) or not expressing KIRs (*e.g.*, YTS and BWZ). Briefly, cells were incubated with various concentrations (typically 0-50 µg/ml) of human anti-KIR mAb1-7F9 in DMEM-medium containing 2% FCS. After incubation, cells were washed and incubated with APC-conjugated secondary antibodies specific for human IgG. All incubations and washing steps were performed at 0-4ºC. Subsequently, cells were washed, resuspended in PBS, and analyzed by flow cytometry on a FACScalibur or a FACScanto (both from Beckton Dickinson). A typical example is shown in Figure 16. For example, 1-7F9 and 1-4F1 were found to not bind to cells transfected with KIR2DS4, whereas NKVSF1 (Pan2D) did (Figure 16).

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Next, the ability of the human anti-KIR mAbs to block the interaction between KIR and its HLA-C ligand was tested by 1) biochemical, direct binding experiments and 2) functional reconsitution of lysis assays.

In the biochemical binding assay, the capacity of human anti-KIR mAbs, including 1-7F9, to block the interaction between HLA-C and KIR-molecules was assessed by competing the binding of soluble, recombinant KIR-Fc fusion proteins to cells expressing HLA-C. The KIR-Fc proteins were produced as described (Wagtmann et al., Immunity 1995;3(6):801-9), except that the human Fc was replaced with murine IgG1 Fc. Soluble KIR-Fc binds to cells expressing the specific HLA-C allotypes that are recognized by KIR2DL1, and this binding can be visualized by flow-cytometry using a secondary fluorochrome-conjugated Ab specific for the murine Fc part of the KIR-Fc protein. For example, KIR2DL1-Fc binds to cells transfected with HLA-Cw*0402 (LCL 721.221-Cw4 transfectants) (Litwin et al., J Exp Med. 1993;178:1321-36) but not to untransfected LCL 721.221 cells (see Figure 11A). To test whether anti-KIR mAbs could prevent this interaction between KIR2DL1-Fc and HLA-Cw4, the KIR2DL1-FC proteins were pre-incubated with increasing concentrations of anti-KIR mAbs, and then added to LCL 721.221-Cw4 cells, incubated at 4 ºC, washed, incubated with an APC-conjugated anti-murine IgG1 Fc, washed, and analyzed by flow cytometry on a FACScalibur, or a FACScanto (Beckton Dickinson), by standard methods. Some murine anti-KIR mAbs, such as DF200, and some human anti-KIR mAbs, namely 1-7F9 and 1-4F1, prevented the binding of KIR2DL1-Fc to the cells expressing HLA-Cw4, showing that these mAbs block the interaction between KIR2DL1 and HLA-Cw4. As an example, Figure 17A shows that DF200 prevents binding of KIR2DL1-Fc to cells expressing HLA-Cw4. Figure 17B shows that the 1-7F9 mAbs prevented KIR2DL1 from binding to HLA-Cw4. In parallel, the anti-KIR mAbs were tested for their ability to prevent the binding of KIR2DL2 to HLA-Cw3. Antibodies that prevented the binding of KIR2DL-Fc to HLA-C-expressing cells in a dose-dependent fashion, as exemplified in Figure 17, were designated "blocking mAbs" and were further tested in functional cytotoxicity assays, as follows.

The therapeutic utility of the human anti-KIR mAbs is linked to their ability to induce KIR-expressing NK cells to kill tumor cells, by preventing inhibitory signaling via KIR. Therefore, it was important to assess whether human KIR2DL cross-reactive and blocking mAbs were able to induce lysis of target cells expressing HLA-C by NK cells expressing KIR2DL. For this purpose, the following experiments were performed:

In ^{51}Cr-release cytotoxicity assays, KIR2DL1-expressing YTS cells (YTS-2DL1) can kill LCL 721.221-Cw3, but not LCL 721.221-Cw4 cells (Figure 18A). In contrast, YTS effector-cells that lack KIRs (YTS) kill both cell-lines efficiently. Thus, YTS-2DL1 effector cells

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cannot kill LCL 721.221-Cw4 cells due to HLA-Cw4-induced inhibitory signaling via KIR2DL1. When YTS-2DL1 cells were pre-incubated with 1-7F9 in ^{51}Cr-release cytotoxicity assays, LCL 721.221-Cw4 cells were killed in a 1-7F9 concentration-dependent fashion (Figure 18B). 3-1F13, a human cross-reactive anti-KIR mAb that binds KIR2DL1 with high affinity, but that can not prevent the interaction between KIR2DL1 and HLA-Cw4, can not induce killing of LCL 721.221-Cw4 cells by YTS-2DL1 cells. Thus, 1-7F9 induces NK-mediated killing of target cells by preventing KIR-HLA-C interactions between NK- and target-cells.

Example 8: Affinity measurements of 1-7F9

The affinity of 1-7F9 for binding to KIR2DL1 and KIR2DL3 was measured by Biacore analysis, using soluble recombinant KIR proteins produced as described in Example 3. The Biacore experiments were performed as described in Example 3, except that the antibody used was the human mAb 1-7F9.

The results of these affinity determinations of binding by 1-7F9 to KIR2DL1 and -3 are shown in Table 5.

Table 5

BIAcore analysis of 1-7F9 mAb binding to immobilized KIR2DL1 and KIR2DL3.

K_D: Dissociation constant.

Protein	K_D (10^{-9} M)
KIR2DL1	0,43
KIR2DL3	0,025

Example 9: Epitope mapping of 1-7F9 onto KIR2DL1

Epitope mapping of a given monoclonal antibody onto a given antigen can be obtained by *in silico* methods when suitable X-ray structures/homology models exist of both the antibody and the antigen by performing protein-protein docking.

A homology-model of an antibody can be obtained by aligning the sequence of the Light and Heavy Chain, respectively, with a selection of antibodies for which a 3D structure exists. The length of the 6 Complementarity Determining Regions (CDRs) can be calculated and the best template selected from antibodies with the same CDR lengths. Using standard techniques a homology model can be build from the selected template.

In the protein-protein docking approach, recently reviewed (Schneidman-Duhovny et al., Curr Med Chem 2004;11:91-107), the two surfaces are represented by features on surfaces. Features include hydrogen bonding capabilities, charges and hydrophobicity. In a grid based method, space is divided into cubes and each cube is given a value according to its

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position relative to the surface (interior, surface, exterior) and assigned the relevant feature set. Brute force matching of the surfaces by a scoring function can now be employed by searching the entire 3 translational and 3 rotational degrees of freedom. Translations are handled by Fast Fourier Transform and rotation is treated as individual calculations within a standard discretization of rotational space. From the top scoring complexes the results can be filtered and scored, by a range of methods, *e.g.*, shape complementarity, Van der Waals interactions, hydrophobicity, electrostatics, desolvation, hydrogen bonding, atomic contact energy, residue-residue pairing statistics and hydrophilic group pairing. The top-scoring complexes can be evaluated in detail and interacting residues and thereby the epitope can be identified.

Methods and Analysis

Residue and domain nomenclature for the KIRs were according to Fan et al. (Nature 1997;389:96-100), so that Domain1 comprised residues 6-101 and Domain 2 comprised amino acids residues 105-200).

A homology-model of 1-7F9 was constructed using 1OM3 as a template. The 1OM3 structure, generated by Calarese et al (Science 2003;300:2065, *et seq.*), was retrieved from the PDB (Protein Data Bank, at World Wide Web (WWW) address rcsb.org/pdb/; 1OM3 VL sequence: SEQ ID NO:34; 1OM3 VH sequence: SEQ ID NO:35). Kabat Numbering and CDR assignment were used. The overall alignment was good and the CDR lengths were identical, so the homology model would be accurate enough to be used in a protein-protein docking experiment.

From the protein-protein docking of the 1-7F9 antibody homology model onto the KIR2DL1 structure, 1NKR.pdb 2000 solutions were obtained. They were filtered for their closeness to D183 (atoms < 6 Å from CD) and R131 (atoms < 12 Å from CZ) and 133 resulting solutions were analyzed in detail.

The highest scoring complex formed was analyzed and a possible interaction between R131 (KIR) and D100C (Heavy chain) was identified and restrained. A new rotamer for F181 was selected and the energy was minimized. This model predicted that the antibody interacted with the following residues on KIR2DL1 (SEQ ID NO:23): S20 (Hyd, CB) (HydAcceptor, OG), E21 (Hyd, CB, CG), M44 (Hyd, SD, CE), F45 (Hyd, CD1, CD1, CZ, CE2), R68 (HydDonor, NH2), T70 (Hyd, CB, CG2), Q71 (Hyd, CG), L104 (Hyd, CB, CG, CD1, CD2), Y105 (Hyd, CG, CD2, CE2), R131 (Ion, NH2) (HydDonor, NH2), S133 (Hyd, CA, CB) (HydDonor/Acceptor, OG), Y134 (Hyd, CD1, CE1), F181 (Hyd, CB, CG, CD1, CD2, CE1, CE2, CZ), and D183 (Hyd, CB).

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Example 10: Biacore competitive binding analysis of murine and human anti-KIR antibodies

Competitive binding of 1-7F9, Pan2D (NKVSF1), and DF200 was evaluated according to the same method described in Example 5.

Results are shown in Tables 6 and 7, where the antibodies designated "first antibody" are listed on vertical column and the 'second antibody' are listed on the horizontal column. For each antibody combination tested, the percentage inhibition of second antibody binding is listed. Where the percentages of inhibition for a certain antibody pair were above 40%, regardless of which antibody was used as first antibody, the antibodies were considered competitive. Table 6 shows binding on a KIR2DL1 chip, and Table 7 shows binding of antibodies to a KIR2DL3 chip.

Briefly, for KIR2DL1 binding, the results show that DF200 and Pan2D are competitive, whereas 1-7F9 is not competitive with either one of DF200 or Pan2D. For KIR2DL3 binding, DF200 and Pan2D were competitive, whereas 1-7F9 was not competitive with Pan2D, but was competitive with DF200.

Table 6

KIR2DL1 competitive binding between first and second antibodies.

First antibody (below)	Second antibody		
	1-7F9	Pan2D	DF200
1-7F9	98 %	4 %	15 %
Pan2D	17 %	92 %	90 %
DF200	17 %	85 %	80 %

Table 7

KIR2DL1 competitive binding between first and second antibodies.

First antibody (below)	Second antibody		
First mAb	1-7F9	Pan2D	DF200
1-7F9	95 %	4 %	60 %
Pan2D	0 %	95 %	95 %
DF200	80 %	50 %	85 %

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Example 11: Crystal structure of KIR2DL1 in complex with HuKIR1-7F9-Fab'

The crystal structure of KIR2DL1 in complex with the Fab' fragment of 1-7F9 was solved and refined to 2.35 Å resolution with X-ray crystallography. The results confirmed that the antibody, when bound to KIR2DL1, will be able to block binding to an MHC Class I molecule (Figure 19).

Materials and Methods

Extracellular KIR2DL1 (amino acid 1-223 of SEQ ID NO:23, with residue 16 being arginine (R) and residue 114 being leucine (L), and including an additional N-terminal methionine (M) residue) and HuKIR1-7F9 Fab' (with the light chain sequence of SEQ ID NO:36 and heavy chain sequence of residues 1-221 of SEQ ID NO:37) were mixed, with a slight excess of KIR2DL1, and the complex was purified on a gel-filtration column. The complex was then concentrated to about 13.5 mg/ml. Crystals were grown with the hanging drop-technique in 10 % PEG6000 and 500 mM citrate buffer with a pH of 4.2. Crystals were flash frozen in liquid N_2 and crystallographic data to 2.35 Å resolution were collected at 100 K using the beam-line BL711I, MAX-lab, Lund, Sweden. Data were integrated by the XDS program (Kabsch, J. Appl. Crystallogr. 1993;26:795-800). For structure determination molecular replacement, using the MOLREP program of the CCP4 suite (Bailey, Acta Crystallogr. Sect. D-Biol. Crystallogr. 1994;50:760-763) and the PDB-deposited structures 1RZJ (the Fab part1) and 1NKR (KIR), were used. Phase improvements were made with the ARP/WARP program (Lamzin and Wilson, Acta Crystallogr. Sect. D-Biol. Crystallogr. 1993;49:129-147) and manual modifications to the X-ray derived structure model were made with the QUANTA program (available from Accelrys Inc., San Diego, CA, USA). Refinement was carried out in the REFMAC5 computer program of the CCP4 suite. Water molecules were added by the ARP/WARP program. The model comprised residues 6-114 and 124-200 of KIR2DL1, 1-212 of the 1-7F9 light chain and 1-136 together with 143-224 of the 1-7F9 heavy chain. In addition, 330 water molecules were placed. R- and R-free for the model were 0.191 and 0.253, respectively.

Results

The contacts were identified by the CONTACT computer program of the CCP4 suite using a cut-off distance of 4.0 Å between the Fab' and KIR2DL1 molecules. The resulting KIR2DL1 epitope for human 1-7F9 was found to comprise the following residues of KIR2DL1 (SEQ ID NO:23): L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88 (Tables 8 and 9). Residues of 1-7F9 involved in interactions with KIR2DL1 included S28, V29, S30, Y32, S92, N93, and W94 of the 1-7F9 variable light (VL) chain (SEQ ID NO:15, Table 8), and T28, F29, S30, F31, I54, F55, E74, S77, G102, S103, Y105, Y106,

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D107, and Y108 of the variable heavy (VH) chain (SEQ ID NO:17). The KIR2DL1 epitope, and the residues involved in hydrogen-binding, are also indicated in the amino-acid sequence of KIR2DL1 in Figure 20. The isotropic displacement factors (also called "temperature factors" or "B-values") obtained from the coordinate refinement showed relatively lower values for the N-terminal domain of KIR2DL1 and the variable domains of the 1-7F9 antibody Fab' fragment, all domains directly involved in the intermolecular binding. This showed that the binding forces between the two molecules in the complex of the crystal were strong and stable, also supporting that the crystal structure depicted a stable KIR2DL1/1-7F9 Fab' complex.

The 1-7F9 Fab' molecule bound to the KIR2DL1 molecule on one side of the C'CFGA' β-sheet of domain D1 but, in addition, touched also one E β-strand residue side-chain of the same domain. Connections to loop residues of the D1 domain are also important for binding (for topology naming conventions, see Fan et al., Nature 1997;389:96-100). More specifically, connections are made to the following topological parts of KIR2DL1; β-strand C (amino acids L38 and R41), the loop between β-strands C and C', L2 (M44, F45 and N46), β-strand C' (D47, T48, L49, R50 and I52), β-strand E (F64), the loop between the E and F β-strands, L3 (D72), β-stand F (Y80) and the loop between the F and G β-strands (P87 and Y88).

While the HLA-Cw4 molecule binds both to the D1 and D2 domain of the KIR2DL1 molecule (Fan et al. Nat. Immunol. 2001; 2: 452-460) the 1-7F9 antibody binds to the KIR2DL1 D1 domain only. There is, however, a spatial overlap between a bound 1-7F9 and a bound HLA-Cw4 molecule, large enough for the 1-7F9 antibody to successfully displace HLA-Cw4. Using the published structure of KIR2DL1 in complex with HLA-Cw4 (PDB accession code 1IM9) contact residues between KIR2DL1 and HLA-Cw4 can be calculated with the CONTACT program. In a calculation using a distance cut-off of 4.0 Å it can be seen that three KIR2DL1 residues involved in contacts with HLA-Cw4 residues are common to contact residues in the 1-7F9/KIR2DL1 complex (Table 8 and 9). Those three KIR2DL1 residues are M44, F45 and D72.

Without being limited to any specific theory, the crystallographic results also indicated that 1-7F9 does not bind the activating NK receptor KIR2DS4 because of amino acids N47 and V72 in the extracellular portion of the KIR2DS4 sequence (SEQ ID NO:38).

Table 8

KIR2DL1 – 1-7F9 Fab' VL chain interactions. A cut-off of 4.0 Å was used. The contacts were identified by the CONTACT computer program of the CCP4 suite. In the last col-

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umn "***" indicates a strong possibility for a hydrogen bond at this contact (distance < 3.3 Å) as calculated by CONTACT, " *" indicates a weak possibility (distance > 3.3 Å). Blank indicates that the program considered there to be no possibility of a hydrogen bond.

KIR2DL1 Atoms		1-7F9 Fab' VL Atoms		Distance (Å)
Arg	41A NH2	Tyr	32L OH	2.73 ***
Met	44A O	Asn	93L CG	3.65
		Asn	93L OD1	3.54 *
		Asn	93L ND2	2.96 ***
Phe	45A CA	Asn	93L OD1	3.50
Phe	45A CB	Tyr	32L CE1	3.59
Phe	45A CG	Tyr	32L CE1	3.93
Phe	45A CE1	Ser	30L CB	3.82
Phe	45A CZ	Ser	28L O	3.77
		Ser	30L CB	3.74
		Ser	28L OG	3.89
		Ser	30L N	3.78
Phe	45A CE2	Ser	28L O	3.76
		Val	29L O	3.98
		Val	29L CA	3.94
		Ser	30L CB	3.87
		Val	29L C	3.72
		Ser	30L N	3.89
Phe	45A CD2	Asn	93L ND2	3.75
		Ser	92L CB	3.85
Phe	45A C	Asn	93L OD1	3.70
Asn	46A N	Asn	93L OD1	2.92 ***
Asn	46A CA	Asn	93L OD1	3.82
Asn	46A CB	Asn	93L OD1	3.52
		Trp	94L CH2	3.86
		Trp	94L CZ2	3.52
Asn	46A CG	Asn	93L OD1	3.31
		Trp	94L CH2	3.41
		Trp	94L CZ2	3.65
Asn	46A OD1	Trp	94L CH2	3.85
Asn	46A ND2	Asn	93L CB	3.99
		Asn	93L CG	3.48
		Asn	93L OD1	2.56 ***
		Trp	94L CZ3	3.52
		Trp	94L CH2	3.25
		Trp	94L CZ2	3.76
Asp	47A CB	Tyr	32L OH	3.63
Asp	47A CG	Tyr	32L OH	3.74
		Tyr	32L CE2	3.79
Asp	47A OD2	Tyr	32L CZ	3.88
		Tyr	32L OH	3.34 *
		Tyr	32L CE2	3.54
Asp	72A CB	Ser	30L OG	3.87
Asp	72A CG	Ser	30L OG	3.62
Asp	72A OD2	Ser	30L CB	3.58
		Ser	30L OG	2.93 ***

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Table 9

KIR2DL1 – 1-7F9 Fab' VH chain interactions. A cut-off of 4.0 Å was used. The contacts were identified by the CONTACT computer program of the CCP4 suite. In the last column "***" indicates a strong possibility for a hydrogen bond at this contact (distance < 3.3 Å) as calculated by CONTACT, " *" indicates a weak possibility (distance > 3.3 Å). Blank indicates that the program considered there to be no possibility of a hydrogen bond.

KIR2DL1 Atoms	1-7F9 Fab' VH Atoms	Distance (Å)
Leu 38A CD1	Ile 54H CD1	3.61
	Phe 31H CB	3.64
Leu 38A CD2	Phe 31H CG	3.70
	Phe 31H CD2	3.74
Asp 47A CG	Ser 103H N	3.73
	Ser 103H OG	3.63
Asp 47A OD1	Gly 102H N	3.99 *
	Gly 102H CA	3.40
	Gly 102H C	3.75
	Ser 103H N	3.09 ***
	Ser 103H OG	3.77 *
Asp 47A OD2	Ser 103H N	3.73 *
	Ser 103H CB	3.36
	Ser 103H OG	2.77 ***
Thr 48A C	Tyr 108H OH	3.74
Thr 48A O	Tyr 108H CZ	3.68
	Tyr 108H OH	2.90 ***
	Tyr 108H CE2	3.65
Leu 49A CA	Tyr 105H O	3.28
Leu 49A CB	Tyr 105H O	3.62
Leu 49A CD2	Tyr 105H O	3.67
	Tyr 105H CB	3.71
	Tyr 105H CD1	3.76
	Ser 103H OG	3.91
Leu 49A C	Tyr 105H O	3.59
Arg 50A N	Tyr 105H C	3.80
	Tyr 105H O	2.94 ***
	Tyr 106H N	3.87 *
	Tyr 108H CE2	3.81
Arg 50A CA	Tyr 105H O	3.97
Arg 50A CB	Tyr 106H O	3.88
	Tyr 108H CE2	3.80
Arg 50A CG	Phe 31H CZ	3.65
	Phe 31H CE2	3.90
	Tyr 106H O	3.99
Arg 50A CD	Phe 31H CZ	3.32
	Phe 31H CE2	3.63
	Tyr 106H O	3.71
Arg 50A NE	Tyr 106H O	2.88 ***
	Asp 107H C	3.94
	Asp 107H O	3.58 *
Arg 50A CZ	Tyr 106H O	3.70
	Asp 107H O	3.74

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Arg	50A	NH2	Tyr 106H O	3.61 *
			Asp 107H CB	3.56
			Asp 107H O	3.86 *
Arg	50A	C	Tyr 106H N	3.86
Arg	50A	O	Tyr 106H O	3.56 *
			Tyr 105H C	3.68
			Tyr 105H O	3.72 *
			Tyr 106H N	2.86 ***
			Tyr 106H CA	3.67
			Tyr 106H CB	3.47
Ile	52A	CD1	Tyr 106H CD1	3.93
			Tyr 106H CE1	3.68
Phe	64A	CD2	Tyr 105H CE1	3.86
Tyr	80A	CZ	Phe 55H CE2	3.90
Tyr	80A	OH	Phe 55H CE2	3.87
Pro	87A	CB	Thr 28H OG1	3.46
Pro	87A	CG	Thr 28H OG1	3.66
Pro	87A	O	Ser 30H CA	3.87
			Ser 30H C	3.88
Tyr	88A	CB	Glu 74H CD	3.39
			Glu 74H OE1	3.38
			Glu 74H OE2	3.40
Tyr	88A	CG	Glu 74H CG	3.65
			Glu 74H CD	3.37
			Glu 74H OE1	3.71
			Glu 74H OE2	3.55
Tyr	88A	CD1	Glu 74H CB	3.85
			Glu 74H CG	3.72
			Glu 74H CD	3.82
			Glu 74H OE1	3.97
Tyr	88A	CE1	Glu 74H CB	3.93
			Glu 74H CG	3.91
			Glu 74H O	3.84
			Ser 77H OG	3.68
			Thr 28H CB	3.97
Tyr	88A	CZ	Ser 77H OG	3.51
			Ser 30H N	3.87
			Thr 28H CB	3.71
			Phe 29H N	3.86
Tyr	88A	OH	Ser 77H CA	3.73
			Ser 77H CB	3.35
			Ser 77H OG	2.49 ***
			Thr 28H CA	3.88
			Thr 28H CB	3.34
			Thr 28H C	3.96
			Phe 29H N	3.07 ***
			Phe 29H CA	3.86
			Phe 29H CB	3.67
			Phe 29H CD2	3.83
Tyr	88A	CE2	Ser 30H N	3.32
			Ser 30H O	3.82
			Phe 29H C	3.92
			Phe 29H CB	3.89
Tyr	88A	CD2	Glu 74H CG	3.81
			Glu 74H CD	3.77
			Glu 74H OE2	3.64

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		Ser	30H N	3.91
		Ser	30H O	3.58
		Pro	53H CB	3.83
Tyr	88A C	Ile	54H CG2	3.93
Tyr	88A O	Ile	54H CG2	3.75

Example 12: Physical stability of 1-7F9

The biophysical properties and stability of human antibody 1-7F9 were studied. The folding and secondary structure of the protein was studied by circular dichroism (CD) and the oligomerization and aggregation by dynamic light scattering (DLS). In order to mimic storage conditions for two years at 5 °C the protein was subjected to incubation at 37 °C with shaking for 14 days.

Materials and Methods

Sample preparation. 2 mg/ml 1-7F9 was prepared in (a) 50 mM Na-Phosphate, 0.001 % Polysorbate 80 (Sigma, P8074), pH 7.0; (b) 50 mM Na-Phosphate, 0.001 % Polysorbate 80, pH 7.0, 0.5 mM Sucrose; (c) 50 mM Citrate, 0.001 % Polysorbate 80, pH 3.0; and d) 50 mM Tris, 0.001 % Polysorbate 80, pH 8.5.

Circular dichroism (CD). CD measurements were performed at 25 °C with a protein concentration of 2.0 mg/ml on a Chirascan circular dichroism spectrometer (Applied Photophysics) equipped with a peltier element for temperature control. 1-7F9 samples were in cylindrical quartz cells with 0.1 mm path length. Buffer scans were recorded and subtracted for each sample spectra.

Dynamic light scattering (DLS). DLS was performed at 25 °C with a protein concentration of 2.0 mg/ml using a Dynapro 99 temperature controlled DLS instrument (Protein Solutions Inc.). Data analysis was performed using the Dynamics software supplied with the instrument.

Results

Whereas the molecular size did not change for the samples at pH 7.0 after 14 days incubation as evaluated by DLS, both the samples formulated at pH 3.0 and pH 8.5 aggregated heavily during a 14 day period.

The CD measurements showed characteristics of an all beta structure and revealed that the samples formulated at pH 7.0 maintained their secondary structure throughout the accelerated study, although there was a slight drop in the signal for the sample containing only Polysorbate 80 as excipient. This might be due to a weak precipitation of the sample since the overall form of the spectra is unchanged. The sample containing sucrose showed no such decrease over time. The CD measurements of the samples formulated at pH 3.0

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and 8.5 showed a strong change in spectral characteristics over time, probably as a result of unfolding or other conformational changes, which could lead to non-functional 1-7F9 protein. The changes were observed immediately and were most significant at pH 3.0.

Overall, 1-7F9 maintained its physical properties and remained stable under stressed conditions (37 °C with shaking) at pH 7.0 with Polysorbate 80 and Sucrose as excipients.

Example 13: Affinity determination (monovalent binding) of 1-7F9 and 1-4F1

The affinities of 1-7F9 and 1-4F1 in monovalent binding to KIR2DL1 and KIR2DL3 antigen, (as opposed to the bivalent binding affinity determinations in Examples 3 and 8), were determined by surface plasmon resonance technology, using a Biacore 3000.

Briefly, an anti-human IgG (Dako RAHIgG, # 0423) was used together with an HBS-EP buffer at a flow rate of 20µL/min. Purified antibodies were diluted to 10µg/mL. For each antibody, KIR2DL1 or KIR2DL3 and buffer was injected. The antigens were tested at concentrations of 2000, 500, 200, 50 og 20nM. Flowrate was maintained at 20µL/min. Regeneration of the surfacec was accomplished by a single 30s injection of Glycine-HCl pH 1.8.

The results are displayed in Table 10. For the binding of 1-4F1 to KIR2DL1, there was a significant shift in baseline. Without being limited to any specific theory, this might represent a buffer effect caused by the matrix, or the binding reaction actually involving 2 steps, *e.g.*, because of a conformational change of the antigen.

Table 10

Monovalent binding affinity of 1-7F9 and 1-4F1 to KIR2DL1 and KIR2DL3. *) indicates fit with RI (response of matrix) fitted globally.

	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi2
1-4F1/2DL1	8,33E+03	5,09E-15	6,11E-19	1,24
1-4F1/2DL1 *)	2,68E+04	0,0132	4,95E-07	9,1
1-4F1/2DL3	4,16E+05	2,94E-03	7,05E-09	0,398
1-7F9/2DL1	1,51E+05	1,56E-03	1,03E-08	4,02
1-7F9/2DL3	1,51E+05	5,20E-04	3,45E-09	1,55

EXAMPLARY ASPECTS OF THE INVENTION

1. An antibody comprising a light chain variable region comprising an amino acid sequence at least 50% identical to SEQ ID NO:15 or SEQ ID NO:39, and/or a heavy chain variable re-

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gion comprising an amino acid sequence at least 50% identical to SEQ ID NO:17 or SEQ ID NO:41.

2. An antibody comprising a light chain variable region comprising an amino acid sequence at least 80% identical to SEQ ID NO:15 or SEQ ID NO:39, and/or a heavy chain variable region comprising an amino acid sequence at least 80% identical to SEQ ID NO:17 or SEQ ID NO:41.

3. An antibody comprising a light chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO:15 or SEQ ID NO:39, and/or a heavy chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO:17 or SEQ ID NO:41.

4. An antibody comprising a light chain variable region comprising an amino acid sequence at least 95% identical to SEQ ID NO:15 or SEQ ID NO:39, and/or a heavy chain variable region comprising an amino acid sequence at least 95% identical to SEQ ID NO:17 or SEQ ID NO:41.

5. An antibody comprising a light chain variable region comprising an amino acid sequence at least 98% identical to SEQ ID NO:15 or SEQ ID NO:39, and/or a heavy chain variable region comprising an amino acid sequence at least 98% identical to SEQ ID NO:17 or SEQ ID NO:41.

6. The antibody of any of the preceding aspects, wherein the light chain variable region comprises at least one conservative amino acid substitution, each amino acid substitution being conservative as compared to the amino acid at the corresponding position in SEQ ID NO:15 or SEQ ID NO:39.

7. The antibody of any of the preceding aspects, wherein the heavy chain variable region comprises at least one conservative amino acid substitution, each amino acid substitution being conservative as compared to the amino acid at the corresponding position in SEQ ID NO:17 or SEQ ID NO:41.

8. The antibody of any of the preceding aspects, comprising at least one of

(a) a light chain CDR1 amino acid sequence at least 90% identical to residues 24-34 of SEQ ID NO:15;

(b) a light chain CDR2 amino acid sequence at least 90% identical to residues 50-56 of SEQ ID NO:15;

(c) a light chain CDR3 amino acid sequence at least 90% identical to residues 89-97 of SEQ ID NO:15;

(d) a heavy chain CDR1 amino acid sequence at least 90% identical to residues 31-35 of SEQ ID NO:17;

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(e) a heavy chain CDR2 amino acid sequence at least 90% identical to residues 50-65 of SEQ ID NO:17; and

(f) a heavy chain CDR3 amino acid sequence at least 90% identical to residues 99-112 of SEQ ID NO:17.

9. The antibody of any of the preceding aspects, comprising at least one of

(a) a light chain CDR1 amino acid sequence corresponding to residues 24-34 of SEQ ID NO:15;

(b) a light chain CDR2 amino acid sequence corresponding to residues 50-56 of SEQ ID NO:15;

(c) a light chain CDR3 amino acid sequence corresponding to residues 89-97 of SEQ ID NO:15.

(d) a heavy chain CDR1 amino acid sequence corresponding to residues 31-35 of SEQ ID NO:17;

(e) a heavy chain CDR2 amino acid sequence corresponding to residues 50-65 of SEQ ID NO:17; and

(f) a heavy chain CDR3 amino acid sequence corresponding to residues 99-112 of SEQ ID NO:17.

10. The antibody of any of the preceding aspects, comprising at least one of

(a) a light chain CDR1 amino acid sequence consisting essentially of residues 24-34 of SEQ ID NO:15;

(b) a light chain CDR2 amino acid sequence consisting essentially of residues 50-56 of SEQ ID NO:15;

(c) a light chain CDR3 amino acid sequence consisting essentially of residues 89-97 of SEQ ID NO:15.

(d) a heavy chain CDR1 amino acid sequence consisting essentially of residues 31-35 of SEQ ID NO:17;

(e) a heavy chain CDR2 amino acid sequence consisting essentially of residues 50-65 of SEQ ID NO:17; and

(f) a heavy chain CDR3 amino acid sequence consisting essentially of residues 99-112 of SEQ ID NO:17.

11. The antibody of any of aspects 8-10, comprising at least 2 of (a) to (f).

12. The antibody of any of aspects 8-10, comprising at least 3 of (a) to (f).

13. The antibody of any of aspects 8-10, comprising at least 4 of (a) to (f).

14. The antibody of any of aspects 8-10, comprising at least 5 of (a) to (f).

15. The antibody of any of aspects 8-10, comprising all of (a) to (f).

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16. The antibody of any of the preceding claims, comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17.

17. The antibody of any of aspects 1-7, comprising at least one of

(g) a light chain CDR1 amino acid sequence at least 90% identical to residues 24-34 of SEQ ID NO:39;

(h) a light chain CDR2 amino acid sequence at least 90% identical to residues 50-56 of SEQ ID NO:39;

(i) a light chain CDR3 amino acid sequence at least 90% identical to residues 89-97 of SEQ ID NO:39;

(j) a heavy chain CDR1 amino acid sequence at least 90% identical to residues 31-35 of SEQ ID NO:41;

(k) a heavy chain CDR2 amino acid sequence at least 90% identical to residues 50-66 of SEQ ID NO:41; and

(l) a heavy chain CDR3 amino acid sequence at least 90% identical to residues 99-113 of SEQ ID NO:41.

18. The antibody of any aspects 1-7 and 17, comprising at least one of

(g) a light chain CDR1 amino acid sequence corresponding to residues 24-34 of SEQ ID NO:39;

(h) a light chain CDR2 amino acid sequence corresponding to residues 50-56 of SEQ ID NO:39;

(i) a light chain CDR3 amino acid sequence corresponding to residues 89-97 of SEQ ID NO:39;

(j) a heavy chain CDR1 amino acid sequence corresponding to residues 31-35 of SEQ ID NO:41;

(k) a heavy chain CDR2 amino acid sequence corresponding to residues 50-66 of SEQ ID NO:41; and

(l) a heavy chain CDR3 amino acid sequence corresponding to residues 99-113 of SEQ ID NO:41.

19. The antibody of any aspects 1-7 and 17-18, comprising at least one of

(g) a light chain CDR1 amino acid sequence consisting essentially of residues 24-34 of SEQ ID NO:39;

(h) a light chain CDR2 amino acid sequence consisting essentially of residues 50-56 of SEQ ID NO:39;

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(i) a light chain CDR3 amino acid sequence consisting essentially of residues 89-97 of SEQ ID NO:39;

(j) a heavy chain CDR1 amino acid sequence consisting essentially of residues 31-35 of SEQ ID NO:41;

(k) a heavy chain CDR2 amino acid sequence consisting essentially of residues 50-66 of SEQ ID NO:41; and

(l) a heavy chain CDR3 amino acid sequence consisting essentially of residues 99-113 of SEQ ID NO:41.

20. The antibody of any of aspects 17-19, comprising at least 2 of (a) to (f).

21. The antibody of any of aspects 17-19, comprising at least 3 of (a) to (f).

22. The antibody of any of aspects 17-19, comprising at least 4 of (a) to (f).

23. The antibody of any of aspects 17-19, comprising at least 5 of (a) to (f).

24. The antibody of any of aspects 17-19, comprising all of (a) to (f).

25. The antibody of any of claims 1-7 and 17-24, comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:39, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:41.

26. The antibody of any of the preceding aspects, which antibody does not bind at least one of KIR2DS4 and KIR2DS3.

27. The antibody of any aspects 1-15 and 26, which antibody blocks KIR2DL1 and KIR2DL2/3 binding to an HLA-C class I molecule.

28. The antibody of any aspects 1-15 and 26-27, which potentiates the lytic activity of an NK cell against a human target cell expressing an HLA-C class I molecule.

29. The antibody of any of aspects 1-15 and 26-28 which antibody is more efficient than DF200 in potentiating the lytic activity of an NK cell against a human target cell expressing an HLA-C Class I molecule.

30. The antibody of any of aspects 1-15 and 26-28, which antibody is more efficient than NKVSF1 (Pan2D) in potentiating the lytic activity of an NK cell against a human target cell expressing an HLA-C Class I molecule.

31. The antibody of any of aspects 1-15 and 26-28, which antibody is more efficient than EB6 in potentiating the lytic activity of an NK cell against a human target cell expressing an HLA-C Class I molecule

32. The antibody of any of the preceding aspects, which is a human or humanized antibody.

33. The antibody of any of the preceding aspects, which is an IgG1, IgG2, IgG3, or IgG4 antibody.

34. The antibody of any of the preceding aspects, which is a human IgG4 antibody.

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35. The antibody of any of aspects 1-34, which is a human IgG2 antibody.

36. A human IgG4 antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17.

37. A human IgG2 antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:39, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:41.

38. An antibody that competes with 1-7F9 in the binding to KIR2DL1 or KIR2DL2/3, wherein the antibody is not 1-4F1, DF200, gl183, A210, A803(g), or EB6.

39. An antibody that competes with 1-4F1 in the binding to KIR2DL1 or KIR2DL2/3, wherein the antibody is not 1-7F9, DF200, gl183, A210, A803(g), or EB6.

40. The antibody of aspect 38 , wherein the antibody is not 1-4F1.

41. The antibody of aspects 39, wherein the antibody is not 1-7F9

42. The antibody of any of aspects 38-39, wherein the antibody is not DF200.

43. The antibody of any of aspects 38-39, wherein the antibody is not gl183.

44. The antibody of any of aspects 38-39, wherein the antibody is not EB6.

45. The antibody of any of the preceding aspects, which has a K_d to KIR2DL1 of no more than about 20 nM.

46. The antibody of any of the preceding aspects, which has a K_d to KIR2DL1 of no more than 10.9 nM.

47. The antibody of any of the preceding aspects, which has a K_d to KIR2DL1 of no more than 0.45 nM.

48. The antibody of any of the preceding aspects, which has a K_d to KIR2DL3 of no more than about 20 nM.

49. The antibody of any of the preceding aspects, which has a K_d to KIR2DL3 of no more than 2.0 nM.

50. The antibody of any of the preceding aspects, which has a K_d to KIR2DL3 of no more than 0.025 nM.

51. The antibody of any of the preceding aspects, which is human.

52. A human or humanized antibody that competes with 1-7F9 in the binding to KIR2DL1.

53. A human or humanized antibody that competes with 1-4F1 in the binding to KIR2DL1.

54. A human or humanized antibody that competes with 1-7F9 in the binding to KIR2DL2.

55. A human or humanized antibody that competes with 1-4F1 in the binding to KIR2DL2.

56. A human or humanized antibody that competes with 1-7F9 in the binding to KIR2DL3.

57. A human or humanized antibody that competes with 1-4F1 in the binding to KIR2DL3.

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58. A human or humanized antibody that competes with 1-7F9 in the binding to KIR2DL1 and KIR2DL2/3.

59. A human or humanized antibody that competes with 1-4F1 in the binding to KIR2DL1 and KIR2DL2/3.

60. The human or humanized antibody of any of aspects 52-59, which does not bind at least one of KIR2DS4 and KIR2DS3.

61. The human or humanized antibody of any of aspects 52-60, which blocks KIR2DL1 or KIR2DL2/3 binding to an HLA-C class I molecule.

62. The human or humanized antibody of any of aspects 52-47, which potentiates the lytic activity of an NK cell against a human target cell expressing an HLA-C class I molecule.

63. The human or humanized antibody of any of aspects 52-62, which antibody comprises a light chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO:15, and a heavy chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO:17, or a light chain variable region at least 90% identical to SEQ ID NO:39, and a heavy chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO:41.

64. The human or humanized antibody of any of aspects 52-63, which is an IgG1, IgG2, IgG3, or IgG4 antibody.

65. The human or humanized antibody of aspect 64, which is an IgG4 antibody.

66. The human antibody of aspect 64, which is an IgG2 antibody.

67. A human or humanized antibody that binds to each one of KIR2DL1, -2, and -3, and which blocks KIR2DL1, -2, or -3 binding to an HLA-C class I molecule.

68. An antibody that interacts with residues M44 and F45 of KIR2DL1.

69. The antibody of aspect 68, which further interacts with one or more of KIR2DL1 residues L38, R41, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88.

70. An antibody that binds to KIR2DL1 exclusively within a region defined by the amino acid residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88.

71. An antibody that binds to KIR2DL1 and KIR2DL2/3 without interacting with amino acid residues outside the region defined by the residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88.

72. The antibody of any of aspects 67-71, which is human antibody 1-7F9.

73. An antibody fragment or antibody derivative comprising at least one light chain CDR of the antibody of any of the preceding aspects.

74. An antibody fragment or antibody derivative comprising at least two light chain CDRs of the antibody of any of the preceding aspects.

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75. An antibody fragment or antibody derivative comprising at least three light chain CDRs of the antibody of any of the preceding aspects.

76. An antibody fragment or antibody derivative comprising a VL region of the antibody of any of the preceding aspects.

77. An antibody fragment or antibody derivative comprising at least one heavy chain CDR of the antibody of any of the preceding aspects.

78. An antibody fragment or antibody derivative comprising at least two heavy chain CDRs of the antibody of any of the preceding aspects.

79. An antibody fragment or antibody derivative comprising at least three heavy chain CDRs of the antibody of any of the preceding aspects.

80. An antibody fragment or antibody derivative comprising a VH region of the antibody of any of the preceding aspects.

81. An antibody fragment or antibody derivative comprising all CDRs of the antibody of any of the preceding aspects.

82. An antibody fragment or antibody derivative comprising the VH and VL regions of the antibody of any of the preceding aspects.

83. A hybridoma producing the antibody of any of the preceding claims.

84. A nucleic acid encoding the antibody or antibody fragment of any of aspects 1-82.

85. A vector comprising the nucleic acid of aspect 84.

86. A cell comprising the vector of aspect 85.

87. The cell of aspect 86, wherein the cell is selected from a simian COS cell, a CHO cell, and a human myeloma cell.

88. A method of producing an anti-KIR antibody or antibody fragment comprising culturing the cell of aspect 86 under conditions suitable for expression of the anti-KIR antibody, antibody fragment, respectively.

89. A method of producing a derivative of an anti-KIR antibody or anti-KIR antibody fragment comprising providing an an anti-KIR antibody or antibody fragment and conjugating at least one derivative moiety thereto.

90. A method of treating a cancer in a subject, which method comprises administering to a subject suffering from cancer an effective amount of the antibody or antibody fragment or derivative of any of aspects 1-82.

91. The method of aspect 90, further comprising administering to the patient an agent selected from a chemotherapeutic, radiotherapeutic, anti-angiogenic, immunomodulatory, and hormonal agent.

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92. A method of treating a disease or disorder caused by a virus in a subject, which method comprises administering to a subject suffering from disease or disorder caused by a virus an effective amount of the antibody or antibody fragment or derivative of any of aspects 1-82.

93. A use of the antibody or antibody fragment or derivative of any of aspects 1-82 in the preparation of a medicament for treating cancer.

94. A use of the antibody or antibody fragment or derivative of any of aspects 1-82 in the preparation of a medicament for treating a disease or disorder caused by a virus.

95. A composition comprising the antibody or antibody fragment or derivative of any of aspects 1-82, and a pharmaceutically acceptable carrier or excipient.

96. The composition of aspect 95, comprising Polysorbate 80.

97. The composition of aspect 95, comprising Sucrose.

98. The composition of aspect 95, comprising Polysorbate 80 and Sucrose.

99. The composition of any of aspects 95-98, further comprising an agent selected from a chemotherapeutic, radiotherapeutic, anti-angiogenic, immunomodulatory, and hormonal agent.

100. The composition of any of aspects 95-99, in which the antibody, antibody fragment, or antibody derivative is covalently conjugated to a tumor-targeting agent.

101. The composition of any of aspects 95-99, in which the antibody, antibody fragment, or antibody derivative is encapsulated in a liposome.

102. The composition of any of aspects 95-99, further comprising an antibody binding to a KIR, wherein the KIR is not KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS1, or KIR2DS2.

103. A method of increasing the lytic activity of a cell selected from a lymphocyte and an NK cell comprising contacting the cell with the antibody, antibody fragment or derivative of any of aspects 1-82.

104. A method of reducing the interaction between a KIR expressed by a cell selected from a lymphocyte, T cell, and an NK cell, and an HLA-C class I molecule expressed by a target cell, the method comprising contacting the cell with the antibody or antibody fragment or derivative of any of aspects 1-82.

105. A method of neutralizing the inhibitory activity of a KIR expressed by an NK cell, the method comprising contacting the NK cell with the antibody or antibody fragment or derivative of any of aspects 1-82.

106. A human antibody that binds to each one of KIR2DL1, KIR2DL2, and KIR2DL3, which antibody blocks the binding of an HLA-Cw4 molecule to KIR2DL1, and the binding of an HLA-Cw3 molecule to at least one of KIR2DL2 or KIR2DL3.

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107. The antibody or antibody fragment or derivative according to any of aspects 1-82, wherein the KIR2DL1 comprises the amino acid sequence of SEQ ID NO:23, the KIR2DL2 comprises the amino acid sequence of SEQ ID NO:24, and/or the KIR2DL3 comprises the amino acid sequence of SEQ ID NO:25.

108. A compound that binds to substantially the same KIR2DL1 epitope as the antibody, antibody fragment, or antibody derivative of any of aspects 1-82.

109. A compound that binds to essentially the same KIR2DL1 epitope as the antibody, antibody fragment, or antibody derivative of any of aspects 1-82.

110. An antibody or antibody derivative comprising a light chain comprising the sequence of SEQ ID NO:36.

111. An antibody or antibody derivative comprising a heavy chain comprising the sequence of SEQ ID NO:37.

112. An antibody or antibody derivative comprising the light chain of aspect 109 and the heavy chain of aspect 110.

113. An antibody consisting of a light chain comprising the sequence of SEQ ID NO:36 and a heavy chain consisting of the sequence of SEQ ID NO:37.

* * *

All references, including publications, patent applications and patents, cited herein are hereby incorporated by reference to the same extent as if each reference was individually and specifically indicated to be incorporated by reference and was set forth in its entirety herein.

All headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way,

Any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

The terms "a" and "an" and "the" and similar referents as used in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein

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are representative of corresponding approximate values (*e.g.*, all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate).

All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context.

The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

The citation and incorporation of patent documents herein is done for convenience only and does not reflect any view of the validity, patentability and/or enforceability of such patent documents,

The description herein of any aspect or embodiment of the invention using terms such as "comprising", "having", "including" or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (*e.g.*, a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

This invention includes all modifications and equivalents of the subject matter recited in the aspects or claims presented herein to the maximum extent permitted by applicable law.

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CLAIMS

1. An isolated human antibody that binds to each one of KIR2DL1, KIR2DL2, and KIR2DL3, but which does not bind KIR2DS4.

2. The isolated human antibody of claim 1, which further does not bind to KIR2DS3.

3. The isolated human antibody of any of the preceding claims, which antibody blocks the binding of at least one of KIR2DL1, KIR2DL2, and KIR2DL3 to an HLA-C class I molecule.

4. The isolated human antibody of any of the preceding claims, which antibody blocks the binding of an HLA-Cw4 molecule to KIR2DL1, and the binding of an HLA-Cw3 molecule to at least one of KIR2DL2 or KIR2DL3.

5. The isolated human antibody of any of the preceding claims, which antibody potentiates the lytic activity of an NK cell against a human target cell expressing an HLA-C class I molecule.

6. The isolated human antibody of any of the preceding claims, which antibody competes with an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17, in the binding to at least one of KIR2DL1, KIR2DL2, and KIR2DL3.

7. The isolated human antibody of any of the preceding claims, which antibody competes with an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17, in the binding to each of KIR2DL1, KIR2DL2, and KIR2DL3.

8. The isolated human antibody of any of the preceding claims, which antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:15.

9. The isolated human antibody of any of the preceding claims, which antibody comprises

(a) a heavy chain CDR1 amino acid sequence corresponding to residues 31-35 of SEQ ID NO:17;

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(b) a heavy chain CDR2 amino acid sequence corresponding to residues 50-65 of SEQ ID NO:17; and

(c) a heavy chain CDR3 amino acid sequence corresponding to residues 99-112 of SEQ ID NO:17.

10. The isolated human antibody of any of the preceding claims, which antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17.

11. The isolated human antibody of any of the preceding claims, which antibody has a dissociation constant (K_d) for KIR2DL1 of no more than about 0.45 nM.

12. The isolated human antibody of any of the preceding claims, which antibody has a K_d for KIR2DL3 of no more than about 0.025 nM.

13. The isolated human antibody of any of the preceding claims, which antibody binds to a KIR2DL1 epitope substantially comprising the amino acid residues L38, R41, M44, F45, N46, D47, T48, L49, R50, I52, F64, D72, Y80, P87, and Y88.

14. The isolated human antibody of any of the preceding claims, which antibody blocks the binding of an HLA-Cw4 molecule to residues M44, F45 and D72 of the extracellular portion of KIR2DL1 (SEQ ID NO:23).

15. The isolated human antibody of any of the preceding claims, which antibody is a monoclonal antibody.

16. The isolated human antibody of any of the preceding claims, which antibody is an IgG1, IgG2, IgG3, or IgG4 antibody.

17. The isolated human antibody of any of the preceding claims, which antibody is an IgG4 antibody.

18. A nucleic acid encoding the human antibody of any of the preceding claims.

19. A vector comprising the nucleic acid of claim 18.

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20. A cell comprising the vector of claim 19.

21. A method of producing a human anti-KIR antibody, comprising culturing the cell of claim 20 under conditions suitable for expression of the anti-KIR antibody.

22. A pharmaceutical composition comprising the human antibody of any of claims 1-17, or the human antibody produced by the method of claim 21, in an amount effective to detectably potentiate NK cell cytotoxicity in a patient, and a pharmaceutically acceptable carrier or excipient.

23. The pharmaceutical composition of claim 22, comprising Polysorbate 80, sucrose, or both Polysorbate 80 and sucrose.

24. A method of potentiating NK cell activity in a subject in need thereof, which method comprises administering to the subject an effective amount of the composition of any of claims 22 to 23.

25. The method of claim 24, wherein the subject is a patient suffering from cancer.

26. The method of claim 25, wherein the patient is suffering from a cancer selected from acute myeloid leukaemia, chronic myeloid leukaemia, multiple myeloma, and non-Hodgkin's lymphoma.

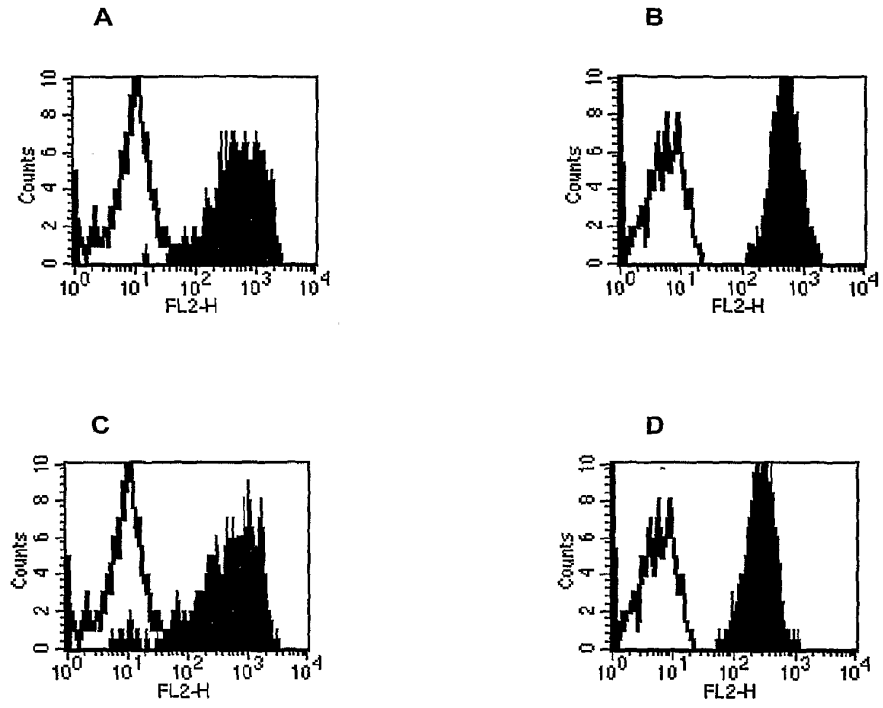
27. The method of claim 25, wherein the patient is suffering from a cancer selected from colorectal cancer, renal cancer, ovarian cancer, lung cancer, breast cancer, and malignant melanoma.

28. The method of claim 24, wherein the subject is a patient suffering from an infectious disease.

29. The method of any of claims 24 to 28, further comprising administering a therapeutic agent selected from an immunomodulatory agent, a hormonal agent, a chemotherapeutic agent, an anti-angiogenic agent, an apoptotic agent, a second antibody that binds to an inhibitory KIR, an anti-infective agent, a targeting agent, and an adjunct compound.

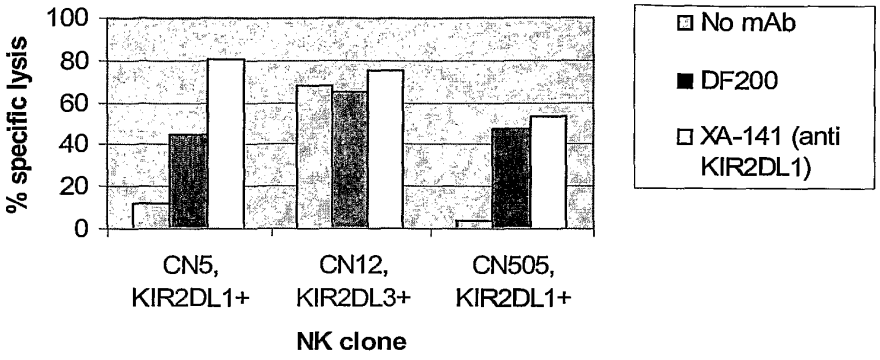
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FIGURE 1



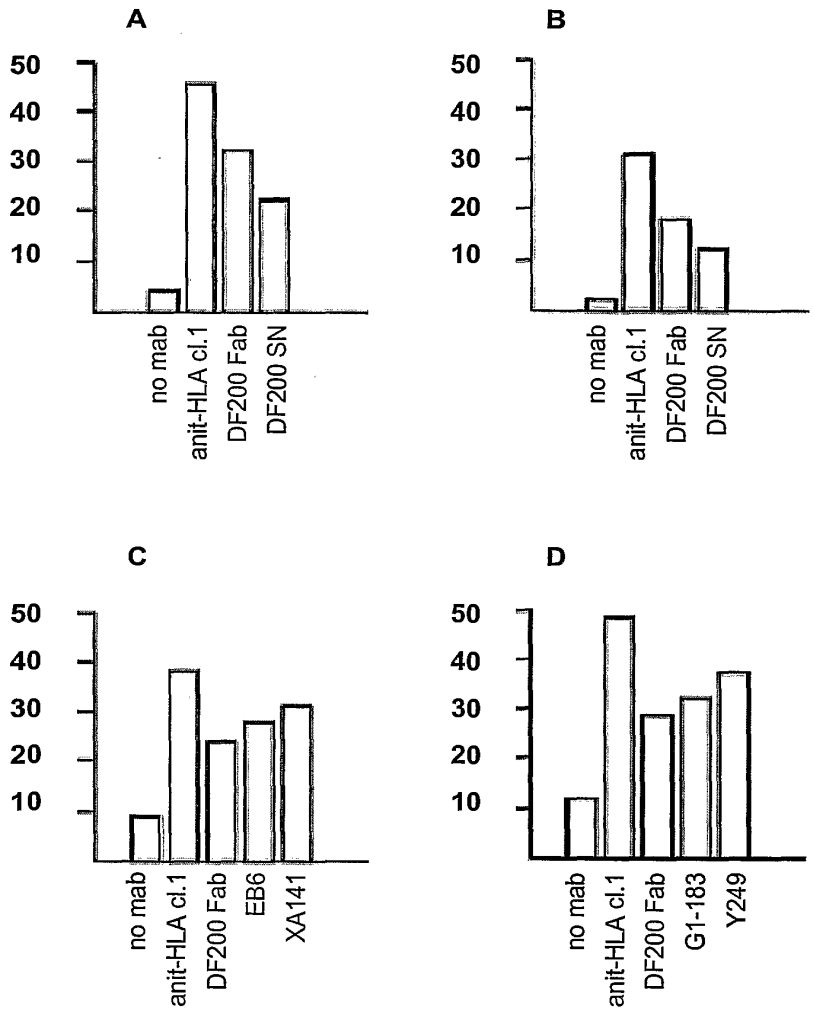
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FIGURE 2



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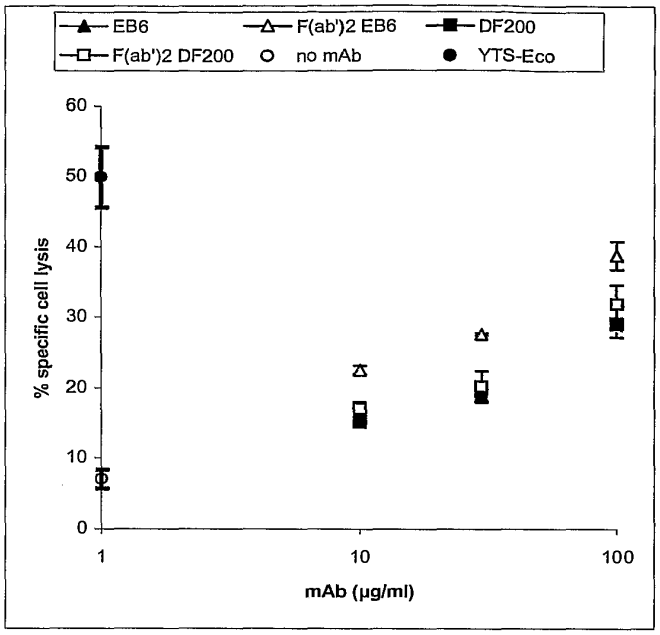
FIGURE 3



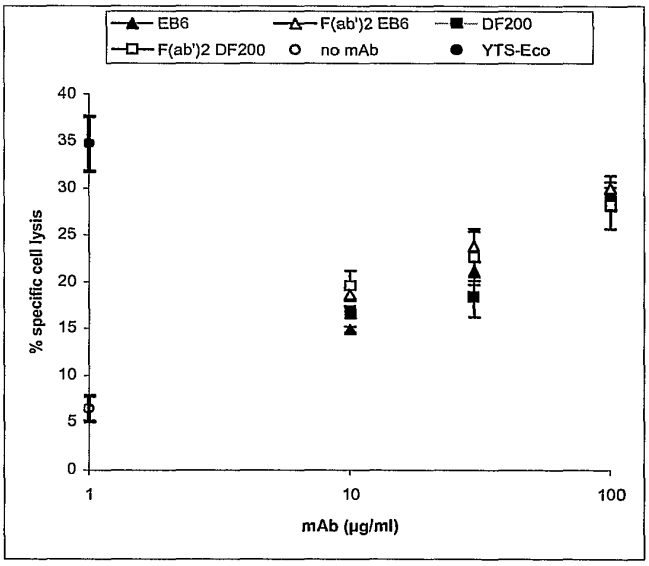
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Page 7018

FIGURE 4

A



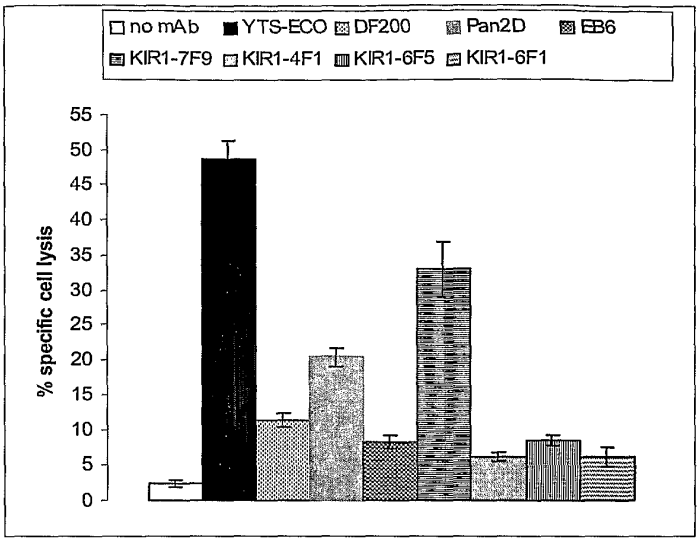
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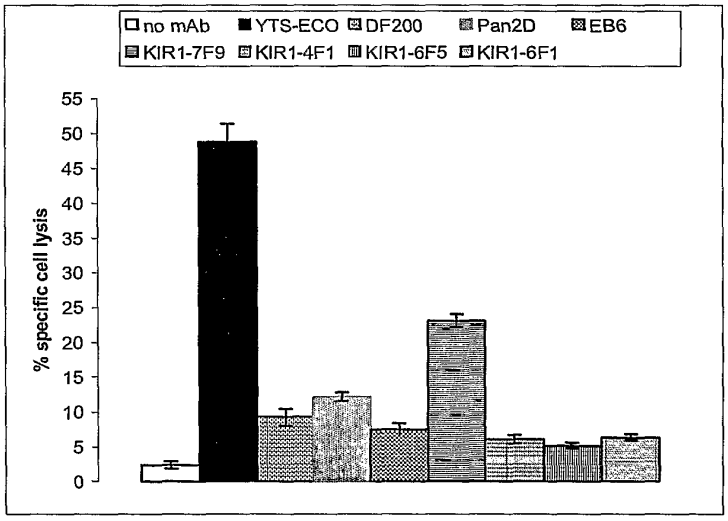
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FIGURE 5

A



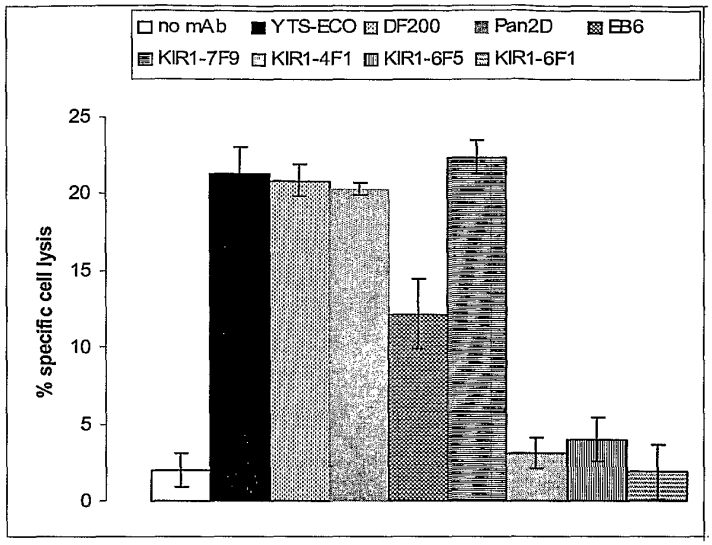
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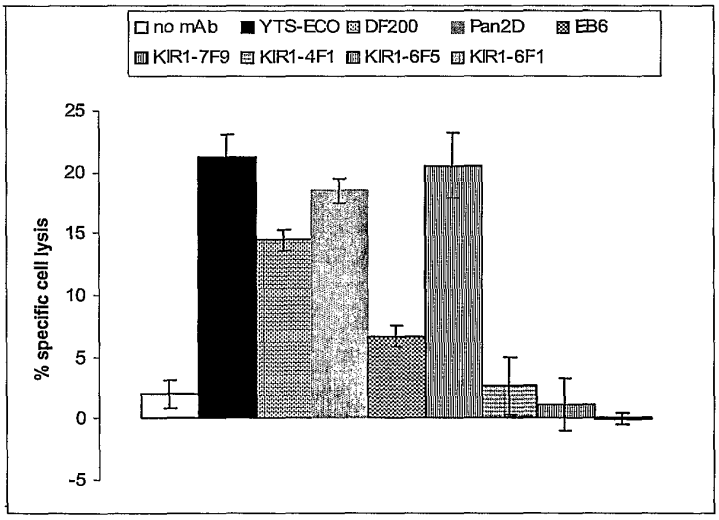
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FIGURE 6

A

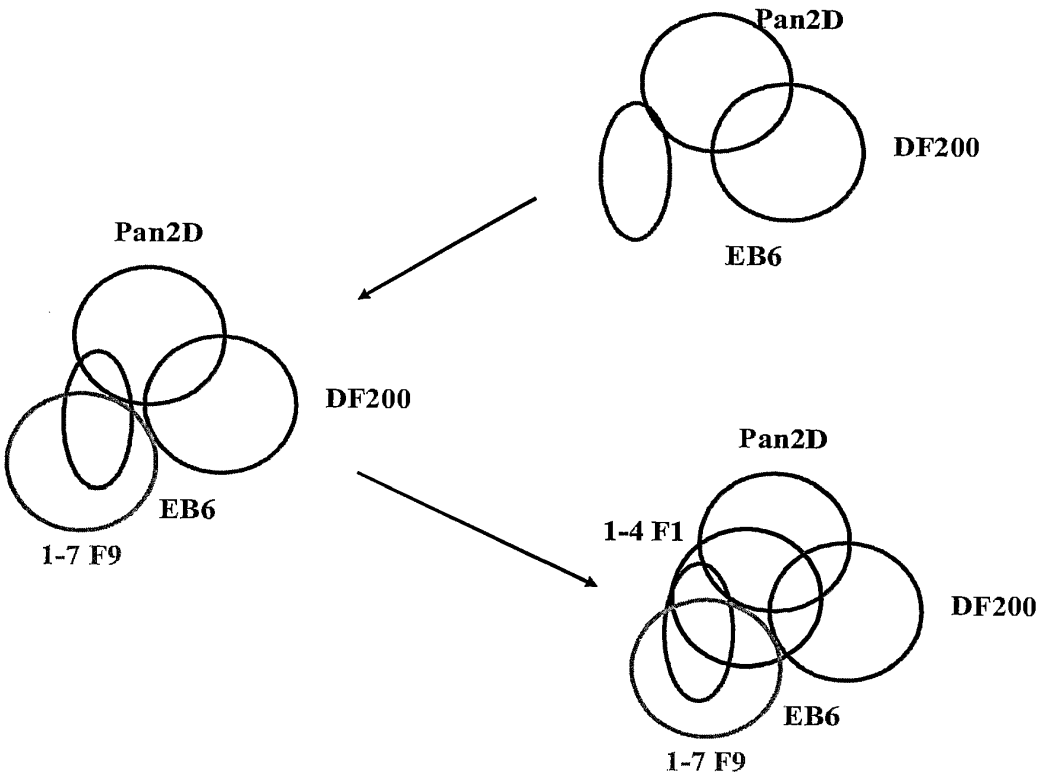


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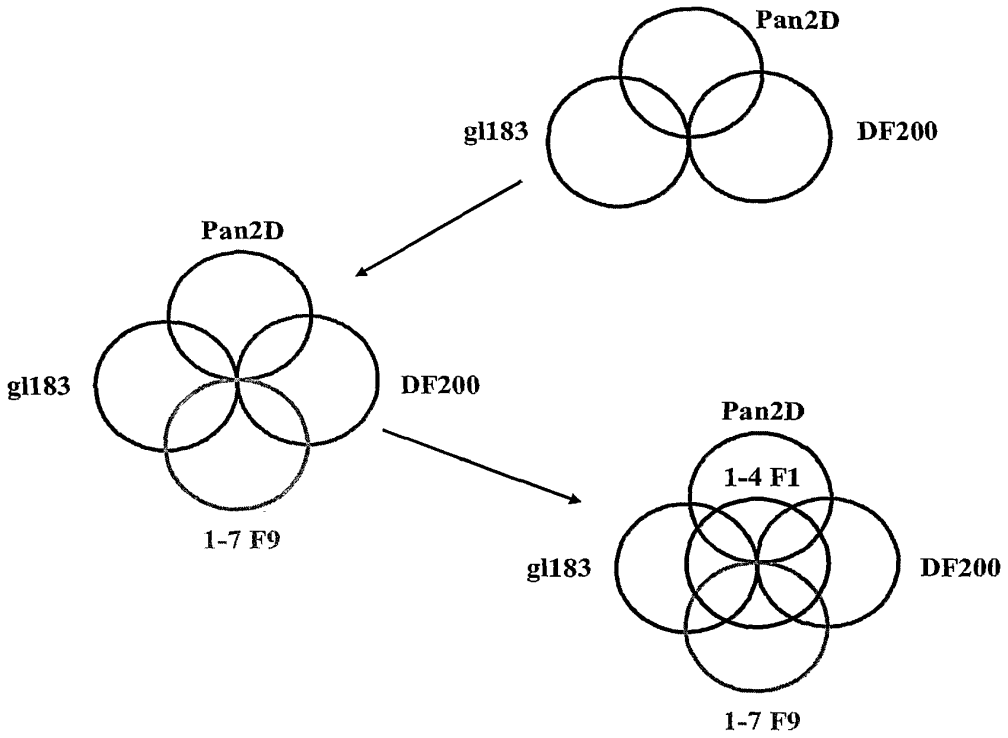
FIGURE 7



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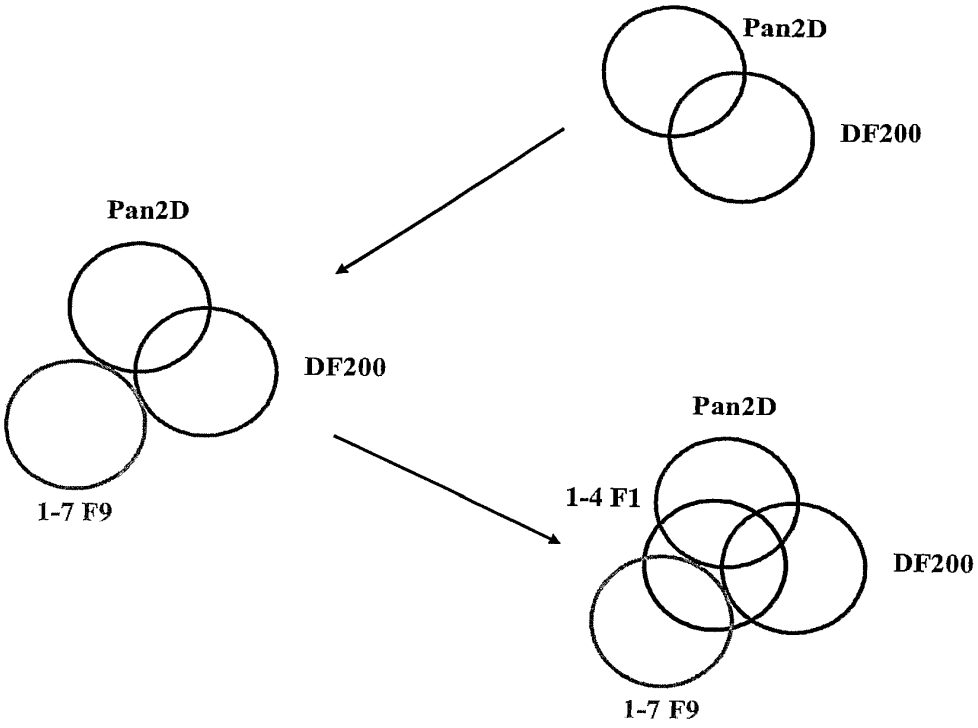
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FIGURE 8



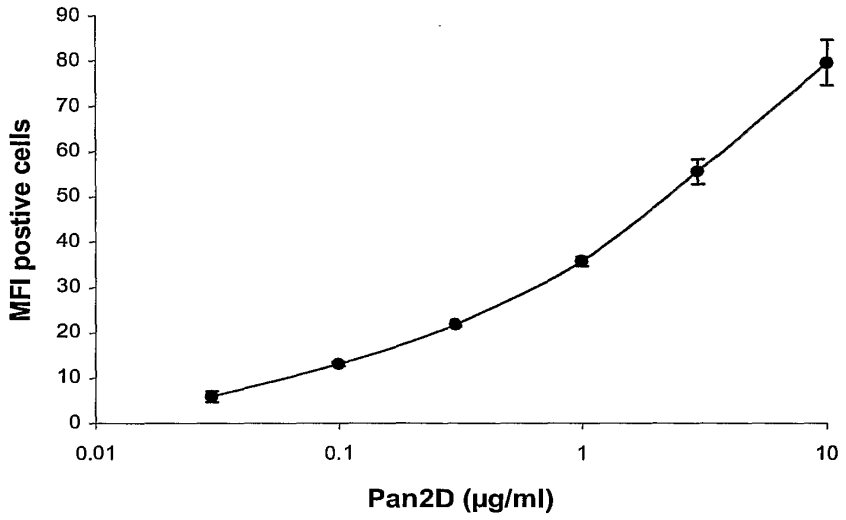
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FIGURE 9



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FIGURE 10

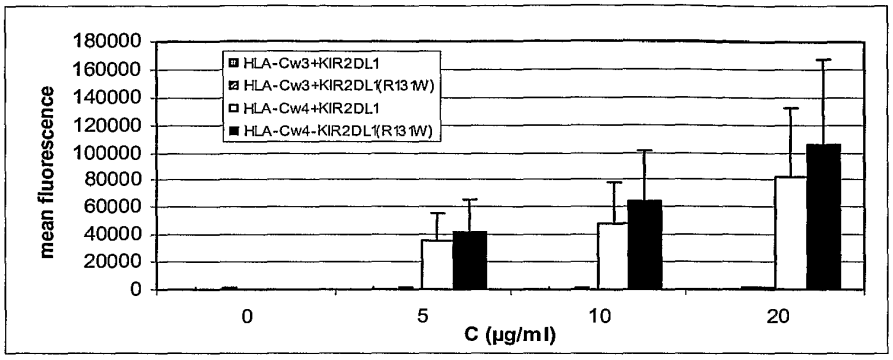


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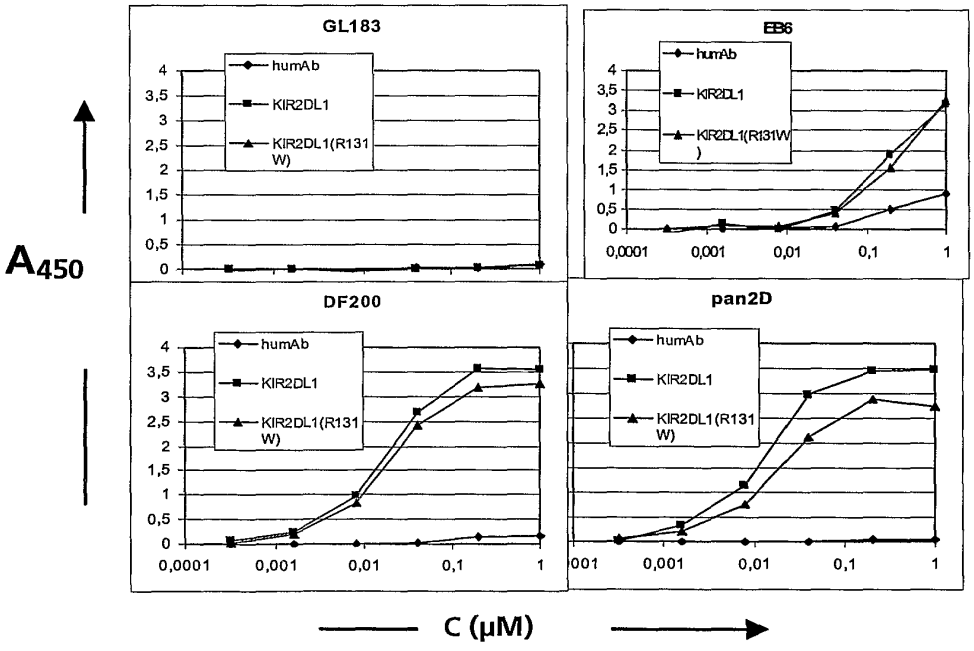
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FIGURE 11

A



B



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FIGURE 12

A

```
                          1                                                  50
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 PAN2D-Light-variable     (1) MDFQVQIFSFLLISASVIMSRGQIVLTQSPASMSASLGERVTMTCTASSS
           Consensus      (1)      Q   FI I   L  A GNIVLTQSP SMS SLGERVTLTC AS
                          51                                                 100
DF-200 light variable     (49) VVL-YVSWYQQKPEQSPKLLIYGASNRYLGVPDRFLGSGSATDFTLTISS
 PAN2D-Light-variable     (51) VSSSYIYWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGISYSLTISS
           Consensus      (51) V S YL WYQQKP  SPKL IY  SN  SGVP RFSGSGSAT FSLTISS
                              101                                131
DF-200 light variable     (98) VQAEDLADYHCGQGVSYPYTFGGGTKLEIKR
 PAN2D-Light-variable     (101)MEAEDAATYVCHQYHRSPPTFGGGTKLEIKR
           Consensus     (101) M AED A YHC Q H  P TFGGGTKLEIKR
```

B

```
DF-200 light variable    (44) KASENVVL-YVS (SEQ ID NO.3)
 PAN2D-Light-variable    (46) TASSSVSSSYIY (SEQ ID NO.4)
           Consensus         AS  V S YL
```

C

```
DF-200 light variable    (70) GASNRYT (SEQ ID NO.5)
 PAN2D-Light-variable    (73) STSNLAS (SEQ ID NO.6)
           Consensus         SN  S
```

D

```
DF-200 light variable    (109) GQGVSYPYT (SEQ ID NO.7)
 PAN2D-Light-variable    (112) HQYHRSPPT (SEQ ID NO.8)
           Consensus          Q H  P T
```


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FIGURE 13

DF200 VH

A

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B

GFSFTPYGVH (SEQ ID NO:10)

C

VIWSGGNTDYNAAFIS (SEQ ID NO:11)

D

NPRPGNYPYGMDY (SEQ ID NO:12)

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FIGURE 14

1-7F9 VL and VH

A

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NO:16)

C

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D

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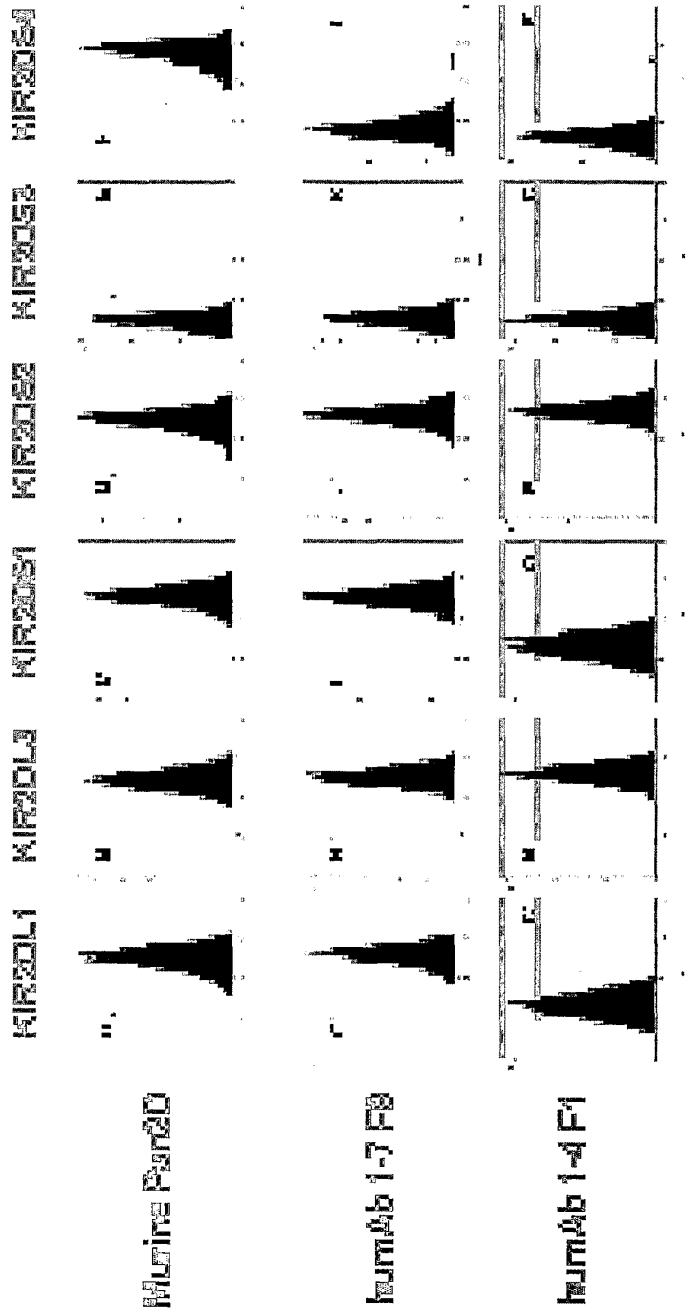
FIGURE 15

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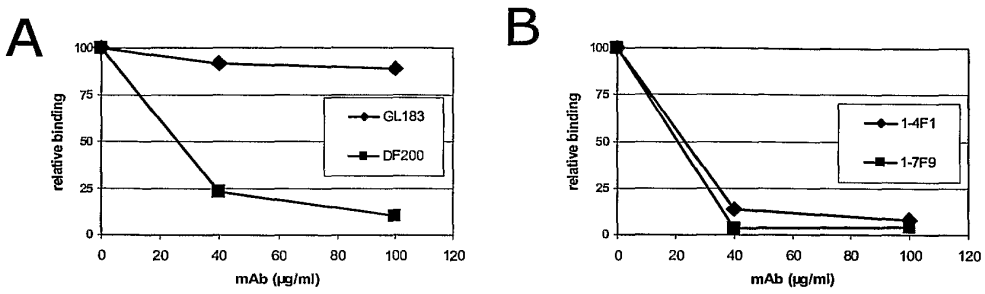
16/20

FIGURE 16



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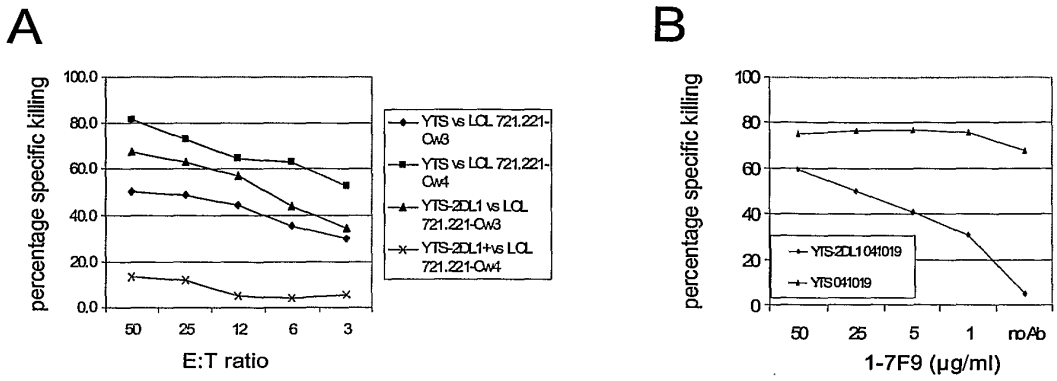
FIGURE 17



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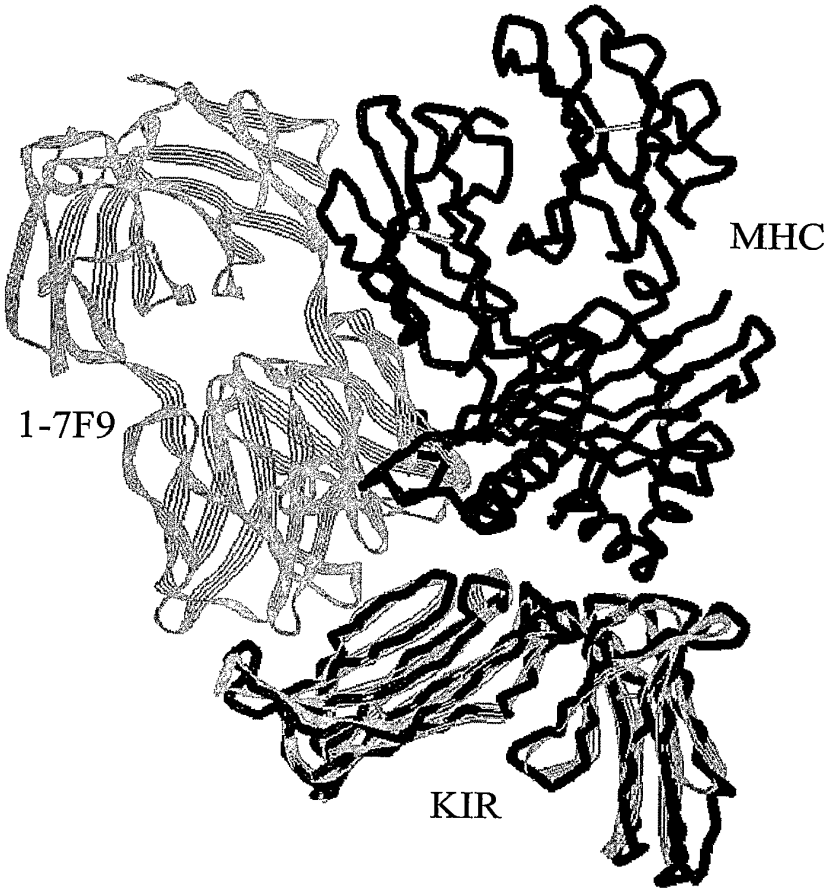
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FIGURE 18



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FIGURE 19



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FIGURE 20

```
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 51   LRGEHHDGVS KANFSISRMT QDLAGTYRCY GSVTHSFYQV SAPSDPLDIV
101   IIGLYEKPSL SAQLGPTVLA GENVTLSCSS RSSYDMYHLS REGEAHERRL
151   PAGPKVNGTF QADFPLGPAT HGGTYRCFGS FHDSPYEWSK SSDPLLVSVT
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35 40 45

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35 40 45

Gly Gly Phe Ile Pro Ile Phe Gly Ala Ala Asn Tyr Ala Gln Lys Phe

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35 40 45

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Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

Lys Met Asn Ser Leu Gln Val Asn Asp Thr Ala Ile Tyr Tyr Cys Ala

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Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val Tyr Tyr Cys
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35 40 45

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Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala
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35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
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Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
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Met Phe Glu His Phe Leu Leu His Arg Glu Gly Met Phe Asn Asp Thr
        35                  40                  45

Leu Arg Leu Ile Gly Glu His His Asp Gly Val Ser Lys Ala Asn Phe
    50                  55                  60

Ser Ile Ser Arg Met Thr Gln Asp Leu Ala Gly Thr Tyr Arg Cys Tyr
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Gly Ser Val Thr His Ser Pro Tyr Gln Val Ser Ala Pro Ser Asp Pro
                85                  90                  95

Leu Asp Ile Val Ile Ile Gly Leu Tyr Glu Lys Pro Ser Leu Ser Ala
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Ala His Glu Arg Arg Leu Pro Ala Gly Pro Lys Val Asn Gly Thr Phe
145                 150                 155                 160

Gln Ala Asp Phe Pro Leu Gly Pro Ala Thr His Gly Gly Thr Tyr Arg
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Cys Phe Gly Ser Phe His Asp Ser Pro Tyr Glu Trp Ser Lys Ser Ser
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20 25 30

Arg Phe Glu His Phe Leu Leu His Arg Glu Gly Lys Phe Lys Asp Thr
35 40 45

Leu His Leu Ile Gly Glu His His Asp Gly Val Ser Lys Ala Asn Phe
50 55 60

Ser Ile Gly Pro Met Met Gln Asp Leu Ala Gly Thr Tyr Arg Cys Tyr
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Gly Ser Val Thr His Ser Pro Tyr Gln Leu Ser Ala Pro Ser Asp Pro
85 90 95

Leu Asp Ile Val Ile Thr Gly Leu Tyr Glu Lys Pro Ser Leu Ser Ala
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Gln Pro Gly Pro Thr Val Leu Ala Gly Glu Ser Val Thr Leu Ser Cys
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Ser Ser Arg Ser Ser Tyr Asp Met Tyr His Leu Ser Arg Glu Gly Glu
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Ala His Glu Cys Arg Phe Ser Ala Gly Pro Lys Val Asn Gly Thr Phe
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165 170 175

Cys Phe Gly Ser Phe Arg Asp Ser Pro Tyr Glu Trp Ser Asn Ser Ser
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<210> 25
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<213> Homo sapiens

<400> 25

His Glu Gly Val His Arg Lys Pro Ser Leu Leu Ala His Pro Gly Pro
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Leu Val Lys Ser Glu Glu Thr Val Ile Leu Gln Cys Trp Ser Asp Val
20 25 30

Arg Phe Gln His Phe Leu Leu His Arg Glu Gly Lys Phe Lys Asp Thr
35 40 45

Leu His Leu Ile Gly Glu His His Asp Gly Val Ser Lys Ala Asn Phe
50 55 60

Ser Ile Gly Pro Met Met Gln Asp Leu Ala Gly Thr Tyr Arg Cys Tyr
65 70 75 80

Gly Ser Val Thr His Ser Pro Tyr Gln Leu Ser Ala Pro Ser Asp Pro
85 90 95

Leu Asp Ile Val Ile Thr Gly Leu Tyr Glu Lys Pro Ser Leu Ser Ala
100 105 110

Gln Pro Gly Pro Thr Val Leu Ala Gly Glu Ser Val Thr Leu Ser Cys
115 120 125

Ser Ser Arg Ser Ser Tyr Asp Met Tyr His Leu Ser Arg Glu Gly Glu
130 135 140

Ala His Glu Arg Arg Phe Ser Ala Gly Pro Lys Val Asn Gly Thr Phe
145 150 155 160

Gln Ala Asp Phe Pro Leu Gly Pro Ala Thr His Gly Gly Thr Tyr Arg
165 170 175

Cys Phe Gly Ser Phe Arg Asp Ser Pro Tyr Glu Trp Ser Asn Ser Ser
180 185 190

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Page 7046

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35 40 45

Lys Ala Ser Thr Leu Lys Thr Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Gly Leu Gln Phe Asp
65 70 75 80

Asp Phe Ala Thr Tyr His Cys Gln His Tyr Ala Gly Tyr Ser Ala Thr
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Phe Gly Gln Gly Thr Arg Val Glu Ile Lys Arg Thr
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20 25 30

Thr Met Asn Trp Val Arg Arg Val Pro Gly Gly Gly Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Thr Ser Ser Thr Tyr Arg Asp Tyr Ala Asp Ala Val

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50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asp Leu Glu Asp Phe Val Tyr
65 70 75 80

Leu Gln Met His Lys Met Arg Val Glu Asp Thr Ala Ile Tyr Tyr Cys
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Ala Arg Lys Gly Ser Asp Arg Leu Ser Asp Asn Asp Pro Phe Asp Ala
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Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Met Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

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Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
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Phe Asn Arg Gly Glu Cys
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Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
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Gly Gly Phe Ile Pro Ile Phe Gly Ala Ala Asn Tyr Ala Gln Lys Phe
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Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ile Pro Ser Gly Ser Tyr Tyr Tyr Asp Tyr Asp Met Asp Val
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Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
    130                 135                 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190
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Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val
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Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys
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Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly
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Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
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Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
        435                 440                 445

Gly Lys
    450
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<212> PRT
<213> Homo sapiens

<400> 38

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35 40 45

Leu His Leu Ile Gly Glu His His Asp Gly Val Ser Lys Ala Asn Phe
50 55 60

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35 40 45

Tyr Xaa Ala Ser Ser Leu Xaa Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Xaa Thr Leu Xaa Ile Ser Ser Leu Gln Pro
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gnnaaagcnc ctaagctcnt natctatnat gcntccagtt tnnaaagtgg ggtcccatca 180

aggttcagcg gcagtggatc tgggacngat tncactctcn ccatcagcag cctgcagcct 240

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20 25 30

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35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

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Gln Gly Arg Val Thr Ile Ser Ala Asp Glu Ser Thr Arg Thr Val Tyr
65 70 75 80

Met Glu Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

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cctggacaag ggcttgagtg gatgggaggg atcatcccta tatttggtac agcaaactac 180

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atggagctga acagtctgag atctgaggat acggccgtgt attactgtgc gagaggatat 300

tacgatattt tcaaggacta ctattacggt atggacgtct ggggccaagg gaccacggtc 360

accgtctcct ca 372

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Pergamon
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HUMANIZED PORCINE VCAM-SPECIFIC MONOCLONAL ANTIBODIES WITH CHIMERIC IgG2/G4 CONSTANT REGIONS BLOCK HUMAN LEUKOCYTE BINDING TO PORCINE ENDOTHELIAL CELLS

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Abstract—Discordant xenografts surviving the initial hyperacute rejection phase may be subject to cellular rejection processes mediated by infiltrating leukocytes including T cells, NK cells and monocytes. The stable adhesion of these cell types to endothelial cells is due to the molecular interaction of the integrins VLA-4 and LFA-1 with their ligands vascular cell adhesion molecule (VCAM) and ICAM-1 present on the endothelial cells. Human VLA-4 binds to porcine VCAM, and blocking mAbs specific for porcine VCAM have been developed. We have localized the epitope of the anti-porcine VCAM blocking mAbs 2A2 and 3F4 to domains 1 and 2, respectively. Humanized antibodies (IgG4 isotype) were constructed from these anti-porcine VCAM antibodies and demonstrated to inhibit adhesion of Ramos, Jurkat and YT cells, as well as purified resting and activated human T cells, to porcine aortic endothelial cells (PAEC). These cell types express both LFA-1 as well as VLA-4, suggesting blockade of human VLA-4 interaction with porcine VCAM may alone be sufficient to significantly impair adhesion of human leukocytes to porcine endothelial cells. The chimeric anti-porcine VCAM (pVCAM) HuG4 antibodies promoted increased adhesion of Fc receptor (FcR) positive cells such as U937 monocytic cells to PAEC. In contrast, chimeric anti-porcine VCAM antibodies created using the CH1 and hinge region from human IgG2 and the CH2 and CH3 regions from human IgG4 (HuG2/G4 antibodies) inhibited binding of FcR positive cells to PAEC. These chimeric anti-pVCAM antibodies should allow delineation of the *in vivo* role of VLA-4/VCAM interaction in porcine-to-primate xenotransplants. Further, the design of the HuG2/G4 antibodies should render them efficacious in multiple settings requiring elimination of FcR binding. © 1997 Elsevier Science Ltd.

Key words: adhesion molecules, antibodies, Fc receptors, inflammation, xenotransplantation.

INTRODUCTION

Although much progress has been made in elucidating the molecular mechanisms of hyperacute rejection of xenotransplants (reviewed in Somerville and D'Apice, 1993), the mechanisms of cell mediated rejection of xenografts remain unclear. The cellular infiltrate observed 3 days following transplantation of guinea pig hearts into complement (C)-depleted rats is comprised predominantly of macrophages and natural killer cells, with low numbers of T lymphocytes present (Blakely *et al.*, 1994). Similarly, the cellular infiltrate present 6 days after transplantation of porcine fetal islet clusters into rats is comprised predominantly of macrophages with low numbers of T lymphocytes present primarily at the periphery of the graft (Wallgren *et al.*, 1995). *In vitro* porcine aortic endothelial cells (PAEC) as well as antigen presenting peripheral blood cells can directly activate human CD4+ and CD8+ T lymphocytes (Rollins *et al.*, 1994*a*; Murray *et al.*, 1994; Yamada *et al.*, 1995). Thus the initial phases of cellular rejection of xenotransplants are likely to involve binding and activation of multiple types of leukocytes including not only T lymphocytes but also natural killer cells and monocytes.

The stable adhesion of lymphocytes, natural killer cells, and monocytes to endothelial cells is mediated predominantly by the interaction of the integrins LFA-1 (CD11a/CD18) and VLA-4 (CD49d/CD29) with their cognate ligands ICAM-1 and VCAM-1 on endothelial cells (Allavena *et al.*, 1991; Jonjic *et al.*, 1992). For both monocytes (Luscinskas *et al.*, 1994) and T lymphocytes (Alon *et al.*, 1995; Luscinskas *et al.*, 1995) the binding of VLA-4 to VCAM is an essential component of the *in vitro* adhesive interactions required for stable arrest under

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conditions of flow. *In vitro*, the transmigration of NK cells and monocytes across human umbilical vein endothelial cell (HUVEC) monolayers also utilizes both LFA-1/ICAM-1- and VLA-4/VCAM-dependent pathways (Bianchi *et al.*, 1993; Meershaert and Furie, 1994; Chuluyan *et al.*, 1995). *In vivo*, the interaction of LFA-1 with ICAM-1 may not be completely essential for extravasation of lymphocytes or monocytes since both of these cell types are present in inflammatory infiltrates of leukocyte adhesion deficiency patients who have a genetic deficiency in CD18 and thus lack integrins such as LFA-1 (Hawkins *et al.*, 1992). Finally, numerous studies have demonstrated the potential of blocking antibodies directed against LFA-1 (Issekutz, 1992; Scheynius *et al.*, 1993), VLA-4 (Issekutz, 1991; Yednock *et al.*, 1992; Chisholm *et al.*, 1993), ICAM-1 (Zeng *et al.*, 1994), or VCAM (Orosz *et al.*, 1993; Silber *et al.*, 1995) to inhibit the accumulation of lymphocytes at inflammatory sites in animals.

For xenogeneic cellular interactions it is not yet fully defined which adhesion molecule interactions will function across species barriers. It is likely that at least some xenogeneic receptor–ligand interactions will be nonfunctional. For example, mouse LFA-1 does not bind to human ICAM-1 (Johnston *et al.*, 1990). To explore the molecular basis of adhesive interactions between distinct classes of adhesion molecules on human and porcine cells we have cloned porcine E-selectin and VCAM-1 and have demonstrated that both are capable of mediating the adhesion of human leukocytes (Rollins *et al.*, 1994*a*; Mueller *et al.*, 1995). Further, we have developed murine monoclonal antibodies (mAbs) specific for porcine VCAM which block binding of human VLA-4 to porcine VCAM (Mueller *et al.*, 1995). The functional blocking activity of these antibodies suggests that they will be useful in suppressing the cellular response to a porcine xenograft. However, murine mAbs are not ideally suited for use in primates due to their short half-life and high immunogenicity (Khazaeli *et al.*, 1994). Here we have utilized antibody engineering strategies to create humanized anti-porcine VCAM antibodies that block adhesion of human natural killer cells, monocytes, and T lymphocytes to activated PAEC. Furthermore, we show the choice of human isotype has critical implications for the potential therapeutic use of humanized antibodies in xenotransplantation.

MATERIALS AND METHODS

Cells

Ramos, Jurkat and U-937 cells were obtained from the American Type Culture Collection, YT cells were the gift of Dr J. M. Wang (National Cancer Institute, Frederick, MD, U.S.A.), porcine aortic endothelial cells (PAEC) were obtained from Cell Systems (Kirkland, WA, U.S.A.) and 293-EBNA cells were obtained from Invitrogen (San Diego, CA, U.S.A.). U-937 cells were maintained in RPMI 1640 supplemented with 15% heat-inactivated FBS and 2 mM L-glutamine. Ramos and Jurkat cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 20 mM HEPES, 50 μM β-mercaptoethanol, 100 units/ml penicillin and 100 μg/ml streptomycin. YT were maintained in RPMI 1640 containing 10% heat-inactivated FBS, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. PAEC were maintained in medium A consisting of M199 supplemented with 20% heat-inactivated FBS, 0.05 mg/ml ECGF (Biomedical Technologies, Stoughton, MA, U.S.A.), 20 mM HEPES and 0.1 mg/ml heparin, and were used within three passages. 293-EBNA cells were grown in Dulbecco's modified Eagle's medium supplemented with 5% heat-inactivated FBS, 100 units/ml penicillin, 100 μg/ml streptomycin and 250 μg/ml G418. Human T cells were purified as described previously (Mueller *et al.*, 1995).

Production of VCAM/Ig fusions

A plasmid containing the human IgG1 Fc region was obtained from Dr M. J. Lenardo (NIH, Bethesda, MD, U.S.A.). DNA fragments containing various domains of human or porcine VCAM were obtained by PCR amplification of appropriate templates and joined in frame to the human IgG1 Fc coding region using standard molecular biology techniques. The cassettes encoding the fusion proteins were ligated into the expression plasmid pAPEX-3 (Evans *et al.*, 1995*a*). VCAM/Ig fusion proteins were produced by transfection of 293-EBNA cells as described previously (Evans *et al.*, 1995*a*). Three days following transfection the media were harvested, centrifuged to remove debris, and stored at 4°C. For ELISA, PolySorp 96-well microtiter plates (Nunc, Naperville, IL, U.S.A.) were coated overnight at 4°C with 100 ng/well goat anti-human IgG Fc (Sigma, St. Louis, MO, U.S.A.) in 0.1 M carbonate buffer, pH 9.6. The plates were then washed three times with PBS supplemented with 0.5% Tween 20 and incubated with blocking buffer (PBS supplemented with 0.05% Tween 20) at 37°C for 1 hr. After three further washes, various dilutions of conditioned media in blocking buffer were added and incubated at 37°C for 1 hr. Following three further washes, 2A2 or 3F4 mAb in blocking buffer was added and incubated for 1 hr at 37°C. After additional washing, peroxidase-conjugated goat anti-mouse IgG Fc (Sigma) diluted 1:2000 in blocking buffer was added and incubated at 37°C for 1 hr. After three final washes the plate was developed for 15 min with 0.4 mg/ml *o*-phenylenediamine dihydrochloride (Sigma). Reactions were stopped by addition of 1 M sulfuric acid. Quantitation was performed by measuring absorbance at 490 nm using a Bio-Rad model 3550 plate reader.

Production of chimeric antibodies

Variable regions were cloned from the 2A2 and 3F4 hybridomas by PCR amplification as described previously (Evans *et al.*, 1995*a*, 1995*b*). These sequences have been submitted to the EMBL/GenBank/DDBL under accession numbers U78799–U78802. The variable regions were joined to genomic DNA cassettes containing

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either the intact human IgG4 constant regions or the first constant region and hinge linker region from human IgG2 followed by the second and third constant regions from human IgG4 using standard molecular biology techniques. Chimeric antibodies were produced in 293-EBNA cells and purified by chromatography on protein A Sepharose as described (Evans *et al.*, 1995*a*). Each chimeric antibody preparation was greater than 95% pure as judged by Coomassie staining of SDS polyacrylamide gels. F(ab')2 was prepared by digestion of the antibodies with ficin (Pierce, Rockford, IL, U.S.A.) followed by protein A chromatography.

Flow cytometry

Cells used were obtained by trypsinization (PAEC and 293-EBNA) or centrifugation (Ramos, Jurkat, YT and U-937 cells). Harvested cells were washed once with 4°C PBS containing 1% heat-inactivated FBS (PBS/1) and aliquoted at 50 000–100 000 cells per well in 96-well V-bottom plates (Nunc). Cells were washed two additional times with PBS/1, and then incubated with PBS/1 containing 10 μg/ml primary Ab or PBS/1 alone for 30 min at 4°C. For some experiments the chimeric HuG4 or HuG2/G4 was centrifuged at $1 \times 10^5 g$ for 1 hr prior to addition to the cells. The cells were then washed twice with PBS/1 and resuspended in PBS/1 containing the appropriate FITC-conjugated secondary antibodies (1:200 dilution) for 30 min at 4°C. The cells were again washed twice with PBS/1 and resuspended in PBS. Cell fluorescence was determined using a Becton Dickinson FACSort.

Adhesion assays

On day 0 PAEC were plated in medium A at a density of 10 000 cells per well in a 96-well flat bottom plate. On day 1 the PAEC were refed medium A supplemented with 1 μg/ml LPS (Sigma, *E. coli* 0111:B4). On day 2, human cells were resuspended in the appropriate growth medium supplemented with 10 μM calcein AM (Molecular Probes, Eugene, OR, U.S.A.) and incubated at 37°C for 30 min. The cells were subsequently washed three times with growth medium and resuspended at 6×10^6 cells/ml in RPMI 1640 supplemented with 1% heat-inactivated FBS (RPMI/1). The PAEC were washed twice with RPMI/1 and refed 50 μl/well of RPMI/1 containing the indicated concentrations of antibodies. After adding calcein-labeled cells at a density of 3×10^5 cells/well (50 μl), the plate was centrifuged at 200*g* for 1 min and incubated at 37°C for 15 min. The nonadherent cells were removed by washing three to five times with RPMI/1. Adherent cells were lysed with HBSS containing 1% SDS and calcein fluorescence was measured with a Millipore CytoFluor 2350 using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Background fluorescence was determined from wells containing PAEC that did not receive calcein-labeled cells. Samples were performed in duplicate in each experiment. Adherence was determined by comparing the residual fluorescence concentration in each well to the input fluorescence concentration. In each experiment the specific fluorescence signal obtained in the absence of competing antibody was defined as 100%.

For adhesion assays using purified soluble pVCAM (Mueller *et al.*, 1995), PolySorp 96-well microtiter plates (Nunc) were coated overnight at 4°C with 200 ng/well purified soluble pVCAM in 0.1 M carbonate buffer, pH 9.6. After blocking the plate with RPMI 1640 supplemented with 10 mg/ml BSA (RPMI/BSA), the plates were incubated with RPMI/BSA containing antibody for 1 hr at 37°C. Subsequently, 3×10^5 calcein-labeled U-937 cells in RPMI/BSA were added to each well. The plate was centrifuged upright for 1 min at 200*g* and incubated at 37°C for 15 min. The plate was then centrifuged inverted at 200*g* for 3 min. Adherent cells were lysed by the addition of HBSS containing 1% SDS and calcein fluorescence was measured with a Millipore CytoFluor 2350 using an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

RESULTS

Identification of porcine VCAM domains recognized by blocking mAbs

Previously, we have identified two murine mAbs (3F4 and 2A2) directed against porcine VCAM (pVCAM) which block VLA-4 dependent adhesion of peripheral blood human T cells to PAEC. These two mAbs are specific for pVCAM and do not recognize human VCAM (hVCAM). Here an initial analysis of the epitope specificity of the 2A2 and 3F4 mAbs was performed using fusion proteins consisting of various domains of pVCAM or hVCAM joined to the Fc region of human IgG1. The VCAM/Ig fusion proteins were produced in 293-EBNA cells and assayed for binding of 2A2 and 3F4 mAbs by ELISA (Fig. 1). Both the 2A2 and 3F4 mAbs bound efficiently to proteins containing the first three domains of pVCAM. The 2A2 mAb bound to fusion proteins containing pVCAM domain 1 such as pD1 + pD2 (porcine domain 1 plus porcine domain 2) and pD1 + hD2 (porcine domain 1 plus human domain 2), but did not bind to fusion proteins lacking pVCAM domain 1 such as pD2 + pD3, hD1 + pD2, or hD1 + hD2. Weak binding of the 2A2 mAb could also be detected to a fusion protein containing only porcine domain 1 (pD1) but not to fusion proteins containing only pD2 or pD3. Conversely, the 3F4 mAb bound to fusion proteins containing pVCAM domain 2, such as pD1 + pD2, pD2 + pD3, and hD1 + pD2, but did not bind to fusion proteins lacking pVCAM domain 2, such as pD1 + hD2 and hD1 + hD2. Binding of the 3F4 mAb could not be detected to fusion proteins containing only a single pVCAM domain. The weak binding of 2A2 and 3F4 to fusion proteins containing only a single VCAM domain is similar to previously characterized blocking antibodies specific for human VCAM and ICAM-1 which require the presence of at least two adhesion molecule domains for binding (Pepinsky *et al.*, 1992; Renz *et al.*, 1994). Together, these results suggested the 2A2 mAb specificity is defined by

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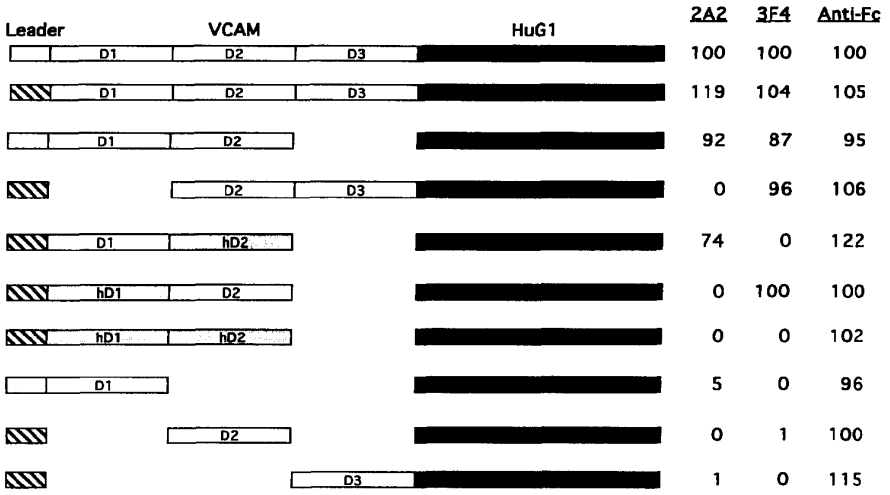


Fig. 1. Binding of 3F4 and 2A2 mAbs to VCAM/Ig fusion proteins. Proteins consisting of domains 1 (D1), 2 (D2), or 3 (D3) from porcine VCAM (open boxes) or human VCAM (gray boxes) joined to the Fc region of human IgG1 (black boxes) were produced in 293-EBNA cells. In some constructs the pVCAM secretory leader sequence was replaced with the secretory leader sequence from human CD59 (striped boxes). The fusion proteins were captured by incubating diluted conditioned media in 96-well plates coated with goat anti-human IgG (Fc specific). After incubation with 2A2 or 3F4 mAb, bound murine mAb was detected using peroxidase-conjugated goat anti-mouse IgG (Fc specific). The total amount of each fusion protein captured per well was quantitated using peroxidase-conjugated goat anti-human IgG (Fc specific). The absorbance signal obtained with the fusion protein containing the intact pVCAM domains 1, 2 and 3 was defined as 100% for each antibody. Binding of the 2A2 and 3F4 mAbs to the remaining fusion proteins is reported as a percentage of this value. Results shown are from a representative experiment of several performed.

pVCAM domain 1 whereas the 3F4 mAb specificity is defined by pVCAM domain 2.

Inhibition of human leukocyte binding to PAEC by chimeric anti-porcine VCAM mAbs

The use of murine antibodies in porcine-to-primate xenotransplantation models or ultimately in porcine-to-human xenotransplants poses several problems including a short half-life of the murine antibody plus development of an immune response directed against the murine antibody. These problems have been significantly reduced by the use of chimeric antibodies consisting of the murine variable regions joined to human constant regions (Khazaeli *et al.*, 1994). To create anti-pVCAM chimeric antibodies we cloned the variable regions from both 2A2 and 3F4. The murine variable heavy regions were joined to the human IgG4 constant region (HuG4) since this isotype does not activate complement (C) (Tao *et al.*, 1993), the predominant mediator of hyperacute rejection of xenografts. The murine variable light regions were joined to the human kappa constant region. Chimeric ch2A2 HuG4 and ch3F4 HuG4 antibodies were produced by coexpression of the chimeric heavy and light chains in 293-EBNA cells and tested for binding to pVCAM by FACS analysis using 293 cells expressing pVCAM (Fig. 2(A)). Both chimeric mAbs produced identical staining profiles as the corresponding murine mAbs, while neither chimeric mAb bound to untransfected 293 cells. Additionally, both the chimeric antibodies and murine mAbs produced identical FACS profiles using LPS-activated porcine aortic endothelial cells (Fig. 2(B)), confirming the chimeric antibodies retained the ability to bind to pVCAM expressed on PAEC.

The ability of the ch2A2 HuG4 and ch3F4 HuG4 antibodies to block the interaction between human VLA-4 and porcine VCAM was assessed by measuring inhibition of Ramos cell binding to activated PAEC (Fig. 3). The murine 3F4 mAb inhibited this binding by 90% when present at concentrations greater than 5 μg/ml. A control humanized antibody directed against human C5 (h5G1.1 HuG4) did not inhibit the binding of Ramos to activated PAEC. The ch2A2 HuG4 and ch3F4 HuG4 produced nearly identical inhibition curves as the murine 3F4 mAb, demonstrating the retention of binding avidity and cell adhesion blocking capabilities in these chimeric antibodies.

The ability of the ch2A2 HuG4 and ch3F4 HuG4 mAbs to inhibit binding of cells expressing both VLA-4 and LFA-1 was next examined using the Jurkat T cell line. Both the ch2A2 HuG4 and ch3F4 HuG4 antibodies inhibited the binding of Jurkat to activated PAEC by up to 90% while the control antibody h5G1.1 HuG4 did not inhibit binding at any concentration (Fig. 4(A)). Similarly, the ch2A2 HuG4 and ch3F4 HuG4 antibodies inhibited the binding of both resting (Fig. 4(B)) and activated (Fig. 4(C)) T cells to activated PAEC, although the

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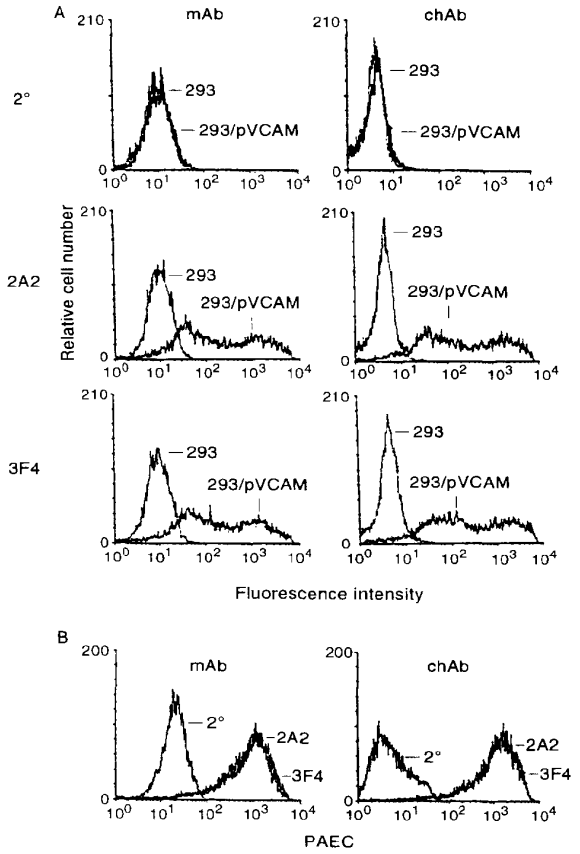


Fig. 2. Binding of 3F4 and 2A2 chimeric antibodies to pVCAM. (A) Chimeric mAbs containing the variable regions of either 2A2 or 3F4 joined to human IgG4 and kappa constant regions were produced in 293-EBNA cells and purified by protein G Sepharose chromatography. Untransfected 293-EBNA cells (293) or 293-EBNA cells expressing pVCAM (293/pVCAM) were incubated with 10 μg/ml purified murine 2A2 mAb, murine 3F4 mAb, ch2A2 HuG4, or ch3F4 HuG4. Bound antibody was detected using either FITC-conjugated goat anti-mouse IgG or FITC-conjugated goat anti-human IgG followed by flow cytometry using a Becton Dickinson FACSort. Data are displayed as histograms with fluorescence intensity plotted on the *x*-axis and relative cell number plotted on the *y*-axis. Background staining by the secondary antibodies is shown in the panels labeled 2°. (B) PAEC were activated by treatment with 1 μg/ml LPS for approximately 16 hr. Cells were then stained with either the murine 2A2 and 3F4 mAbs (left panel) or the ch2A2 HuG4 and ch3F4 HuG4 antibodies (right panel). Background staining by the secondary antibodies (2°) is indicated.

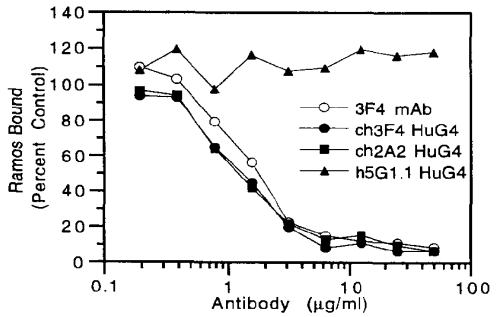


Fig. 3. Binding of Ramos cells to PAEC in the presence of mAbs. On day 0, PAEC were plated at 10 000 cells per well in medium A in 96-well plates. On day 1 the PAEC were activated by adding fresh medium A supplemented with 1 μg/ml LPS. After 16 to 24 hr incubation, the PAEC were washed and refed RPMI 1640 containing 1% FCS plus the indicated concentrations of the murine 3F4 mAb (3F4 mAb), the chimeric 2A2 and 3F4 mAbs (ch2A2 HuG4 and ch3F4 HuG4) or a humanized negative control antibody (h5G1.1 HuG4). Calcein-labeled Ramos cells were added and allowed to adhere to the PAEC under static conditions for 15 min. After nonadherent cells were removed by washing, the remaining bound cells were lysed with SDS. Calcein fluorescence of the SDS supernatant was measured with a Millipore CytoFluor 2350. In each experiment the binding of Ramos cells to PAEC in the absence of antibody was defined as 100%. Results shown are the mean of two to four experiments, with each value determined in duplicate in each experiment.

maximal inhibition achieved was reduced to approximately 60%. Addition of a blocking mAb directed against human VLA-4 also maximally inhibited adhesion by 60% (Fig. 4(C)), confirming the binding inhibition observed was due to blockade of the molecular interaction between human VLA-4 and porcine VCAM.

The potential of the ch2A2 HuG4 and ch3F4 HuG4 antibodies to inhibit binding of natural killer cells and monocytes to activated PAEC was examined using the cell lines YT (Yodio *et al.*, 1985) and U-937 (Sundstron and Nilsson, 1976), respectively. Binding of YT cells to activated PAEC was strongly inhibited by concentrations of 10 μg/ml ch2A2 HuG4 and ch3F4 HuG4 antibodies (Fig. 5(A)). In contrast, the ch2A2 HuG4 and ch3F4 HuG4 antibodies did not inhibit binding of U-937 cells to activated PAEC (Fig. 5(B)). One possible explanation for this failure to inhibit U-937 cell binding could be the presence of elevated levels of VLA-4 on U-937 cells. However, FACS analysis revealed the presence of comparable levels of CD49d and activated CD29 on U-937 cells as on other cell lines such as Jurkat whose adhesion to PAEC was inhibited by the chimeric anti-pVCAM antibodies (Fig. 6). Interestingly, the adhesion of U-937 to PAEC was consistently augmented by approximately 50% in all experiments. This increase was specific for the anti-pVCAM antibodies as no increase in U-937 binding to PAEC was detected in the presence of the control h5G1.1 HuG4 antibody (Fig. 5(B)). This suggested that binding of U-937 to PAEC could be increased through FcR binding to the anti-pVCAM HuG4 antibodies on the PAEC surface. In support of this hypothesis, FACS analysis confirmed the presence of significant levels of FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) on the surface of U-937 cells. In contrast, Jurkat and YT cells had no detectable Fcγ receptors while Ramos cells showed the presence of only low levels of FcγRII (Fig. 6).

Binding of the HuG4 chimeric antibodies to U-937 could be directly demonstrated by flow cytometry (Fig.

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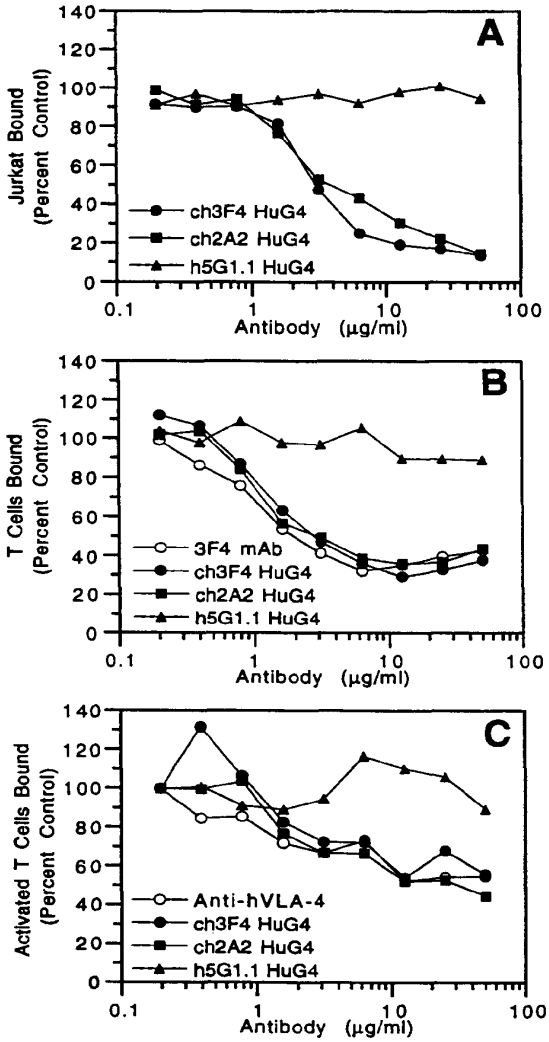


Fig. 4. Binding of Jurkat and human T cells to PAEC in the presence of HuG4 antibodies. Binding experiments were performed as described in Fig. 3 using calcein-labeled Jurkat cells (A) or calcein-labeled purified human T cells (B and C). Human T cells were either assayed immediately after purification (B) or cultured in the presence of 1 μg/ml PHA for 5 days followed by 1 day without PHA stimulation (C) prior to performing the adhesion assay. Results shown are the mean of two to four experiments (A and B) or from an individual experiment (C). Anti-hVLA-4 denotes the blocking murine mAb HP2/1 directed against the α4 subunit of human VLA-4.

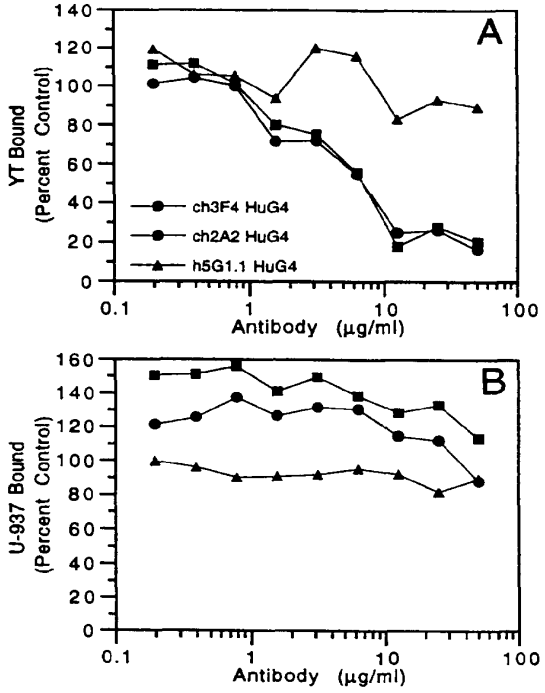


Fig. 5. Binding of YT and U-937 to PAEC in the presence of HuG4 mAbs. Adherence of calcein-labeled YT cells (A) or U-937 cells (B) to PAEC was assayed as described in Fig. 3. Results shown are the mean of two to four experiments.

7). Both the HuG4 antibodies as well as the anti-human C5 HuG4 antibody bound to U-937 cells. In contrast, 293/pVCAM cells bound only the anti-pVCAM antibodies but not the anti-human C5 HuG4 antibody. No binding of the HuG4 chimeric antibodies could be detected with Ramos, Jurkat, or YT cells. These results suggest that the HuG4 chimeric antibodies bound to U-937 cells through interaction of the HuG4 Fc region with the Fc receptors (FcRs) present on U-937 cells. Finally, F(ab')2 fragments prepared from ch3F4 HuG4 strongly inhibited binding of U-937 cells to PAEC (Fig. 8) whereas F(ab')2 fragments prepared from h5G1.1 HuG4 did not inhibit the binding. This result demonstrates that, if antibody crosslinking is prevented, blockade of the interaction between human VLA-4 and porcine VCAM can effectively inhibit the adhesion of human monocytes to PAEC.

Inhibition of FcR binding of humanized mAbs with altered constant regions

Although F(ab')2 fragments proved capable of inhibiting adhesion of U-937 cells, antibody fragments are suboptimal for chronic treatments, as they have much shorter *in vivo* half-lives than intact antibodies (Covell *et al.*, 1986). The use of intact antibodies with human constant regions as anti-rejection agents is complicated by the capacity of such antibodies to fix C and/or to bind FcRs. Human antibodies of the G4 isotype are devoid of C activity (Tao *et al.*, 1993; Smith *et al.*, 1995). Human antibodies of the G2 isotype do not activate C or bind FcRs (Canfield and Morrison, 1991; Burton and Woof, 1992). Rather than altering the specific residues known to mediate C fixation and FcR binding, we explored the possibility of creating an antibody with a composite human constant region. To create chimeric mAbs which would not activate C or bind to FcRs, we utilized the overall similarity in the human G2 and G4 primary

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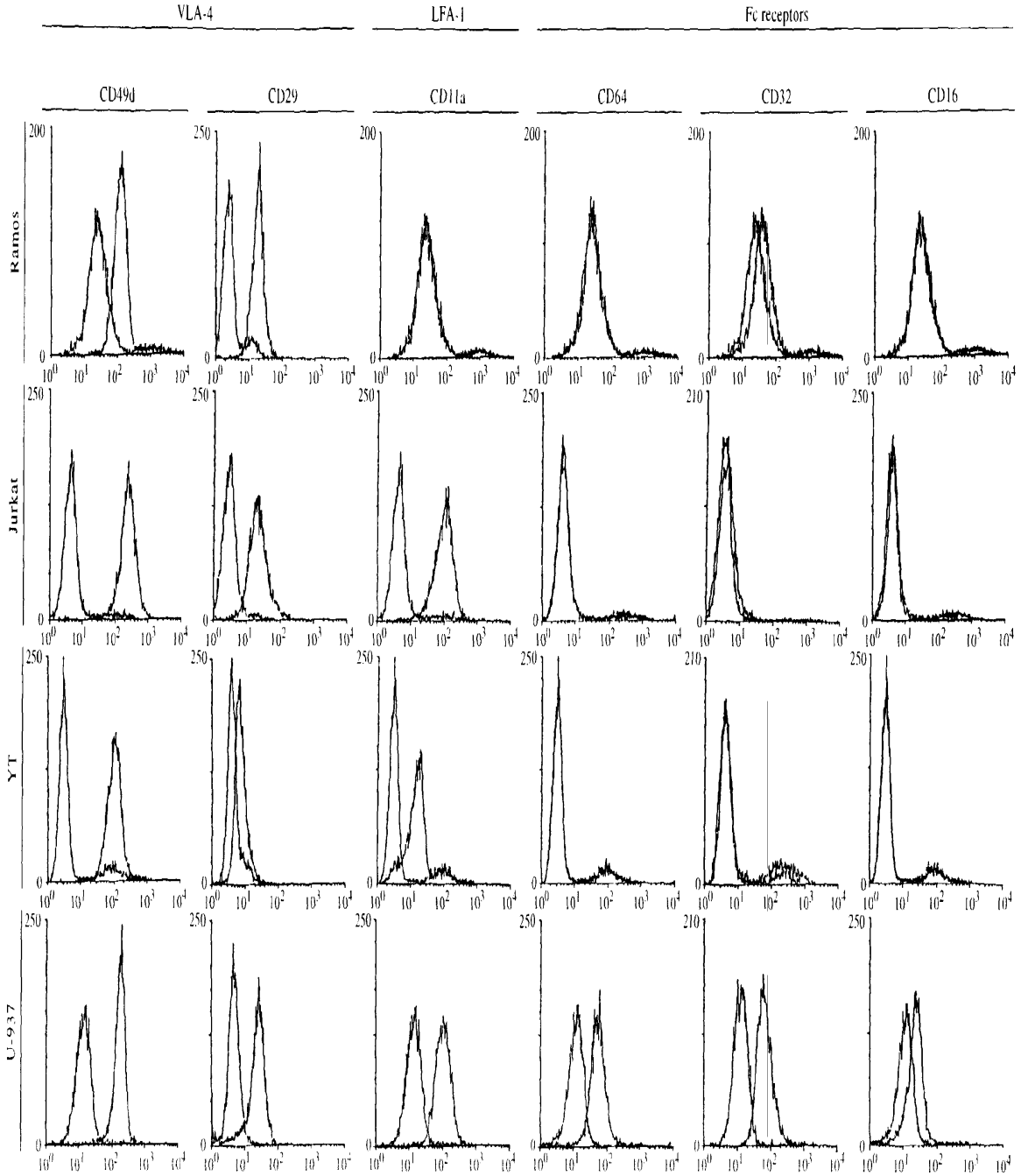


Fig. 6. Expression of VLA-4, LFA-1 and FcRs on the surface of Ramos, Jurkat, YT and U-937 cells. Cells were incubated with 10 μg/ml of the following primary antibodies: mouse anti-human CD49d, rat anti-activated human CD29, mouse anti-human CD11a, mouse anti-human CD64 ($Fc_\gamma RI$), mouse anti-human CD32 ($Fc_\gamma RII$) or mouse anti-human CD16 ($Fc_\gamma RIII$). After washing, cells were incubated with either FITC-conjugated goat anti-mouse IgG or FITC-conjugated goat anti-rat IgG as appropriate. Each panel presents the results obtained in the presence of primary plus secondary antibody or in the presence of secondary antibody alone.

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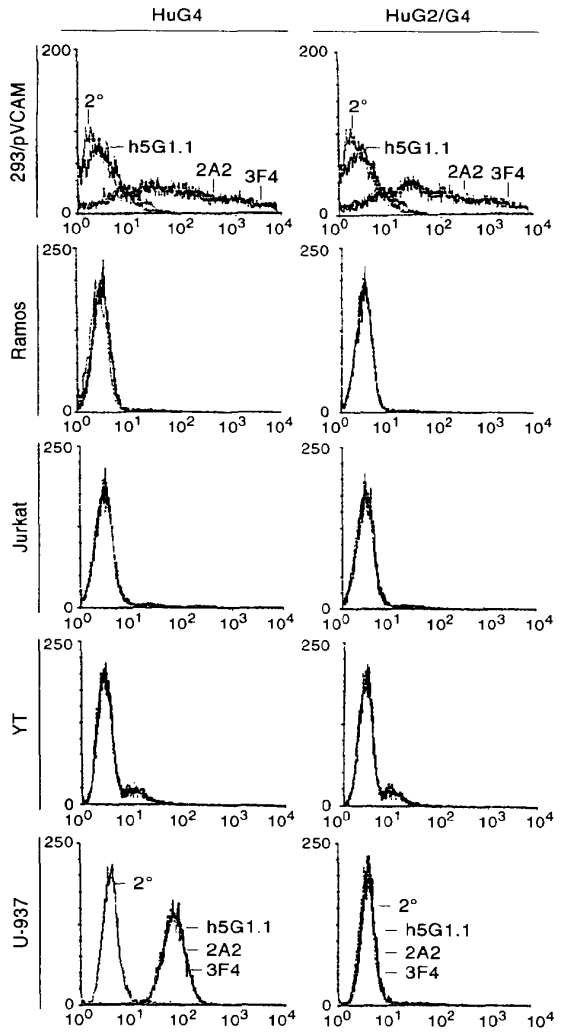


Fig. 7. Binding of chimeric antibodies to 293/pVCAM, Ramos, Jurkat, YT and U-937 cells. Cells were incubated with 10 μg/ml h5G1.1 HuG4, ch2A2 HuG4, or ch3F4 HuG4 (left panels) or 10 μg/ml h5G1.1 HuG2/G4, ch2A2 HuG2/G4, or ch3F4 HuG2/G4 (right panels). After washing, cells were incubated with FITC-conjugated goat anti-human IgG except for Ramos cells which were incubated with FITC-conjugated goat anti-human IgG (γ chain specific). Each panel presents the results obtained with the indicated cell line for h5G1.1 HuG4, ch2A2 HuG4 and ch3F4 HuG4 mAbs as well as the secondary antibody alone (2°) control.

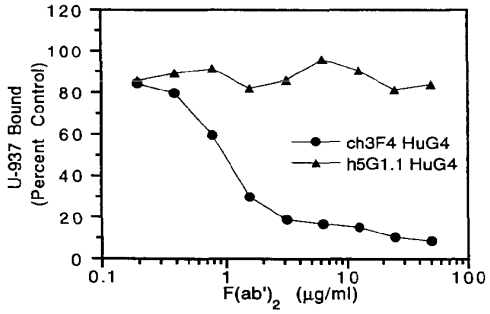


Fig. 8. Binding of U-937 cells to PAEC in the presence of F(ab')2 fragments. F(ab')2 fragments were prepared from h5G1.1 HuG4 and ch3F4 HuG4 by digestion with ficin. Undigested antibody and Fc fragments were removed by protein A chromatography. Adherence of calcein-labeled U-937 cells to PAEC in the presence of F(ab')2 was assayed as described in Fig. 3. Results shown are the mean of five experiments.

sequences to design a HuG2/G4 composite antibody consisting of the first constant region domain and the hinge region from the HuG2 isotype joined to the second and third constant region domains from the HuG4 isotype (Fig. 9). The resulting mAb did not contain the antibody sequences necessary for FcR binding, nor any new epitopes that would likely be immunogenic.

Binding to FcR was examined by using the monocytic cell line U937. FACS analysis demonstrated that the resulting ch3F4 HuG2/G4, ch2A2 HuG2/G4 and h5G1.1 HuG2/G4 antibodies did not bind to U-937 cells (Fig. 7). The ch3F4 HuG2/G4 and ch2A2 HuG2/G4 antibodies maintained the ability to bind pVCAM as demonstrated both by binding to 293/pVCAM cells (Fig. 7) and by inhibition of Jurkat and YT binding to activated PAEC (Fig. 10). For both Jurkat and YT cells inhibition of binding with the chimeric HuG2/G4 was equivalent to the HuG4 or original murine anti-pVCAM antibodies. The binding of U-937 cells to PAEC was now potently inhibited by the presence of either ch2A2 HuG2/G4 or ch3F4 HuG2/G4 antibodies. Finally, the chimeric antibodies were examined for the ability to block the adhesion of U-937 cells to purified soluble pVCAM (Fig. 11). Addition of the ch3F4 HuG4 antibody failed to inhibit binding of U-937 cells to pVCAM unless very high concentrations of antibody were added. In contrast the ch3F4 HuG2/G4 antibody potently inhibited the binding of U-937 cells to pVCAM, while the negative control h5G1.1 HuG2/G4 antibody did not block binding. Together, these results demonstrate the anti-pVCAM HuG2/G4 antibodies specifically inhibited the adhesive interaction of human VLA-4 with porcine VCAM and that disruption of this interaction was sufficient to inhibit binding of numerous human cell types to porcine endothelial cells.

DISCUSSION

Current success in allotransplantation has resulted from the development of effective global immunosuppressive therapies that prevent cellular graft rejection. Recent attempts to develop more specific immunosuppressive strategies have focused on inhibiting interactions between integrins and costimulatory molecules on T cells with their ligands on graft endothelial cells and other antigen presenting cells (reviewed in Faustman, 1995; Guinan *et al.*, 1994). The success of allotransplantation has been limited by the resulting shortage of human donor organs. Recent progress in overcoming the initial

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HuG2      STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV
HuG2/G4   STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV
HuG4      STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV

HuG2      HTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVE
HuG2/G4   HTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVE
HuG4      HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE

                                  235
HuG2      RKCCVECPPCPAPPV- AGPSVFLF
HuG2/G4   RKCCVECPPCPAPPV- AGPSVFLF
HuG4      SKYGPPCPSCPAPEFLGGPSVFLF

HuG2      PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR
HuG2/G4   PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
HuG4      PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR

                                                      331
HuG2      EEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKG
HuG2/G4   EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG
HuG4      EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG
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Fig. 9. Sequence of the CH1, hinge and CH2 regions of human IgG2 (HuG2), human IgG4 (HuG4), and the chimeric HuG2/G4 construct. Positions where differences occur between the three molecules are double underlined. The amino acid sequence at position 235/236 determines ability to bind to Fcγ receptors (Canfield and Morrison, 1991; Duncan *et al.*, 1988; Alegre *et al.*, 1992; Burton and Woof, 1992) while the presence of a serine at position 331 confers the inability to activate C (Tao *et al.*, 1993).

hyperacute rejection of discordant xenografts suggests the potential for use of porcine xenografts as a clinical option to circumvent this chronic shortage. Although there are limited data regarding the fate of discordant xenografts which survive the initial hyperacute rejection phase, xenograft recipients will likely require continued cellular immunosuppression for the prevention of graft rejection. In this light, xenografts offer a unique opportunity for the development of blocking reagents specific for the graft, thereby avoiding global immunosuppression of the recipient.

To investigate the interactions occurring between human leukocytes and porcine endothelial cells, we have cloned the porcine VCAM molecule and created mAbs specific for porcine VCAM that do not bind to human VCAM (Mueller *et al.*, 1995). Two of these antibodies (3F4 and 2A2) are capable of blocking the interaction between human VLA-4 and porcine VCAM. Here we demonstrate that the binding specificity for 2A2 is located within domain 1 of pVCAM while the binding specificity for 3F4 is located within domain 2. This is identical to the results found with human VCAM, where blocking antibodies have also been shown to be directed against either domains 1 or 2 (Osborn *et al.*, 1992; Vonderheide and Springer, 1992).

The use of murine mAbs in humans and primates presents numerous problems including a short antibody half-life and a high degree of immunogenicity (Khazaeli *et al.*, 1994). To optimize the use of the anti-porcine VCAM antibodies in porcine-to-primate xenograft experiments we developed chimeric antibodies using the variable regions cloned from the 2A2 and 3F4 antibodies joined to the constant regions of the human IgG4,κ isotype. Given the extensive activation of C which occurs within xenografts, the human IgG4 isotype was chosen due to its inability to activate C. The resultant chimeric antibodies (ch2A2 HuG4 and ch3F4 HuG4 antibodies) retained specificity for porcine VCAM and were as potent as the original murine mAbs at blocking adhesion of Ramos cells to activated PAEC by greater than 90% (Fig. 3). In contrast to Ramos, the binding of resting or activated human T cells to activated PAEC was inhibited only approximately 50% by either the chimeric antibodies or the original mAbs (Fig. 4). Activation of human T cells by PAEC can be blocked by antibodies directed against either CD2 or LFA-1 (Rollins *et al.*, 1994*b*), suggesting either of these molecules could mediate the remaining adhesion by interacting with porcine CD58 or porcine ICAM-1, respectively.

The adhesion of human lymphocytes, monocytes or NK cells to activated HUVEC is dependent on the interaction of both LFA-1 with ICAM-1 and VLA-4 with VCAM (Allavena *et al.*, 1991; Jonjic *et al.*, 1992; Vonderheide and Springer, 1992). For each cell type, blocking the VLA-4/VCAM interaction by itself produces only a small reduction in adhesion, while antibodies which disrupt the LFA-1/ICAM-1 interaction provide a greater, although still partial, inhibition of adhesion. Addition of antibodies blocking both VLA-4/VCAM and LFA-1/ICAM-1 is required to completely block adhesion of these cell types to HUVEC. Surprisingly, binding of LFA-1$^+$–VLA$^+$4 Jurkat or YT cells to activated PAEC could be inhibited nearly completely by the ch3F4 HuG4 and ch2A2 HuG4 antibodies. One possible explanation for this could be the lack of interaction between human LFA-1 and porcine ICAM. The conservation of ICAM-1 is relatively low between species, with human and mouse ICAM-1 being only 50% conserved at the amino acid level (reviewed in Carlos and Harlan, 1994), and *in vitro* mouse LFA-1 does not bind to human ICAM-1. Alternatively, human LFA-1 may interact with porcine ICAM-1, but with a reduced affinity. For example, human VLA-4 binds to human VCAM at two independent sites, one within domain 1 and one within

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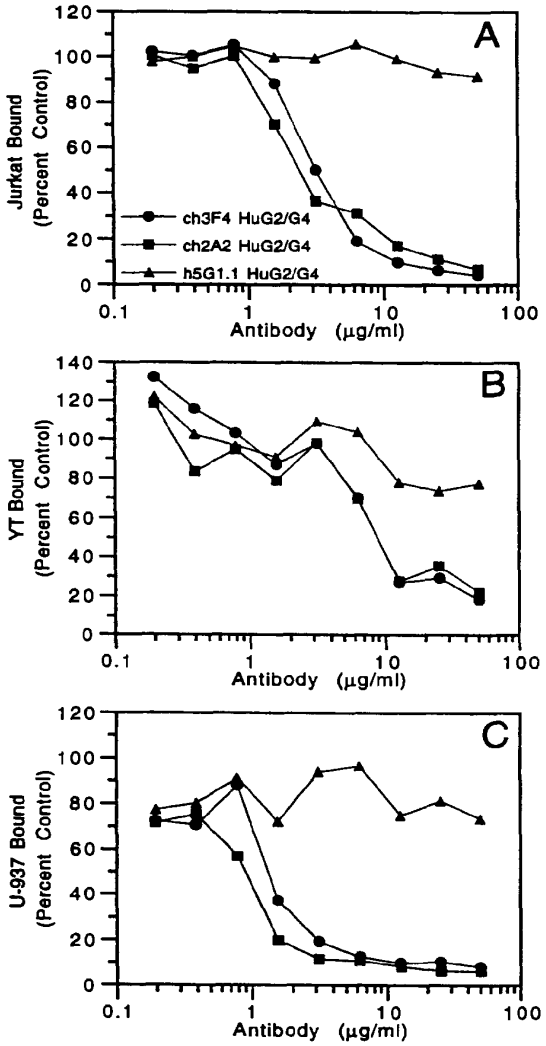


Fig. 10. Binding of Jurkat, YT and U-937 to PAEC in the presence of HuG2/G4 mAbs. Adherence of calcein-labeled Jurkat (A), YT (B) and U-937 (C) cells to PAEC was assayed as described in Fig. 3. Results shown are the mean of two to four experiments.

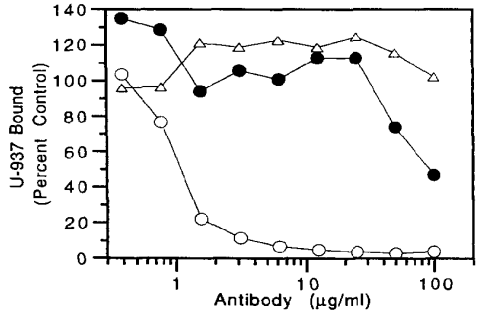


Fig. 11. Binding of U-937 to purified pVCAM. Microtiter plate wells were coated with purified soluble pVCAM. After blocking with FCS, wells received the indicated concentrations of either ch3F4 HuG4 (●), ch3F4 HuG2/G4 (○) or h5G1.1 HuG2/G4 (△) followed by 3×10^5 calcein-labeled U-937 cells. The cells were allowed to adhere at 37°C for 15 min after which non-adherent cells were removed by washing. Adherence of U-937 cells in the absence of competitor antibody was defined as 100%. Results shown are from an individual representative experiment.

domain 4 (Osborn *et al.*, 1992; Vonderheide *et al.*, 1994). In contrast, porcine VCAM lacks domain 4, suggesting the interaction between human VLA-4 and porcine VCAM may be weaker than the interaction between human VLA-4 and human VCAM. Reduced ligand affinities could make the binding of human effector cells to porcine endothelial cells more susceptible to disruption of any one of multiple weak interactions. These observations suggest the potential for significant differences in the therapeutic efficacy achieved by inhibiting particular receptor–ligand interactions with xenografts as compared to allografts.

Although the ch3F4 HuG4 and ch2A2HuG4 blocked binding of Jurkat and YT to activated PAEC, both antibodies failed to block binding of the monocytic U-937 cells to activated PAEC (Fig. 5). The levels of activated $\beta 1$ were comparable on Jurkat and U937 cells indicating an increased level of functional VLA-4 was not the mechanism behind the lack of inhibition of U-937 binding. Additionally, flow cytometry demonstrated the direct binding of the chimeric HuG4 antibodies to U-937 cells (Fig. 7). This binding was not dependent on the antigen specificity of the variable region, suggesting the interaction occurred through the Fc portion of the antibody. Finally, F(ab′)2 prepared from 3F4 HuG4 were able to block the adhesion of U-937 cells to activated PAEC by greater than 80% (Fig. 8). Together these results were consistent with a model in which the chimeric anti-pVCAM HuG4 mAbs crosslinked U-937 cells to activated PAEC by simultaneously binding FcRs and cell surface VCAM present on the PAEC. This is comparable to the situation encountered with the anti-CD3 murine monoclonal antibody (mAb) OKT3, which when first administered to patients produces crosslinking of T cells to FcR bearing cells, resulting in the activation of both cell types (Ceuppens *et al.*, 1985; Kan *et al.*, 1986) with resultant toxicity to the patient.

The interaction of antibodies with Fcγ receptors has been found to be dependent on the sequence of the hinge linker region of the CH2 domain (Duncan *et al.*, 1988; Alegre *et al.*, 1992; Burton and Woof, 1992) as well as an additional bend located between two β strands within the CH2 domain (Canfield and Morrison, 1991). In the case of a humanized OKT3 antibody (human IgG4 constant regions), binding to Fcγ receptors has been eliminated by the introduction of amino acid mutations in the hinge linker (Alegre *et al.*, 1992, 1994). In either case the resulting human IgG4 constant region contains amino acid differences which could be immunogenic. Here, we have created chimeric antibodies (designated HuG2/G4) containing the CH1 domain and hinge linker region of

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human IgG2 followed by the CH2 and CH3 domains of the human IgG4 constant region. Due to the similarities between these two isotypes at the amino acid level, the resultant antibody contained no novel peptides less than 30 amino acids in length likely to be presented by MHC class II molecules and invoke an immune response. The chimeric HuG2/G4 antibodies no longer bound to U-937 cells. Moreover, the ch2A2 HuG2/G4 and ch3F4 HuG2/G4 antibodies blocked the binding of Jurkat and YT cells to activated PAEC as potently as the chimeric HuG4 antibodies, demonstrating the alteration of the Fc region did not reduce the antibody avidities. Moreover, the chimeric anti-pVCAM HuG2/G4 recombinant antibodies nearly completely blocked the binding of U-937 cells to activated PAEC and to purified pVCAM. Finally, as predicted from the low affinity of IgG2 and IgG4 for human C1q, our preliminary results indicate that the HuG4 and HuG2/G4 recombinant antibodies show little or no C1q binding (data not shown).

In summary, we have developed humanized mAbs specific for porcine VCAM which potently block the adhesion of multiple human inflammatory cell types to porcine endothelial cells. The lack of binding of these antibodies to human VCAM leaves intact the interaction of human leukocytes with human endothelial cells. The adhesion-blocking properties of these antibodies will allow delineation of the role of VLA-4/VCAM interactions in cellular mediated rejection of porcine-to-primate xenografts. Additionally, the HuG2/G4 antibody design should prove useful in humanization of other antibodies intended for human use where elimination of FcR binding and C activation may be desirable.

REFERENCES

Alegre M.-L., Collins A. M., Pulito V. L., Brosius R. A., Olson W. C., Zivin R. A., Knowles R., Thistlethwaite J. R., Jolliffe L. K. and Bluestone J. A. (1992) Effect of a single amino acid mutation on the activating and immunosuppressive properties of a "humanized" OKT3 mAb. *J. Immun.* **148,** 3461–3468.

Alegre M.-L., Peterson L. J., Xu D., Sattar H. A., Jeharajah D. R., Kowalkowski K., Thistlethwaite J. R., Zivin R. A., Jolliffe L. and Bluestone J. A. (1994) A non-activating "humanized" anti-CD3 mAb retains immunosuppressive properties *in vivo*. *Transplantation* **57,** 1537–1543.

Allavena P., Paganin C., Martìn-Padura I., Peri G., Baboli M., Dejana E., Marchisio P. C. and Montovani A. (1991) Molecules and structures involved in the adhesion of natural killer cells to vascular endothelium. *J. Exp. Med.* **173,** 439–448.

Alon R., Kassner P. D., Carr M. W., Finger E. B., Hemlet M. E. and Springer T. A. (1995) The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J. Cell Biol.* **128,** 1243–1253.

Bianchi G., Sironi M., Ghibaudi E., Selvaggini C., Elices M., Allavena P. and Montovani A. (1993) Migration of natural killer cells across endothelial cell monolayers. *J. Immun.* **151,** 5135–5144.

Blakely M. L., Werf W. J. van der, Berndt M. C., Dalmasso A. P., Bach F. H. and Hancock W. W. (1994) Activation of intragraft endothelial and mononuclear cells during discordant xenograft rejection. *Transplantation* **58,** 1059–1066.

Burton D. R. and Woof J. M. (1992) Human antibody effector function. *Adv. Immun.* **51,** 1–18.

Canfield S. M. and Morrison S. L. (1991) The binding affinity of human IgG for its high affinity FcR is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. *J. Exp. Med.* **173,** 1483–1491.

Carlos T. M. and Harlan J. M. (1994) Leukocyte-endothelial adhesion molecules. *Blood* **84,** 2068–2101.

Ceuppens J. L., Bloemmen F. J. and Wauwe J. P. van (1985) T cell unresponsiveness to the mitogenic activity of OKT3 antibody results from a deficiency of monocyte Fcγ receptors for murine IgG2a and inability to cross-link the T3–Ti complex. *J. Immun.* **135,** 3882–3886.

Chisholm P. L., Williams C. A. and Lobb R. R. (1993) Monoclonal antibodies to the integrin α4 subunit inhibit the murine contact hypersensitivity response. *Eur. J. Immun.* **23,** 621–628.

Chuluyan H. E., Osborn L., Lobb R. and Issekutz A. C. (1995) Domains 1 and 4 of vascular cell adhesion molecule-1 (CD106) both support very late activation antigen-4 (CD49d/CD29)-dependent monocyte transendothelial migration. *J. Immun.* **155,** 3135–3144

Covell D. G., Barbet J., Holton O. D., Black C. D., Parker P. J. and Weinstein J. N. (1986) Pharmacokinetics of monoclonal immunoglobulin G1, F(ab')2, and Fab' in mice. *Cancer Res.* **46,** 3969–3978.

Duncan A. R., Woof J. M., Partridge L. J., Burton D. R. and Winter G. (1988) Localization of the binding site for the human high-affinity FcR on IgG. *Nature* **332,** 563–564.

Evans M. J., Hartman S. L., Wolff D. W., Rollins S. A. and Squinto S. P. (1995*a*) Rapid expression of an anti-human C5 chimeric Fab utilizing a vector that replicates in COS and 293 cells. *J. Immun. Meth.* **184,** 123–138.

Evans M. J., Rollins S. A., Wolff D. W., Rother R. P., Norin A. J., Therrien D. M., Grijalva G. A., Mueller J. P., Nye S. H., Squinto S. P. and Wilkins J. A. (1995*b*) *In vitro* and *in vivo* inhibition of C activity by a single-chain Fv fragment recognizing human C5. *Mol. Immun.* **32,** 1183–1195.

Faustman D. (1995) Strategies for circumventing transplant rejection: modification of cells, tissues and organs. *Trends Biotech.* **13,** 100–105.

Guinan E. C., Gribben J. G., Boussiotis V. A., Freeman G. J. and Nadler L. M. (1994) Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. *Blood* **84,** 3261–3282.

Hawkins H. K., Heffelfinger S. C. and Anderson D. C. (1992) Leukocyte adhesion deficiency: clinical and postmortem observations. *Ped. Pathol.* **12,** 119–130.

Issekutz T. B. (1991) Inhibition of *in vivo* lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 mAb: a likely role for VLA-4 *in vivo*. *J. Immun.* **147,** 4178–4184.

Issekutz T. B. (1992) Inhibition of lymphocyte endothelial adhesion and *in vivo* lymphocyte migration to cutaneous inflammation by TA-3, a new mAb to rat LFA-1. *J. Immun.* **149,** 3394–3402.

Johnston S. C., Dustin M. L., Hibbs M. L. and Springer T. A. (1990) On the species specificity of the interaction of LFA-1 with intercellular adhesion molecules. *J. Immun.* **145,** 1181–1187.

Jonjic N., Jilec P., Bernasconi S., Peri G., Martin-Padura I., Cenzuales S., Dejana E. and Mantovani A. (1992) Molecules involved in the adhesion and cytotoxicity of activated monocytes on endothelial cells. *J. Immun.* **148,** 2080–2083.

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Kan E. A. R., Wright S. D., Welte K. and Wand C. Y. (1986) FcRs on monocytes cause OKT3-treated lymphocytes to internalize T3 and to secrete IL-2. *Cell. Immun.* **98,** 181–185.

Khazaeli M. B., Conry R. M. and LoBuglio A. F. (1994) Human immune response to mAbs. *J. Immunother.* **15,** 42–52.

Luscinskas F. W., Kansas G. S., Ding H., Pizcueta P., Schleiffenbaum B. E., Tedder T. F. and Gimbrone M. A. (1994) Monocyte rolling, arrest and spreading on IL-4-activated vascular endothelium under flow is mediated via sequential action of L-selectin, β1-integrins, and β2-integrins. *J. Cell Biol.* **125,** 1417–1427.

Luscinskas F. W., Ding H. and Lichtman A. H. (1995) P-selectin and vascular cell adhesion molecule 1 mediate rolling and arrest, respectively, of CD4+ T lymphocytes on tumor necrosis factor α-activated vascular endothelium under flow. *J. Exp. Med.* **181,** 1179–1186.

Meershaert J. and Furie M. B. (1994) Monocytes use either CD11/CD18 or VLA-4 to migrate across human endothelium *in vitro*. *J. Immun.* **152,** 1915–1926.

Mueller J. P., Evans M. J., Cofiell R., Rother R. P., Matis L. A. and Elliott E. A. (1995) Porcine vascular cell adhesion molecule (VCAM) mediates endothelial cell adhesion to human T cells. *Transplantation* **60,** 1299–1306.

Murray A. G., Khodadoust M. M., Pober J. S. and Bothwell A. L. (1994) Porcine aortic endothelial cells activate human T cells: direct presentation of MHC antigens and costimulation by ligands for human CD2 and CD28. *Immunity* **1,** 57–63.

Orosz C. G., Ohye R. G., Pelletier R. P., van Buskirk A. M., Huang E., Morgan C., Kincade P. W. and Ferguson R. M. (1993) Treatment with anti-vascular cell adhesion molecule 1 mAb induces long-term murine cardiac allograft acceptance. *Transplantation* **56,** 453–460.

Osborn L., Vassallo C. and Benjamin C. D. (1992) Activated endothelium binds lymphocytes through a novel binding site in the alternately spliced domain of vascular cell adhesion molecule-1. *J. Exp. Med.* **176,** 99–107.

Pepinsky B., Hession C., Chen L.-L., Moy P., Burkly L., Jakubowski A., Chow E. P., Benjamin C., Chi-Rosso G., Luhowskyj S. and Lobb R. (1992) Structure/function studies on vascular cell adhesion molecule-1. *J. Biol. Chem.* **267,** 17820–17826.

Renz M. E., Chiu H. H., Jones S., Fox J., Kin K. J., Presta L. G. and Fong S. (1994) Structural requirements for adhesion of soluble recombinant murine vascular cell adhesion molecule-1 to $\alpha 4\beta 1$. *J. Cell Biol.* **125,** 1395–1406.

Rollins S. A., Evans M. J., Johnson K. K., Elliott E. A., Squinto S. P., Matis L. A. and Rother R. P. (1994*a*) Molecular and functional analysis of porcine E-selectin reveals a potential role in xenograft rejection. *Biochem. Biophys. Res. Commun.* **204,** 763–771.

Rollins S. A., Kennedy S. P., Chodera A. J., Elliott E. A., Zavoico G. B. and Matis L. A. (1994*b*) Evidence that activation of human T cells by porcine endothelium involves direct recognition of porcine SLA and costimulation by porcine ligands for LFA-1 and CD2. *Transplantation* **57,** 1709–1716.

Scheynius A., Camp R. L. and Puré E. (1993) Reduced contact sensitivity reactions in mice treated with mAbs to leukocyte function-associated molecule-1 and intercellular adhesion molecule-1. *J. Immun.* **150,** 655–663.

Silber A., Newman W., Sasseville V. G., Pauley D., Beall D., Walsh D. G. and Ringler D. J. (1995) Recruitment of lymphocytes during cutaneous delayed hypersensitivity in non-human primates is dependent on E-selectin and VCAM-1. *J. Clin. Invest.* **93,** 1554–1563.

Smith R. I. F., Coloma M. J. and Morrison S. L. (1995) Addition of a μ-tailpiece to IgG results in polymeric antibodies with enhanced effector functions including C-mediated cytolysis by IgG4. *J. Immun.* **154,** 2226–2236.

Somerville C. A. and D'Apice A. J. F. (1993) Future directions in transplantation: xenotransplantation. *Kidney Int.* **42,** S112.

Sundstron C. and Nilsson K. (1976) Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int. J. Cancer* **17,** 565–569.

Tao M.-H., Smith R. I. F. and Morrison S. L. (1993) Structural features of human immunoglobulin G that determine isotype-specific differences in C activation. *J. Exp. Med.* **178,** 661–667.

Vonderheide R. H., Springer T. A. (1992) Lymphocyte adhesion through very late antigen 4: evidence for a novel binding site in the alternatively spliced domain of vascular cell adhesion molecule 1 and an additional a4 integrin counter-receptor on stimulated endothelium. *J. Exp. Med.* **175,** 1422–1433.

Vonderheide R. H., Tedder T. F., Springer T. A. and Staunton D. E. (1994) Residues within a conserved amino acid motif of domains 1 and 4 of VCAM-1 are required for binding to VLA-4. *J. Cell Biol.* **125,** 215–222.

Wallgren A. C., Karlssonparra A. and Korsgren O. (1995) The main infiltrating cell in xenograft rejection is a CD4(+) macrophage and not a T lymphocyte. *Transplantation* **60,** 594–601.

Yamada K., Sachs D. H. and DerSimonian H. (1995) Human anti-porcine xenogeneic T cell response. Evidence for allele specificity of mixed leukocyte reaction and for both direct and indirect pathways of recognition. *J. Immun.* **155,** 5249–5256.

Yednock T. A., Cannon C., Fritz L. C., Sanchex-Madrid F., Steinman L. and Karin N. (1992) Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4\beta 1$ integrin. *Nature* **356,** 63–66.

Yodio J., Teshigawara K., Nikaido T., Fukui J. K., Noma T., Honjo T., Takigawa M., Minato M., Tsudo M., Uchiyama T. and Maeda M. (1985) TCGF (IL-2)-receptor inducing factor(s) I. Regulation of IL-2 receptor on a natural killer-like cell line (YT cells). *J. Immun.* **164,** 1623–1627.

Zeng Y., Gage A., Montag A., Rothlein R., Thistlethwaite J. R. and Bluestone J. A. (1994) Inhibition of transplant rejection by pretreatment of xenogeneic pancreatic islet cells with anti-ICAM-1 antibodies. *Transplantation* **58,** 681–689.

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Submission in opposition proceedings

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Opponent/representative's reference	198 441 u6/ne

The information given below is pertaining to the following patent in opposition proceedings:

Patent No.	EP2359834
Application No.	EP11001632.6
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Facts and arguments	2018-11-20 Submission before oral proceedings.PDF	OPPO.PDF

Evidence filed subsequently:

D39	Other evidence	ICH Harmonised Tripartite Guideline on Stability Testing of Biological Products original file name: D39 ICH Guidelines.PDF attached as: Other-evidence-1.PDF

D40	Non-patent literature - article	Mueller, John P. et al., "Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells" Molecular Immunology, 1997 original file name: D40 Mueller et al. (1997).pdf attached as: Published-Evidence-2.pdf
D41	Patent document	WO 2004/071439 (A2) , 26.08.2004 original file name: D41 WO 2004071439 A2.pdf attached as: Published-Evidence-3.pdf
D42	Patent document	WO 2005/092073 (A2) , 06.10.2005 original file name: D42 WO 2005092073 A2.pdf attached as: Published-Evidence-4.pdf
D43	Patent document	WO 2006/003179 (A2) , 12.01.2006 original file name: D43 WO 2006003179 A2.pdf attached as: Published-Evidence-5.pdf

Signatures

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INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

QUALITY OF BIOTECHNOLOGICAL PRODUCTS: STABILITY TESTING OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS

Q5C

Current *Step 4* version

dated 30 November 1995

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

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Q5C
Document History

First Codification	History	Date	New Codification **November 2005**
Q5C	Approval by the Steering Committee under *Step 2* and release for public consultation.	29 March 1995	Q5C

Current *Step 4* version

Q5C	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	30 November 1995	Q5C

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QUALITY OF BIOTECHNOLOGICAL PRODUCTS: STABILITY TESTING OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS

Annex to the Tripartite ICH Guideline for the Stability Testing of New Drug Substances and Products

ICH Harmonised Tripartite Guideline

Having reached *Step 4* of the ICH Process at the ICH Steering Committee meeting on 30 November 1995, this guideline is recommended for adoption to the three regulatory parties to ICH

1. PREAMBLE

The guidance stated in the ICH harmonised tripartite guideline "Stability Testing of New Drug Substances and Products" (27 October 1993) applies in general to biotechnological/biological products. However, biotechnological/biological products do have distinguishing characteristics to which consideration should be given in any well-defined testing program designed to confirm their stability during the intended storage period. For such products, in which the active components are typically proteins and/or polypeptides, maintenance of molecular conformation and, hence of biological activity, is dependent on noncovalent as well as covalent forces. The products are particularly sensitive to environmental factors such as temperature changes, oxidation, light, ionic content, and shear. In order to ensure maintenance of biological activity and to avoid degradation, stringent conditions for their storage are usually necessary.

The evaluation of stability may necessitate complex analytical methodologies. Assays for biological activity, where applicable, should be part of the pivotal stability studies. Appropriate physicochemical, biochemical and immunochemical methods for the analysis of the molecular entity and the quantitative detection of degradation products should also be part of the stability program whenever purity and molecular characteristics of the product permit use of these methodologies.

With the above concerns in mind, the applicant should develop the proper supporting stability data for a biotechnological/biological product and consider many external conditions which can affect the product's potency, purity and quality. Primary data to support a requested storage period for either drug substance or drug product should be based on long-term, real-time, real-condition stability studies. Thus, the development of a proper long-term stability program becomes critical to the successful development of a commercial product. The purpose of this document is to give guidance to applicants regarding the type of stability studies that should be provided in support of marketing applications. It is understood that during the review and evaluation process, continuing updates of initial stability data may occur.

2. SCOPE OF THE ANNEX

The guidance stated in this annex applies to well-characterised proteins and polypeptides, their derivatives and products of which they are components, and which are isolated from tissues, body fluids, cell cultures, or produced using rDNA technology. Thus, the document covers the generation and submission of stability data for products such as cytokines (interferons, interleukins, colony-stimulating factors, tumour necrosis factors), erythropoietins, plasminogen activators, blood

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plasma factors, growth hormones and growth factors, insulins, monoclonal antibodies, and vaccines consisting of well-characterised proteins or polypeptides. In addition, the guidance outlined in the following sections may apply to other types of products, such as conventional vaccines, after consultation with the appropriate regulatory authorities. The document does not cover antibiotics, allergenic extracts, heparins, vitamins, whole blood, or cellular blood components.

3. TERMINOLOGY

For the basic terms used in this annex the reader is referred to the "Glossary" in the ICH harmonised tripartite guideline "Stability Testing of New Drug Substances and Products" (27 October 1993). However, since manufacturers of biotechnological/biological products sometimes use traditional terminology, traditional terms are specified in parentheses to assist the reader. A supplemental glossary is also included that explains certain terms used in the production of biotechnological/biological products.

4. SELECTION OF BATCHES

4.1. Drug Substance (Bulk Material)

Where bulk material is to be stored after manufacture but prior to formulation and final manufacturing, stability data should be provided on at least 3 batches for which manufacture and storage are representative of the manufacturing scale of production. A minimum of 6 months stability data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested. For drug substances with storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis. Data from pilot-plant scale batches of drug substance produced at a reduced scale of fermentation and purification may be provided at the time the dossier is submitted to the regulatory agencies with a commitment to place the first 3 manufacturing scale batches into the long-term stability program after approval.

The quality of the batches of drug substance placed into the stability program should be representative of the quality of the material used in preclinical and clinical studies and of the quality of the material to be made at manufacturing scale. In addition, the drug substance (bulk material) made at pilot-plant scale should be produced by a process and stored under conditions representative of that used for the manufacturing scale. The drug substance entered into the stability program should be stored in containers which properly represent the actual holding containers used during manufacture. Containers of reduced size may be acceptable for drug substance stability testing provided that they are constructed of the same material and use the same type of container/closure system that is intended to be used during manufacture.

4.2. Intermediates

During manufacture of biotechnological/biological products, the quality and control of certain intermediates may be critical to the production of the final product. In general, the manufacturer should identify intermediates and generate in-house data and process limits that assure their stability within the bounds of the developed process. While the use of pilot-plant scale data is permissible, the manufacturer should establish the suitability of such data using the manufacturing scale process.

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4.3. Drug Product (Final Container Product)

Stability information should be provided on at least 3 batches of final container product representative of that which will be used at manufacturing scale. Where possible, batches of final container product included in stability testing should be derived from different batches of bulk material. A minimum of 6 months data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested. For drug products with storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis. Product expiration dating will be based upon the actual data submitted in support of the application. Since dating is based upon the real-time/real-temperature data submitted for review, continuing updates of initial stability data should occur during the review and evaluation process. The quality of the final container product placed on stability studies should be representative of the quality of the material used in the preclinical and clinical studies. Data from pilot-plant scale batches of drug product may be provided at the time the dossier is submitted to the regulatory agencies with a commitment to place the first 3 manufacturing scale batches into the long term stability program after approval. Where pilot-plant scale batches were submitted to establish the dating for a product and, in the event that product produced at manufacturing scale does not meet those long-term stability specifications throughout the dating period or is not representative of the material used in preclinical and clinical studies, the applicant should notify the appropriate regulatory authorities to determine a suitable course of action.

4.4. Sample Selection

Where one product is distributed in batches differing in fill volume (e.g., 1 millilitre (ml), 2 ml, or 10 ml), unitage (e.g., 10 units, 20 units, or 50 units), or mass (e.g., 1 milligram (mg), 2 mg, or 5 mg) samples to be entered into the stability program may be selected on the basis of a matrix system and/or by bracketing.

Matrixing, i.e., the statistical design of a stability study in which different fractions of samples are tested at different sampling points, should only be applied when appropriate documentation is provided that confirms that the stability of the samples tested represents the stability of all samples. The differences in the samples for the same drug product should be identified as, for example, covering different batches, different strengths, different sizes of the same closure and possibly, in some cases, different container/closure systems. Matrixing should not be applied to samples with differences that may affect stability, such as different strengths and different containers/closures, where it cannot be confirmed that the products respond similarly under storage conditions.

Where the same strength and exact container/closure system is used for 3 or more fill contents, the manufacturer may elect to place only the smallest and largest container size into the stability program, i.e., bracketing. The design of a protocol that incorporates bracketing assumes that the stability of the intermediate condition samples are represented by those at the extremes. In certain cases, data may be needed to demonstrate that all samples are properly represented by data collected for the extremes.

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5. STABILITY-INDICATING PROFILE

On the whole, there is no single stability-indicating assay or parameter that profiles the stability characteristics of a biotechnological/biological product. Consequently, the manufacturer should propose a stability-indicating profile that provides assurance that changes in the identity, purity and potency of the product will be detected.

At the time of submission, applicants should have validated the methods that comprise the stability-indicating profile and the data should be available for review. The determination of which tests should be included will be product-specific. The items emphasised in the following subsections are not intended to be all-inclusive, but represent product characteristics that should typically be documented to adequately demonstrate product stability.

5.1. Protocol

The dossier accompanying the application for marketing authorisation should include a detailed protocol for the assessment of the stability of both drug substance and drug product in support of the proposed storage conditions and expiration dating periods. The protocol should include all necessary information which demonstrates the stability of the biotechnological/biological product throughout the proposed expiration dating period including, for example, well-defined specifications and test intervals. The statistical methods that should be used are described in the tripartite guideline on stability.

5.2. Potency

When the intended use of a product is linked to a definable and measurable biological activity, testing for potency should be part of the stability studies. For the purpose of stability testing of the products described in this guideline, potency is the specific ability or capacity of a product to achieve its intended effect. It is based on the measurement of some attribute of the product and is determined by a suitable quantitative method. In general, potencies of biotechnological/biological products tested by different laboratories can be compared in a meaningful way only if expressed in relation to that of an appropriate reference material. For that purpose, a reference material calibrated directly or indirectly against the corresponding national or international reference material should be included in the assay.

Potency studies should be performed at appropriate intervals as defined in the stability protocol and the results should be reported in units of biological activity calibrated, whenever possible, against nationally or internationally recognised standard. Where no national or international reference standards exist, the assay results may be reported in in-house derived units using a characterised reference material.

In some biotechnological/biological products, potency is dependent upon the conjugation of the active ingredient(s) to a second moiety or binding to an adjuvant. Dissociation of the active ingredient(s) from the carrier used in conjugates or adjuvants should be examined in real-time/real-temperature studies (including conditions encountered during shipment). The assessment of the stability of such products may be difficult since, in some cases, *in vitro* tests for biological activity and physicochemical characterisation are impractical or provide inaccurate results. Appropriate strategies (e.g., testing the product prior to conjugation/binding, assessing the release of the active compound from the second moiety, *in vivo* assays) or the use of an appropriate surrogate test should be considered to overcome the inadequacies of *in vitro* testing.

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5.3. Purity and Molecular Characterisation

For the purpose of stability testing of the products described in this guideline, purity is a relative term. Due to the effect of glycosylation, deamidation, or other heterogeneities, the absolute purity of a biotechnological/biological product is extremely difficult to determine. Thus, the purity of a biotechnological/biological product should be typically assessed by more than one method and the purity value derived is method-dependent. For the purpose of stability testing, tests for purity should focus on methods for determination of degradation products.

The degree of purity, as well as individual and total amounts of degradation products of the biotechnological/biological product entered into the stability studies, should be reported and documented whenever possible. Limits of acceptable degradation should be derived from the analytical profiles of batches of the drug substance and drug product used in the preclinical and clinical studies.

The use of relevant physicochemical, biochemical and immunochemical analytical methodologies should permit a comprehensive characterisation of the drug substance and/or drug product (e.g., molecular size, charge, hydrophobicity) and the accurate detection of degradation changes that may result from deamidation, oxidation, sulfoxidation, aggregation or fragmentation during storage. As examples, methods that may contribute to this include electrophoresis (SDS-PAGE, immunoelectrophoresis, Western blot, isoelectrofocusing), high-resolution chromatography (e.g., reversed-phase chromatography, gel filtration, ion exchange, affinity chromatography), and peptide mapping.

Wherever significant qualitative or quantitative changes indicative of degradation product formation are detected during long-term, accelerated and/or stress stability studies, consideration should be given to potential hazards and to the need for characterisation and quantification of degradation products within the long-term stability program. Acceptable limits should be proposed and justified, taking into account the levels observed in material used in preclinical and clinical studies.

For substances that cannot be properly characterised or products for which an exact analysis of the purity cannot be determined through routine analytical methods, the applicant should propose and justify alternative testing procedures.

5.4. Other Product Characteristics

The following product characteristics, though not specifically relating to biotechnological/biological products, should be monitored and reported for the drug product in its final container:

> Visual appearance of the product (colour and opacity for solutions/suspensions; colour, texture and dissolution time for powders), visible particulates in solutions or after the reconstitution of powders or lyophilised cakes, pH, and moisture level of powders and lyophilised products.

Sterility testing or alternatives (e.g., container/closure integrity testing) should be performed at a minimum initially and at the end of the proposed shelf-life.

Additives (e.g., stabilisers, preservatives) or excipients may degrade during the dating period of the drug product. If there is any indication during preliminary stability studies that reaction or degradation of such materials adversely affect the quality of the drug product, these items may need to be monitored during the stability program.

The container/closure has the potential to adversely affect the product and should be carefully evaluated (see below).

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6. STORAGE CONDITIONS

6.1. Temperature

Since most finished biotechnological/biological products need precisely defined storage temperatures, the storage conditions for the real-time/real-temperature stability studies may be confined to the proposed storage temperature.

6.2. Humidity

Biotechnological/biological products are generally distributed in containers protecting them against humidity. Therefore, where it can be demonstrated that the proposed containers (and conditions of storage) afford sufficient protection against high and low humidity, stability tests at different relative humidities can usually be omitted. Where humidity-protecting containers are not used, appropriate stability data should be provided.

6.3. Accelerated and Stress Conditions

As previously noted, the expiration dating should be based on real-time/real-temperature data. However, it is strongly suggested that studies be conducted on the drug substance and drug product under accelerated and stress conditions. Studies under accelerated conditions may provide useful support data for establishing the expiration date, provide product stability information for future product development (e.g., preliminary assessment of proposed manufacturing changes such as change in formulation, scale-up), assist in validation of analytical methods for the stability program, or generate information which may help elucidate the degradation profile of the drug substance or drug product. Studies under stress conditions may be useful in determining whether accidental exposures to conditions other than those proposed (e.g., during transportation) are deleterious to the product and also for evaluating which specific test parameters may be the best indicators of product stability. Studies of the exposure of the drug substance or drug product to extreme conditions may help to reveal patterns of degradation; if so, such changes should be monitored under proposed storage conditions. While the tripartite guideline on stability describes the conditions of the accelerated and stress study, the applicant should note that those conditions may not be appropriate for biotechnological/biological products. Conditions should be carefully selected on a case-by-case basis.

6.4. Light

Applicants should consult the appropriate regulatory authorities on a case-by-case basis to determine guidance for testing.

6.5. Container/Closure

Changes in the quality of the product may occur due to the interactions between the formulated biotechnological/biological product and container/closure. Where the lack of interactions cannot be excluded in liquid products (other than sealed ampoules), stability studies should include samples maintained in the inverted or horizontal position (i.e., in contact with the closure), as well as in the upright position, to determine the effects of the closure on product quality. Data should be supplied for all different container/closure combinations that will be marketed.

In addition to the standard data necessary for a conventional single-use vial, the applicant should demonstrate that the closure used with a multiple-dose vial is capable of withstanding the conditions of repeated insertions and withdrawals so that the product retains its full potency, purity, and quality for the maximum period

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specified in the instructions-for-use on containers, packages, and/or package inserts. Such labelling should be in accordance with relevant national/regional requirements.

6.6. Stability after Reconstitution of Freeze-Dried Product

The stability of freeze-dried products after their reconstitution should be demonstrated for the conditions and the maximum storage period specified on containers, packages, and/or package inserts. Such labelling should be in accordance with relevant national/regional requirements.

7. TESTING FREQUENCY

The shelf-lives of biotechnological/biological products may vary from days to several years. Thus, it is difficult to draft uniform guidelines regarding the stability study duration and testing frequency that would be applicable to all types of biotechnological/biological products. With only a few exceptions, however, the shelf-lives for existing products and potential future products will be within the range of 0.5 to 5 years. Therefore, the guidance is based upon expected shelf-lives in that range. This takes into account the fact that degradation of biotechnological/biological products may not be governed by the same factors during different intervals of a long storage period.

When shelf-lives of 1 year or less are proposed, the real-time stability studies should be conducted monthly for the first 3 months and at 3 month intervals thereafter.

For products with proposed shelf-lives of greater than 1 year, the studies should be conducted every 3 months during the first year of storage, every 6 months during the second year, and annually thereafter.

While the testing intervals listed above may be appropriate in the pre-approval or pre-licence stage, reduced testing may be appropriate after approval or licensure where data are available that demonstrate adequate stability. Where data exist that indicate the stability of a product is not compromised, the applicant is encouraged to submit a protocol which supports elimination of specific test intervals (e.g., 9 month testing) for post-approval/post-licensure, long-term studies.

8. SPECIFICATIONS

Although biotechnological/biological products may be subject to significant losses of activity, physicochemical changes, or degradation during storage, international and national regulations have provided little guidance with respect to distinct release and end of shelf-life specifications. Recommendations for maximum acceptable losses of activity, limits for physicochemical changes, or degradation during the proposed shelf-life have not been developed for individual types or groups of biotechnological/biological products but are considered on a case-by-case basis. Each product should retain its specifications within established limits for safety, purity, and potency throughout its proposed shelf-life. These specifications and limits should be derived from all available information using the appropriate statistical methods. The use of different specifications for release and expiration should be supported by sufficient data to demonstrate that clinical performance is not affected as discussed in the tripartite guideline on stability.

9. LABELLING

For most biotechnological/biological drug substances and drug products, precisely defined storage temperatures are recommended. Specific recommendations should be stated, particularly for drug substances and drug products that cannot tolerate

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freezing. These conditions, and where appropriate, recommendations for protection against light and/or humidity, should appear on containers, packages, and/or package inserts. Such labelling should be in accordance with relevant national/regional requirements.

10. GLOSSARY

Conjugated Product

A conjugated product is made up of an active ingredient (for example, peptide, carbohydrate) bound covalently or noncovalently to a carrier (for example, protein, peptide, inorganic mineral) with the objective of improving the efficacy or stability of the product.

Degradation Product

A molecule resulting from a change in the drug substance (bulk material) brought about over time. For the purpose of stability testing of the products described in this guideline, such changes could occur as a result of processing or storage (e.g., by deamidation, oxidation, aggregation, proteolysis). For biotechnological/biological products some degradation products may be active.

Impurity

Any component of the drug substance (bulk material) or drug product (final container product) which is not the chemical entity defined as the drug substance, an excipient, or other additives to the drug product.

Intermediate

For biotechnological/biological products, a material produced during a manufacturing process which is not the drug substance or the drug product but whose manufacture is critical to the successful production of the drug substance or the drug product. Generally, an intermediate will be quantifiable and specifications will be established to determine the successful completion of the manufacturing step prior to continuation of the manufacturing process. This includes material which may undergo further molecular modification or be held for an extended period of time prior to further processing.

Manufacturing Scale Production

Manufacture at the scale typically encountered in a facility intended for product production for marketing.

Pilot-Plant Scale

The production of the drug substance or drug product by a procedure fully representative of and simulating that to be applied at manufacturing scale. The methods of cell expansion, harvest, and product purification should be identical except for the scale of production.

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Acknowledgement of receipt

We hereby acknowledge receipt of the following submission by the opponent:

Submission number	6927342
Application number	EP11001632.6
Patent number	EP2359834
Date of receipt	20 November 2018
Your reference	198 441 u6/ne
Opponent	Hoffmann Eitle Patent- und Rechtsanwälte PartmbB
Title	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement

Documents submitted	package-data.xml ep-oppo.pdf (2 p.) Published-Evidence-2.pdf\D40 Mueller et al. (1997).pdf (12 p.) Published-Evidence-4.pdf\D42 WO 2005092073 A2.pdf (50 p.) Other-evidence-1.PDF\D39 ICH Guidelines.PDF (10 p.)	ep-opposition-data.xml OPPO.PDF\2018-11-20 Submission before oral proceedings.PDF (16 p.) Published-Evidence-3.pdf\D41 WO 2004071439 A2.pdf (32 p.) Published-Evidence-5.pdf\D43 WO 2006003179 A2.pdf (153 p.)

Submitted by	CN=Nicole Egloffstein-Hein 56945
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Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

J A • K E M P

21 November 2018

Our Ref: C411599EP

SUBMISSION UNDER R116

Patent No:	2359834
Application No:	11001632.6
Patentee:	Alexion Pharmaceuticals, Inc.
Opponents:	(1) Amgen Inc. (2) Hoffmann Eitle

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1 REQUESTS

1.1 The Patent Proprietor requests that the Opposition Division maintains the Patent as granted.

1.2 If the Opposition Division is not able to grant the Main Request, even after discussion at Oral Proceedings, then the Patent Proprietor requests that the patent is maintained on the basis of one of Auxiliary Requests 1 or 2 enclosed herewith. Also enclosed, to assist the Opposition Division, are tracked copies of Auxiliary Requests 1 and 2 showing the amendments which have been made compared with the Main Request.

1.3 It is further requested that, should any minor alterations be required to these Auxiliary Requests, an opportunity be provided to make such amendments at the Oral Proceedings.

1.4 We shall speak in English at the Oral Proceedings. We shall require interpretation into English should any other party speak in a language other than English.

1.5 We shall be accompanied at the hearing by Todd Spalding and Tara Rahemba of Alexion Pharmaceuticals, Inc and Dr Dino Miano, also of Alexion Pharmaceuticals, Inc and author of declaration D33. I request permission for Dr Miano to speak at the oral proceedings, should this be appropriate. Dr Miano's qualifications are set out in D33. Dr Miano may speak in relation to the commercial product, Soliris. Dr Miano may also discuss the subject matter contained in his declaration, in particular the stability data presented therein, as well as the content of Dr Treuheit's declaration D23.

2 DOCUMENTS

2.1 The following additional documents are cited and enclosed herewith:

D39 Declaration of Dr Alexander Klibanov.

D40 Rational Design of Stable Protein Formulations, Pharmaceutical Biotechnology Volume 13, 2002 Kluwer Academic/Plenium Publishers; Chapters 1, 5 and 8.

D41 Alberts *et al*, Molecular Biology of the Cell, Third Edition, 1994, page 585.

2.2 We also enclose a consolidated list of all the documents cited by both Opponents and the Patent Proprietor, including the additional documents mentioned above.

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3 Declaration of Dr Klibanov and Inventive Step

3.1 Dr Klibanov is an expert in the formulation of therapeutic proteins, including antibodies. In his declaration, Dr Klibanov provides his expertise on the issues which the skilled person in this field would have faced around 2007, the filing date of the present patent. Dr Klibanov particularly comments on the complexities and challenges which the skilled person would have faced in 2007, when developing a therapeutic antibody formulation.

3.2 In the Summons, the Opposition Division summarise the arguments of the Patent Proprietor in the submission of 22 March 2018 and comment briefly on the Proprietor's position that formulating antibodies was a complex area. It is the Proprietor's position that the difficulties in antibody formulation in 2007 cannot be overestimated and it is considered important that the significance of these issues is fully appreciated. Accordingly, Dr Klibanov, who is an expert in this field and was working in this area in 2007, has been asked to provide his expert opinion.

3.3 The relevance of Dr Klibanov's declaration to the Patent Proprietor's position on inventive step will be explained further below with reference to the problem and solution approach:

3.4 The Opposition Division set out their view that D1 can be taken as the closest prior art document. The difference between the claimed subject matter and that of D1 is at least the following:

- The formulation is a single use 300mg dosage form;
- The concentration is 10 mg/ml;
- The volume is 30ml;
- The solution is preservative free.

3.5 As explained in detail in section 5.3 of the 22 March 2018 submission, the technical effect of the claimed composition is extremely good long term stability, such that the antibody maintains its integrity and biological activity in the composition. Further, the 10 mg/ml concentration provides this enhanced stability in a low-volume liquid formulation which is easy for the clinician to store and use.

3.6 Accordingly, the objective technical problem to be solved over D1 is to provide a composition of eculizumab, an antibody having a unique, engineered structure, which provides the ideal and necessary balance of properties for use in the treatment of PNH, including enhanced maintenance of biological integrity following prolonged storage, whilst also providing a low-volume liquid formulation.

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3.7 The solution to this problem is the 300 mg single-use dosage form of eculizumab, comprising 30 ml of a 10 mg/ml preservative free solution.

3.8 The skilled person could not have predicted, at the filing date of the patent, that the 10 mg/ml composition would provide the required balance of low volume formulation combined with high stability. Indeed, as Dr Klibanov explains in his declaration at paragraph 4, achieving long term stability was a major challenge at the time:

"One of the major challenges in developing a successful pharmaceutical formulation of a therapeutic antibody (and of any therapeutic protein) around 2007 was, and still is today, being able to create a composition that is stable enough for sufficiently long periods of time such that the antibody does not undergo deleterious physical or chemical degradation reactions or form aggregates prior to administration."

3.9 Dr Klibanov comments that a skilled person faced with the task of formulating a new therapeutic protein would not have expected to be able to develop a liquid composition at all, due to these potential instabilities (see paragraph 6, D39). Dr Klibanov goes on to discuss the difficulties of balancing the various causes of protein instability, concluding in paragraph 10:

"Given the complexity and the sheer number of the various modes of protein instability and the diverse mechanisms underlying each of them, there was no universal approach to successfully formulating an antibody in 2007 (nor is there one now). My both academic and industrial experience at the time was that the field of antibody formulation was difficult, case-specific, and uncertain. For these reasons, a person skilled in the art aiming to develop a liquid formulation of a new antibody with a sufficient storage stability at a high concentration would not have been able to predict which, if any, such formulation would meet the necessary requirements."

3.10 Dr Klibanov's view therefore confirms the unpredictability of the field. The Opponents suggest that the composition of eculizumab described in claim 1 would have been obvious, based on disclosures relating to formulations of different antibodies, in particular D20 and D21. Dr Klibanov's experience, however, demonstrates that this is not the case: as he confirms, in fact, each antibody needs to be considered individually. This requires a uniquely-designed, detailed research program to determine the ideal composition for a given antibody. A person skilled in the art would not have had any confidence that applying a formulation found to be successful for a different antibody would also be successful with eculizumab.

3.11 In particular, in paragraphs 8 and 9 of his declaration, Dr Klibanov explains that each of D20 and D21 referred to by the Opponents highlight the uniqueness of every antibody and the unpredictable nature of formulating a stable antibody composition. D20 teaches that *"each antibody seems to have a unique personality related to its requirements for stability"* (see

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paragraph 9 of D39), and D21 stresses *"that one formulation excipient stabilizing a specific antibody may not be suitable for another because of the difference in their sequence"* (see paragraph 9 of D39).

3.12 Further, eculizumab itself would have presented particular difficulties to the skilled person in developing a suitable formulation. As set out in paragraph 3.6 above, the objective technical problem relates to providing a suitable formulation for eculizumab, which has a unique, engineered structure. Dr Klibanov explains at paragraphs 7 to 9 of D39 that even small structural differences between antibodies can lead to significant differences in behaviour in liquid compositions. For instance, at paragraph 7, Dr Klibanov refers to a 2002 text book:

"*The book explains that 'the exquisite sensitivity of protein structure, function, and stability to the primary sequence does not readily lend itself to a generic approach for protein formulation' and that '[e]ven for closely related proteins, the relative stability and major pathways for degradation might be quite different.' (Ex. 2 at 185-186.) In the 2007 time-frame, researchers like myself understood that therapeutic antibody (or protein in general) formulations, especially liquid ones, are* ***not*** *amenable to a 'one size fits all' approach."*

3.13 The sensitivity of protein stability to primary sequence, which Dr Klibanov here refers to, would have been particularly relevant to eculizumab, given its uniquely-engineered structure.

3.14 Dr Klibanov's declaration therefore demonstrates that the skilled person in the art in 2007, faced with the issue of developing a pharmaceutical composition for eculizumab which provides the right balance of a low volume formulation with high stability, would not have been able to predict whether a liquid composition would be possible at all, let alone be able to predict, with a reasonable expectation of success, that a 10 mg/ml formulation in particular would provide the required balance of properties. For these reasons, the Proprietor maintains that the claimed subject matter is in accordance with Article 56 EPC.

4 Insufficiency

4.1 The skilled person would have had no difficulty in carrying out the invention as claimed, which is to provide a single-use dosage form of a sterile and preservative free solution comprising 30ml of a 10mg eculizumab/ml solution and to use it to treat PNH patients. This is because the full heavy and light chain sequences of eculizumab are disclosed in SEQ ID NOs: 2 and 4 in the patent, enabling the skilled person to produce eculizumab.

4.2 Although SEQ ID NO: 4 in the patent is the *immature* light chain sequence of eculizumab and thus contains the signal peptide, the signal peptide would be cleaved off during antibody production in the cell, and this would have been known to the skilled person as part of his common general

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knowledge at the effective filing date (see, for example, D41, an extract from the 1994 textbook, Alberts *et al*). Therefore, the skilled person would have been able to make (the mature form of) eculizumab, and hence a single-use dosage form of a sterile and preservative free solution comprising 30ml of a 10mg eculizumab/ml solution, without an undue burden in 2007.

5 Auxiliary Requests

5.1 Auxiliary Request 1 specifies that administration is by intravenous infusion. Basis for this amendment can be found on page 22 of the application as filed. For instance, page 22 lines 18 to 20 indicate that intravenous administration is the most preferable route of administration, and page 22, line 32 also specifies intravenous infusion.

5.2 Auxiliary Request 2 specifies that administration is via IV infusion and that the composition is diluted to a final concentration of 5 mg/ml prior to administration. Basis for this amendment can be found at page 22, lines 27 to 29 of the application as filed.

5.3 Each of these Auxiliary Requests is filed in order to address the arguments raised by the Opponents in relation to inventive step.

6 Summary

6.1 For the reasons discussed above and in our previous response, the Proprietor requests that the patent is maintained as granted and the oppositions are dismissed.

Signed

AMANDA SIMONS
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ASSOCIATION NO. 811

PAMELA TUXWORTH
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November 21, 2018

Opposition against European patent EP 2 359 834
(Application No. 11 001 632.6)
Title: Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor: Alexion Pharmaceuticals, Inc.
Opponent: Amgen Inc.
Our Ref.: AMG17203OP

In preparation of Oral Proceedings and in Reply to Proprietor's Submission dated March 22, 2018

I. Requests

1. We maintain our request pursuant to Article 101(2) EPC that EP 2 359 834 (hereinafter "the opposed patent") be revoked in its entirety.

2. We further maintain our request for oral proceedings pursuant to Article 116(1) EPC, in case the Opposition Division does not consider the above request justified on the basis of our written submissions.

II. Newly submitted documents

3. We herewith submit the following documents in response to the Proprietor's Observations:

D39 Tacken, P.J. *et al. Blood* **2005**, *106(4)*, 1278-1285;

D40 WO 2004/050017 A2 (Bowdish, K. S. *et al.*, June 17, 2004);

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D41 WO 2004/108158 A1 (Rother, R. P. *et al.*, December 16, 2004);

D42 U.S. Patent No. 6,407,213;

D43 Emery *et al.*, *Arthritis & Rheumatism* 2006, *54(5)*, 1390-1400;

D44 Rituximab 1997 Product Data Sheet;

D45 Declaration by Prof. Ronald Taylor, including CV (Appendix A); and

D46 Hillmen *et al.*, *N. Eng. J. Med.* 1995, 333:1253-8.

III. Inadmissible Amendments (Art. 100(c) EPC / Art. 76(1) EPC, 123(2) EPC) and Insufficiency of Disclosure (Art. 83 EPC)

4. We have noted the OD's preliminary views expressed under items 6 and 8 of the Preliminary Opinion according to which the requirements of Articles 76(1) and 83 EPC are met. We are grateful for the OD's guidance in this regard, although we respectfully disagree. We shall expand on the issue of compliance with the requirements of Articles 100(b) and (c) EPC in the course of oral proceedings.

IV. No Inventive Step (Article 100(a) EPC / Article 56 EPC)

5. As argued in our Notice of Opposition, **D1** may be regarded as the closest prior art, which was not disputed by the Proprietor. In the alternative, **D2** to **D4**, **D11** and **D18** all represent promising springboards for the claimed subject matter. The allegedly distinguishing features of the claimed subject matter over **D1** are

- the single-use dosage form of 300 mg,
- in a concentration of 10 mg eculizumab/mL
- resulting in a total volume of 30 mL
- wherein the solution is preservative free.

6. The application is silent about supposed technical effects of <u>any of these features</u>. As argued before (sections 36 to 42 of our Notice of Opposition), it is plainly obvious and clearly most convenient for the person of skill in the art to select an amount of 300 mg eculizumab in order to provide a composition that is suitable for the administration of individual doses of 600 mg or 900 mg, as disclosed *inter alia* in **D1** (e.g., [0082], [0088], [0091]). We also enclose a Declaration by Prof. Taylor (**D45**) which confirms this, referring to the example of the use of a single-use dosage form of rituximab of 500 mg for doses of 500 or 1000 mg to treat

rheumatoid arthritis (see **D43** and **D44**). We also note that a similar view was (rightly) taken by the Primary Examiner during prosecution of the opposed patent (Communication dated July 21, 2011, p. 3, 6th paragraph).

7. Further, based on obvious medical considerations, but also regulatory guidelines (**D19**, p. 1/9, 4th and 5th paragraph), the skilled person would avoid using preservatives in multi-dose preparations, whenever possible. Once an amount and concentration of active agent have been determined, the total volume is inherently defined. This leaves the antibody **concentration of 10 mg/mL** as the only possibly relevant distinguishing feature over the prior art.

8. Notably, and in agreement with our position, the Proprietor has focused on discussing the alleged technical effects of the concentration of 10 mg/mL.

9. On a side note, the Proprietor argues that PNH would require that patients start treatment very quickly after having been diagnosed (e.g., sections 4.2.2 and 4.2.3). This allegedly requires that eculizumab must be stored *"on site at a location nearby"* to be ready for use when a patient is identified. It is unclear why the specific *"nature of PNH"* would demand such storage nearby, though. In the absence of any tangible evidence that there is a difference in this regard between PHN and any other disease evoking significant symptoms, this remains a completely unsupported allegation which is contested.

10. Indeed, many of the documents cited in our Notice of Opposition or during prosecution contain general descriptions of PNH and the repercussions the disease has on a patient's life. None of the documents mentions that it would be necessary to start treatment immediately after diagnosis. It seems worth pointing out that PNH is a chronic condition and the treatment with eculizumab aims at improving the quality of life of afflicted patients (see, e.g., [0030] of **B1**). The therapy does not aim at curing an acutely life-threatening condition. Accordingly, trials described in the prior art commonly involved observation periods of up to 13 weeks after identification of eligible patients and before start of the treatment (cf. **D18**, p. 1234, right column 1st and 3rd full paragraph; but also [0070] of **B1**). This would hardly appear to be ethically justified if treatment would indeed have to start immediately, as purported by the Proprietor. We also note that **D18** reports the need to vaccinate PHN patients prior to treatment against *N. meningitides* (page 1234, right column, end of 2nd paragraph), again indicating that treatment was not initiated immediately upon diagnosis. Further, we refer to new reference **D46** (Hillmen, enclosed herewith), whose authors followed a group of 80 PHN patients (see Abstract) for up to 48 years and reported a median survival of 10 years (see the Discussion). None of the statement in this article indicates that PHN patients, once diagnosed, would need more immediate treatment than other chronically ill patients. The Proprietor's

allegations in this regard are thus not only unsupported but also contrary to the facts emanating from the documents on file.

11. Any requirements derived from this alleged need for immediate treatment (such as stability over 48 months) must therefore be rated as being artificially construed. The requirements a pharmaceutical composition suitable for use in the treatment of a patient suffering from PNH would have to meet are no different from the requirements pharmaceutical compositions for the treatment of other chronic diseases would have to satisfy.

IV.1 The concentration constitutes an arbitrary choice

12. We maintain that the concentration of 10 mg/mL, presented by the applicant as allegedly providing "the ideal balance" of properties (e.g. section 5.3.2, 5.3.10, 5.4.8, etc. of the submission), constitutes an arbitrary choice among numerous equally (or possibly even more) suitable alternatives.

13. It is established case law that a feature which is considered an arbitrary choice among equivalent variations cannot contribute to inventiveness (**T 206/91** (item 5.5) and **T 37/82** (item 3).

14. In this regard, we first of all note that the concept of "the *ideal balance of properties"* advanced by the Proprietor should be seen for what it is: a red herring which has been artificially created in the hope to successfully argue for an allegedly unobvious technical effect resulting from meeting said artificial (and wholly arbitrary) requirement. The suggestion under item 5.3.2 of the Observations that the allegedly "ideal" balance purportedly achieved by the claim features would have been instrumental to *"the approval of the claimed composition as a treatment for PNH"* has no basis whatsoever. There is no evidence that the regulatory bodies specifically preferred the "balance of properties" offered by the approved formulation over the "balance of properties" displayed by any other formulation. In fact, there is no such thing as an objectively "ideal balance" of properties of any pharmaceutical, since any "balancing" will be based on subjective elements and preferences.

15. As an aside, the approval obviously did not relate to *"the claimed composition"* but to an antibody that has been recombinantly produced in specific cells following a specific manufacturing and purification protocol, and which has been formulated in specific buffers and excipients, and at a specific pH. None of this is mentioned in the claim. There is no evidence that the "balance of properties" displayed by the approved product is shared by each and every formulation falling within the claim; rather, the contrary is to be assumed, as will be explained further below.

16. According to a more realistic proposal by the Proprietor, the underlying problem of the original filing lies in the provision of a(n alternative) useful treatment for patients suffering from PNH (section 5.10.2 of the submission). If anything, a pharmaceutical composition to serve this purpose must be suitable (or useful) for use in the treatment of patients suffering from PNH. Any requirement going beyond this (such as stability during storage over 48 months, maintaining over 98% monomer content, etc.) is – in the absence of any evidence to the contrary – arbitrary, and thus, cannot be invoked as justification for an inventive step.

17. With regard to the 98% monomer threshold we further note that according to **D20**, the standard of the World Health Organization (WHO) for commercial intravenous immunoglobulin products was an aggregate level of less than 5% (**D20**, p. 9, left column, 1st paragraph). This means that, according to the WHO, any antibody solution comprising at least 95% antibody monomer would be suitable for intravenous delivery and could thus be released into the market. It seems obvious that the Proprietor tries to generate an artificially high barrier to make an insignificant difference (if there is any) appear significant. The 98% monomer threshold is therefore not technically meaningful.

18. The Proprietor also failed to provide any evidence that would show that a monomer content of 98% was essential for maintaining biologic activity in an antibody formulation. As noted above there is no evidence that this exact threshold is technically relevant. Why would 96% or 95% monomer content be insufficient? Perhaps more importantly, there is no evidence of a correlation between aggregation and loss-of-activity for this specific antibody! This is even more relevant for the alleged requirement of stability for 48 months. It is apparent from the Proprietor's own allegations that Soliris® obtained market approval by the FDA with a shelf life of 24 months, as will be discussed in more detail below. This means that both, the Proprietor and the regulatory authorities considered a formulation with a shelf life of 24 months, when properly stored, to be sufficiently stable for use in the treatment of PNH.

19. In fact, the concentration of 10 mg/mL cannot serve to distinguish the claimed subject matter from the prior art for the purposes of Article 56 EPC, as there is no particular technical effect conferred by this specific concentration. This is *inter alia* evident from **D23** and **D25** and even from the Proprietor's own data.

20. **D23** presents data on solutions of eculizumab in the buffer which is used for the commercial formulations of the drug, using three different analytical methods, size-exclusion ultra high-performance liquid chromatography (SE-UHPLC), anion exchange high-performance liquid chromatography (AEX-HPLC), and reduced sodium dodecyl sulfate capillary electrophoresis (rCE-SDS). These methods allow for assessing both chemical and structural stability of an antibody solution.

21. The Proprietor has argued that the data presented with the declaration by Dr. Michael Treuheit may not be informative, as stability of the solution was tested *"only over a 13 week period"*, which would be significantly less than the 48-month stability allegedly demonstrated in **D33**. We note in this regard that the Proprietor (and his party expert Dr. Miano) has at the same time admitted that stability testing at elevated temperatures would provide a good indication of effects which might be seen at 2 to 8°C over a longer period of time (see section 5.3.5 of the submission). This is consistent with the statement of Dr. Treuheit (cf. section 4 of **D23**). The data presented in **D23** for elevated temperatures thus provides a good estimate of the behavior of the tested antibody solutions over a longer period of time than the tested 13 weeks.

22. The Proprietor finds the data presented in **D23** to be in line with the data submitted as part of the Miano declaration (**D33**), by selectively interpreting the data to suit his line of arguments. This selective and self-serving interpretation is, however, misleading.

23. Antibody degradation occurs via multiple pathways, some of which are interdependent, such as denaturation, aggregation, surface adsorption, chemical degradation (oxidation, deamidation, formation of acidic species), etc. This has been correctly noted by the Proprietor under section 4.3.3 of the submission. One particular degradation pathway does not necessarily dominate for any one antibody (see also section 4.3.4 of the submission), however, Dr. Miano has solely analyzed aggregation of the antibody at merely two different concentrations (cf. section 3 of **D33**). **D23**, in contrast, provides data on aggregation (SE-HPLC, rCE-SDS) and chemical degradation (AEX-HPLC, rCE-SDS) for five different concentrations. The rCE-SDS analysis, in particular, provides a good overview over different degradation pathways, as it allows for separation of both chemically and structurally altered species with very high resolution (cf. section 7 of **D23**).

24. A closer look at the data of **D23** reveals that all tested concentrations of eculizumab (i.e., 2, 5, 10, 30 and 50 mg/mL) were equally stable at 4°C over the tested period, as measured by SE-HPLC (**D23**, Appendix A). At elevated temperature (25°C), there is slight aggregation visible for 30 mg/mL and 50 mg/mL, but this is at levels that are hardly significant. The lower concentrations (10, 5 and 2 mg/mL) show levels of monomer at the latest time point that are higher than at the earliest time point. This may be taken as an indicator of experimental noise in the measurement and the observed decrease in the monomer content at 30 mg/mL and 50 mg/mL is well within these limits.

25. In any case, all concentrations show a monomer content that is above 98% at any time point and would thus be suitable for release into the market according to the (we believe arbitrary) limits defined by the Proprietor (section 3 of **D33** and e.g. sections 5.3.4, 5.3.6 of the submission) and even more so within the limit defined by the WHO (95% monomer). The

Proprietor alleged that the 30 mg/mL and 50 mg/mL concentrations would fall *"below the release parameter for commercial lots of eculizumab"* (section 5.4.5 of the submission), but this is clearly incorrect, as all tested solutions exhibit a monomer content that is comfortably above the 98% threshold at any time point.

26. In addition, the lower concentrations of 5 and 2 mg/mL exhibit a monomer content at the latest time point that is higher than for the 10 mg/mL solution. Thus, if one follows the interpretation of the Proprietor and takes these very small differences into account, 10 mg/mL is actually less stable than either 5 mg/mL or 2 mg/mL and thus obviously not the "ideal" concentration. If the observed increase of monomer content for the 5 mg/mL and 2 mg/mL is interpreted as noise, they are at least as stable as the 10 mg/mL solution, i.e., the concentration of 10 mg/mL is of no advantage.

27. We note at this point that the commercial formulation of eculizumab is diluted to a concentration of 5 mg/mL prior to administration to the patient (section 5.3.9 of the submission and **D24**, p. 2 under *"Preparation and Administration"*), which means that the higher concentration of 10 mg/mL is, in any case, of no benefit to the patient, when compared to 5 mg/mL. The infusion volume (and thus infusion time) would be identical no matter if the antibody was stored at a concentration of 10 mg/mL or 5 mg/mL (or alternatively 6, 7, 8 or 9 mg/mL).

28. When looking at the chemical degradation, as measured by AEX-HPLC in **D23** (Appendix B), there is, again, no significant difference between the tested concentrations. Slight degradation is visible for all samples at elevated temperatures, but no discernible advantage of any concentration is apparent. If anything, when taking minimal changes into account as the Proprietor did, the solution of 30 mg/mL shows slightly less chemical degradation at the latest time point (25°C), compared to 10 mg/mL. In addition, the AEX-HPLC analysis also makes clear that chemical degradation appears to be the more relevant degradation pathway for eculizumab, as all samples show markedly faster degradation than in the SE-HPLC assay.

29. The overall picture (chemical and physical degradation), as determined by rCE-SDS is in accordance with this analysis. If anything, the higher concentrations of 50 mg/mL and 30 mg/mL are slightly more stable than 10 mg/mL (purity at the latest time point) at 4°C. At 25°C some degradation takes place in all samples, but there is no difference between 10, 30 and 50 mg/mL. If anything, 5 mg/mL is slightly worse than the other concentrations.

30. In summary, **D23** shows that, leaving experimental noise aside, there is no difference in stability between solutions of eculizumab, formulated in the commercial buffer at concentrations of 2, 5, 10, 30 and 50 mg/mL. Even if the OD was inclined to take the minimal differences into consideration that are visible in this raw data, there is no clear picture. All

concentrations are well within the specifications purported by the Proprietor (at least 98% monomer content) and 10 mg/mL shows, if anything, slightly faster chemical degradation than 30 mg/mL. The concentration of 10 mg/mL provides no advantage, and hence provides no technical effect that may be taken into account for an assessment of inventive step.

31. According to the Proprietor, the Miano declaration (**D33**) shows the benefits of the concentration of 10 mg/mL. We note that **D33** greatly exaggerates entirely insignificant differences to make a minimal effect appear technically relevant (see in particular the scale on the y-axis). In addition, **D33** only analyzes two concentrations, 10 mg/mL and 30g/mL and therefore cannot serve as evidence that 10 mg/mL is in fact "ideal", or even just better than concentrations of, e.g., 25, 15, 9, 8, 5 mg/mL, etc. Two data points are obviously insufficient to draw such a conclusion.

32. As explained by the Proprietor, the initial shelf life of commercial formulations of eculizumab was determined to be 24 months, which was later extended to 30 months, when stored at 2°C to 8°C (see FDA letters annexed to **D33**). Any discussion relating to stability of the "bulk" drug prior to final formulation is immaterial, as no feature relating to repackaging of bulk product is recited in the claim as granted. This means that both the Proprietor and the regulatory agencies found a shelf life of 24 months to be sufficient for Soliris® to be approved and enter the market at the time the opposed patent was filed.

33. More importantly, as the current shelf life is specified to be 30 months, any final eculizumab drug product older than 30 months would have to be discarded. No responsible physician would administer eculizumab compositions older than the specified shelf life (**D24** at p. 6, under *"How Supplied/Storage and Handling"* explicitly states the obvious: *"Do not use beyond the expiration date stamped on the carton."*). It is thus entirely immaterial, if the composition claimed by the opposed patent is hypothetically stable for 36, 48, or even 100 months. Any stability beyond 24 months, the shelf life which Soliris® was initially approved with, or at most 30 months, makes no difference whatsoever in the context of the original disclosure.

34. Hence, any solution of eculizumab providing sufficient stability over 24 months, when stored properly, would be equally suitable for use in the treatment of patients suffering from PNH. The data provided in **D33** shows that both tested solutions provide this degree of stability (see Fig. 2 of **D33**). Even when the required shelf life is extended to 30 months, Fig. 2 of **D33** shows that both, 10 mg/mL and 30 mg/mL, exhibit a monomer content that is distinctly above 98% after 30 months of storage (and, of course above 95%). This means there is no technically relevant difference between the two concentrations, even if the arbitrary 98% threshold purported by the Proprietor was accepted as a measure.

35. If one assumes a correlation between antibody concentration and aggregation tendency (which the person of skill would do, as further discussed below), this would also be true for concentrations of, e.g., 25, 20 and 15 mg/mL and even more so for concentrations between 10 and 5 mg/mL. As noted above, Soliris® is diluted to 5 mg/mL prior to administration, so reducing the concentration to 5 mg/mL would not be detrimental to the patient in any way.

36. This means that both tested concentrations (and the hypothetical alternatives) are equally suitable for the provision of a solution of eculizumab for use in the treatment of PNH. Even if there were slight differences with regard to the formation of aggregates, they would be insignificant in the context of the original disclosure (and any technical problem retrospectively derived from it), which relates to methods of treatment for PNH.

37. Again we note as an aside that Dr. Miano solely analyzed aggregation and only by one particular analytical method. As explained above, aggregation is only one of numerous different degradation pathways that may affect the activity of an antibody drug. Concluding from this very limited data that 10 mg/mL would provide an "ideal" concentration, in particular with regard to biologic activity (sections 4.2.3, 4.3.1, 4.6.9 of the submission) is complete speculation. It is perfectly possible and, when taking **D23** into consideration, indeed not unlikely that chemical degradation at 10 mg/mL occurs at least as fast as at 30 mg/mL. In any case, biological activity was not tested by Dr. Miano and it is thus readily conceivable that the solutions lost their biologic activity without aggregating.

38. The Proprietor thus failed to submit any evidence that the claimed pharmaceutical composition actually provides any particular effect that is relevant in the context of providing a pharmaceutical composition for use in the treatment of PNH.

39. Lastly, **D25** represents further evidence that there is no actual difference in the stability of eculizumab when stored at concentrations between 10 mg/mL up to more than 100 mg/mL. **D25** is a patent application, filed by the Proprietor of the opposed patent and directed to high concentration formulations of anti-C5 antibodies, particularly eculizumab. The Examples of **D25** disclose a wealth of data regarding numerous criteria of antibody stability for eculizumab (**D25**, [0229]) solutions at concentrations of *inter alia* 10, 30 and 105 mg/mL (**D25**, [0244] to [0265]).

40. In section 5.4.6 of the submission, the Proprietor alleged that there was no indication of the relative stability of high concentration formulations compared to the claimed 10 mg/mL composition in **D25**. This is plainly incorrect. Solution V of **D25** is a solution of eculizumab in a buffer comprising 10 mM sodium phosphate, 150 mM sodium chloride and 0.02% polysorbate 80, at pH 7.0 (**D25**, [0262] to [0265]). This composition is identical to the commercial formulation of Soliris® (cf. section 2 of **D33**).

41. This solution is tested against four other solutions comprising eculizumab in concentrations of either 30 mg/mL or 105 mg/mL with regard to, e.g., appearance and osmolality, SDS-gel band pattern, biologic activity but also monomer content via SE-HPLC (**D25**, table 4). The analysis shows that all tested solutions exhibited a monomer content of at least 98% after storage for 24 months at 2 to 8°C, including 30 mg/mL in two different buffers (see also [0282] of **D25**). Thus, all tested solutions would comply with the requirements formulated by the Proprietor after storage for 2 years. There is no significant difference between any of the tested solutions with regard to monomer content. This is equally true with regard to biologic activity as is evident from Table 6 of **D25** (see also [0306]). The authors conclude that

> *"These results indicate that the formulations described herein maintain the functional as well as structural stability of the anti-C5 antibodies dissolved therein for at least 2 years at 2°C to 8°C."* (**D25**, [0306])

42. In summary, **D25** shows that eculizumab may be formulated at a range of different concentrations, particularly at 30 mg/mL and higher, with very good stability over at least 24 months. Moreover, **D25** shows that there are no meaningful differences in terms of stability and biologic activity between solutions at 10 mg/mL, 30 mg/mL and 105 mg/mL. With regard to stability of the antibody, any one of the solutions disclosed in **D25** would thus be equally suitable for being used in the treatment of patients suffering from PNH.

43. The Proprietor stated that the buffer was changed in **D25**, when moving from the stock solution of 10 mg/mL to the higher concentrations and the different solutions would therefore not be comparable (section 5.4.6 of the submission). Notably, the granted claim contains no limitation with regard to the buffer. This means that the purported technical effect ("ideal" stability) must be provided by the allegedly distinguishing feature (i.e., the concentration of 10 mg/mL) over the whole scope claimed (i.e., in any buffer, at any pH, etc.).

44. Further, if differences in the buffer would have a significant impact with regard to long term stability, which the Proprietor appears to suggest, it would be *prima facie* not credible that the technical problem (or technical effect) put forward by the Proprietor is solved (or achieved) over the whole scope claimed (again, this is also discussed in more detail below).

45. In the present case, the Proprietor explicitly relies on the concentration of 10 mg/mL as the sole feature allegedly providing the solution to the technical problem. The technical effect must therefore be provided by the concentration only and not depend on further features that are not recited in the claims (such as choice of buffer, excipients, pH, etc.). The solutions disclosed in **D25** are therefore perfectly comparable to the formulation as claimed, no matter which buffer was used. **D25** shows that the concentration of 10 mg/mL cannot serve as a

distinguishing feature, as it does not provide any technical effect over the other tested concentrations.

46. Notably, **D25**, which is specifically concerned with high concentration formulations of *inter alia* eculizumab and was filed by the Proprietor of the opposed patent, makes no note whatsoever of any challenges or unusual properties of the antibody arising from its allegedly unique and unusual structure. This sheds considerable doubt on the Proprietor's allegations in this regard.

47. In summary, the concentration of 10 mg/mL provides no relevant technical effect over other concentrations (such as 30 mg/mL), and therefore constitutes an arbitrary choice.

IV.2 The skilled person would arrive at the claimed solution without inventive activity

48. Even assuming a concentration of 10 mg/mL would provide any superior stability of eculizumab over other concentrations, which we contest, the skilled person would arrive at this concentration without inventive activity.

49. Starting from **D1**, the person of skill in the art would essentially be confronted with the problem of identifying a suitable concentration for eculizumab in a pharmaceutical composition for use in the treatment of PNH. Based on common general knowledge (such as **D20**, **D21**), the skilled artisan would be aware that antibodies are commonly formulated for intravenous administration at concentrations ranging from 1 mg/mL to 50 mg/mL (**D21**, table 1) or 0.5 mg/mL to 100 mg/mL (**D20**, table 1).

50. From **D22**, the skilled person would furthermore be aware that specifically eculizumab may be formulated at concentrations between 1 mg/mL and 30 mg/mL (**D22**, Fig. 10 and par. [0170] to [0172]). The Proprietor argued that the skilled artisan would not consider **D22**, as it relates to formulations for nebulization. While we agree that formulations for different routes of administration may be different in certain aspects, factors such as long term stability of the active substance and maintenance of biologic activity are clearly relevant for formulations for any route of administration. Thus, in the absence of any alternative guidance as to which concentration would be suitable for the specific antibody in question, the skilled person would take **D22** as an indication that eculizumab, formulated at a concentration of 1 to 30 mg/mL, would at the very least be sufficiently stable and biologically active to be used as a drug.

51. Importantly, even the buffers disclosed as being suitable in **D22** encompass the buffer used in the commercial formulation of eculizumab (Fig. 10, **D22**: 10 mM sodium phosphate, 150 mM sodium chloride, pH 6-8 and 0.001 to 0.02% Tween 80, compare section 2 of **D33**). "Tween 80" is synonymous for polysorbate 80 (see also [0069] of **D22**).

52. **D22** also discloses that eculizumab maintains its integrity after being formulated according to the teaching of **D22** and subsequent nebulization, as shown by SDS-PAGE and HPLC ([0173] of **D22**).

53. Thus, when faced with the task of identifying a concentration for eculizumab in a pharmaceutical composition for the treatment of PNH, the skilled person would not disregard **D22**, as it shows that eculizumab may be formulated at a concentration of 1 to 30 mg/mL while maintaining its structural integrity, i.e., without aggregating or precipitating.

54. The Proprietor tries to create the impression that **D22** would solely teach eculizumab formulations of 30 mg/mL and therefore teach away from the claimed subject matter (section 5.8.2 of the submission). This is evidently not the case. Fig. 10 of **D22** clearly shows that the concentration of the antibody may range from 1 mg/mL to 30 mg/mL, which obviously encompasses the claimed concentration of 10 mg/mL. Further, paragraphs [0171] and [0172] of **D22** teach that the *"Mab"* referred to in Fig. 10 is eculizumab.

55. Since eculizumab is a humanized monoclonal antibody with a hybrid IgG2/IgG4 constant domain, the skilled person would also readily refer to **D42** (entitled "Method for Making Humanized Antibodies"; submitted herewith) for guidance regarding concentrations. As noted by Prof. Taylor in **D45**, **D42** states that such humanized *"antibodies will typically be formulated … at concentrations of about 1 mg/ml to 10 mg/ml"* (**D42**, col. 45, li. 57, to col. 46, li. 2). **D42** relates to methods for making and using humanized antibodies, including antibodies with hybrid constant domains (**D42**, col. 8, ll. 47-51). Thus, selecting 10 mg/ml as concentration for a humanized mAb with a hybrid IgG2/IgG4 constant domain, such as eculizumab, would have been a typical, arbitrary choice among known concentrations.

56. We therefore submit that starting from **D1**, in combination with either **D22** or **D42** the skilled person would have had a reasonable expectation of success that a pharmaceutical composition comprising eculizumab in a concentration of 10 mg/mL would be useful in the treatment of PNH.

57. At the very least, the skilled person would have adopted a "try-and-see" attitude. We again refer to Board of Appeal decision **T 1241/03**. This case is indeed highly similar to the present one. The patent in suit *inter alia* related to *"aqueous liquid hGH formulations which are stable during long-term storage, namely 6 to 18 months at 2-8°C"* (**T 1241/03**, point 24 of the reasons, emphasis added). A document cited in the proceedings (document (7)) disclosed stable aqueous protein solutions, comprising *"growth and differentiation factors"* (point 28 of the reasons), i.e., not even the precise protein the patent in suit related to. Still, the competent Board found that

> *"[…] the skilled person, although knowing that the development of liquid stable protein formulations is technically unpredictable and does not allow to rely on stability predictions obtained from other, different samples, would not have been deterred from testing the stability of an hGH containing solution comprising the components indicated in document (7).*
>
> *Obviousness is not only at hand when the results are clearly predictable but also when there is a reasonable expectation of success (cf. decision T 149/93 of 23 March 1995; point (5.2) of the reasons). A reasonable expectation of success does not require certainty (cf. decision T 338/97, of 7 February 2000; point (14) of the reasons).*
>
> *Thus, in spite of the understandable uncertainties which always characterize experiments using biologic compounds like proteins, the skilled person had no reason to adopt a sceptical attitude. He/she would have had either some expectations of success or, at worst, no particular expectations of any sort, but only a "try and see" attitude, which - as pointed out in decisions T 333/97 of 5 October 2000; point (13) of the reasons - does not equate with the absence of an reasonable expectation of success (cf. decision T 1045/98 of 22 October 2001; point (17) of the reasons)."*
> (**T 1241/03**, point 31 of the reasons, emphasis added)

58. This approach has been endorsed in further Board of Appeal decisions, such as **T 1149/09** and **T 499/09**.

59. The Proprietor argued that formulation of antibody drugs was regarded *"at the time"* as being a highly complex and unpredictable issue. We note in this regard that **T 1241/03** was concerned with a patent that had a priority date in 1992, i.e., 15 years before the filing date of the opposed patent. Obviously, a task that was regarded to be within the scope of routine experimentation for a skilled artisan in 1992 would have been routine experimentation 15 years later.

60. The Proprietor has further argued that *"a "try and see" situation is considered by the Boards to be limited to particular situations, i.e. where the prior art clearly envisages a specific feature, and the skilled person merely has to determine by routine tests whether such feature does indeed provide a desired effect."* (section 5.5.4 of the submission, emphasis added). This is in fact exactly the case here. **D22** describes formulations of the same antibody (eculizumab) at concentrations ranging between 1 and 30 mg/mL, even in the same buffer. All the skilled person had to do was test a number of concentrations falling within the range disclosed in **D22** (in the very same buffers disclosed in **D22**) and determine their suitability, thereby immediately arriving at concentrations such as 10 mg/mL. Similar considerations apply to **D42**.

61. The Proprietor has stressed that it is essential to consider what the skilled person would have done instead of considering what the skilled person could have done (sections 5.5.1 and 5.5.2 of the submission). Indeed, the skilled person would have been aware of the inherently empirical nature of the task at hand, namely identifying (a) suitable concentration(s) for eculizumab. With this in mind, the skilled person would have looked for any prior art that would indicate a suitable starting point or springboard, which would have undoubtedly led to **D22**. The Proprietor argued that the skilled person would disregard **D22**, but if the alternative would be merely guessing a starting point for the empirical analysis it seems much more likely that the skilled person would have considered the teaching of **D22**, as it teaches stable pharmaceutical formulations of the same antibody. The Proprietor failed to provide any convincing argument why the skilled person would have disregarded **D22**. Further, the skilled person would have alternatively readily considered **D42** for the reasons explained above.

62. Thus, in view of the prior art, the skilled person would have envisaged a range of concentrations (1 to 30 mg/mL) and would subsequently have determined by routine testing which of said concentrations had the desired effect. When testing a limited number of concentrations in the relevant range, for example 1, 10, 20 and 30 mg/mL, and without modifying the teaching of **D22** in any way (i.e., in the same buffer) the skilled person would have instantly arrived at the claimed concentration of 10 mg/mL. No further modification would have been necessary. Alternatively, **D42** would have led the way to the claimed concentration.

63. The skilled person would have been further guided towards the alleged invention by his/her common general knowledge, particularly the known fact that aggregation propensity generally increases with concentration. The authors of review article **D20**, for example note that

> *"Increasing the concentration of antibodies often increases the aggregation tendency of the protein"* (**D20**, p. 9, left column, 2nd full paragraph).

While this differs from the Proprietor's interpretation of the disclosure of **D20** (cf. section 4.6.8 of the submission), the above quote from the document shows that the authors of **D20** would not share the Proprietor's view.

64. Thus, when testing concentrations within the limited range of 1 to 30 mg/mL disclosed in **D22**, the skilled person would have had a clear motivation and incentive based on the common general knowledge to not only test eculizumab at 30 mg/mL, but also at lower concentrations in the expectation of being able to reduce any aggregation tendency that might be found at 30 mg/mL.

65. Thus, the allegedly inventive feature of a concentration of 10 mg/mL would have been arrived at in an obvious manner by following routine experimentation.

66. The Proprietor tried to explain at some length why finding a suitable concentration for eculizumab was a tedious task, necessitating an inventive activity. First, the Proprietor suggested that the formulation of antibodies was a *"complex challenge"* (section 4.3 of the submission). In the absence of any features in the claim further defining the formulation we note that the only challenge the skilled person was faced with in the current case was the identification of suitable concentrations within the range of 1 to 30 mg/mL disclosed in **D22**, (or the range of 1 to 10 mg/ml disclosed in **D42**, for that matter) which would have been achieved (and the claimed solution thereby arrived at) by means of routine experimentation.

67. This is not changed by the fact that *"each antibody is unique"* (section 4.4 of the submission). Even if this was the case, it does not render finding a suitable antibody concentration inventive. Finding a suitable concentration belongs to the steps which are routinely undertaken when identifying a useful formulation for a specific antibody. According to established case law, work *"involving mere routine experiments, such as merely conventional trial-and-error experimentation without employing skills beyond common general knowledge, lack[s] inventive step (**T 455/91**, **T 104/92**)"* (Case Law of the Boards of Appeal, 8th Edition, section 9.18.5). It is not apparent which skills beyond the average and which insights going beyond mere common general knowledge would have been involved in finding suitable (or even the claimed allegedly *"ideal"*) concentration(s) for eculizumab.

68. Notably, many passages of the Proprietor's observations relate to the identification of, e.g., the right *"formulation components"* (section 4.4.1 of the submission), pH value (section 4.4.2 of the submission), *"pH and excipients"* (section 4.5.1 of the submission), lyoprotective additives (ibid.), and so on. Importantly, however, no features related to such parameters are recited in the granted claim. The Proprietor tries to draw a picture of the skilled person being confronted with a complex, multi-dimensional problem. This attempt must fail already in view of the lack of corresponding features of the claim of the opposed patent, and the lack of evidence of an ambitious technical problem having been solved. On the contrary, the skilled person would have been confronted with a rather straightforward task, namely the identification of suitable concentrations. Of course, the determination of suitable concentrations would have to be performed in any case, as the skilled person anyway would have to make a choice in this regard (and would be able to rely on simple and routine measures in this regard) when formulating an antibody.

69. With regard to antibody concentrations used in earlier formulations (section 4.6 of the submission, also section 47 of our Notice of Opposition), the Proprietor argued that the concentration of 10 mg/mL would not clearly emerge as the single most common concentration for pharmaceutical antibody formulations prior to the filing date of the opposed patent. This is, however, not necessary for a finding of obviousness. As noted above,

obviousness is not only at hand when the results are clearly predictable, but also when there is a reasonable expectation of success (**T 149/93**), which does not require certainty (**T 338/97**). This of course means that it is equally not necessary that a skilled person is able to predict a suitable formulation from his/her common general knowledge (section 4.6.7 of the submission). It is similarly immaterial that *"[t]here is no single technique or set of excipients and buffer/pH conditions, which formulation experts have at their disposal, which is guaranteed to lead to stability of any particular antibody product"* (section 4.6.8 of the submission, emphasis added).

70. As correctly summarized by the Proprietor, an observation that can be made based on the antibody products on the market before the filing date of the patent is that *"[t]hose products that can be stored in solution form include five products having a concentration from 1 to 2 mg/ml; four products having a concentration of 10 mg/ml; and three products having a concentration of 20 mg/ml or above"* (section 4.6.3 of the submission). Thus, even assuming 10 mg/mL was not the single most used concentration, it was undoubtedly a very common one. It is not apparent what would have deprived the skilled person of his/her reasonable expectation of success regarding the stability of eculizumab when being formulated at a concentration of 10 mg/mL, particularly not when taking the teaching of **D22** into consideration, which is specific for eculizumab.

71. Finally, the Proprietor puts heavy emphasis on the allegedly unique structure of eculizumab, which supposedly made finding a suitable concentration more challenging, compared to other antibodies. As noted above **D22** specifically discloses suitable concentrations for eculizumab and therefore any (anyway completely hypothetical and speculative) alleged problems due to the uniqueness of the antibody are irrelevant when taking **D22** as prior art into consideration. It further seems worth pointing out that the application as filed does not mention any challenges arising from the structure of eculizumab either. To the contrary, the original disclosure merely states that

> *"Formulations suitable for injection are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed. (1985)."* (**WO**, p. 22, li. 21/22)

The application as filed thus confirms that eculizumab may be formulated according to common procedures. It was also known prior to the filing date of the opposed patent that eculizumab comprises a chimeric IgG2/IgG4 constant region, as evidenced by **D39**, submitted herewith (**D39**, p. 1279, left column, under *"Recombinant antibodies"*). Contrary to the Proprietors allegation, there are other antibodies disclosed in the prior art which contain the same engineered heavy chain constant region (see **D15**, e.g., p. 8, point 9); **D16**, throughout

the document, e.g., p. 5, 10, 11, etc.; but also **D40**, SEQ ID 67 and **D41** p. 18, submitted herewith).

72. None of these documents mentions any complication arising from the structure of the heavy chain constant region, or teaches that the IgG2/IgG4-containing antibody would behave differently when formulating them from other antibodies. To the contrary, all of them contain statements that the disclosed antibodies may be formulated according to standard procedures (**D15**, p. 24, 3rd full paragraph; **D16**, p. 17/18; **D40**, paragraph bridging p. 36/37; **D41**, p. 16). As noted above, this is equally true for **D25**, which specifically relates to high concentration, long-term stable formulations of anti-C5 antibodies, such as eculizumab. Eculizumab was used in all examples of **D25**. Yet, **D25** makes no mention of any difference between eculizumab and other anti-C5 antibodies which would be due to its allegedly unusual heavy chain.

73. Notably, all of the above-mentioned patent applications **D15**, **D16**, **D25**, **D40** and **D41** were filed by Alexion, the Proprietor of the opposed patent.

74. Thus, based on the state of the art at the filing date of the opposed patent, there was nothing to suggest that eculizumab would require conditions for its formulation that would be significantly different from commonly used conditions. It is therefore not apparent that the skilled person would have been discouraged from testing commonly used concentrations and confirming the expected usefulness of such concentrations, including the concentration as claimed. Indeed, the concentration which according to the Proprietor is "ideal", can by no means be considered to be unusual.

75. In summary, the claimed subject matter is wholly obvious in view of **D1** in combination with **D22** (or **D42**) and/or common general knowledge.

IV.3 <u>The technical problem as formulated by the proprietor is not derivable from the application as filed and has in any event not been credibly solved</u>

76. When justifying the reformulation of the technical problem, the Proprietor pointed to the Guidelines of Examination (section 5.10.1 of the submission), which state that it may be possible to rely on new effects *"submitted subsequently during the proceedings by the applicant, provided that the skilled person would recognize these effects <u>as implied by or related to the technical problem initially suggested</u> (see G−VII, 11 and T 184/82)"* (emphasis added). Under section 5.10.3 of the submission, the Proprietor then recites the example at G-VII-11 of the Guidelines where pharmaceutical activity and toxicity are considered to be sufficiently related for the technical problem to be reformulated accordingly. However, the

opposed patent, and the application underlying it utterly fail to provide a sufficient basis for the reformulation of the technical problem attempted in the present case.

77. The problem initially suggested was the provision of a treatment for patients suffering from PNH using eculizumab:

> *"In certain aspects, the application provides a method of treating a patient suffering from paroxysmal nocturnal hemoglobinuria by administering a pharmaceutical composition comprising an antibody that binds C5 or an active antibody fragment thereof. In certain embodiments, the antibody that binds C5 or an active antibody fragment thereof is eculizumab."* (**WO**, p. li. 24 to 28)

This is supported by various similar statements throughout the original disclosure. In accordance with this interpretation, the Proprietor formulated the underlying problem of the original filing to lie in the provision of a useful treatment for patients suffering from PNH (see also again section 5.10.2 of the submission).

78. The Proprietor then alleges that the application as filed would justify reformulating the technical problem as the *"provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced stability and thus maintenance of biological integrity following prolonged storage in the absence of preservative, whilst also providing a low-volume liquid formulation having acceptable infusion times and which is convenient to store"* (section 5.11.1 of the submission).

79. We note that this is not only a wholly artificial but also a highly specific problem, and one that is simply not derivable from the application as filed. Even if, as in the example given in the Guidelines at G-VII-11, pharmaceutical activity and toxicity were to be considered sufficiently related to allow for changing the technical problem accordingly, this is not so in the case of pharmaceutical activity and, e.g., enhanced stability, maintenance of biological integrity following prolonged storage in the absence of preservative, low-volume liquid formulation having acceptable infusion times and convenience to store, **let alone the provision of the allegedly "ideal combination" of all of the above** (which would obviously require some comparison to the prior art). Rather, these are galenic questions. The opposed patent is, however, not concerned with galenic questions at all.

80. In this regard, we refer to the Case Law Book which notes at page 458 of the English version (under item II.E.1.14) that reformulating the technical problem is not in breach of Art. 123(2) EPC provided the problem as clarified and the solution proposed could have been deduced from the application as a whole. In this regard, the Case Law Book cites **T 871/08** which

rejected the reformulation of the technical problem vis-à-vis the prior art requested in that case as follows:

> *4.3.4 … The board observes that a reformulation of the problem taking into account the above arguments would only be allowable, if the new problem could be deduced from the application as filed when considered in the light of the closest state in the art (see in this respect T 0013/84, OJ EPO 1986, 253; T 0530/90, point 4.3 of the reasons). In the present case, there is no basis in the application as filed for the provision of the so-called "safety margin for stable operation" that the respondent emphasised during the oral proceedings, nor can the latter be deduced from the application as filed by a person skilled in the art.*
>
> *4.3.5 It follows that the problem is to be reformulated in less ambitious terms, namely, to provide an alternative process for the production of hydrogen peroxide according to the anthraquinone process."* (Emphasis added)

Similarly to the concept of the "*safety margin for stable operation"* which the proprietor in **T 871/08** creatively (but eventually unsuccessfully) invented, there is no basis in the application as filed in the light of the closest prior art for reformulating the technical problem to the convoluted construct proposed by the Proprietor.

81. Hence the Proprietor's artificial and arbitrarily defined "ideal balance of properties" must not be used as basis for the discussion of inventive step already for legal reasons.

82. In any case, the data comprised in the original filing, but also in **D33**, by no means show that the claimed composition provides an ideal balance of properties. It is not quite clear what properties the Proprietor has in mind when making this bold claim, but undoubtedly one property that would appear to be highly relevant for a patient suffering from PNH is the biologic activity / drug activity of the active agent (cf. e.g. sections 4.3.1, 4.6.9, 5.3.1, 5.3.3 of the submission). In section 5.3.3 of the submission, the Proprietor states that

> *"[i]t is, of course, critical that the antibody maintains its biological integrity and activity in the period between manufacture and use."* (emphasis added)

83. However, neither the application as filed, nor **D33** contain any information on the activity of the antibody in the tested solutions. No comprehensive data on biological integrity is provided, either. As the Proprietor stated (section 4.3.3 of the submission) and as further discussed below, biological integrity depends on numerous different degradation pathways one of which is aggregation. The mere fact that an antibody does not aggregate over a certain period of time is by no means sufficient to prove that it actually retained its activity, let alone that a

solution exhibits an *"ideal balance of properties"*. Not even biological integrity can be reliably evaluated by this single parameter.

84. According to **D20** (p. 8 to 14), the major degradation pathways for antibodies are physical degradation (i.e., denaturation and aggregation) and chemical degradation (i.e., disulfide bond formation/exchange, nonreducible cross-linking, deamidation, isomerization, oxidation, formation of acidic or basic species, C-terminal clipping, fragmentation and/or Maillard reaction). Of all these pathways, the Proprietor only analyzed one, namely aggregation.

85. **D21** notes in this regard that

> *"Probably the most important aspect of identifying a successful antibody drug formulation is in the identification of truly detrimental alterations rather than all of the modifications that might occur. Although, from a regulatory standpoint, it may be beneficial to understand all of the potential degradation events associated with an antibody formulation, it is the recognition of a stability-indicating parameter (or several parameters) that allows the formulation scientist to focus in on what is critical to development of a successful product. Here, a series of physico-chemical and bioanalytical assays can be used to identify these parameters [32]. Although in vivo bioassays may ultimately be required to validate a stability-indicating formulation parameter, the most critical tools at the disposal of the formulation scientist are rapid and reproducible assays to measure protein changes due to oxidation, deamidation, aggregation, fragmentation and other forms of chemical modification that could lead to a loss of function of the antibody drug."* (**D21**, p. 690, right column, 1st full paragraph, emphasis added)

86. In the present case, the proprietor picked one out of many aspects and chose to present it as being indicative of an *"ideal balance of properties"*. This is not credible by any means when judged in view of the prior art, or in view of the Proprietor's own statements. In fact, it is not even clear if aggregation belongs to the *"truly detrimental alterations"* described in **D21**, or if it is of minor significance for the specific antibody in question, eculizumab.

87. To evaluate any *"balance of properties"*, it would obviously be necessary to evaluate more than one property. At the very least it would appear appropriate to determine *"oxidation, deamidation, aggregation, fragmentation and other forms of chemical modification"* (**D21**, see above). One analysis that may be used to deliver a more complete picture is the rCE-SDS assay performed by Dr. Treuheit (**D23**, Annex C). It shows that there is no significant difference between the tested concentrations. If anything, it shows that 5 mg/mL is slightly less stable than the other tested samples. It does, however, not show any advantage in terms of overall stability (physical and chemical degradation) for the concentration of 10 mg/mL.

88. In summary, it is not credible from the application as filed, or the subsequently filed data (**D33**) that the technical problem as formulated by the Proprietor ("*provision of a composition of eculizumab which demonstrates the ideal balance of properties for use in the treatment of PNH, including enhanced stability and thus maintenance of biological integrity following prolonged storage […]*") has been solved. The original disclosure contains no data in this regard at all and **D33** does not in fact provide evidence for the alleged maintenance of biological integrity, but at best a minute effect, if any, on aggregation, which does not seem to be the most relevant variable for the maintenance of the biological integrity of eculizumab. Any assertion about "ideal" properties is mere speculation.

89. Hence, the technical problem put forward by the Proprietor cannot be derived from (or is insufficiently related to) to original disclosure and in any event has not been credibly solved. Thus, it cannot be accepted as basis for the evaluation of inventive step.

IV.4 The problem has in any event not been credibly solved over the whole scope claimed

90. Even if a concentration of 10 mg/mL was indeed accepted to provide any benefit over other concentrations in the specific formulation that was tested, and even if it were assumed that finding this solution would require an inventive effort (which it should not be for the above reasons), the technical problem would not be credibly solved over the whole scope claimed.

91. The Proprietor alleged in great detail that finding a suitable concentration for eculizumab was a highly challenging endeavor. While we disagree with this assessment, we note that if all of the Proprietor's arguments were indeed accepted as true, one would have to conclude that numerous relevant and in fact essential features are missing from the claim as granted.

92. The Proprietor argued that *"it is very important to maintain the integrity of the antibody and to minimize any possible degradation pathways"* (section 4.3.1 of the submission, emphasis added). Such degradation pathways would include *"denaturation, aggregation, surface adsorption, disulphide bond formation/exchange, non-reducible cross-linking, deamidation, isomerisation, oxidation, formation of acidic or basic species, C-terminal clipping, fragmentation and Maillard reaction. The skilled person looking to formulate an antibody therefore has a multitude of different problems to consider, each of which will apply in different ways to each individual antibody."* (Section 4.3.3 of the submission). As noted before, the data provided by the proprietor relates to only one of these pathways, namely aggregation.

93. The Proprietor further states that

> *"[…] the strategies available to minimize the various degradation pathways may conflict with one another. For instance, deamidation, a major degradation pathway in*

> *antibodies, has been shown to have a strong pH-dependence, with less deamidation occurring at pH 4 compared to pH 7 (**D20**, page 12, right-hand column penultimate paragraph). In contrast, antibody precipitation can be a significant problem at low pH values. **D20** reports (page 11, left-hand column first paragraph) that incubation of two mouse monoclonal antibodies (lgG2a and lgG 1) at 37° C for 32 days caused precipitation at pH 3 and pH 4 solutions for both antibodies. It is therefore difficult to create a stable antibody formulation due to the numerous degradation pathways possible, and the potentially conflicting strategies available to the formulation chemist for dealing with each of them in the context of each individual antibody."* (Section 4.3.5 of the submission)

94. Thus, if the Proprietor's statements are accepted, then optimization of the pH value of an antibody formulation is absolutely crucial to provide long term stability. Due to contrary tendencies with regard to different degradation pathways (deamidation and precipitation), such optimization is allegedly a challenging task. In any case, varying pH values will have a significant impact on the stability of the antibody in solution.

95. Another critical variable appears to be the buffer of the solution, according to the passage from **D21**, recited by the Proprietor in section 4.3.6:

> *"One approach is to find a pH where the minimal amount of degradation events such as deamidation and oxidation will occur. Another is to identify a buffer system that can stabilize the formulation at that pH without damaging the antibody."* (**D21**, p. 701, right hand column, emphasis added)

96. Thus, certain buffer systems may damage an antibody in solution, according to the Proprietor's submission, citing **D21**.

97. Further, specific salt concentrations may lead to dimerization of one particular antibody while not leading to aggregation of a closely related antibody (section 4.4.2 of the submission).

98. The fact that many stress factors may influence aggregation of an antibody during *inter alia* formulation and long term storage is summarized in **D34**, which was submitted by the Proprietor:

> *"Throughout these steps the antibody molecule may encounter several stress factors that can dramatically increase its propensity to aggregate (including variations of temperature, pH, protein concentrations, ionic strength, exposure to air-water interfaces and mechanical stress)."* (**D34**, p. 270, left column, 1st paragraph, emphasis added)

99. Thus, according to the Proprietor and the cited prior art, an individual antibody will be stable only at a specific concentration, at a specific pH, in a specific buffer with a specific amount of certain salts being present. In order to *"determine a suitable composition for the uniquely-engineered eculizumab antibody, the skilled person would have to conduct a significant research program to fully investigate the stability and biological integrity of the antibody, as well as the various factors (and their interplay) affecting each"* (section 5.5.5 of the submission, emphasis added).

100. However, of all the various factors, only the concentration is defined in the granted claim. Consequently, the alleged technical effect must solely be provided by the claimed concentration, at any pH, in any buffer, using any concentration of any salt, etc.

101. These parameters (including concentration) may in our view be determined by means of routine experimentation, particularly when starting from such a promising springboard as **D1** in combination with **D22** or **D42**. If, however, the OD was inclined to follow the Proprietor's argument that the identification of a suitable concentration requires inventive activity, this would equally apply to the other essential variables, such as buffer, pH and salt concentration of the antibody composition. In this case it would in fact appear that the vast majority of embodiments falling under the ambit of the claims could not possibly provide the claimed technical effect and thus could not be assumed to solve the problem, while at the same time the identification of functional embodiments would require an undue burden.

102. The Boards of Appeal have clearly decided, in particular in cases where the Applicant/Proprietor tries to reformulate the technical problem to be solved based on a subsequently invoked technical effect, that this can only be accepted if the problem was credibly/plausibly solved over the whole ambit of the claims. Further, the burden of proof in such a case lies with the Proprietor (see e.g. **T 1188/00**, point 4.5 of the reasons, **T 1417/05**, point 9.4 of the reasons, **T 1034/14**, point 5.4 of the reasons, **T 235/04** point 4.6 to 4.8 of the reasons). However, the Proprietor utterly failed to provide any evidence that the alleged technical effect is in fact provided over the whole scope of the claims.

103. As it is by no means credible/plausible that the technical effect of superior, ideal or merely suitable antibody stability over long periods of storage is provided by the concentration of 10 mg/mL over the whole range of conceivable pH values, buffers and salt concentrations, in particular not in light of the Proprietor's own arguments and the documents on file, the alleged technical effect must be disregarded for the assessment of inventive step (**T 668/94** point 8.3 of the reasons). Any other approach would be in stark contrast to the long standing principle that the extent of a patent monopoly, as defined by the claims, should reflect the technical contribution to the art (e.g. **T 939/92**, point 2.4.2 of the reasons, together with **T 409/91**, point 3.3 and 3.4 of the reasons).

104. The claim as granted lacks an inventive step at least because the technical problem has not been credibly / plausibly solved over the whole ambit of the claims, and for the reasons detailed above.

V. No Priority Entitlement (Article 87 EPC)

105. We have explained in our Notice of Opposition why the opposed patent is not entitled to the claimed priority of US 783070 P. We note that the Proprietor has not submitted any arguments regarding this issue. The filing date of the opposed patent, i.e. March 15, 2007 is thus considered to be the relevant date for any consideration of prior art.

VI. Summary

106. In view of all of the above, revocation of the patent in its entirety is fully justified.

Respectfully,

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European Patent Attorney

UD/SI/KS

Enclosures
Documents **D39** to **D46**

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(54) **Title:** DE-IMMUNIZED ANTI-CD3 ANTIBODY

(57) **Abstract:** Antibodies or functional antibody fragments that recognize or interfere with the production of a component of the CD3 antigen complex are de-immunized.

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DE-IMMUNIZED ANTI-CD3 ANTIBODY

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application 60/475,155 filed June 2, 2003, the entire disclosure of which is incorporated herein by this reference.

BACKGROUND

1. Technical Field

The present disclosure relates to the field of genetically engineered antibodies. More specifically this disclosure relates to anti-CD3 antibodies which have been structurally altered to eliminate binding to HLA proteins, thereby potentially reducing immunogenicity.

2. Background of Related Art

Antibodies are produced by B lymphocytes and defend against infections. The basic structure of an antibody consists of two identical light polypeptide chains and two identical heavy polypeptide chains linked together by disulphide bonds. The first domain located at the amino terminus of each chain is highly variable in amino acid sequence, providing the vast spectrum of antibody binding specificities found in each individual. These are known as variable heavy (VH) and variable light (VL) regions. The other domains of each chain are relatively invariant in amino acid sequence and are known as constant heavy (CH) and constant light (CL) regions.

The interaction between the antigen and the antibody takes place by the

formation of multiple bonds and attractive forces such as hydrogen bonds, electrostatic forces and Van der Waals forces. Together these form considerable binding energy which allows the antibody to bind the antigen. Antibody binding affinity and avidity have been found to affect the physiological and pathological properties of antibodies.

The advent of genetic engineering technology has led to various means of producing unlimited quantities of uniform antibodies (monoclonal antibodies) which, depending upon the isotype, exhibit varying degrees of effector function. For example, certain murine isotypes (IgG1, IgG2) as well as human isotypes (particularly IgG1) can effectively bind to Fc receptors on cells such as monocytes, B cells and NK cells, thereby activating the cells to release cytokines. Such antibody isotypes are also potent in activating complement, with local or systemic inflammatory consequences. The anti-CD3 murine antibody OKT3 is one antibody that has been observed to cause significant cytokine release leading to cytokine release syndrome (CRS). The human CD3 antigen consists of at least four invariant polypeptide chains, which are non-covalently associated with the T cell receptors (TCR) on the surface of T-cells, typically referred to as the CD3 antigen complex. The CD3 antigen complex plays an important role in the T-cell activation upon antigen binding to the T cell receptor. Some anti-CD3 antibodies can activate T-cells in the absence of antigen-TCR ligation, but such activation also depends on the interaction of the Fc portion of the mAb and the Fc receptors on accessory cells to crosslink CD3 complexes on the T-cells. The importance of Fc interactions in anti-CD3 mediated T-cell activation is illustrated by the observation that "mitogenic" anti-CD3 antibodies such as OKT3 do not stimulate T-cells to proliferate in vitro unless they are bound to plastic (which permits CD3 cross-leaking) or bound to Fc

receptor bearing cells.

Antibodies to the CD3 ε signaling molecule of the T-cell receptor complex have proven to be powerful immunosuppressants. For example, OKT3 is a mouse IgG2a/k MAb which recognizes an epitope on the T-cell receptor-CD3 epsilon chain and has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection. The binding of OKT3 to CD3 results in a coating and/or modulation of the entire TcR complex, which mediates TcR blockade and may be one mechanism by which alloantigen and cell-mediated cytotoxicity are inhibited.

The murine OKT3 antibody has been in use therapeutically since its approval in 1985. However, in view of the murine nature of this MAb, a significant human anti-mouse antibody (HAMA) response, with a major anti-idiotype component occurs, which severely limits the dosing potential of this antibody. A HAMA response is initiated when T cells from an individual make an immune response to the administered antibody. The T cells then recruit B cells to generate specific "anti-antibody" antibodies. Thus the HAMA response, though mediated by B cell-generated antibodies directed against mouse antibodies, depends upon an initial T cell response to occur. Clearly, it would be highly desirable to diminish or abolish this HAMA response by suitable humanization or other recombinant DNA manipulation of this very useful antibody and thus enlarge its area of use.

Several techniques have been employed to address the HAMA problem and thus enable the use of therapeutic murine-derived monoclonal antibodies in humans. A common aspect of these methodologies has been the introduction into the therapeutic

antibody, which in general is of rodent origin, of significant tracts of sequence identical to that present in human antibody proteins. Such alterations are also usually coupled to alteration of particular single amino acid residues at positions considered critical to maintaining the antibody-antigen binding interaction. For antibodies, this process is possible due to the very high degree of structural (and functional) conservation between antibody molecules of different species. However for potentially therapeutic proteins where no structural homologue may exist in the host species (e.g. human) for the therapeutic protein, such processes are not applicable.

The term humanized antibody describes a molecule having certain components of the antigen binding site called complementarity determining regions (CDRs) derived from an antibody from a non-human species, while the remaining regions of the antigen binding site (called framework regions) are derived from human antibodies. The antigen binding site may also comprise complete non-human variable regions fused onto human constant domains (a "chimeric" antibody). Since a primary function of an antibody is to bind its target antigen, it is important that the original features of the antibody are preserved in such a way that the antigen specificity and affinity are maintained. Unfortunately, however, humanization of non-human antibodies has unpredictable effects on antibody-antigen interactions, e.g., antigen binding properties. This means that in therapeutic applications, more of the humanized antibody may be required per dose resulting in a higher cost of treatment and potentially greater risk of adverse events. In addition, both fully human and humanized antibodies can provoke an immune response or be immunogenic when administered to certain individuals.

According to another method, an antibody is rendered non-immunogenic, or less

immunogenic, to a given species, by first determining at least part of the amino acid sequence of the protein and then identifying in that amino acid sequence one or more potential epitopes to which T cells from the given species can react. Next, the amino acid sequence of the antibody is modified to eliminate at least one of the T cell epitopes identified to reduce the immunogenicity of the protein or part thereof when exposed to the immune system of the given species. Unlike antibodies, which can recognize and bind to soluble antigens, T cells must encounter their antigen targets through the activity of specialized antigen-presenting cells (APC's) such as dendritic cells and macrophages. APC's ingest foreign antigens and process them into peptides, which are then complexed to the HLA proteins and expressed on the surface of the APC. T cells can only recognize antigen fragments "presented" in the context of HLA. In the method which has been termed "de-immunization" and is described herein, amino acids within the antibody sequence that are predicted to bind effectively to HLA molecules are changed such that they no longer bind HLA and thus can no longer stimulate a T cell response. The lack of a T cell response to antigen translates into a reduction or elimination of a HAMA response.

SUMMARY

Antibodies in accordance with this disclosure recognize the CD3 antigen complex or interfere with the cell –surface expression of a component of the CD3 antigen complex. The anti-CD3 antibodies are also de-immunized (that is, rendered non-immunogenic, or less immunogenic, to a given species). In a particularly useful embodiment, de-immunization is achieved by first determining at least part of the amino acid sequence of the protein and then identifying in the amino acid sequence one or

more potential epitopes for T cells ("T cell epitopes") which are able to bind to HLA proteins of the given species. Next, the amino acid sequence of the antibody is modified to eliminate at least one of the T cell epitopes identified in order to reduce the immunogenicity of the protein or part thereof when exposed to the immune system of the given species.

In another aspect, this disclosure relates to a process for producing an antibody which includes the steps of: (a) producing an expression vector having a DNA sequence which includes a sequence that encodes an anti-CD3 antibody, at least a portion of which has been de-immunized; (b) transfecting a host cell with the vector; and (c) culturing the transfected cell line to produce the engineered antibody molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show the complete nucleotide and amino acid sequences of the OKT3 heavy chain variable region (GenBank Accession number A22261) and light chain variable region (GenBank Accession number A22259), respectively.

Fig. 2 schematically shows expression cassettes for the heavy and light chain variable regions as *Hind*III to *Bam*H1 fragments.

Figure 3 shows the complete nucleotide (SEQ ID NO: 1) and amino acid sequences of the murine OKT3 heavy chain variable region, including the murine immunoglobulin promoter, a murine signal sequence with intron at the 5' ends, and a splice donor site (Bam HI) at the 3' ends. Restriction enzyme sites are indicated.

Figure 4 shows the complete nucleotide (SEQ ID NO: 3) and amino acid sequences of the murine OKT3 light chain variable region, including the murine immunoglobulin promoter, a murine signal sequence with intron at the 5' ends, and a

splice donor site (Bam HI) at the 3' ends. Restriction enzyme sites are indicated.

Figure 5A shows a graphic map of the vector APEX-1 3F4V_HHuGamma4.

Figure 5B shows the complete nucleotide sequence of the vector (SEQ ID NO: 5) and indicates the amino acid and nucleotide sequences of the hIgG4 insert adjacent to an irrelevant VH region (labeled 3F4VH). The locations of the signal sequence, CH1, hinge, CH2 and CH3 regions are indicated.

Figure 6A shows a graphic map of the vector APEX-1 3F4V_HHuG2/G4.

Figure 6B shows the nucleotide sequence of the vector (SEQ ID NO: 7) and the amino acid and nucleic acid sequence of the G2/G4 insert, and indicates the locations of the signal sequence, irrelevant Vh (herein labeled 3F4Vh), CH1, hinge, CH2 and CH3 regions.

Figure 7 shows a graphic map of the heavy chain expression vector pSVgptHuG2/G4.

Figure 8 shows the complete nucleotide sequence (SEQ ID NO: 9).of the HuG2/G4 fragment excised from the APEX-1 3F4V_HHuG2/G4 vector and modified for insertion into a PUC 19 cloning vector by the addition, at the 5' end, of a Bam HI site and 5' untranslated inron sequences from native human IgG4 and, at the 3' end, of a Bgl II site and 3' untranslated sequence from natural human IgG4.

Figure 9 shows a graphic map of the expression vector pSVgptHuCk and indicates the position of the light chain variable and constant regions.

Figure 10 shows the amino acid sequences of the murine OKT3 variable heavy chain (SEQ ID NO: 10) and the amino acid sequences of several of the deimmunized heavy chain variable regions (SEQ ID NOS: 11-17) constructed in the Example.

Figure 11 shows the amino acid sequences of the murine OKT3 variable light chain (SEQ ID NO: 18) and the amino acid sequences of two deimmunized light chain variable regions (SEQ ID NOS: 19 and 20) constructed in the Example.

Figure 12 shows the mutagenic oligonucleotides primers used to construct the designed de-immunized sequences by mutagenesis using overlapping PCR.

Figure 13 shows the nucleic acid (SEQ ID NO: 21) and amino acid sequences for the de-immunized VH expression cassette OKT3DIVHV1.

Figure 14 shows the nucleic acid (SEQ ID NO: 23) and amino acid sequences for the de-immunized Vκ expression cassette OKT3DIVKV1

Figure 15 shows bidning of murine OKT3 and chimeric OKT3 to Jurkat, JRT3 and HPB-ALL cells.

Figures 16 and 17 are tables showing the binding of de-immunized anti-CD3 antibodies to HPB-ALL and JRT3 CELLS.

Figures 18, 19, 20 and 21 show the results of competition assays measuring the affinity of the de-immunized antibodies relative to that of chimeric OKT3 and murine OKT3.

Figure 22 is a table summarizing the IC50 of the de-immunized antibodies relative to that of murine OKT3.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

De-immunized anti-CD3 antibodies are described. The term "anti-CD3 antibodies" means any antibody or functional antibody fragment that recognizes the CD3 antigen complex or interferes with the cell surface expression of a component of

the CD3 antigen complex. The anti-CD3 antibody can be recombinant or naturally occurring. The anti-CD3 antibody can be human, non-human, chimeric or humanized. Anti-CD3 antibodies are known to those skilled in the art and include, for example, the antibodies described in U.S. Patent No. 5,527,713 entitled "Methods for inducing a population of T cells to proliferate using agents which recognize TCR/CD3 and ligands which stimulate an accessory molecule on the surface of the T cells"; U.S. Patent No. 6,352,694 entitled "Methods for inducing a population of T cells to proliferate using agents which recognize TCR/CD3 and ligands which stimulate an accessory molecule on the surface of the T cells"; U.S. Patent No. 6,406,696 entitled "Methods of stimulating the immune system with anti-CD3 antibodies"; U.S. Patent No. 6,143,297 entitled "Methods of promoting immunopotentiation and preparing antibodies with anti-CD3 antibodies"; U.S. Patent No. 6,113,901 entitled "Methods of stimulating or enhancing the immune system with anti-CD3 antibodies"; U.S. Patent No. 6,491,916 entitled "Methods and materials for modulation of the immunosuppresive activity and toxicity of monoclonal antibodies"; U.S. Patent No. 5,929,212 entitled "CD3 specific recombinant antibody"; U.S. Patent No. 5,834,597 entitled "Mutated nonactivating IgG2 domains and anti CD3 antibodies incorporating the same"; U.S. Patent No. 5,527,713 entitled "Anti-CD3 antibody-aminodextran conjugates for induction of T-cell activation and proliferation"; U.S. Patent No. 5,316,763 entitled "Short-term anti-CD3 stimulation of lymphocytes to increase their in vivo activity"; U.S. Patent No. 5,821,337 entitled "Immunoglobulin variants". Each of these patents is incorporated herein in its entirety by this reference.

The anti-CD3 antibody is de-immunized. De-immunization renders the anti-CD3 antibody non-immunogenic, or less immunogenic, to a given species. De-immunization can be achieved through structural alterations to the anti-CD3 antibody. Any de-immunization technique known to those skilled in the art can be employed. One suitable technique for de-immunizing antibodies is described, for example, WO 00/34317 published June 15, 2000, the disclosure of which is incorporated herein in its entirety. In summary, a typical protocol within the general method described therein includes the following steps.

1. Determining the amino acid sequence of the antibody or a part thereof (if modification ofonly of a part is required);

2. Identifying potential T cell epitopes within the amino acid sequence of the antibody by any method including determination of the binding of peptides to MHC molecules, determination of the binding of peptide:HLA complexes to the T cell receptors from the species to receive the therapeutic protein, testing of the antibody or parts thereof using transgenic animals with HLA molecules of the species to receive the therapeutic protein, or testing such transgenic animals reconstituted with immune system cells from the species to receive the therapeutic protein;

3. By genetic engineering or other methods for producing modified antibodies, altering the antibody to remove one or more of the potential T cell epitopes and producing such an altered antibody for testing;

4. Optionally within step 3, altering the antibody to remove one or more of the potential B cell epitopes;

5. Testing altered antibodies with one or more potential T cell epitopes (and optionally B cell epitopes) removed in order to identify a modified antibody which has retained all or part of its desired activity but which has lost one or more T cell epitopes. Potential T-cell epitopes herein are defined as specific peptide sequences which either are predicted to or that bind with reasonable efficiency to HLA class II molecules (or their equivalent in a non-human species), or which in the form of peptide:HLA complexes bind strongly to the T cell receptors from the species to receive the therapeutic protein or which, from previous or other studies, show the ability to stimulate T-cells via presentation on HLA class II molecules present on antigen presenting cells from the species to receive the therapeutic antibody.

This de-immunization method recognizes that an effective T cell-dependant immune response to a foreign protein requires activation of the cellular arm of the immune system. Such a response requires the uptake of the therapeutic (foreign) protein (i.e. therapeutic antibody) by antigen presenting cells (APCs). Once inside such cells, the protein is processed and fragments of the protein form a complex with MHC class II molecules and are presented at the cell surface. Should such a complex be recognized by binding of the T cell receptor from T-cells, such cells can be, under certain conditions, activated to produce stimulatory cytokines. The cytokines will elicit differentiation of B-cells to mature antibody producing cells. In addition, such T cell responses may also mediate other deleterious effects on the patient such as

inflammation and possible allergic reaction.

The whole anti-CD3 antibody or only a portion thereof (e.g., the variable portions of the anti-CD3 antibody) can be de-immunized. De-immunization of only a portion of the anti-CD3 antibody is particularly useful where the anti-CD3 antibody is a chimeric antibody (e.g. one with human constant regions).

The term "antibody" as used herein includes whole polyclonal and monoclonal antibodies, single chain antibodies, and other functional antibody fragments. Whole, monoclonal antibodies are preferred.

In general, the construction of the antibodies disclosed herein is achieved by using recognized manipulations utilized in genetic engineering technology. For example, techniques for isolating DNA, making and selecting vectors for expressing the DNA, purifying and analyzing nucleic acids, specific methods for making recombinant vector DNA (e.g. PCR), cleaving DNA with restriction enzymes, ligating DNA, introducing DNA, including vector DNA, into host cells by stable or transient means, culturing the host cells in selective or non-selective media, to select and maintain cells that express DNA, are generally known in the field.

The monoclonal antibodies disclosed herein may be derived using the hybridoma method (Kohler et al., Nature, 256:495, 1975), or other recombinant DNA methods well known in the art. In the hybridoma method, a mouse or other appropriate host animal is immunized with a protein which elicits the production of antibodies by the lymphocytes. Alternatively, lymphocytes may be immunized in vitro. The lymphocytes produced in response to the antigen are then are fused with myeloma cells using a suitable fusing

agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986). The hybridoma cells are then seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. Preferred myeloma cells are those that fuse efficiently, support stable production of antibody by the selected antibody-producing cells, and are not sensitive to a selective medium such as HAT medium (Sigma Chemical Company, St. Louis, Mo., Catalog No. H-0262). Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-20, NS0 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA.

The hybridoma cells are grown in a selective culture medium (e.g., HAT) and surviving cells expanded and assayed for production of monoclonal antibodies directed against the antigen. The binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by assays, such as, immunoprecipitation, radioimmunoassay (RIA), flow cytometry or enzyme-linked immunoabsorbent assay (ELISA).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986). In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal. The monoclonal antibodies secreted by

the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, or mammalian cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells.

Antibodies or antibody fragments can also be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., Nature, 348:552-554 (1990). Other publications have described the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., Bio/Technology, 10:779-783, 1992), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., Nuc. Acids. Res., 21:2265-2266, 1993). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of antigen-specific monoclonal antibodies.

In another aspect, this disclosure provides recombinant expression vectors which include the synthetic, genomic, or cDNA-derived nucleic acid fragments necessary to produce a de-immunized anti-CD3 antibody. The nucleotide sequence coding for any

de-immunized anti-CD3 antibody in accordance with this disclosure can be inserted into an appropriate vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Any suitable host cell vector may be used for expression of the DNA sequences coding for the de-immunized anti-CD3 antibody. Bacterial (e.g. E.coli) and other microbial systems may be used. Eukaryotic (e.g. mammalian) host cell expression systems may also be used to obtain antibodies of the present disclosure. Suitable mammalian host cell include COS cells and CHO cells (Bebbington C R (1991) Methods 2 136-145); and myeloma or hybridoma cell lines (for example NSO cells; Bebbington, et al., Bio Technology, 10, 169-175. 1992).

The de-immunized anti-CD3 antibodies can also be used as separately administered compositions given in conjunction with therapeutic agents. For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the de-immunized anti-CD3 antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the de-immunized antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme co-factors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

The present de-immunized anti-CD3 antibodies can be administered to a patient in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the antibodies to the patient. Sterile water, alcohol, fats, waxes, and inert solids may be included in the

carrier. Pharmaceutically accepted adjuvants (buffering agents, dispersing agent) may also be incorporated into the pharmaceutical composition.

The antibody compositions may be administered to a patient in a variety of ways. Preferably, the pharmaceutical compositions may be administered parenterally (e.g., subcutaneously, intramuscularly or intravenously). Thus, compositions for parental administration may include a solution of the antibody, antibody fragment or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody or antibody fragment in these formulations can vary widely, e.g., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Actual methods for preparing parenterally administrable compositions and adjustments necessary for administration to subjects will be known or apparent to those skilled in the art and are described in more detail in, for example, *Remington's Pharmaceutical Science*, 17th Ed., Mack Publishing Company, Easton, Pa (1985), which is incorporated herein by reference.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

EXAMPLE 1

De-immunized, Chimeric Anti-CD3 Antibody

A de-immunized, chimeric anti-CD3 antibody was prepared. The variable regions selected were derived from the known mouse anti-human CD3 antibody OKT3. The variable regions were de-immunized and combined with an engineered human constant region to prepare the chimeric, de-immunized anti-CD3 antibody. The procedures used to prepare and test the chimeric, de-immunized anti-CD3 antibodies are described below.

The murine OKT3 heavy and light chain variable regions were constructed synthetically by gene synthesis using overlapping 40 mer oligonucleotides and a polymerase chain reaction. The sequences of the heavy and light chain variable regions of this antibody have been previously determined and deposited in the GenBank database (Accession numbers A22261 and A22259 respectively; see Figure 1). Sequences, including the murine immunoglobulin promoter and a murine signal sequence with intron, were added at the 5' ends, and sequences including the splice donor site were added at the 3' end by PCR to form expression cassettes (see Figure 2) for the heavy and light chain variable regions as *Hind*III to *Bam*HI fragments. The entire sequences of the expression cassettes were confirmed to be correct. The complete DNA and amino acid sequences of the murine OKT3 heavy and light chain expression cassettes are shown in Figures 3 and 4, respectively. The heavy chain

constant region was engineered to include a human IgG2 portion and a human IgG4 portion ("HuG2G4 constant region"). This constant region was prepared as follows: First, the genomic DNA encoding the human IgG4-derived portions (part of CH2 and CH3 regions) was inserted into the bacterial carrier plasmid pBR322, a plasmid derived from an E. coli species (ATCC 37017; Mandel, M. et al. (1970) J. Mol. Biol. 53, 154). The IgG4-derived insert was released from the plasmid by performing a restriction digest with Hind III and Xho I. The insert was gel purified, excised, and subjected to further restriction analysis to confirm the published sequence of the human IgG4 genomic DNA. The individual genomic IgG4 insert (HindIII/SmaI restriction fragment; the SmaI site is in the 3' untranslated region approximately 30 bp 3' of the translation stop site for each insert) was then subcloned by ligation into the expression cassette APEX-1 to yield APEX-1 3F4 VH HuGamma 4 (see Figure 5). DNA sequence analysis was performed to confirm the correct sequence of the human IgG4 desired regions.

The above procedure was also performed with a pBR322 bacterial plasmid which carried genomic DNA encoding the human IgG2 CH1, hinge region and first part of CH2, which were excised with PmlII and Bst EII and subcloned into APEX-1 3F4 VH HuGamma 4 to replace the corresponding IgG4-derived sequences.. The sequence of the resulting chimeric IgG2/IgG4 human constant region is shown in Figure 6 (APEX-1 3F4 VH G2/G4).

Construction of modified G2G4 constant region

The HuG2G4 constant region was modified for insertion into a heavy chain expression vector as follows:

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The 5' end of the HuIgG4 constant region (native HuIgG4 5' intron sequence with BamH1 site at 5' end) up to the start of the coding region is amplified in reaction 1. The Hu G2G4 coding sequence (including intron) is amplified from APEX-1 3F4 VH Hu G2/G4 vector in reaction 2 (from start of CH1 to end of CH3 region). The 3' end of native HuIgG4 3' sequence from the end of the CH3 coding region is amplified in reaction 3, using a 3' primer designed to introduce a 3' Bam HI site with a Bgl II site just inside the Bam H1 and an Eco R1 site just inside the Bgl II site. The products of these 3 reactions (which overlap) are combined in a 4th PCR reaction using 5' and 3' primers. The combined product is cloned into the BamH1 site of pUC19 and the DNA sequence of the modified HuG2G4 fragment confirmed. The G2G4 gene is cut out with Bgl II and Bam HI to give a fragment with Bam HI at the 5' end and Bgl II at the 3' end. This is cloned into a heavy chain expression vector cut with Bam HI. A clone with the constant region inserted in the correct orientation (Bam HI site reformed at 5' end, hybrid Bam HI / BglII site at 3' end) is selected (Figure 7). The complete sequence of the Bam HI to Bgl II fragment is shown in Figure 8. Antibody Variable regions can be cloned in directly as Hind III to Bam HI fragments.

Primers (SEQ ID NOS:77 - 83):

TTGTGAGCGGATAACAATTTC M13 –50 REVERSE
GTTTTCCCAGTCACGACGTTGTA M13 –40 FORWARD
CTTGCAGCCTCCACCAAGGGCCCATCCGTC G2G4-1
CCCTTGGTGGAGGCTGCAAGAGAGG G2G4-2
GAGCCTCTCCCTGTCTCTGGGTAAATGAGTGCC G2G4-3
TCATTTACCCAGAGACAGGGAGAGGCTCTTCTGTG G2G4-4
TACCCGGGGATCCAGATCTGAATTCCTCATGTCAC G2G4-6

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The light chain constant region was the human kappa constant region. This is included in the expression vector pSV hyg HuCκ as shown in Figure 9.

The amino acid sequence of the variable regions of the murine anti-CD3 antibody OKT3 were analyzed for potential T cell epitopes (MHC Class II binding peptides) by using the peptide threading software as detailed in WO 02/069232 published September 6 2002 and other *in silico* techniques. De-immunized sequences were designed to eliminate the potential T cell epitopes, as far as possible by making conservative amino acid changes. In order to test the effect on antibody binding of alternative substitutions designed to remove T cell epitopes, several versions of the de-immunized heavy and light chain variable region were constructed, as shown in Figures 10 and 11.

The murine OKT3 heavy and light chain variable region cassettes were used as templates for construction of the designed de-Immunized sequences by mutagenesis using overlapping PCR with mutagenic oligonucleotides primers (see Figure 12 The vectors VH-PCR1 and VK-PCR1 (Riechmann et al., 1988) were used as templates to introduce 5' flanking sequences including the leader signal peptide sequence, the leader intron and the murine immunoglobulin promoter, and 3' flanking sequence including the splice site and intron sequences. Sets of mutagenic primer pairs were synthesized encompassing the regions to be altered, such that the target DNA sequence is amplified as a set of fragments.

Adjacent oligos were designed so that the sequences overlap by at least 15bp. The number of these depends on the number of sites to be mutated.

PCR amplifications for each primer pair were set up using the following reagents:

1µL template DNA
1 µL (25pmol) forward primer
1 µL (25pmol) reverse primer
1 µL 10mM dNTPs
5 µL 10 x Pfu polymerase buffer
0.5 µL (1unit) Pfu DNA polymerase
H_2O to 50µL

All reagents except the enzyme were mixed in a 0.5 ml thin wall PCR tube and heated to 94°C on the PCR block. The Pfu enzyme was added then samples cycled: 94°C/2min, 15-20 cycles of 94°C/30s, 50°C/30s, 75°C/1 min (depending on the length of extension required), finishing with 75° C 5 min. The annealing temperature may be lower or higher than 50°C depending on the Tm of the oligos.

5µL of each reaction were run on an agarose gel to check that the PCRs had given products of the expected size. If not, I the annealing temperature was lowered by 5°C and/or the number of cycles of PCR was increased and/or the $MgCl_2$ concentration was increased to 5mM. If a primary PCR gave multiple bands, the band of the correct size was gel-purified.

The products were joined in a second PCR using the 2nd round 5' and 3' primers only. This sequence was not present in the original template so only mutagenised DNA can be amplified at this stage. The templates for the 2nd joining PCR were the fragments produced in the first round. The quantities of these were adjusted to add approximately equal amounts. The reagents for the 2nd round PCR were:

Products of 1st round PCR
2 µL (50 pmol) 5' 2nd round primer
2 µL (50 pmol) 3' 2nd round primer
1 µL 10 mM dNTPs
5 µL 10xPfu polymerase buffer
0.5 µL (1 unit) Pfu DNA polymerase
H_2O to 50 µL

All reagents except the enzyme were mixed in a 0.5 ml thin wall PCR tube and heated to 94°C on the PCR block. The Pfu enzyme was added then samples cycled: 94°C/2min, 15 cycles of 94°C/ 30s, 50°C/30s, 75°C/ 1min (depending on the length of extension required), finishing with 75°C 5 min.

5 µL of each reaction was run on an agarose gel to check that the PCRs had given products of the expected size (approximately 820 bp for VH expression cassettes and 650 bp for VK expression cassettes). If not, the 2nd round PCR was repeated lowering the annealing temperature by 5°C and/or increasing the number of cycles of PCR.

The remainder of the PCR product was phenol/chloroform extracted and ethanol precipitated, digested with the required enzymes (usually *Hind*111 and *Bam*H1 for expression cassettes) and loaded onto a 1.5% low-melting point agarose gel. DNA bands of the correct size were excised and purified.

The de-immunized VH and Vκ expression cassettes produced (Figure 2) were cloned into the vector pUC19 and the entire DNA sequence was confirmed to be correct for each de-immunized VH and Vκ. As an example, the DNA and amino acid sequences for the de-immunized OKT3 VH and Vk 1 expression cassettes OKT3 DIV H V1 (version 1) and OKT3 DIVK V1 (version 1) are shown in Figures 13 and 14, respectively.

The de-immunized heavy and light chain V-region genes were excised from the vector pUC19 as *Hind*III to *Bam*HI expression cassettes. These were transferred to the expression vectors pSVgpt HuG2G4 and pSVhyg HuCκ (Figures 7 and 9, respectively), which include the previously described HuG2G4 or human κ constant regions,

respectively, and markers for selection in mammalian cells. The DNA sequence was confirmed to be correct for the de-immunized VH and Vκ in the expression vectors.

The original murine OKT3 heavy and light chain light chain variable region cassettes were also transferred to the expression vectors pSVgpt HuG2G4 and pSVhyg HuCκ as described above, to generate a chimeric antibody with the murine variable region genes linked to the human constant region G2/G4 construct. This chimeric antibody was used as an isotype matched control for binding experiments with the de-immunized antibodies, as it has with the same effector functions and the same secondary detection reagents are used as for the de-immunized antibodies.

The host cell line for antibody expression was NSO, a non-immunoglobulin producing mouse myeloma, obtained from the European Collection of Animal Cell Cultures, Porton UK (ECACC No 85110503). The heavy and light chain expression vectors were co-transfected into NSO cells by electroporation. The transfection was accomplished as follows: DNA to be transfected was linearized to improve efficiency. PvuI digests of about 3 and 6 mg of the plasmids pSVgpt HuG2/G4 and pSV hyg HUCK, respectively, were prepared. The digested DNA was ethanol precipitated and dissolved in 50 ml dH_2O. Recipient NSO cells were resuspended from a semi-confluent 75 cm^2 flask and collected by centrifugation at 1000 rpm for 5 min. The supernatant was discarded. The cells were resuspended in 0.5 ml DMEM and transferred to a Gene Pulser cuvette (Bio-Rad). The DNA was mixed with the cells by gentle pipetting and left on ice for 5 minutes. The cuvette was inserted between the electrodes of the Bio-rad Gene Pulser and a single pulse of 170 V, 960 mF was applied. The cuvette was then returned to ice for 20 minutes. The cell suspension was transferred to a 75 cm^2 flask

containing 20 ml DMEM and allowed to recover for 1-2 days. Cells were harvested and resuspended in 80 ml selective DMEM and a 200 μL aliquot was added to each well of 96-well plates. Selective DMEM is Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) foetal calf serum, 250μg/ml xanthine, 0.8μg/ml mycophenolic acid. Approximately 10 days from the start of selection, colonies were visible to the naked eye. 20 μL of medium from each well was assayed for the presence of human antibodies. On the basis of the level of antibody production and the number of cells in the well, wells were chosen for expansion. To resuspend the cells from the designated wells, the tip of a Gilson P200 pipette (with yellow tip) was rubbed across the surface and the medium transferred to a well of a 24-well tissue culture plate containing 1.5 ml of fresh selective DMEM. Cells were expanded to 25 cm^2 and larger tissue culture flasks in order to lay down liquid nitrogen stocks and to provide medium for antibody purification and testing.

Each of the 7 de-immunized heavy chain genes (Fig. 10) was paired with each of 2 de-immunized light chain genes (Fig. 11) to give a total of 14 de-immunized OKT3 antibodies to be produced. The chimeric heavy and light chain vectors were co-transfected to produce the chimeric antibody. Colonies expressing the gpt gene were selected using selective DMEM. Transfected cell clones were screened for production of human antibody by ELISA for human IgG as follows: An ELISA plate (Dynatech Immulon 2) was coated at 100 μL per well with sheep anti-human κ antibody (The Binding Site Cat No: AU015) diluted 1:1000 in carbonate/bicarbonate coating buffer pH9.6 (Sigma Cat: C-3041). The samples were incubated at 4°C overnight. After

washing 3 times with a solution of PBS with 0.05% Tween 20 (RTM), Where transfections were plated into 96-well plates, screening was conducted using 25μL samples of culture medium from each well by transfer into an assay plate containing 75μL per well of PBS/Tween (PBST) solution. Where transfected cells were cultured using 24-well plates, 12.5 μL was added to 87.5 μL in the first well and a doubling dilution series set out across the plate. For all assays, blank wells received PBST only. The samples were incubated at room temperature for 1 hour and washed 3 times with the PBS/Tween solution.. The secondary antibody, a peroxidase-conjugated sheep anti-human IgG γ chain specific reagent (The Binding Site Cat No: AP004), was added at a ratio of 1:1000 in the PBS/Tween solution in an amount of 100 μL per well. The samples were again incubated at room temperature for 1 hour and washed 3 times with the PBS/Tween solution. To prepare the colour substrate , one tablet (20mg) of OPD (o-PHENYLENE DIAMINE) (Sigma Cat No: P-7288) was dissolved in 45ml of H_2O plus 5 ml 10x peroxidase buffer (make 10 x peroxidase buffer with Sigma phosphate citrate buffer tablets pH 5.0, Cat No: P-4809), and 10mL 30% (w/w) hydrogen peroxide (Sigma Cat No: H-1109) was added just before use. The substrate was added at 100 μL per well and incubated at room temperature for 5 min. or as required. When colour developed, the process was stopped by adding 25 μL 12.5% H_2SO_4. The result was read at 492 nm. The standard antibody employed was Human IgG1/κ purified myeloma protein (The Binding Site Cat No: BP078).

Cell lines secreting antibody were expanded and the highest produces selected. De-immunized and chimeric antibodies were purified using Prosep ®-A (Bioprocessing

Ltd, Durham, UK) as follows: The NSO transfectoma cell line producing antibody was grown in DMEM 5% FCS in Nunc Triple layer flasks, 250 ml per flask (total volume 1L) for 10-14 days until nearing saturation. The conditioned medium was collected and spun at 3000 rpm for 5 minutes in a bench centrifuge to remove cells. One tenth the volume of 1M Tris-HCL pH8 (Sigma Cat: T3038) was added to the cell supernatant to make this 0.1M Tris-HCL pH8. 0.5 to ml of Prosep A (Millipore Cat: 113111824) was added and stirred overnight at room temperature. Prosep A was collected by spinning at 3000 rpm for 5 minutes then packed into a Biorad Poly-Prep column (Cat: 731-1550). The column was washed with 10ml PBS, then eluted in 1ml fractions with 0.1 M Glycine pH3.0. Each fraction was collected into a tube containing 100μL 1M Tris-HCL pH8 (Sigma, as above). The absorbance of each fraction measured at 280 nm. The fractions containing antibody were pooled and dialysed against PBS overnight at room temperature. The preparation was sterilized by filtration through a 0.2 micron syringe filter and the A_{280} was measured. The concentration was determined by ELISA for human IgG1κ.

Evaluation of Binding of De-immunized Antibodies to Cells Expressing CD3

Each de-immunized antibody was evaluated for its ability to bind the CD3 molecule on T cells as it was possible that the mutations introduced during de-immunization could affect antibody specificity or affinity. Cells of the HPB-ALL (Human peripheral blood acute lymphocytic leukemia) line were obtained from the Cell Resource Center for Biomedical Research, Tohoku University, Japan. Jurkat and J.RT3 cell lines were obtained from American Type Tissue Culture (ATCC), Rockville, MD. Cells were

cultured at 2 x 10^5 – 2 x 10^6 cells/ml in RPMI 1640 (Cellgro) containing 10% heat-activated fetal bovine serum (Atlas Biologicals), 1% penicillin-streptamycin, 0.01M Hepes (Sigma), 0.2mM 1-glutamine and 5 x 10^{-5}M 2-mercaptoethanol (Sigma) at 37°C in a humidified chamber containing 5% CO_2 in air. HPB-ALL and Jurkat cells bear high levels of the TCR/CD3 complex on their cell surface, while the J.RT3 cells are a variant of the Jurkat line that do not express CD3. Preparations of the murine OKT3, chimeric OKT3 and de-immunized OKT3 G2/G4 antibodies were evaluated for binding to cells of all three lines by immunocytochemistry and flow cytometry. Briefly, 10^6 cells were plated in individual wells of a 96-well plate and reacted with 1μg of each test antibody or with appropriate human or mouse isotype controls for 20 minutes at 4°C. The cells were then washed three times with PBS containing 2% fetal bovine serum (Atlas Biologicals) followed by reactivity for 20 minutes at 4°C with a phycoerthrin-conjugated secondary antibody (R-PE Affinity pure F(ab)2 goat anti-human IgG (H+L) (Jackson ImmunoResearch, Bar Harbor, Maine) for the detection of the chimeric and de-immunized antibodies and G2/G4 isotype control, and R-PE-conjugated goat anti-mouse IgG (Pharmingen), for the detection of the murine OKT3 and murine IgG2a isotype control). The cells were washed three times with PBS-FBS as above, and then resuspended in PBS for analysis on a flow cytometer (FACs Calibur, Becton Dickenson).

Both the chimeric OKT3 HuG2/G4κ antibody and the murine OKT3 antibody bound comparably to Jurkat and HPB-All cells that express CD3, but showed no binding to the CD3 negative cell line J.RT3. The matched murine and human isotype controls showed no binding to any of the cell types (Figure 15)

Conditioned media from NSO cell lines expressing de-immunised OKT3 antibodies were also tested in the flow cytometry binding assay using HPB-ALL and J.RT3 cells. The concentration of antibody in the conditioned medium was determined by ELISA for human IgG using the ELISA procedure previously described. The immunocytochemistry and flow cytometry procedures were performed as described above. The results for the 7 versions of de-immunised heavy chain combined with de-immunised OKT3 light chain version 1 are shown in Figure 16. The results for the de-immunised heavy chains combined with de-immunised OKT3 light chain version 2 are shown in Figure 17. A number of the de-immunised OKT3 antibodies demonstrated binding to HPB-ALL cells equivalent to that observed for murine and chimeric antibodies. However, several of the de-immunized antibodies showed a significantly lower level of binding to HPB-ALL cells (e.g. antibodies derived from clones 24C12, 48G3, and 55B2). The ability of a given antibody to bind to HPB-ALL cells did not correlate with the usage of a particular version of kappa light chain; i.e., both mutated kappa chains, in combination with some, but not all of the mutated VH regions, showed comparable binding in this assay.

Competition Assay Comparing Binding Of Murine, Chimeric And De-Immunized OKT3 Antibodies

To obtain more discrimination between the various de-immunized antibodies, and to determine their binding affinity relative to that of OKT3, a competition binding assay was carried out. Because CD3 is part of a cell-surface complex of proteins, affinities could not be measured by BIACORE analysis, but instead were measured by flow cytometry. Murine OKT3 was biotinylated using EZ-Link Sulfo-NHS-LC Biotin from

Perbio Science, catalogue number 21335, following the protocol provided by the manufacturer. The amount of biotinylated OKT3 to use was determined by titrating HPB-ALL cells with decreasing amounts of antibody. A suitable sub-saturating concentration was determined to be 10 ng of biotinylated antibody per 10^6 cells. This concentration was then used in all experiments. The secondary detection reagent was avidin-FITC, Sigma catalogue number A2050. Competition with dilutions of test (de-immunized or chimeric) antibodies from 100 pg to 1 µg was tested. The results are expressed as percent inhibition of maximal fluorescence activity (determined by binding of biotinylated murine OKT3 in the absence of blocking antibody) and are shown in Figures 18, 19, 20 and 21.

The results show that the chimeric OKT3 antibody and the six de-immunized antibodies OKT3 DIVHv5 to DIVGv7/DIVKv1 and OKT3 DIVH5 to DIVH7/DIVK2 can compete with the binding of the biotinylated murine OKT3 antibody either as efficiently as the murine antibody itself or within two to three fold of it.

Throughout this specification, various publications and patent disclosures are referred to. The teachings and disclosures thereof, in their entireties, are hereby incorporated by reference into this application to more fully describe the state of the art to which the present invention pertains.

Although preferred and other embodiments of the invention have been described herein, further embodiments may be perceived by those skilled in the art without departing from the scope of the invention as defined by the following claims.

We claim:

1. A de-immunized anti-CD3 antibody.

2. A de-immunized anti-CD3 antibody heavy chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 11, 12, 13, 14, 15, 16 and 17.

3. A de-immunized anti-CD3 antibody light chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 19 and 20.

4. A method comprising:

selecting an anti-CD3 antibody; and

rendering the anti-CD3 antibody less immunogenic to a given species.

5. A method as in claim 4 wherein the step of rendering the anti-CD3 antibody less immunogenic to a given species comprises the steps of:

a) determining at least part of the amino acid sequence of the antibody;

(b) identifying in the amino acid sequence one or more potential epitopes for T cells ("T cell epitopes") which are found in an endogenous protein of the given species; and

(c) modifying the amino acid sequence to eliminate at least one of the T cell epitopes identified in step (b) thereby to reduce the immunogenicity of the antibody or part thereof when exposed to the immune system of the given species.

6. A method comprising the steps of:

(a) producing an expression vector having a DNA sequence which includes a sequence that encodes an anti-CD3 antibody, at least a portion of which has been a de-immunized;

(b) transfecting a host cell with the vector; and

(c) culturing the transfected cell line to produce a de-immunized anti-CD3 antibody molecule.

7. A pharmaceutical composition comprising a de-immunized anti-CD3 antibody and a pharmaceutically acceptable carrier.

8. A composition as in claim 7 wherein the de-immunized anti-CD3 antibody includes a heavy chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 11, 12, 13, 14, 15, 16 and 17.

9. A composition as in claim 7 wherein the de-immunized anti-CD3 antibody includes a light chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 19 and 20.

10. A method comprising administering an anti-CD3 antibody, the anti-CD3 antibody including an engineered heavy chain constant region having a first portion derived from one or more human IgG2 antibodies and a second portion derived from

one or more human IgG4 antibodies, at least a portion of the antibody being de-immunized.

11. A method as in claim 10 wherein at least the light chain variable region of the antibody is de-immunized.

12. A method as in claim 10 wherein at least the heavy chain variable region of the antibody is de-immunized.

13. A method as in claim 10 wherein both the light and heavy chain variable regions of the antibody are de-immunized.

14. A method as in claim 10 wherein the anti-CD3 antibody includes a heavy chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 11, 12, 13, 14, 15, 16 and 17.

15. A method as in claim 10 wherein the anti-CD3 antibody includes a light chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 19 and 20.

16. A method as in claim 10 wherein at least a portion of the antibody is de-immunized by a process comprising the steps of:

rendering the antibody, or part thereof, non-immunogenic, or less immunogenic,

to a given species by

a) determining at least part of the amino acid sequence of the antibody;

(b) identifying in the amino acid sequence one or more potential epitopes for T cells ("T cell epitopes") which are found in an endogenous protein of the given species; and

(c) modifying the amino acid sequence to eliminate at least one of the T cell epitopes identified in step (b) thereby to reduce the immunogenicity of the antibody or part thereof when exposed to the immune system of the given species.

17. Nucleic acid encoding a de-immunized anti-CD3 antibody.

18. Nucleic acid in accordance with claim 17 which encodes an antibody heavy chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 11, 12, 13, 14, 15, 16 and 17.

19. Nucleic acid in accordance with claim 17 which encodes an antibody light chain variable region comprising a sequence selected from the group consisting of SEQ. ID NOS: 19 and 20.

20. A pharmaceutical composition comprising an anti-CD3 antibody encoded by the nucleic acid in accordance with any of claims 17 through 19 and a pharmaceutically acceptable carrier.

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Amino Acid and nucleotide sequence of the murine OKT3 heavy chain variable region (Accession #A222621)

MERHWIFLLLLSVTAGVHSQVQLQQSGAELARPGASVKMSCKASYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTN
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KKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQ
TQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTL
TCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFS
RTPGK

ORIGIN

```
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     61 tctactcctg ttgtcagtaa ctgcaggtgt ccactcccag gtccagctgc agcagtctgg
    121 ggctgaactg gcaagacctg gggcctcagt gaagatgtcc tgcaaggctt ctggctacac
    181 ctttactagg tacacgatgc actgggtaaa acagaggcct ggacagggtc tggaatggat
    241 tggatacatt aatcctagcc gtggttatac taattacaat cagaagttca aggacaaggc
    301 cacattgact acagacaaat cctccagcac agcctacatg caactgagca gcctgacatc
    361 tgaggactct gcagtctatt actgtgcaag atattatgat gatcattact gccttgacta
    421 ctggggccaa ggcaccactc tcacagtctc ctcagccaaa acaacagccc catcggtcta
    481 tccactggcc cctgtgtgtg gagatacaac tggctcctcg gtgactctag gatgcctggt
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FIG. 1A

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 601 tgtgcacacc ttcccagctg tcctgcagtc tgacctctac accctcagca gctcagtgac
 661 tgtaacctcg agcacctggc ccagccagtc catcacctgc aatgtggccc acccggcaag
 721 cagcaccaag gtggacaaga aaattgagcc cagagggccc acaatcaagc cctgtcctcc
 781 atgcaaatgc ccagcaccta acctcttggg tggaccatcc gtcttcatct tccctccaaa
 841 gatcaaggat gtactcatga tctccctgag ccccatagtc acatgtgtgg tggtggatgt
 901 gagcgaggat gacccagatg tccagatcag ctggtttgtg aacaacgtgg aagtacacac
 961 agctcagaca caaacccata gagaggatta caacagtact ctccgggtgg tcagtgccct
1021 ccccatccag caccaggact ggatgagtgg caaggagttc aaatgcaagg tcaacaacaa
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1261 aacagagcta aactacaaga acactgaacc agtcctggac tctgatggtt cttacttcat
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1441 taaatgagct cagcacccac aaaactctca ggtccaaaga gacacccaca ctcatctcca
1501 tgcttccctt gtataaataa agcacccagc aatgcctggg accatgtaaa aaaaaaaaaa
1561 aaaggaattc
```

FIG. 1A (Cont.)

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Amino Acid and nucleotide sequence of the murine OKT3 light chain variable region (Accession #A22259)

MDFQVQIFSFLLISASVIISRGQIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYD
TSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRADTAPTVSIFPPS
SEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSY
TCEATHKTSTSPIVKSFNRNEC

ORIGIN

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        ctaatcagtg
     61 cctcagtcat aatatccaga ggacaaattg ttctcaccca gtctccagca
        atcatgtctg
    121 catctccagg ggagaaggtc accatgacct gcagtgccag ctcaagtgta
        agttacatga
    181 actggtacca gcagaagtca ggcacctccc ccaaaagatg gatttatgac
        acatccaaac
    241 tggcttctgg agtccctgct cacttcaggg gcagtgggtc tgggacctct
        tactctctca
    301 caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
        tggagtagta
    361 acccattcac gttcggctcg gggacaaagt tggaaataaa ccgggctgat
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FIG. 1B

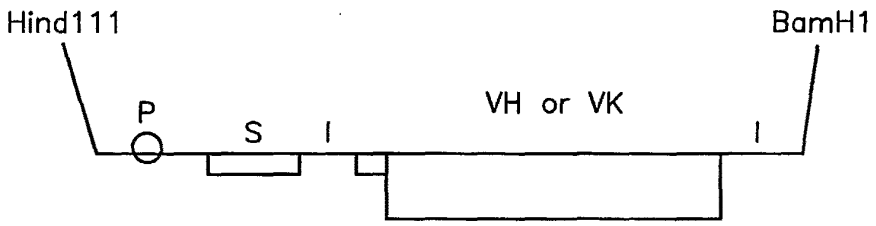
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    aggaatgagt
721 gttagagaca aaggtcctga gacgccacca ccagctccca gctccatcct
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    atttgcagaa
901 aatattcaat aaagtgagtc tttgccttga aaaaaaaaaa aaa
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FIG. 1B (Cont.)

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P	Mouse heavy chain Ig promoter
S	Signal peptide sequence
I	Intron

FIG. 2

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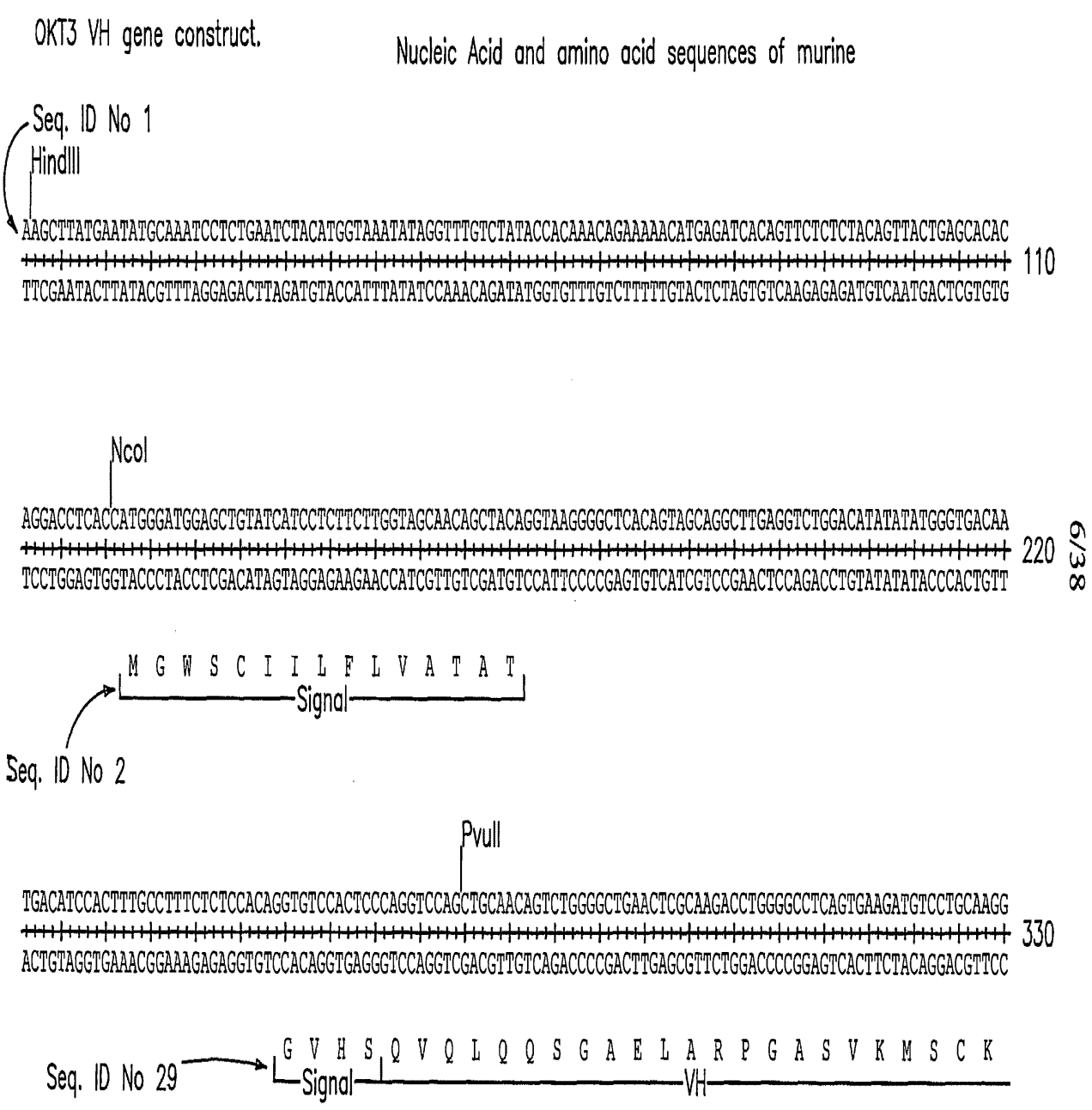


FIG. 3

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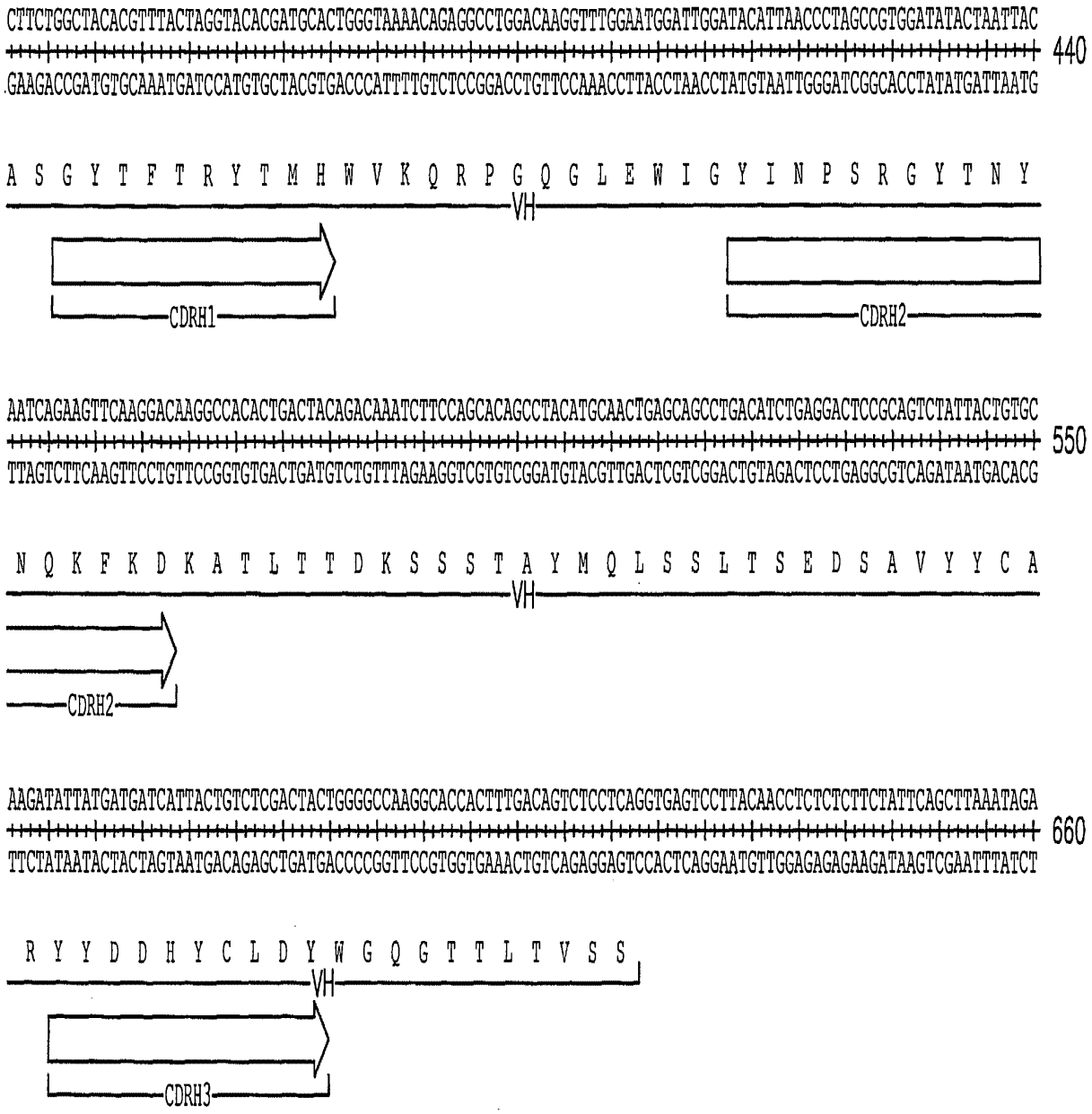


FIG. 3 (Cont.)

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FIG. 3 (Cont.)

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OKT3 VL gene construct. Nucleic acid and amino acid sequences of murine

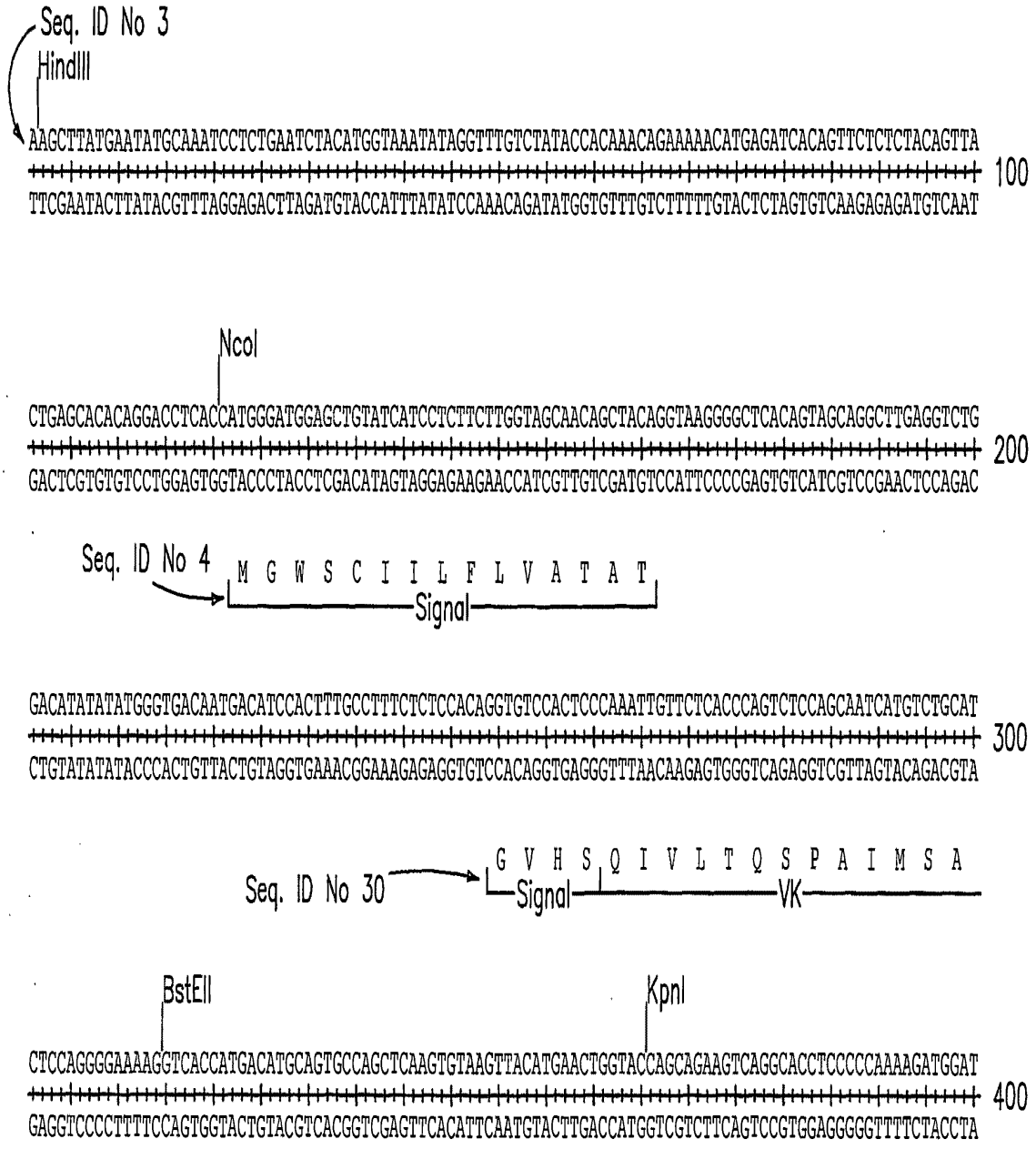


FIG. 4

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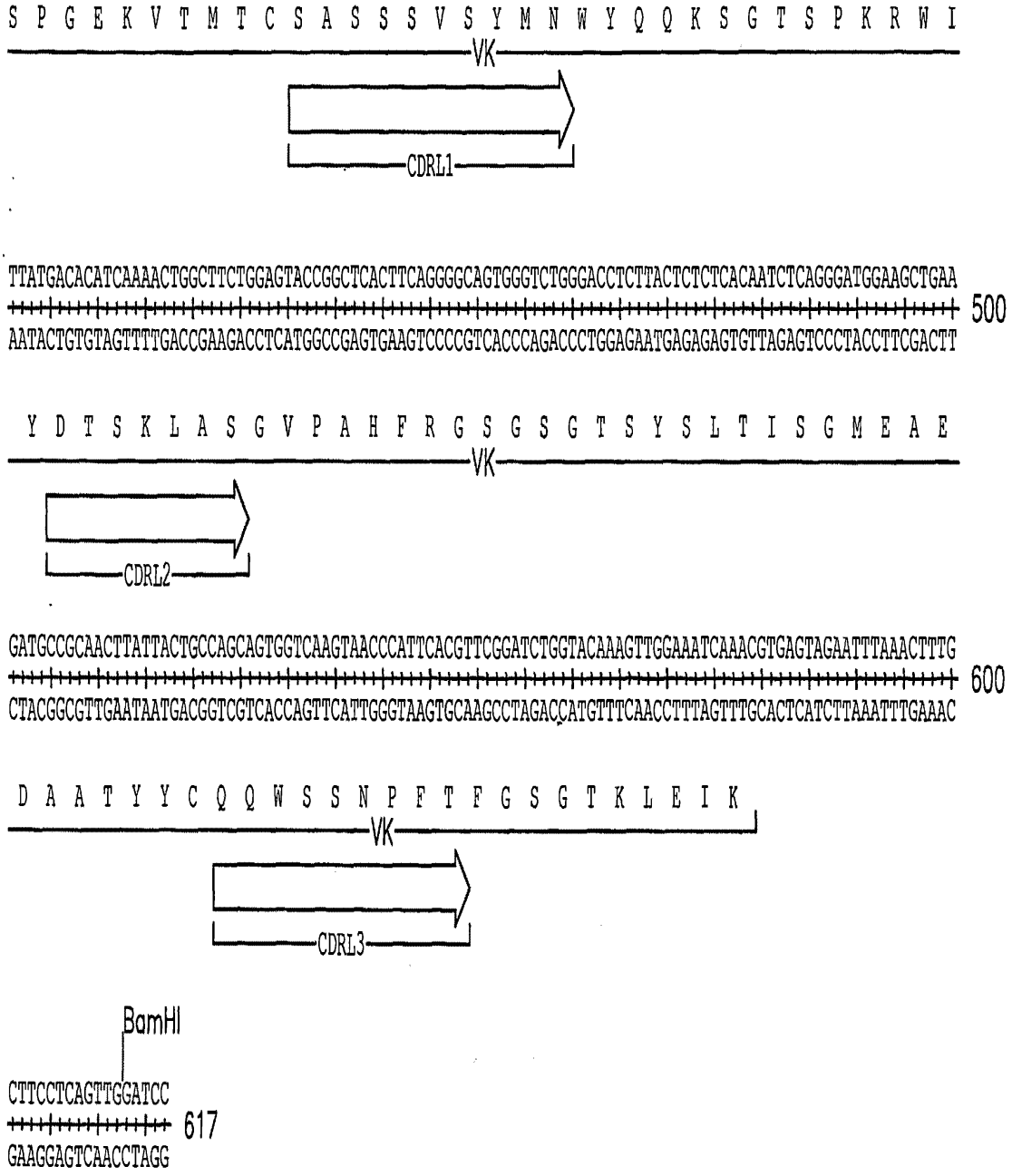


FIG. 4 (Cont.)

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Schematic map of the vector Apex−1 3F4V_HHuGamma4.

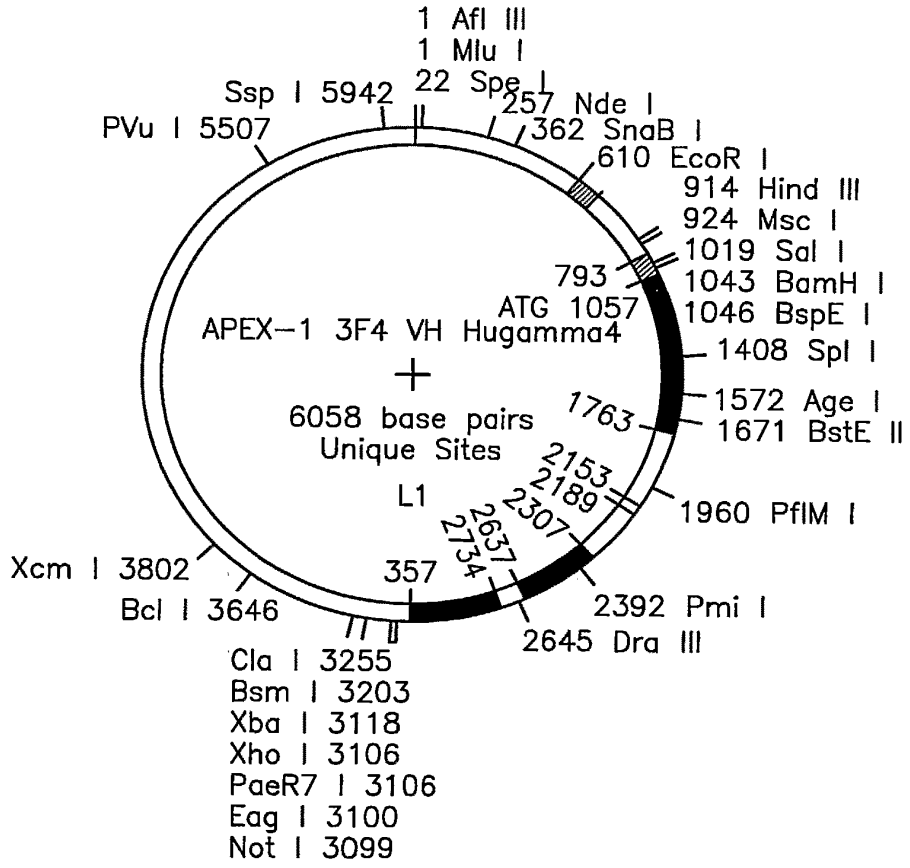


FIG. 5A

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Vector sequence (APEX-1 3F4V$_H$HuGamma4)

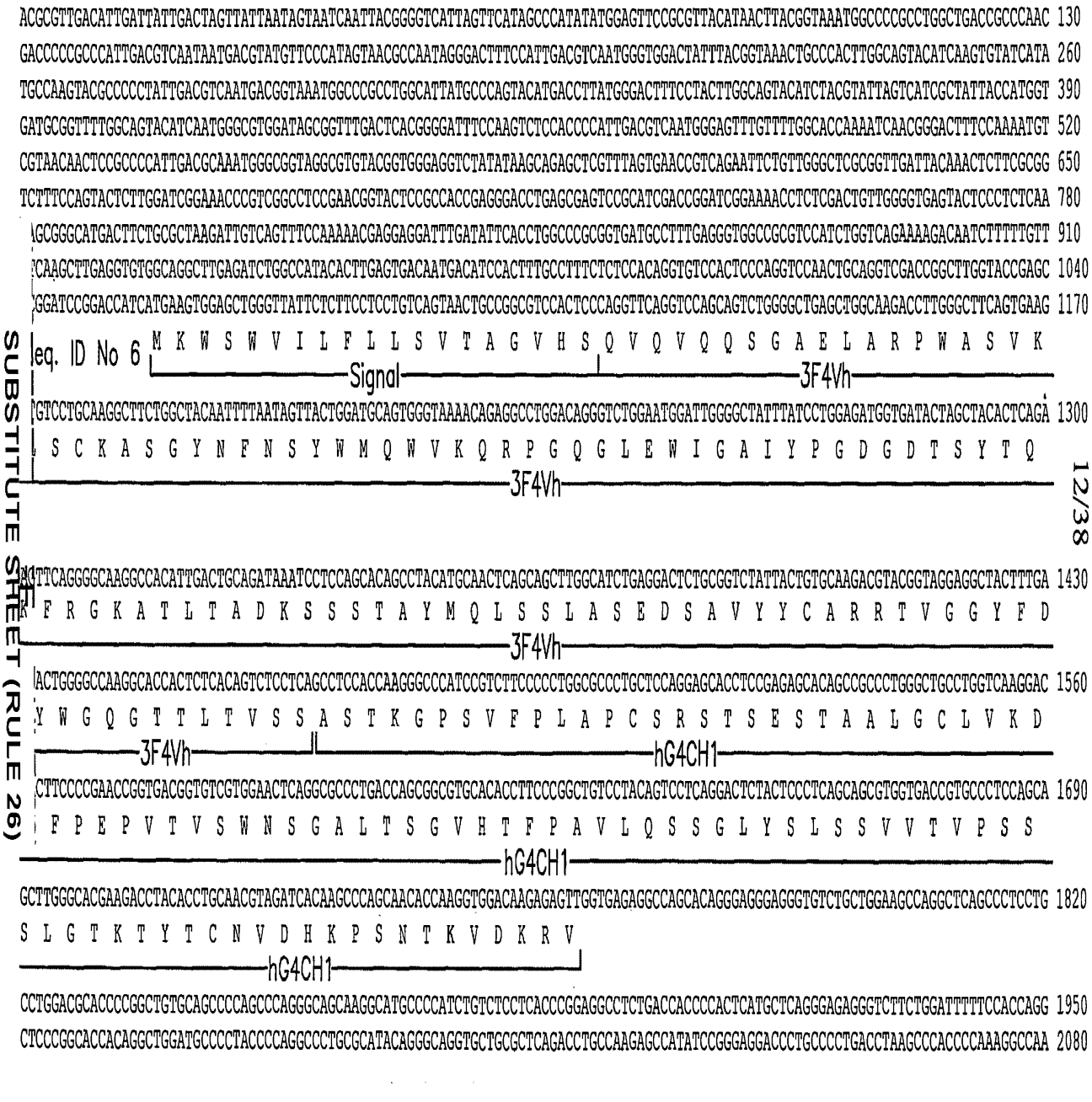


FIG. 5B

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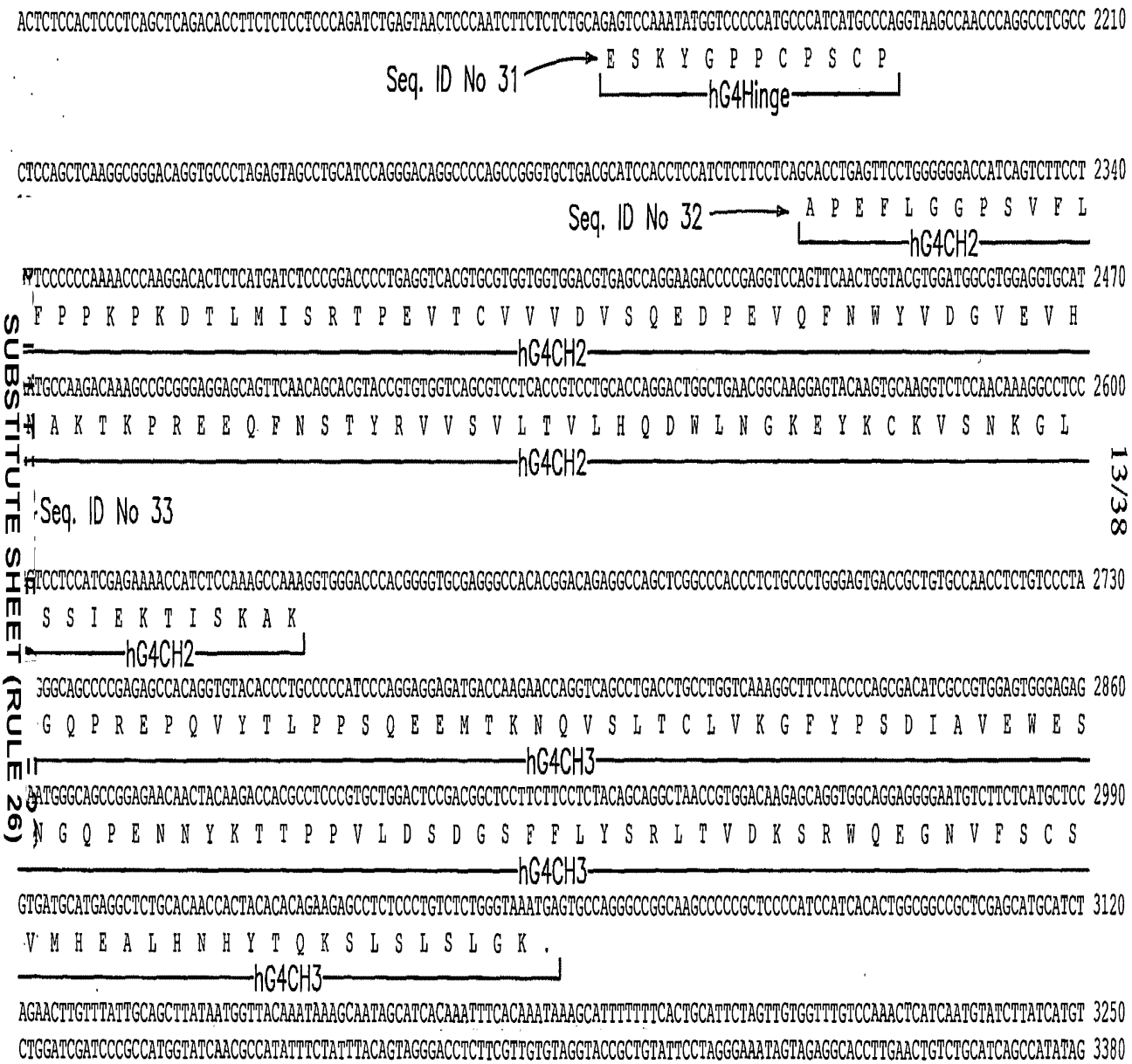


FIG. 5B (Cont.)

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CCTCTTTTAATTAGTTGCTAGGCAACGCCCTCCAGAGGGCGTGTGGTTTTGCAAGAGGAAGCAAAAGCCTCTCCACCCAGGCCTAGAATGTTTCCACCCAATCATTACTATGACAACAGCTGTTTTTTTT 3900
GCTATTAAGCAGAGGCCGGGGACCCCTGGGCCCGCTTACTCTGGAGAAAAAGAAGAGAGGCATTGTAGAGGCTTCCAGAGGCAACTTGTCAAAACAGGACTGCTTCTATTTCTGTCACACTGTCTGGCCC 4030
GTCACAAGGTCCAGCACCTCCATACCCCCTTTAATAAGCAGTTTGGGAACGGGTGCGGGTCTTACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATT 4160
TTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGGAGCTCCCAGCAAAAGGCCAGGAACCGTAAAAAG 4290
GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCC 4420
CGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAA 4550
TGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC 4680
GAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCT 4810
TTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAA 4940
CAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAC 5070
ATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGA 5200
CCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC 5330
GAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACA 5460
TCATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT 5590
GTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAA 5720
AGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTT 5850
TCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGA 5980
GCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTG 6058

FIG. 5B (Cont.)

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Schematic map of the vector Apex−1 3F4V_HHuG2/G4.

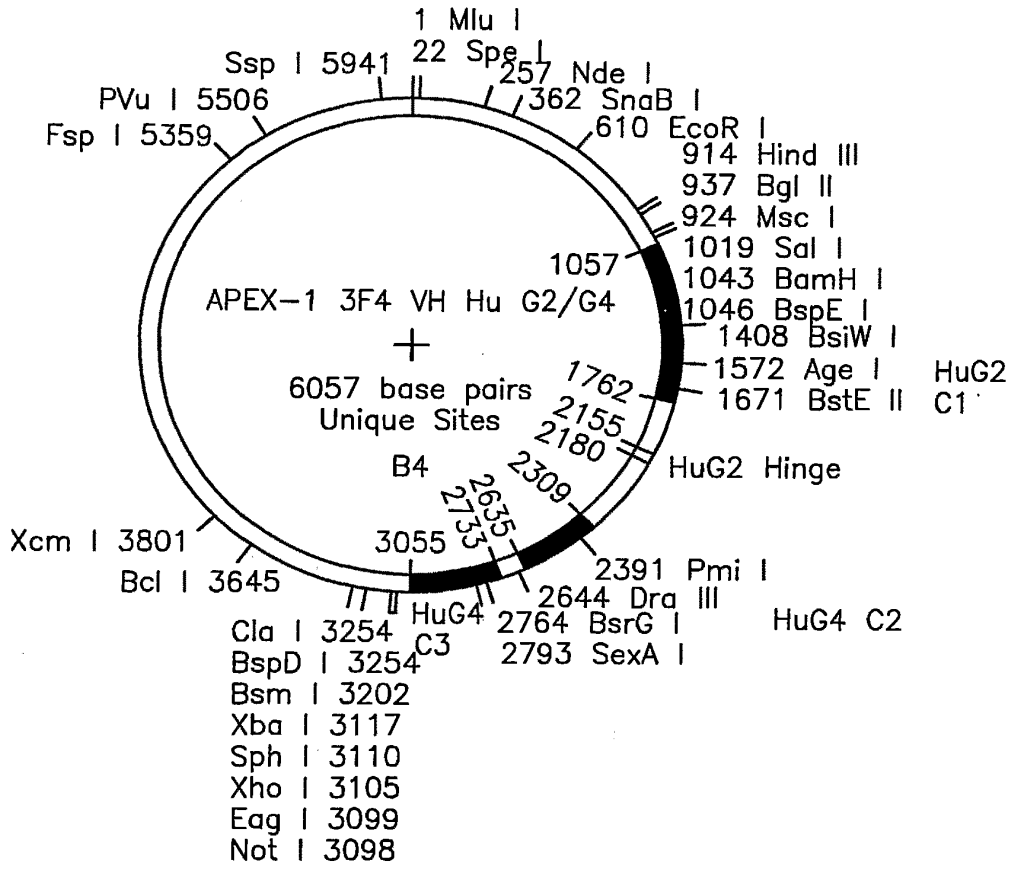


FIG. 6A

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SUBSTITUTE SHEET (RULE 26)

Vector Sequence (APEX-13F4V$_H$HuG2/G4)

```
ACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCCGCCTGGCTG 120
ACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGC 240
AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACAT 360
CTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAG 480
TTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGT 600
GAACCGTCAGAATTCTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACCCGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGC 720
AGTCCGCATCGACCGGATCGGAAAACCTCTCGACTGTTGGGGTGAGTACTCCCTCTCAAAAGCGGGCATGACTTCTGCGCTAAGATTGTCAGTTTCCAAAAACGAGGAGGATTTGATAT 840
CACCTGGCCCGCGGTGATGCCTTTGAGGGTGGCCGCGTCCATCTGGTCAGAAAAGACAATCTTTTTGTTGTCAAGCTTGAGGTGTGGCAGGCTTGAGATCTGGCCATACACTTGAGTGA 960
AATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGGTCCAACTGCAGGTCGACCGGCTTGGTACCGAGCTCGGATCCGGACCATCATGAAGTGGAGCTGGGTTATTCTC 1080
                                                    (Seq. ID No: 8) → M K W S W V I L
                                                                      Signal
TCCTCCTGTCAGTAACTGCCGGCGTCCACTCCCAGGTTCAGGTCCAGCAGTCTGGGGCTGAGCTGGCAAGACCTTGGGCTTCAGTGAAGTTGTCCTGCAAGGCTTCTGGCTACAATTTT 1200
F L L S V T A G V H S Q V Q V Q Q S G A E L A R P W A S V K L S C K A S G Y N F
Signal ——— 3F4Vh
ATAGTTACTGGATGCAGTGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGATTGGGGCTATTTATCCTGGAGATGGTGATACTAGCTACACTCAGAAGTTCAGGGGCAAGGCCACA 1320
N S Y W M Q W V K Q R P G Q G L E W I G A I Y P G D G D T S Y T Q K F R G K A T
3F4Vh
TGACTGCAGATAAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCTTGGCATCTGAGGACTCTGCGGTCTATTACTGTGCAAGACGTACGGTAGGAGGCTACTTTGACTACTGGGGC 1440
L T A D K S S S T A Y M Q L S S L A S E D S A V Y Y C A R R T V G G Y F D Y W G
3F4Vh
CAAGGCACCACTCTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGAC 1560
Q G T T L T V S S A S T K G P S V F P L A P C S R S T S E S T A A L G C L V K D
3F4Vh ——— G2G4CH1
```

FIG. 6B

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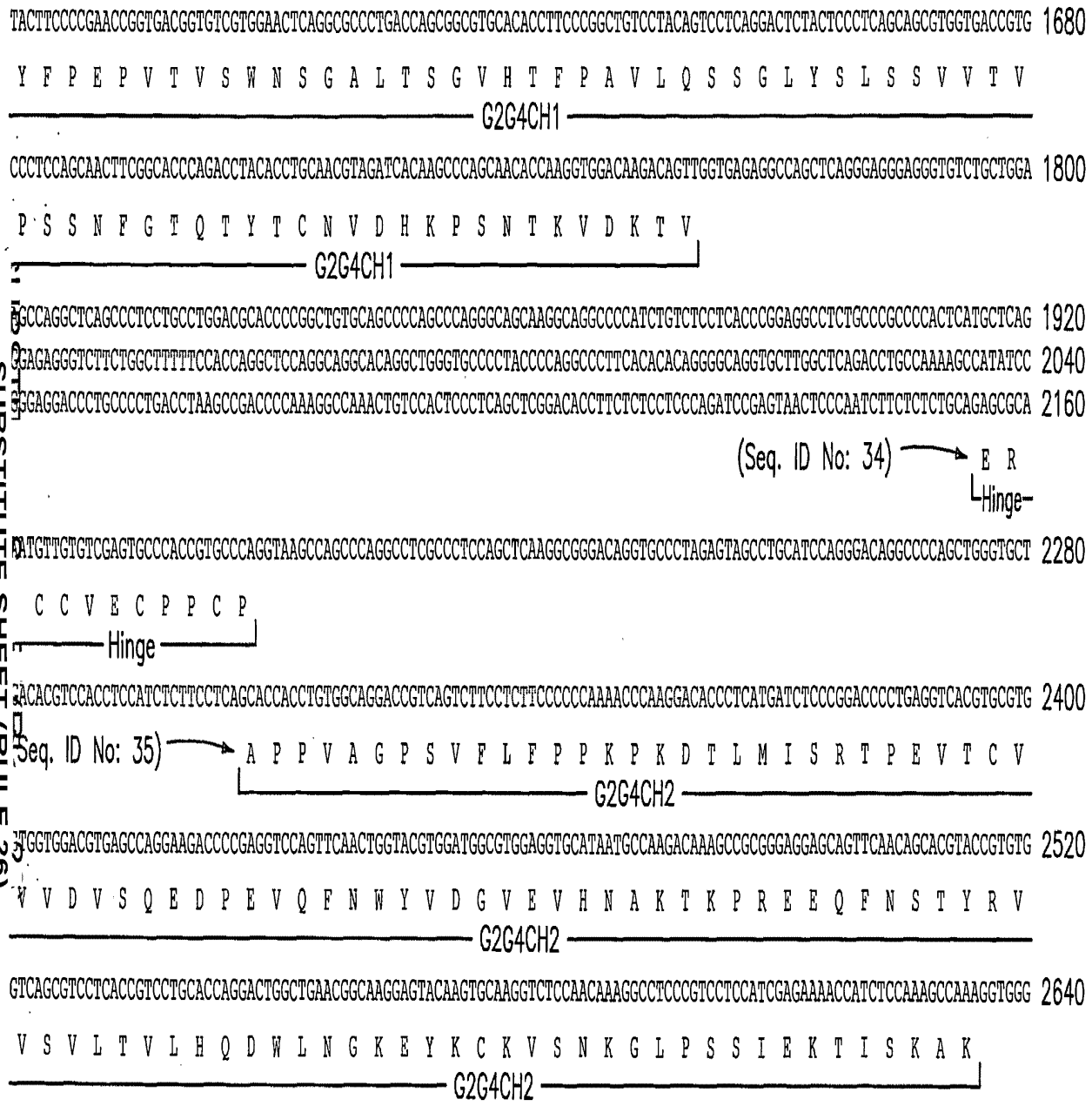
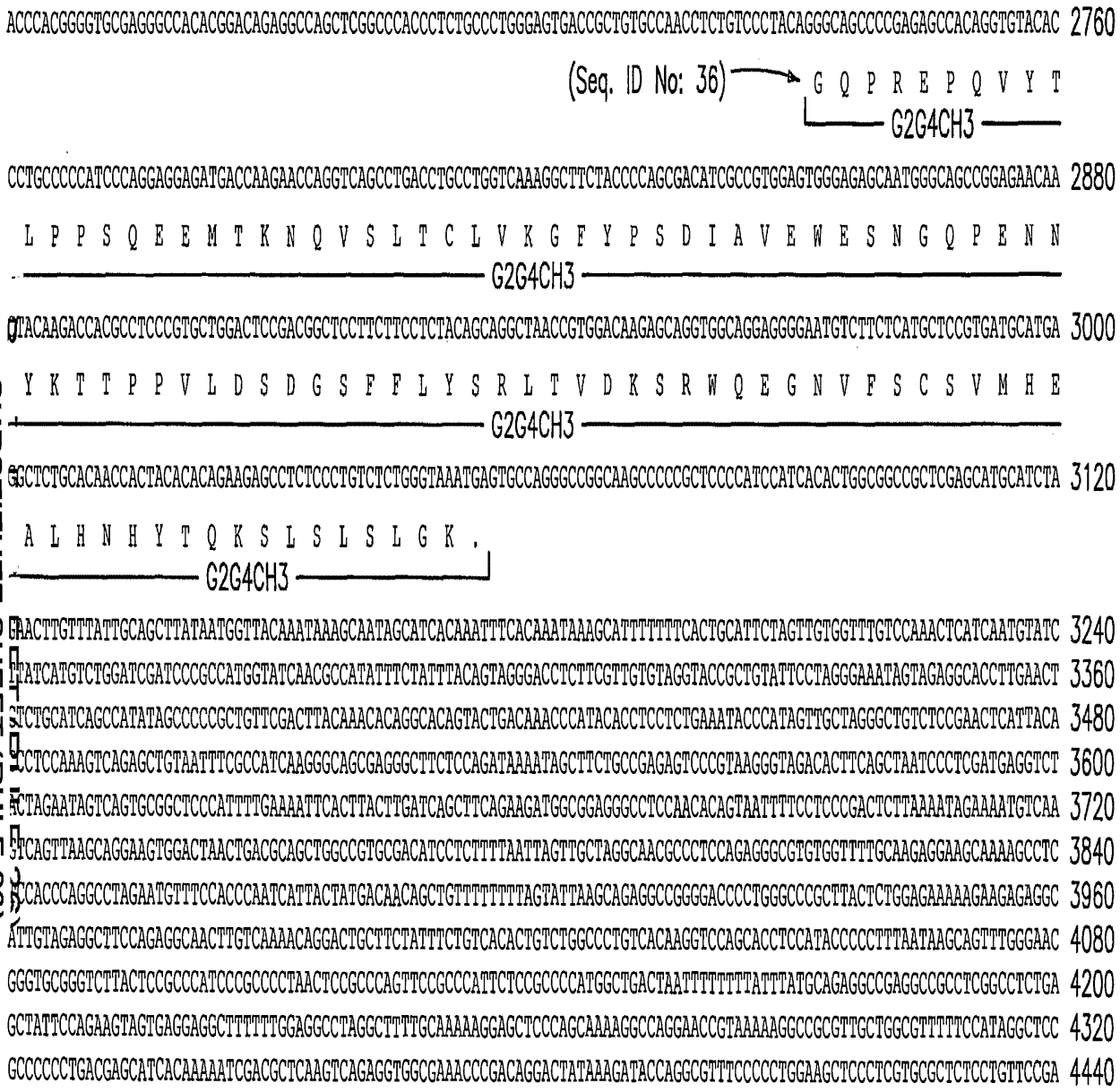


FIG. 6B (Cont.)

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FIG. 6B (Cont.)

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CCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG 4560

GCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCA 4680

GAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAG 4800

TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGG 4920

GGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTA 5040

ATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGA 5160

TACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC 5280

CTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCAC 5400

CTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAA 5520

TAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAG 5640

AATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCT 5760

CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATG 5880

CGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTA 6000

TTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTG 6057

FIG. 6B (Cont.)

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Map of the heavy chain expression vector pSVgptHuG2/G4 used in

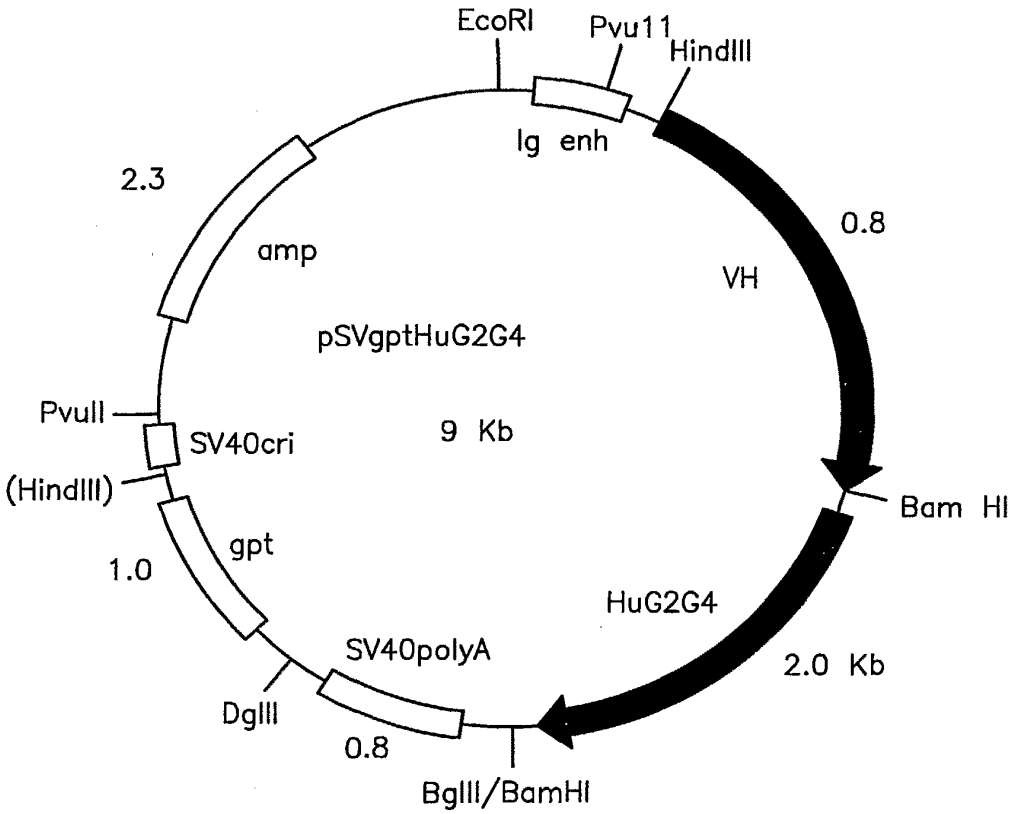


FIG. 7

(Seq. ID No. 9)

Bam HI

5' untranslated intron from native IgG4

GGATCCTCTAGATTGAGCTTTCTGGGGCAGGCCAGGCCTGACCTTGGCTGGG
GGCAGGGAGGGGGCTAAGGTGACGCAGGTGGCGCCAGCCAGGTGCACACCC
AATGCCCATGAGCCCAGACACTGGACCCTGCATGGACCATCGCGGATAGACA
AGAACCGAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCCACACCGCGGTC
ACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCCGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCT
GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC
CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTA
CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACC
TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACA
GTTGGTGAGAGGCCAGCTCAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTC
AGCCCTCCTGCCTGGACGCACCCCGGCTGTGCAGCCCCAGCCCAGGGCAGCA
AGGCAGGCCCCATCTGTCTCCTCACCCGGAGGCCTCTGCCCGCCCCACTCATG
CTCAGGGAGAGGGTCTTCTGGCTTTTTCCACCAGGCTCCAGGGAGGCACAGG
CTGGGTGCCCCTACCCCAGGCCCTTCACACACAGGGGCAGGTGCTTGGCTCA
GACCTGCCAAAAGCCATATCCGGGAGGACCCTGCCCCTGACCTAAGCCGACC
CCAAAGGCCAAACTGTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCCCAGA
TCCGAGTAACTCCCAATCTTCTCTCTGCAGAGCGCAAATGTTGTGTCGAGTGC
CCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCGGG
ACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCTGGGTGCTG
ACACGTCCACCTCCATCTCTTCCTCAGCACCACCTGTGGCAGGACCGTCAGTC
TTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA
GGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTC
AACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGG
GAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGC
ACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAG
GCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACCCA
CGGGGTGCGAGGGCCACATGGACAGAGGTCAGCTCGGCCCACCCTCTGCCCT
GGGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGGCAGCCCCGAGAGCCA
CAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCA
GCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTG
GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCT
GGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGC
AGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAATGAGTGCC
AGGGCCGGCAAGCCCCCGCTCCCCGGGCTCTCGGGGTCGCGCGAGGATGCTT

3' untranslated region from native IgG4

GGCACGTACCCCGTCTACATACTTCCCAGGCACCCAGCATGGAAATAAAGCA
CCCACCACTGCCCTGGGCCCCTGTGAGACTGTGATGGTTCTTTCCACGGGTCA
GGCCGAGTCTGAGGCCTGAGTGACATGAGGaAttCAGAtctGGatCC

Bgl II

FIG. 8

Map of the light chain expression vector pSVgptHuCK

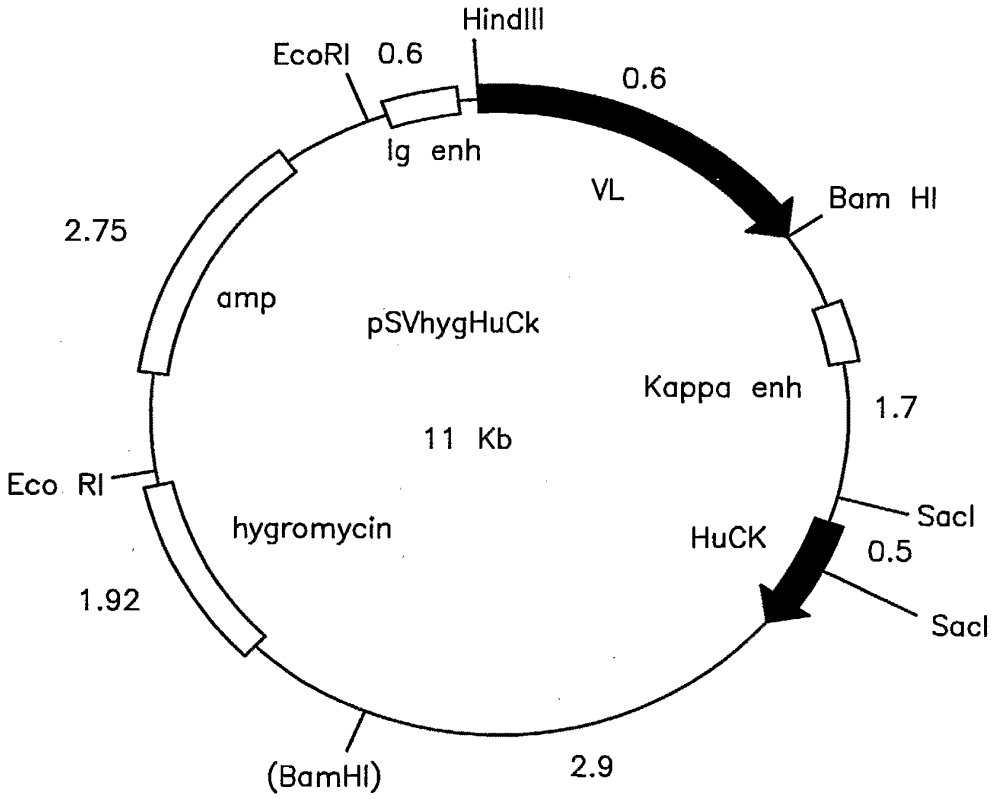


FIG. 9

Amino Acid sequences of DeImmunised OKT3 heavy chain variable regions

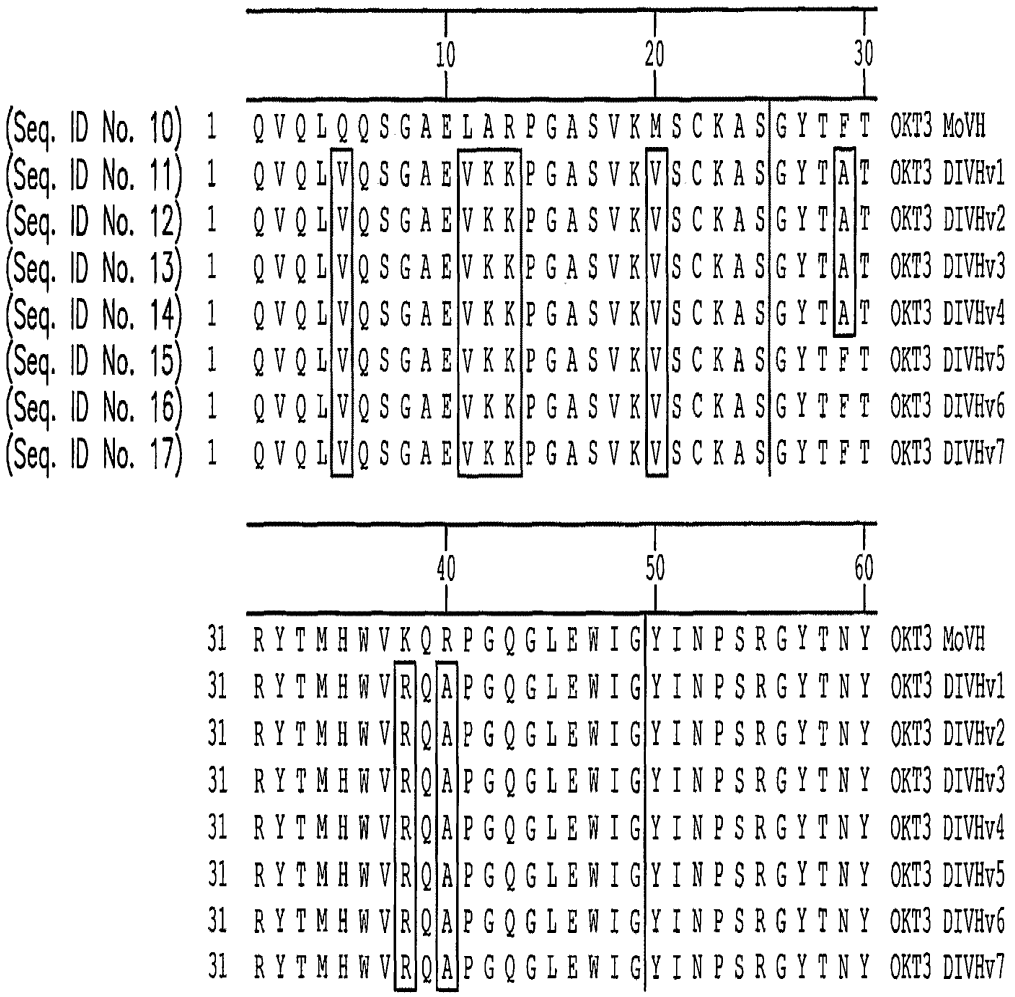


FIG. 10

SUBSTITUTE SHEET (RULE 26)

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SUBSTITUTE SHEET (RULE 26)

```
                    70                  80                  90
61  NQKFKDKATLTTDKSSSTAYMQLSSLTSED  OKT3 MoVH
61  AQKFQDRVTITTDKSSSTAYLQMNSLKTED  OKT3 DIVHv1
61  ADSVKGRFTITTDKSSSTAYLQMNSLKTED  OKT3 DIVHv2
61  NQKFKDRVTITTDKSSSTAYLQMNSLKTED  OKT3 DIVHv3
61  NQKVKDRFTITTDKSSSTAYLQMNSLKTED  OKT3 DIVHv4
61  NQKFKDRVTITTDKSSSTAYLQMNSLKTED  OKT3 DIVHv5
61  AQKFQDRVTITTDKSSSTAYLQMNSLKTED  OKT3 DIVHv6
61  NQKVKDRFTITTDKSSSTAYLQMNSLKTED  OKT3 DIVHv7

                   100                 110
91  SAVYYCARYYDDHYCLDYWGQGTTLTVSS   OKT3 MoVH
91  TAVYYCARYYDDHYCLDYWGQGTTVTVSS   OKT3 DIVHv1
91  TAVYYCARYYDDHYCLDYWGQGTTVTVSS   OKT3 DIVHv2
91  TAVYYCARYYDDHYCLDYWGQGTTVTVSS   OKT3 DIVHv3
91  TAVYYCARYYDDHYCLDYWGQGTTVTVSS   OKT3 DIVHv4
91  TAVYYCARYYDDHYCLDYWGQGTTVTVSS   OKT3 DIVHv5
91  TAVYYCARYYDDHYCLDYWGQGTTVTVSS   OKT3 DIVHv6
91  TAVYYCARYYDDHYCLDYWGQGTTVTVSS   OKT3 DIVHv7
```

FIG. 10 (Cont.)

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Amino Acid sequences of DeImmunised OKT3 light chain variable regions

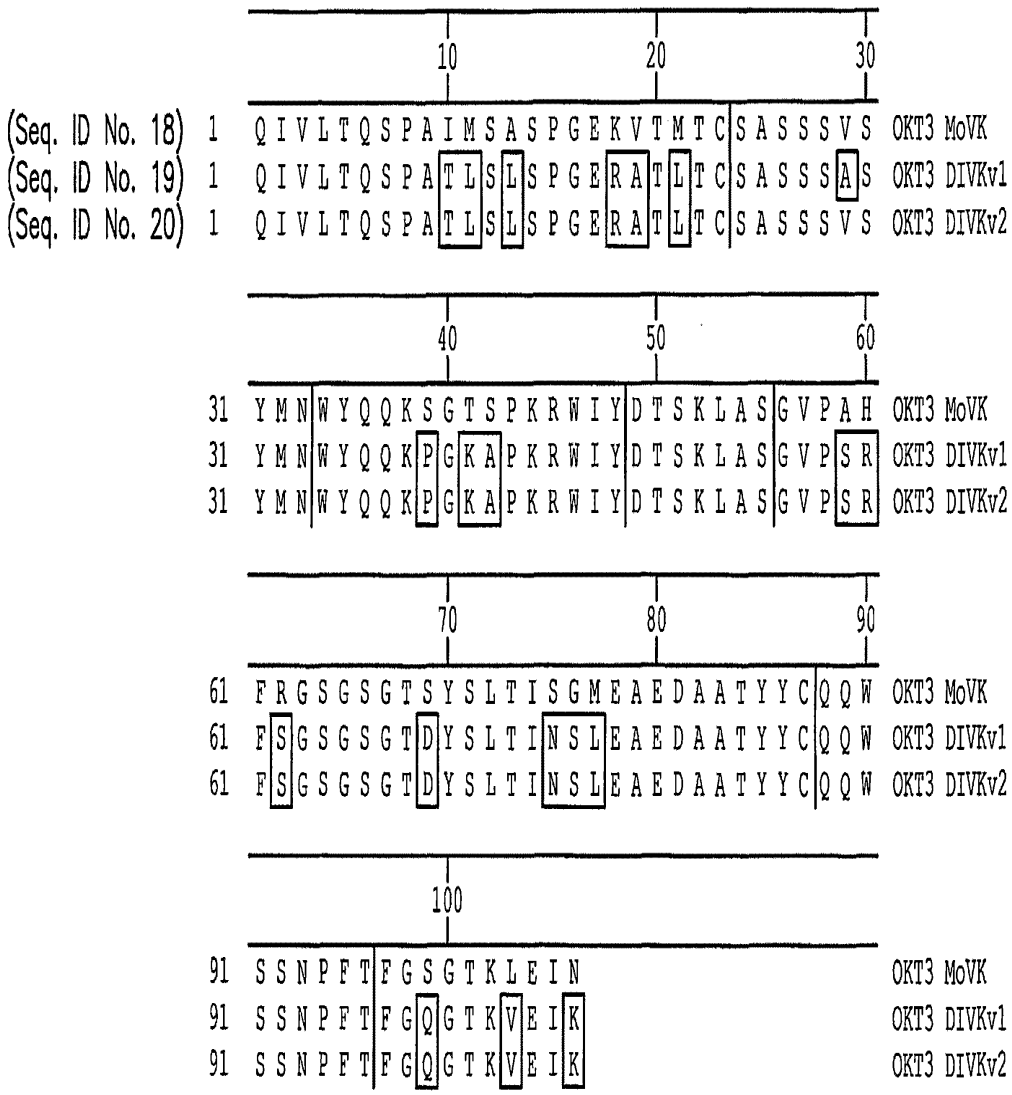


FIG. 11

Samsung Bioepis Exhibit No. 1002
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OLIGOS FOR CONSTRUCTION OF DIVHs (SEQ ID NOS: 37–57)

KTDIVH1 GAAGTCAAGAAACCTGGGGCCTCAGTGAAGGTGTCCTGCAAGG
KTDIVH2
GCCCCAGGTTTCTTGACTTCAGCCCCAGACTGTACCAGCTGGACCTG
KTDIVH3 TGGGTAAGACAGGCGCCTGGACAAGGTTTGG
KTDIVH4 GTCCAGGCGCCTGTCTTACCCAGTGCATC
KTDIVH4A
AGGCGCCTGTCTTACCCAGTGCATCGTGTACCTAGTAGCCGTGTAGCC
KTDIVH5 CAATCAGAAGTTCAAGGACAGGGTCACAATCACTACAGACAAA
KTDIVH5A CGCTCAGAAGTTCCAGGACAGGGTCACAATCACTACAGACAAA
KTDIVH5B CGCTGACAGTGTCAAGGGCAGGTTCACAATCACTACAGACAAA
KTDIVH5C CAATCAGAAGGTCAAGGACAGGTTCACAATCACTACAGACAAA
KTDIVH6 GTCCTTGAACTTCTGATTGTAATTAGTATATCCACGG
KTDIVH6A GTCCTGGAACTTCTGAGCGTAATTAGTATATCCACGG
KTDIVH6B GCCCTTGACACTGTCAGCGTAATTAGTATATCCACGG
KTDIVH6C GTCCTTGACCTTCTGATTGTAATTAGTATATCCACGG
KTDIVH7 AGCCTGAAAACTGAGGACACCGCAGTCTATTACTG
KTDIVH8 GTCCTCAGTTTTCAGGCTGTTCATTTGCAAGTAGGCTGTGCT
KTDIVH9 CCAAGGCACCACTGTGACAGTCTCCTCAGG
KTDIVH10 CCTGAGGAGACTGTCACAGTGGTGCCTTGG
KT3VHY GGTGTCCACTCCCAGGTCCAGCTG
KT3VHZ CAGCTGGACCTGGGAGTGGACACCTGTGG
VHVK1 GCATGTTGACCCTGACGCAAGCTTATGAATATGCAAA
VH12 GCGATAGCTGGACTGAATGGATCCTATAAATCTCTG

OLIGOS FOR CONSTRUCTION OF DIVKs (SEQ ID NOS: 58–74)

KTDIVK1 CCCTCTCTCTTTCTCCAGGGGAACGCGCCACCTTGACATGCAGTG
KTDIVK2 CCTGGAGAAAGAGAGAGGGTTGCTGGAGACTGGGTG
KTDIVK3
CATGAACTGGTACCAGCAGAAGCCCGGCAAAGCTCCCAAAAGATGGAT
KTDIVK4 CGGGCTTCTGCTGGTACCAGTTCATGTAACTTACACTT
KTDIVK4A CTTCTGCTGGTACCAGTTCATGTAACTTGCACTTGAGC
KTDIVK5
GGGTCTGGGACCGATTACTCTCTCACCATCAATAGTCTGGAAGCTGAAG
KTDIVK6
GTAATCGGTCCCAGACCCACTGCCACTGAAGCGAGACGGTACTCCAG
KTDIVK7 TTCACGTTCGGACAAGGTACAAAGGTGGAAATCAAACG
KTDIVK8 CTTTGTACCTTGTCCGAACGTGAATGGGTTACTTGACC
KKT22 GCGGATCCAGTCGACGAAGCA
KT3VKX CTGAATGGATCCAACTGAGGAAGCAAAGTTTAAATTCTACTCACG
KT3VKY CAAATTGTTCTCACCCAGTCTCCAGCAA
KT3VKZ TTGCTGGAGACTGGGTGAGAACAATTTGGGAG
KT3VKZ2 TGGAGACTGGGTGAGAACAATTTGGGAGTGGACACCTGTGG
KT3VKZ3 AGAGAGGGTTGCTGGAGACTGGGTGAGAACAATTTG
VHVK1 GCATGTTGACCCTGACGCAAGCTTATGAATATGCAAA
VK12 GCGATAGCTGGACTGAATGGATCCAACTGAGGAAGC

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DNA and Amino acid sequence of DeImmunised OKT3 VH version 1.

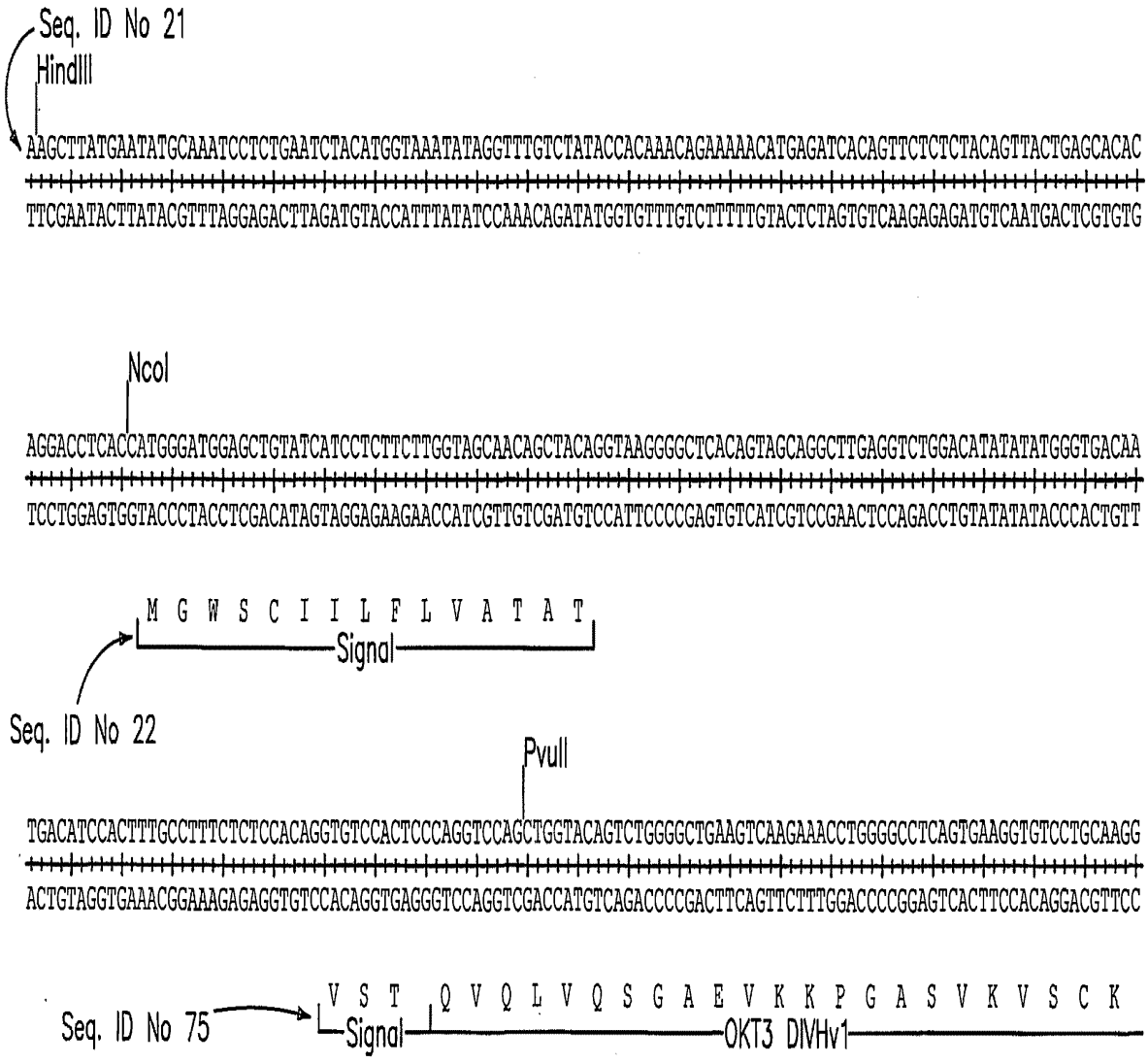


FIG. 13

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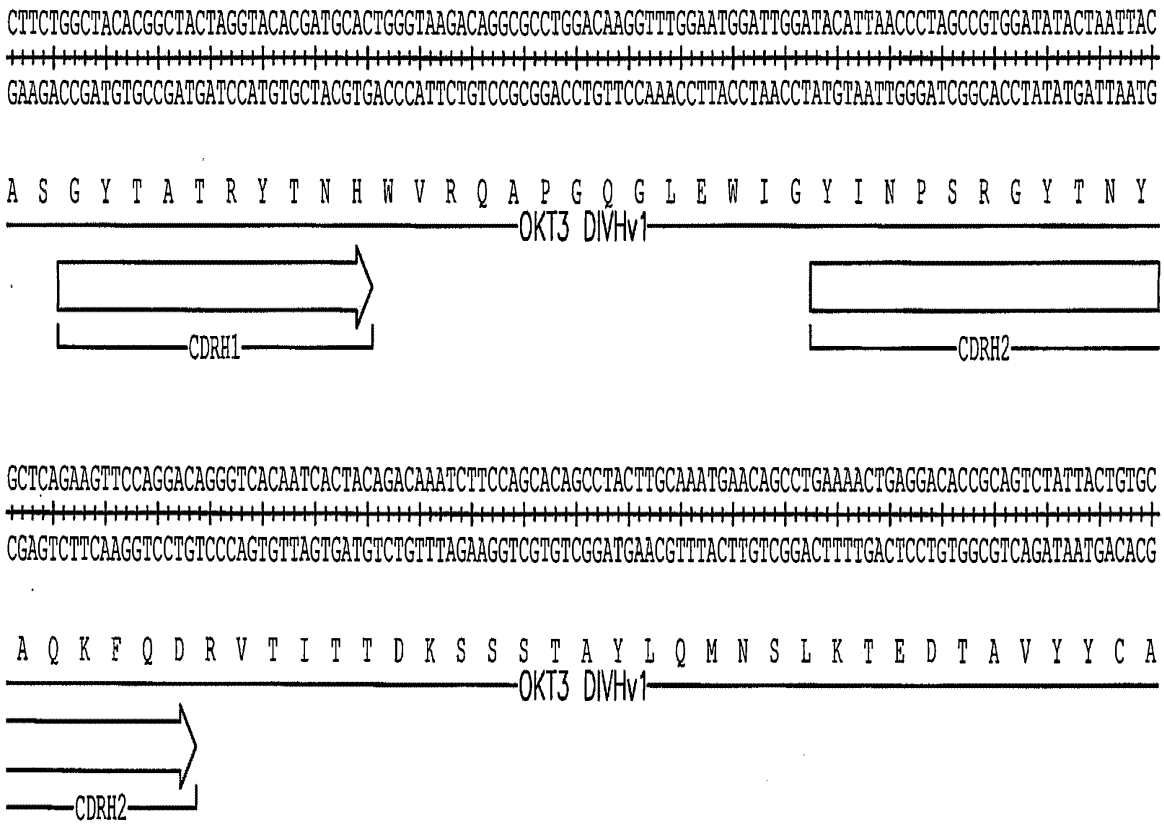


FIG. 13 (Cont.)

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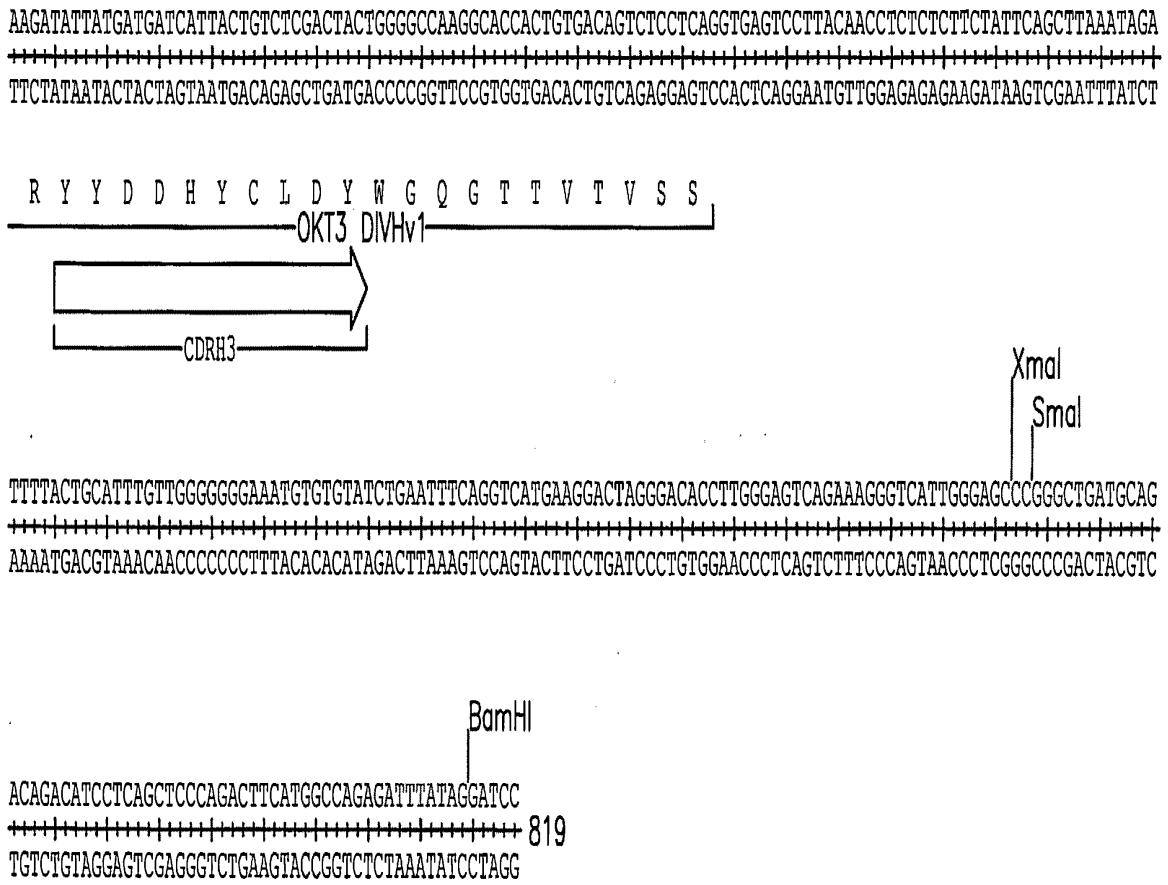


FIG. 13 (Cont.)

Samsung Bioepis Exhibit No. 1002
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DNA and Amino Acid Sequence of DeImmunised OKT3 VK version 1.

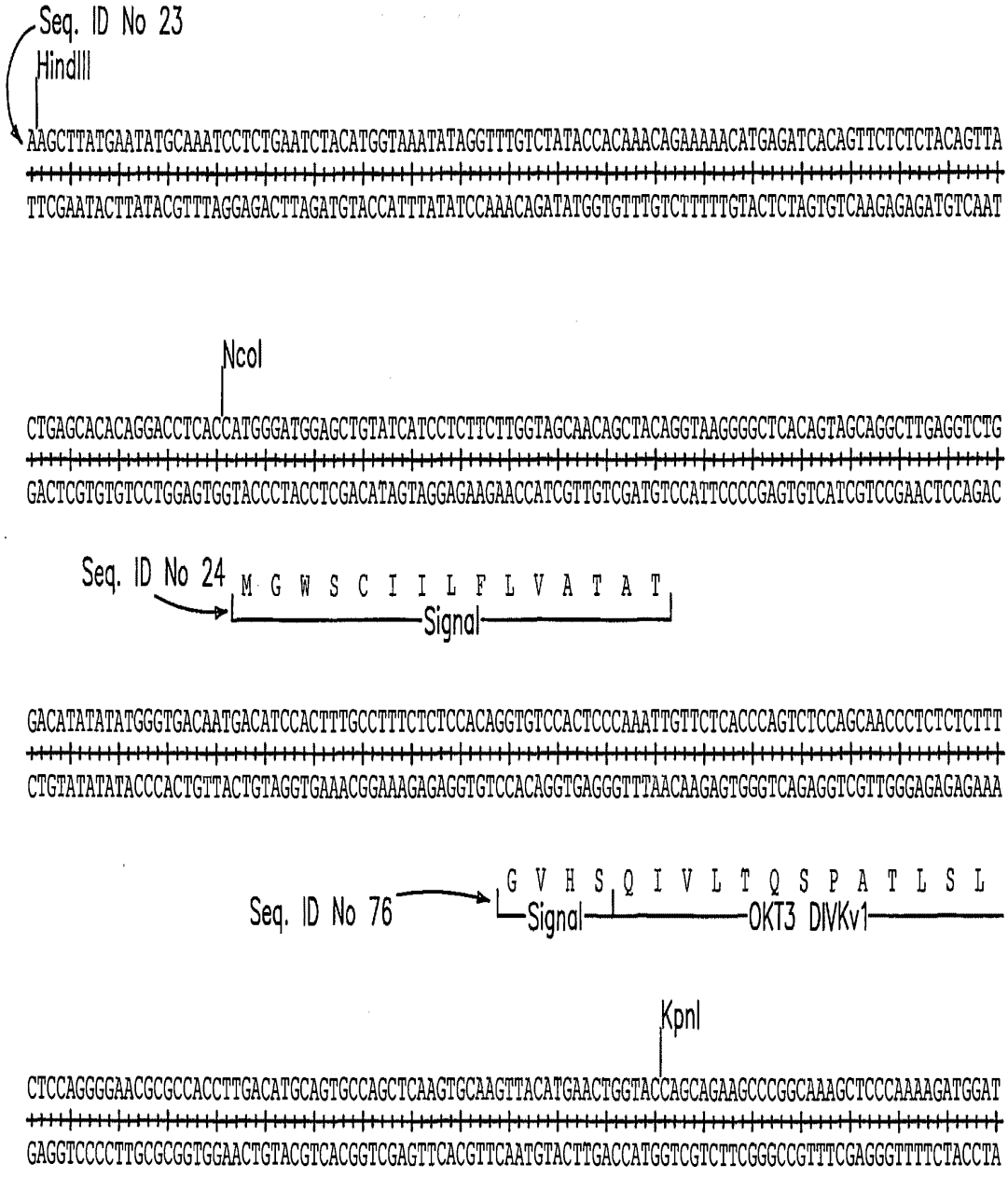


FIG. 14

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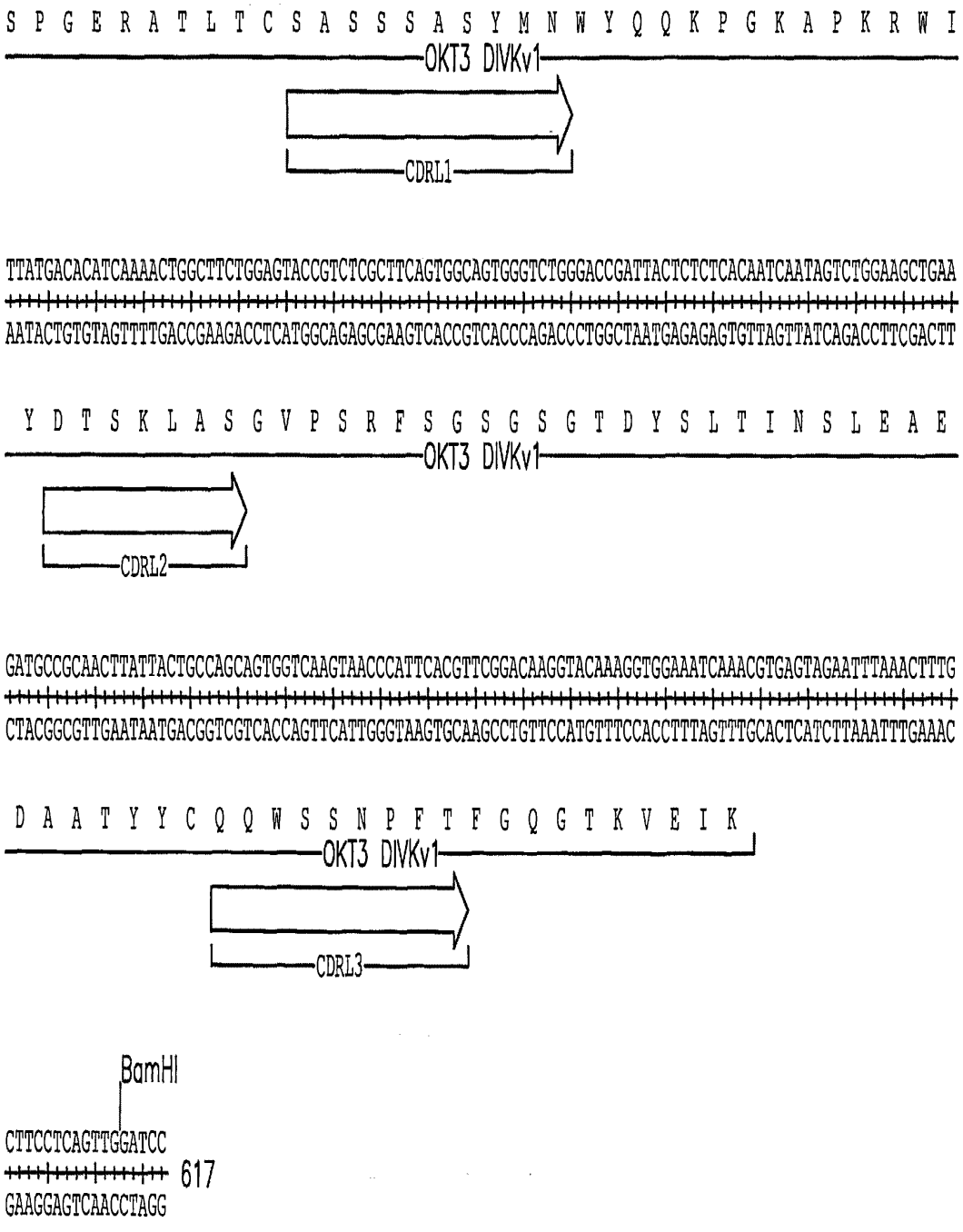


FIG. 14 (Cont.)

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Murine and Chimaeric OKT3 binding to Jurkat, JRT3 and HPB–ALL cells
Values represent the positive % of gated cells in M1

Cell Type	Passage #	Murine OKT3	Mouse Isotype Control	Chimaeric OKT3	Human Isotype Control
Jurkat	12	81.20	0.5	94.68	0.44
JRT3	14	3.45	0.26	4.56	0.43
HPB–ALL	10	99.63	0.62	99.39	0.29

FIG. 15

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Antibody	Clone No.	% Cells in M1	
		HPB-ALL	JRT3
Chimaeric OKT3	N/A	99.74	7.74
Control no OKT3 no PE	N/A	2.22	2.3
Control no OKT3 with PE	N/A	2.3	2.21
DMEM Control	N/A	1.91	2.42
DIVH1/DIVK1	19D6	93.87	2.16
DIVH2/DIVK1	24C12	28.47	2.34
DIVH3/DIVK1	27F6	84.75	2.28
DIVH4/DIVK1	30F7	93.06	2.65
DIVH5/DIVK1	35F2	98.15	2.77
DIVH6/DIVK1	37E9	97.85	3.08
DIVH7/DIVK1	42E7	98.62	3.12

FIG. 16

Table 3:

Antibody	Clone No.	% Cells in M1	
		HPB-ALL	JRT3
Chimaeric OKT3	N/A	99.95	0.1
Control no OKT3 no PE	N/A	0.1	0.02
DIVHv1/DIVK2	48G3	20.18	0.1
DIVHv2/DIVK2	52B8	90.04	0.25
DIVHv3/DIVK2	55G5	84.73	0.14
DIVHv4/DIVK2	55B2	69.26	0.13
DIVHv6/DIVK2	66C6	98.16	0.53
DIVHv7/DIVK2	70G10	95.57	0.66

FIG. 17

Competition assay. Inhibition of binding biotinylated mouse OKT3 by chimaeric and Delmmunised OKT3 antibodies, DIVHv1/DIVKv1, DIVHv3/DIVKv1, DIVHv5/DIVKv1, DIVHv6/DIVKv1, OKT3DIVH7/DIVKv1.

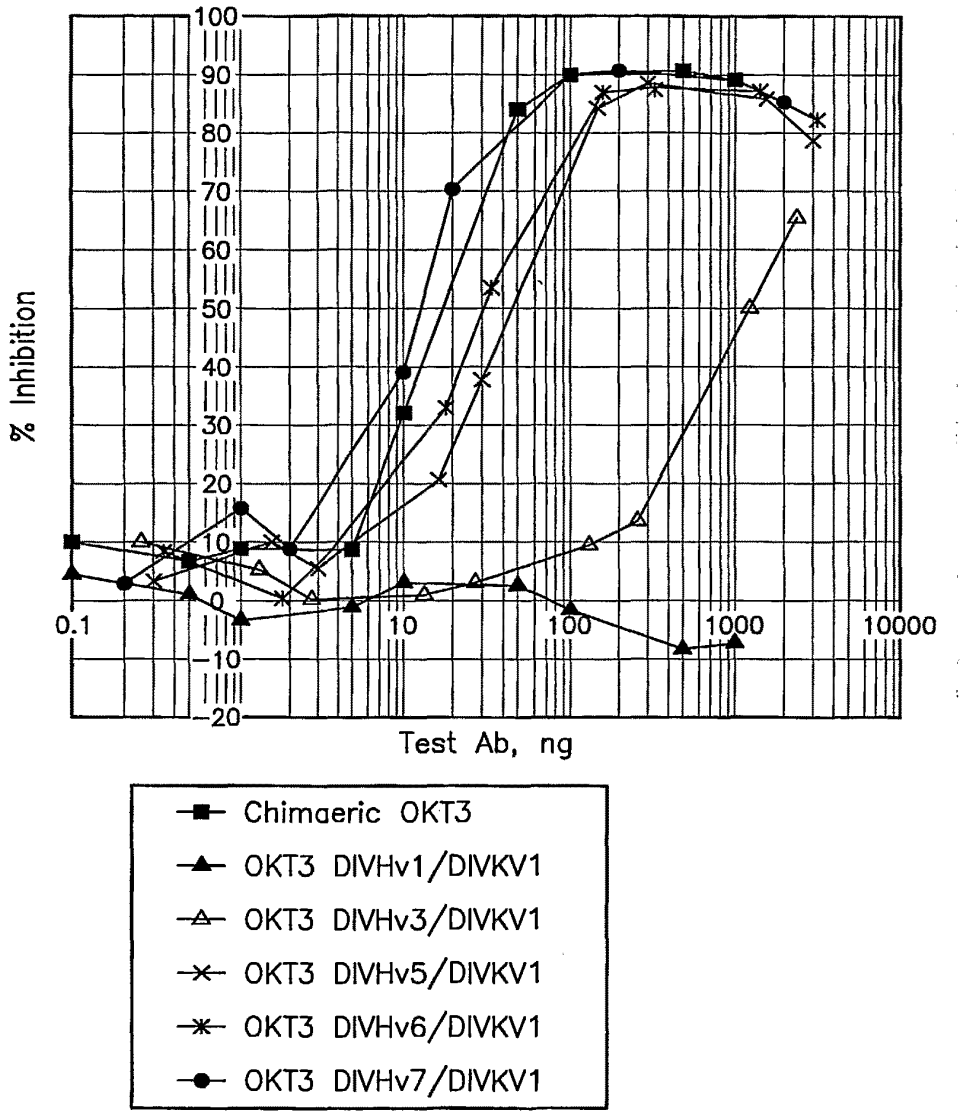


FIG. 18

Competition assay. Inhibition of binding biotinylated mouse OKT3 by mouse, chimaeric and DeImmunised OKT3 antibodies DIVHv2/DIVKv1, DIVHv4/DIVKv1.

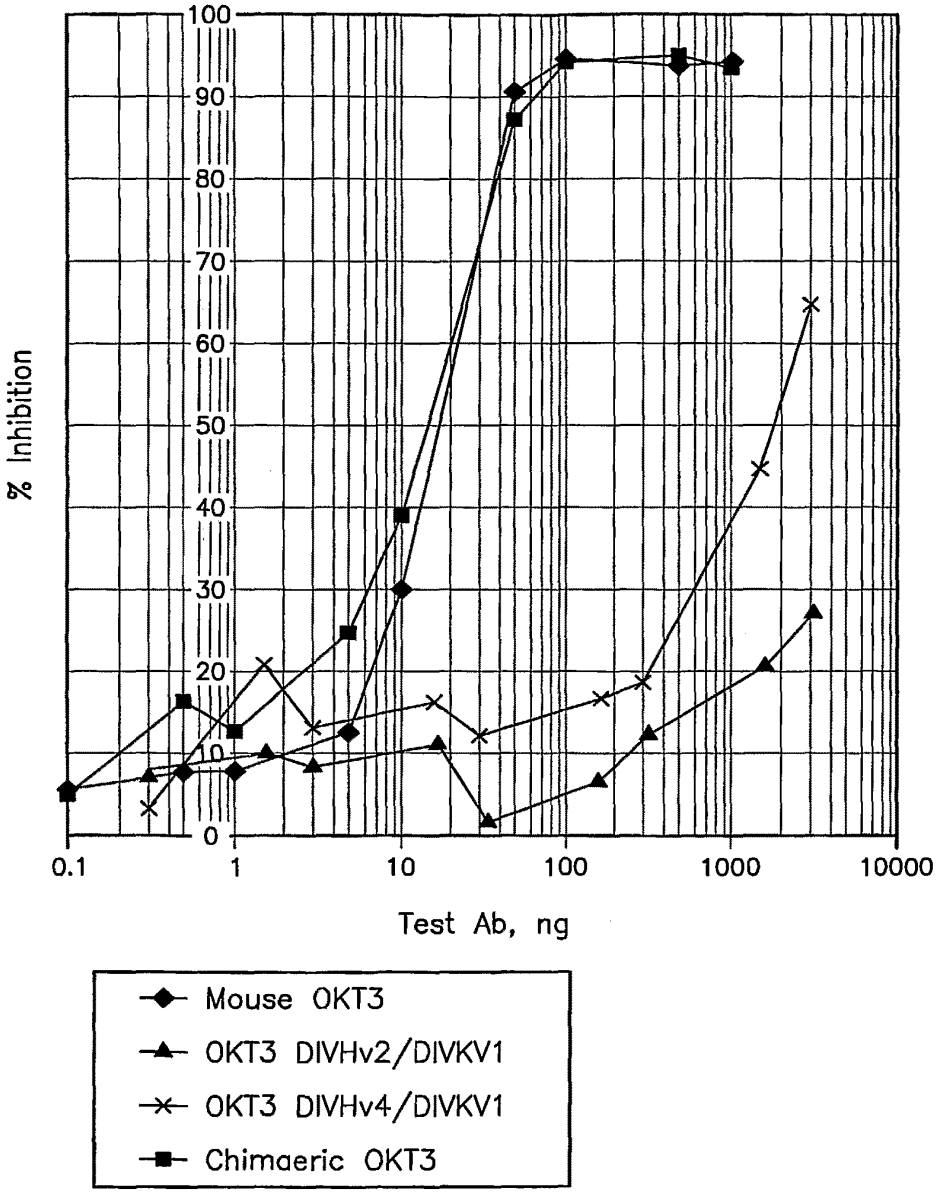


FIG. 19

Competition assay. Inhibition of binding biotinylated mouse OKT3 by mouse, chimaeric and DeImmunised OKT3 antibodies DIVHv3/DIVKv2, DIVHv7/DIVKv2.

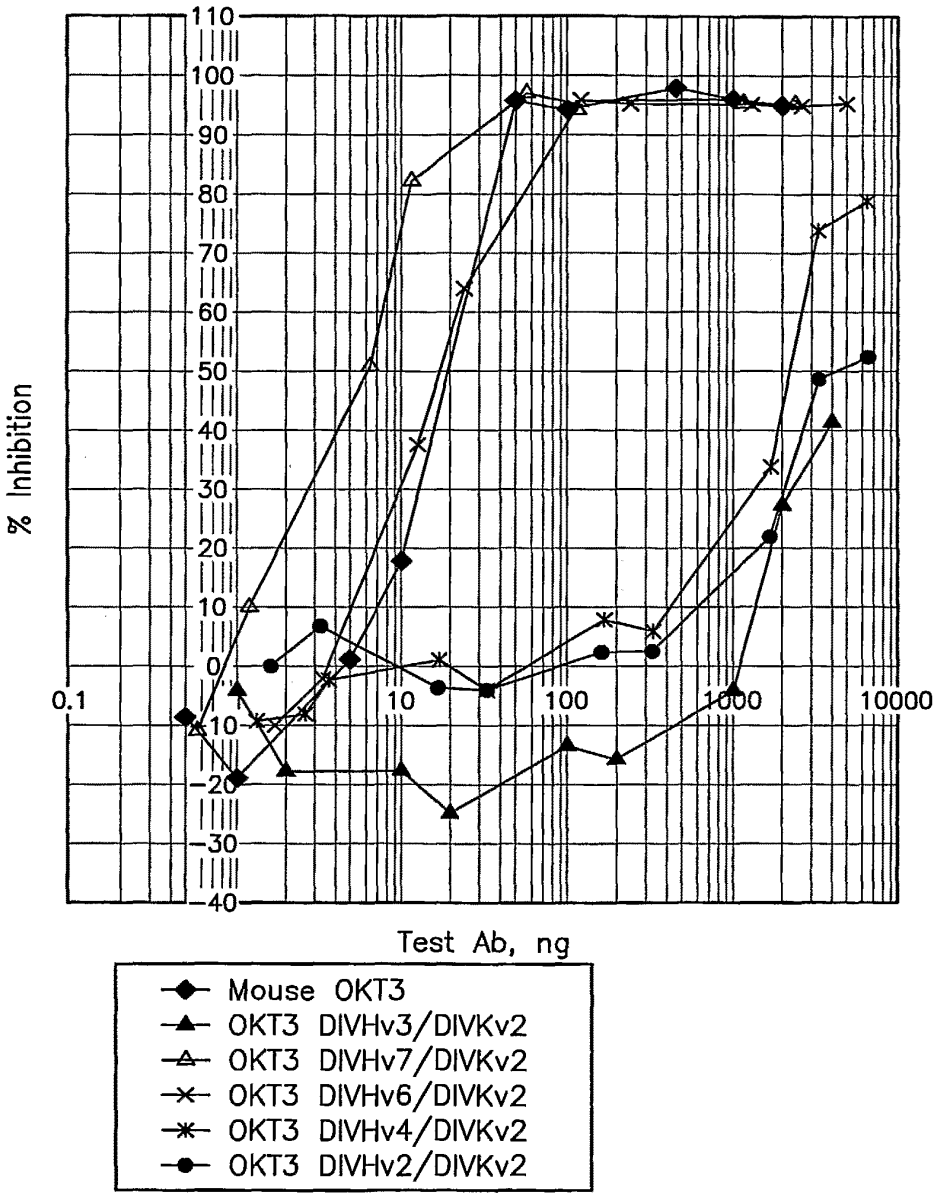


FIG. 20

Competition assay. Inhibition of binding biotinylated mouse OKT3 by mouse, chimaeric and DeImmunised OKT3 antibodies DIVHv1/DIVKv2, DIVHv1/DIVKv1, DIVHv5/DIVKv2, DIVHv5/DIVKv1.

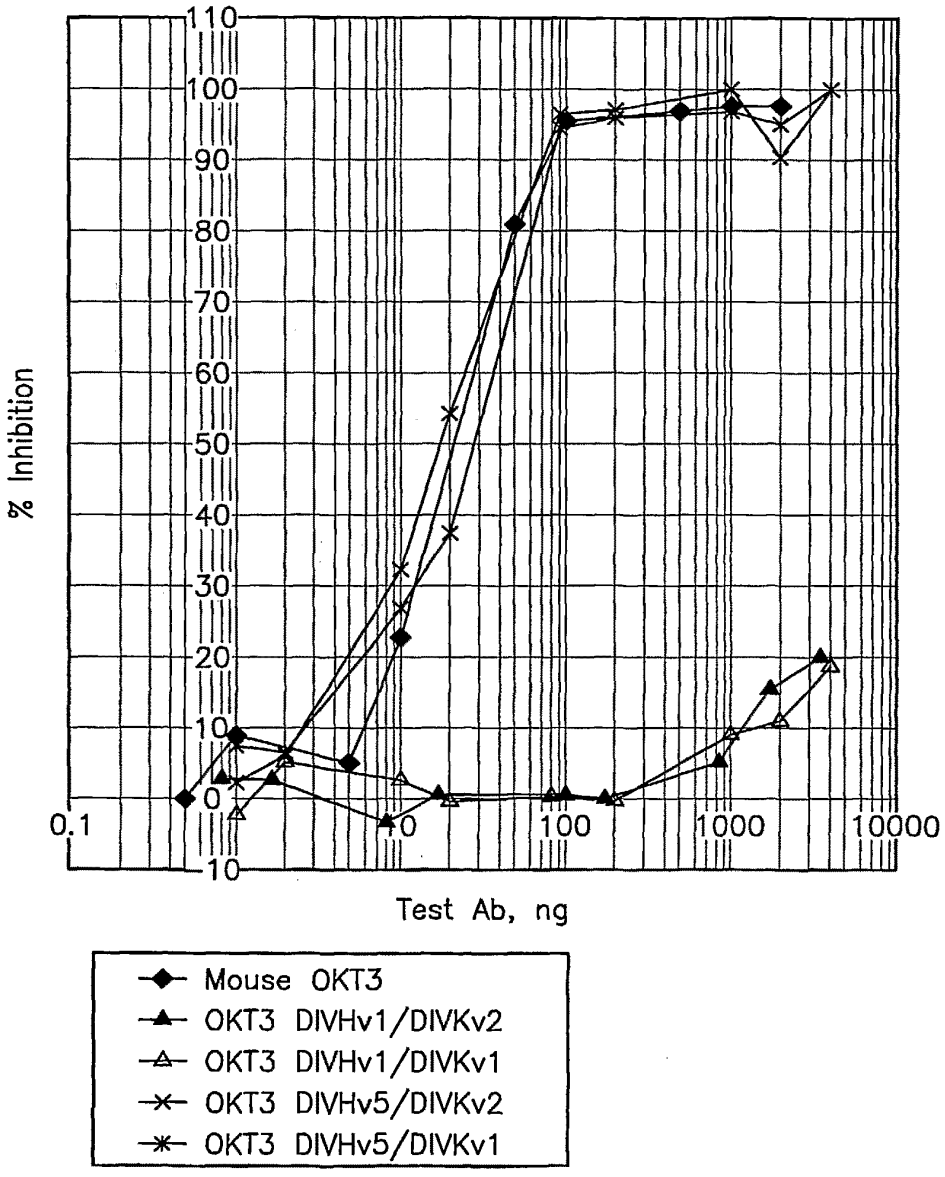


FIG. 21

The IC_{50} determinted from these plots are shown in Table 4.

Table 4:

ANTIBODY	IC50 (nq)
Murine OKT3 1	18
Murine OKT3 2	19
Murine OKT3 3	20
Chimeric OKT3 1	18
Chimeric OKT3 2	15
Di-immunized OKT3 DIVHv1/DIVKv1	N/A
Delm OKT3 DIVHv1/DIVKv1 2nd prep	>2000
De-immunized OKT3 DIVHv2/DIVKv1	>3000
De-immunized OKT3 DIVHv3/DIVKv1	1250
De-immunized OKT3 DIVHv4/DIVKv1	1900
De-immunized OKT3 DIVHv5/DIVKv1	45
Delm OKT3 DIVHv5/DIVKv1 2nd prep	19
De-immunized OKT3 DIVHv6/DIVKv1	30
De-immunized OKT3 DIVHv7/DIVKv1	12
De-immunized OKT3 DIVHv1/DIVKv2	>2000
De-immunized OKT3 DIVHv2/DIVKv2	>3000
De-immunized OKT3 DIVHv3/DIVKv2	>4000
De-immunized OKT3 DIVHv4/DIVKv2	2100
De-immunized OKT3 DIVHv5/DIVKv2	28
De-immunized OKT3 DIVHv6/DIVKv2	18
De-immunized OKT3 DIVHv7/DIVKv2	6

FIG. 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/17219

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 39/395; CO7K 16/00; C12N 15/13
US CL : 530/387.3; 424/133.1; 536/23.53; 435/69.6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/387.3; 424/133.1; 536/23.53; 435/69.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,491,916 B1 (BLUESTONE et al.) 10 December 2002 (10.12.2002), see entire document.	1,4,6,7,10-13,17
---		---
Y		5,16
Y	US 2003/0100741 A1 (MULLER et al.) 29 May 2003 (29.05.2003), see entire document.	5,16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
22 September 2004 (22.09.2004)

Date of mailing of the international search report
19 OCT 2004

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet) (January 2004)

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Continuation of B. FIELDS SEARCHED Item 3:
WEST 2.1, MEDICINE/BIOTECH (compendium database on DIALOG) search terns: inventor names, cd3, okt3, de immuniz?, deimmuniz?, reduc?, immunogenic?, anticd3, epitop?, remov?, delet?

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/17219

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 2,3,8,9,14,15 and 18-20
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The aformentioned claims were not examined because they recite sequences and no sequence listing has been submitted.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(2)) (January 2004)

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US006407213B1

D42

(12) **United States Patent**
Carter et al.

(10) **Patent No.: US 6,407,213 B1**
(45) **Date of Patent: Jun. 18, 2002**

(54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**

(75) Inventors: **Paul J. Carter**; **Leonard G. Presta**, both of San Francisco, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/146,206**

(22) PCT Filed: **Jun. 15, 1992**

(86) PCT No.: **PCT/US92/05126**

§ 371 (c)(1),
(2), (4) Date: **Nov. 17, 1993**

Related U.S. Application Data

(63) Continuation-in-part of application No. 07/715,272, filed on Jun. 14, 1991, now abandoned.

(51) **Int. Cl.**[7] **C07K 16/00**
(52) **U.S. Cl.** **530/387.3**; 435/69.6; 435/69.7; 435/70.21; 435/91; 536/23.53; 424/133.1
(58) **Field of Search** 435/69.6, 69.7, 435/70.21, 91, 172.2, 240.1, 240.27, 252.3, 320.1, 328; 536/23.53; 424/133.1; 530/387.3

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567	A	3/1989	Cabilly et al.	
4,845,198	A	7/1989	Urdal et al.	530/388.22
5,132,405	A	7/1992	Huston et al.	530/387.3
5,225,539	A	7/1993	Winter	530/389.3
5,530,101	A	6/1996	Queen et al.	530/387.3
5,558,864	A	9/1996	Bendig et al.	424/133.1
5,585,089	A	12/1996	Queen et al.	424/133.1
5,677,171	A	10/1997	Hudziak et al.	435/7.23
5,693,762	A	* 12/1997	Queen et al.	530/387.2
5,714,350	A	2/1998	Co et al.	435/69.6
5,772,997	A	6/1998	Hudziak et al.	424/130.1
5,821,337	A	10/1998	Carter et al.	530/387.3
5,834,598	A	11/1998	Lowman et al.	530/399
5,859,205	A	1/1999	Adair et al.	530/387.3

FOREIGN PATENT DOCUMENTS

AU	85058/91	3/1992	C07K/15/12
EP	120694	10/1984	
EP	125023 A1	11/1984	
EP	0 239 400	* 9/1987	C12N/15/00
EP	323806 A1	7/1989	
EP	328404 A1	8/1989	A61K/39/395
EP	338745 A1	10/1989	
EP	365209 A2	4/1990	
EP	365997 A2	5/1990	
EP	368684	5/1990	
EP	403156 A1	12/1990	
EP	438310 A2	7/1991	
EP	438312 A2	7/1991	
EP	440351 A2	8/1991	
EP	0 460 167 B1	12/1991	
EP	0 519 596 A1	12/1992	
EP	0 592 106 A1	4/1994	
EP	0 620 276	10/1994	
EP	682040 A1	11/1995	
EP	451216 B1	1/1996	C12P/21/08
EP	432249 B1	9/1996	
GB	2 188941	10/1987	
WO	WO 87/02671	5/1987	
WO	WO 88/09344	12/1988	
WO	WO 89/01783	3/1989	
WO	WO 89/06692	7/1989	
WO	WO 89/09622	10/1989	
WO	WO 90/07861	7/1990	
WO	90/07861	* 7/1990	C12P/21/00
WO	WO 91/07492	5/1991	
WO	WO 91/07500	5/1991	
WO	WO 91/09966	7/1991	C12P/21/08
WO	WO 91/09968	7/1991	C12P/21/08
WO	WO 91/09967	11/1991	
WO	WO 92/01047	1/1992	
WO	WO 92/04380	3/1992	
WO	WO 92/04381	3/1992	
WO	WO 92/05274	4/1992	
WO	WO 92/11383	7/1992	
WO	WO 92/11018	9/1992	A61K/35/14
WO	WO 92/15683	9/1992	
WO	WO 92/16562	10/1992	
WO	WO 92/22653	12/1992	
WO	WO 93/02191	2/1993	
WO	94/11509	5/1994	
WO	WO 94/12214	6/1994	

OTHER PUBLICATIONS

Riechmann et al. [Nature 332:323–327 (1988)].*
Queen et al. [Proc. Natl. Acad. Sci. 86:10029–10033 (1989)].*
Roitt [*Immunology*, published 1985, by Gower Medical Publishing Ltd. (London, England) p. 5.5].*
Tramontano et al. [J. Mol. Biol. 215:175–182 (1990)].*
"Biosym Technologies" in New Products, Chemical Design Automation 3 (Dec. 1988).
"Polygen Corporation" in New Products, Chemical Design Automation 3 (Nov. 1988).
Adair et al., "Humanization of the murine anti-human CD3 monoclonal antibody OKT3" *Hum. Antibod. Hybridomas* 5:41–47 (1994).
Chothia et al., "Principles of protein–protein recognition" *Nature* 256:705–708 (1975).
Chothia et al., "Transmission of conformational change in insulin" *Nature* 302:500–505 (1983).
Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" *J. Mol. Biol.* 235:53–60 (1994).

(List continued on next page.)

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Assistant Examiner—Minh-Tam Davis
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

82 Claims, 9 Drawing Sheets

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Page 7187

OTHER PUBLICATIONS

Couto et al., "Anti–BA46 Monoclonal Antibody Mc3 Humanization Using a Novel Positional Consensus and in Vivo and in Vitro Characterization" *Cancer Research Supplement* 55:1717–1722 (1995).

Couto et al., "Humanization of KC4G3, an Anti–Human Carcinoma Antibody" *Hybridoma* 13:215–219 (1994).

Ellis et al., "Engineered Anti–CD38 Monoclonal Antibodies for Immunotherapy of Multiple Myeloma" *The Journal of Immunology* pp. 925–937 (1995).

Hieter et al., "Evolution of Human Immunoglobulin K J Region Genes" *The Journal of Biological Chemistry* 257:1516–1522 (1982).

Lesk, Arthur M., "How Different Amino Acid Sequences Determine Similar Protein Structures: The Structure and Evolutionary Dynamics of the Globins" *J. Mol. Biol.* 135:225–270 (1980).

Matsumura et al., "Hydrophobic stabilization in T4 lysozyme determined directly by multiple substitutions of Ile 3" *Nature* 334:406–410 (1988).

Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies" *Science* 229:1202–1207 (Sep. 20, 1985).

Nakatani et al., "Humanization of mouse anti–human IL–2 receptor antibody B–B10" *Protein Engineering* 7:435–443 (1994).

Ohtomo et al., "Humanization of Mouse ONS–M21 Antibody with the Aid of Hybrid Variable Regions" *Molecular Immunology* 32:407–416 (1995).

Rodrigues et al., "Engineering a humanized bispecific $F(ab')_2$ fragment for improved binding to T cells" *Int. J. Cancer* (Suppl.) 7:45–50 (1992).

Sha et al., "A Heavy–Chain Grafted Antibody that Recognizes the Tumor–Associated TAG72 Antigen" *Cancer Biotherapy* 9:341–349 (1994).

Tempest et al., "Identification of framework residues required to restore antigen binding during reshaping of a monoclonal antibody against the glycoprotein gB of human cytomegalovirus" *Int. J. Biol. Macromol.* 17:37–42 (1995).

Tramontano, "Structural Determinants of the Conformations of Medium–Sized Loops in Proteins" *Proteins* 6:382–394 (1989).

Uchiyama et al., "A Monoclonal Antibody (Anti–Tac) Reactive with Activated and Functionally Mature Human T Cells" *Journal of Immunology* 126:1393–1397 (1981).

Vincenti et al., "Interleukin–2–Receptor Blockade with Daclizumab to Prevent Acute Rejection in Renal Transplantation" *New Engl. J. Med.* 338:161–165 (1998).

Vitetta et al., "Redesigning Nature's Poisons to Create Anti–Tumor Reagents" *Science* 238:1098–1104 (1987).

Waldmann et al., "Interleukin 2 Receptor (Tac Antigen) Expression in HTLV–1–associated Adult T–Cell Leukemia" *Cancer Research* 45:4559s–4562s (1985).

Waldmann, Thomas A., "The Structure, Function, and Expression of Interleukin–2 Receptors on Normal and Malignant Lymphocytes" *Science* 232:727–732 (1986).

Wu et al., "An Analysis of the Sequences of the Variable Regions of Bence Jones Proteins and Myeloma Light Chains and Their Implications for Antibody Complementarity" *Journal of Experimental Medicine* 132:211–250 (1970).

Rhodes, P., "Recombinant antibodies from CHO cells" *Abstr Pap Am Chem Soc* (Abstract No. 60 from the 199th American Chemical Society National Meeting held in Boston, MA Apr. 22–27, 1990) 199(1–2):BIOT 60 (Apr. 1990).

Amzel and Poljak, "Three–dimensional structure of immunoglobulins" *Ann. Rev. Biochem.* 48:961–967 (1979).

Bindon et al., "Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q" *Journal of Experimental Medicine* 168(1):127–142 (Jul. 1988).

Boulianne, G. L. et al., "Production of functional chimaeric mouse/human antibody" *Nature* 312(5995):643–646 (Dec. 1984).

Brown et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991).

Bruccoleri, "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* (erratum to article in Nature 335(6190):564–568 and) 336:266 (1988).

Bruggemann, M. et al., "Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies" *Journal of Experimental Medicine* 166:1351–1361 (1987).

Burgess et al., "Possible Dissociation of the Heparin–binding and Mitogenic Activities of Heparin–binding (Acidic Fibroblast) Growth Factor–1 from Its Receptor–binding Activities by Site–directed Mutagenesis of a Single Lysine Residue" *Journal of Cell Biology* 111:2129–2138 (1990).

Carter et al., "Humanization of an anti–$p185^{HER2}$ antibody for human cancer therapy" *Proc. Natl. Acad. Sci.* 89:4285–4289 (1992).

Cheetham, J., "Reshaping the antibody combining site by CDR replacement–tailoting or tinkering to fit?" *Protein Engineering* 2(3):170–172 (1988).

Chothia and Lesk, "Canonical Structures for the Hypervariable Regions" *J. Mol. Biol.* 196:901–917 (1987).

Chothia et al., "The predicted structure of immunoglobulin D1.3 and its comparison with the crystal structure" *Science* 233:755–758 (Aug. 15, 1986).

Chothia, C. et al., "Conformations of immunoglobulin hypervariable regions" *Nature* 342(6252):877–883 (1989).

Chothia, Cyrus, "Domain association in immunoglobulin molecules: The packing of variable domains" *J. Mol. Biol.* 186:651–663 (1985).

Clark et al., "The improved lytic function and in vivo efficacy of monovalent monoclonal CD3 antibodies" *European Journal of Immunology* 19:381–388 (1989).

Co et al., "Humanized antibodies for antiviral therapy" *Proc. Natl. Acad. Sci. USA* 88:2869–2873 (1991).

Coussens et al., "Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene" *Science* 230:1132–1139 (1985).

Daugherty, BL et al., "Polymerase chain reaction facilitates the cloning, CDR–grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins" *Nucleic Acids Research* 19(9):2471–2476 (May 11, 1991).

Davies, D. R. et al., "Antibody–Antigen Complexes" *Ann. Rev. Biochem.* 59:439–473 (1990).

Epp et al., "The molecular structure of a dimer composed of the variable portions of the Bence–Jones protein REI refined at 2.0–A resolution" *Biochemistry* 14(22):4943–4952 (1975).

Fendly et al., "Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product" *Cancer Research* 50:1550–1558 (1990).

Samsung Bioepis Exhibit No. 1002
Page 7188

Furey et al., "Structure of a novel Bence–Jones protein (Rhe) fragment at 1.6 A resolution" *J. Mol. Biol.* 167(3):661–692 (Jul. 5, 1983).

Gorman, SD et al., "Reshaping a therapeutic CD4 antibody" *Proc. Natl. Acad. Sci. USA* 88(10):4181–4185 (May 15, 1991).

Gregory et al., "The solution conformations of the subclasses of human IgG deduced from sedimentation and small angle X–ray scattering studies" *Molecular Immunology* 24(8):821–829 (Aug. 1987).

Hale et al., "Remission induction in non–hodgkin lymphoma with reshaped human monoclonal antibody campath–1H" *Lancet* 1:1394–1399 (1988).

Harris and Emery, "Therapeutic antibodies—the coming of age" *Tibtech* 11:42–44 (Feb. 1993).

Huber et al., "Crystallographic structure studies of an IgG molecule and an Fc fragment" *Nature* 264:415–420 (Dec. 2, 1976).

Hudziak et al., "p185^{HER2} Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor" *Molecular & Cellular Biology* 9(3):1165–1172 (1989).

Jaffers, G. J. et al., "Monoclonal antibody therapy. Anti–idiotypic and non–anti–idiotypic antibodies to OKT3 arising despite intense immunosuppression" *Transplantation* 41(5):572–578 (May 1986).

Jones, P. T. et al., "Replacing the complementarity–determining regions in a human antibody with those from a mouse" *Nature* 321(6069):522–525 (1986).

Junghans et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders" *Cancer Research* 50(5):1495–1502 (Mar. 1, 1990).

Kabat et al. *Sequences of Proteins of Immunological Interest,* Bethesda, MD:National Institutes of Health pp. iii–xxvii, 41–176 (1987).

King et al., "Amplification of a Novel v–erbB–Related Gene in a Human Mammary Carcinoma" *Science* 229:974–976 (1985).

Lazar et al., "Transforming Growth Factor α: Mutation of Aspartic Acid 47 and Leucine 48 Results in Different Biological Activities" *Molecular & Cellular Biology* 8(3):1247–1252 (1988).

Love et al, "Recombinant antibodies possessing novel effector functions" *Methods in Enzymology* 178:515–527 (1989).

Lupu et al., "Direct interaction of a ligand for the erbB2 oncogene product with the EGF receptor and p185^{erbB2}" *Science* 249:1552–1555 (1990).

Margni RA and Binaghi RA, "Nonprecipitating asymmetric antibodies" *Ann. Rev. Immunol.* 6:535–554 (1988).

Margolies et al., "Diversity of light chain variable region sequences among rabbit antibodies elicited by the same antigens." *Proc. Natl. Acad. Sci. USA* 72:2180–84 (Jun. 1975).

Marquart et al., "Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen–binding fragment at 3.0 A and 1.0 A resolution" *J. Mol. Biol.* 141(4):369–391 (Aug. 25, 1980).

Mian, IS et al., "Structure, function and properties of antibody binding sites" *J. Mol. Biol.* 217(1):133–151 (Jan. 5, 1991).

Miller, R. et al., "Monoclonal antibody therapeutic trials in seven patients with T–cell lymphoma" *Blood* 62:988–995 (1983).

Morrison, S. L. et al., "Chimeric human antibody molecules: mouse antigen–binding domains with human constant region domains" *Proc. Natl. Acad. Sci. USA* 81(21):6851–6855 (Nov. 1984).

Neuberger et al., "Recombinant antibodies possessing novel effector functions" *Nature* 312(5995):604–608 (Dec. 1984).

Neuberger, M. S. et al., "A hapten–specific chimaeric IgE antibody with human physiological effector function" *Nature* 314(6008):268–270 (Mar. 1985).

Novotny and Haber, "Structural invariants of antigen binding: comparison of immunoglobulin V_L–V_H and V_L–V_L domain dimers" *Proc. Natl. Acad. Sci. USA* 82(14):4592–4596 (Jul. 1985).

Pluckthun, Andreas, "Antibody engineering: advances from the use of *Escherichia coli* expression systems" *Biotechnology* 9:545–51 (1991).

Queen, M. et al., "A humanized antibody that binds to the interleukin 2 receptor" *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989).

Riechmann, L. et al., "Reshaping human antibodies for therapy" *Nature* 332:323–327 (1988).

Roitt et al. *Immunology* (Gower Medical Publishing Ltd., London, England) pp. 5.5 (1985).

Saul et al., "Preliminary refinement and structural analysis of the Fab fragment from human immunoglobulin new at 2.0 A resolution" *Journal of Biological Chemistry* 253(2):585–597 (Jan. 25, 1978).

Schroff, R. et al., "Human anti–murine immunoglobulin responses in patients receiving monoclonal antibody therapy" *Cancer Research* 45:879–885 (1985).

Segal et al., "The three–dimensional structure of a phosphorylcholine–binding mouse immunoglobulin Fab and the nature of the antigen binding site" *Proc. Natl. Acad. Sci. USA* 71(11):4298–4302 (Nov. 1974).

Shalaby et al., "Development of humanized bispecific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene" *Journal of Experimental Medicine* 175(1):217–225 (Jan. 1, 1992).

Shepard and Lewis, "Resistance of tumor cells to tumor necrosis factor" *J. Clin. Immunol.* 8(5):333–395 (1988).

Sheriff et al., "Three–dimensional structure of an antibody–antigen complex" *Proc. Natl. Acad. Sci. USA* 84(22):8075–8079 (Nov. 1987).

Sherman et al., "Haloperidol binding to monoclonal antibodies" *Journal of Biological Chemistry* 263:4064–4074 (1988).

Silverton et al., "Three–dimensional structure of an intact human immunoglobulin" *Proc. Natl. Acad. Sci. USA* 74:5140–5144 (1977).

Slamon et al., "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER–2/neu Oncogene" *Science* 235:177–182 (1987).

Slamon et al., "Studies of the HER–2/neu proto–oncogene in human breast and ovarian cancer" *Science* 244:707–712 (1989).

Snow and Amzel, "Calculating three–dimensional changes in protein structure due to amino–acid substitutions: the variable region of immunoglobulins" *Protein: Structure, Function, and Genetics,* Alan R. Liss, Inc. vol. 1:267–279 (1986).

Sox et al., "Attachment of carbohydrate to the variable region of myeloma immunoglubulin light chains" *Proc. Natl. Acad. Sci. USA* 66:975–82 (Jul. 1970).

Samsung Bioepis Exhibit No. 1002
Page 7189

Spiegelberg et al., "Localization of the carbohydrate within the variable region of light and heavy chains of human γG myeloma proteins" *Biochemistry* 9:4217–23 (Oct. 1970).
Takeda et al., "Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences" *Nature* 314(6010):452–454 (Apr. 1985).
Tao et al., "Role of Carbohydrate in the Structure and Effector Functions Mediated by the H uman IgG Constant Region" *J. Immunol.* 143(8):2595–2601 (1989).
Tramontano et al., "Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins" *J–Mol–Biol* 215(1):175–182 (Sep. 5, 1990).
Verhoeyen, M. et al., "Reshaping human antibodies: grafting an antilysozyme activity" *Science* 239(4847):1534–1536 (Mar. 25, 1988).
Waldmann, T., "Monoclonal antibodies in diagnosis and therapy" *Science* 252:1657–1662 (1991).
Wallick et al., "Glycosylation of a VH residue of a monoclonal antibody against alpha (1——6) dextran increases its affinity for antigen" *Journal of Experimental Medicine* 168(3):1099–1109 (Sep. 1988).
Winter and Milstein, "Man–made antibodies" *Nature* 349(6307):293–299 (Jan. 24, 1991).
Yamamoto et al., "Similarity of protein encoded by the human c–erb–B–2 gene to epidermal growth factor receptor" *Nature* 319:230–34 (1986).
Carter et al., "High level *escherichia coli* expression and production of a bivalent humanized antibody fragment" *Bio/Technology* 10:163–167 (1992).
Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" *J. Mol. Biol.* 224:487–499 (1992).
Foote, J., "Humanized Antibodies" *Nova acta Leopoldina* 61(269):103–110 (1989).
Kabat et al., "Sequences of Proteins of Immunological Interest", Bethesda, MD:National Institute of Health pp. 14–32 (1983).
Kettleborough et al., "Humanization of a Mouse Monoclonal Antibody by CDR–grafting: the Importance of Framework Residues on Loop Conformation" *Protein Engineering* 4(7):773–783 (1991).
Maeda et al., "Construction of Reshaped Human Antibodies with HIV–neutralizing Activity" *Hum. Antibod. Hybridomas* 2:124–134 (Jul. 1991).
Riechmann et al, "Expression of an Antibody Fv Fragment in Myeloma Cells" *J. Mol. Biol.* 203:825–828 (1988).
Routledge et al., "A Humanized Monovalent CD3 Antibody which Can Activate Homologous Complement" *European Journal of Immunology* 21:2717–2725 (1991).
Shearman et al., "Construction, Expression and Characterization of Humanized Antibodies Directed Against the Human α/β T Cell Receptor" *J. Immunol.* 147(12):4366–4373 (Dec. 15, 1991).
Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" *Bio/Technology* 9:266–271 (Mar. 1991).
Brown, Jr. et al., "Anti–Tac–H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival" *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991).
Casale et al., "Use of an anti–IgE humanized monoclonal antibody in ragweed–induced allergic rhinitis" *J. Allergy Clin. Immunol.* 100:110–121 (1997).
Fahy et al., "The Effect of an Anti–IgE Monoclonal Antibody on the Early– and Late–Phase Responses to Allergen Inhalation in Asthmatic Subjects" *Am J. Respir. Crit. Care Med* 155:1828–1834 (1997).
Mathieson et al., "Monoclonal–Antibody Therapy in Systemic Vasculitis" *New England J. of Medicine* pp. 250–254 (Jul. 1990).
Presta et al., "Humanization of an anti–vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders" *Cancer Research* 57(20):4593–4599 (Oct. 15, 1997).
Amit et al., "Three–Dimensional Structure of an Antigen–Antibody Complex at 2.8 A Resolution" *Science* 233:747–753 (Aug. 1986).
Amzel et al., "The Three Dimensional Structure of a Combining Region–Ligand Complex of Immunglobulin New at 3.5–A Resolution" *Proc. Natl. Acad. Sci. USA* 71(4):1427–1430 (Apr. 1974).
Baselga et al., "Phase II Study of Weekly Intravenous Recombinant Humanized Anti–p185/HER2 Monoclonal Antibody in Patients With HER2/neu–Overexpressing Metastatic Breast Cancer" *J. Clin. Oncol.* 14(3):737–744 (1996).
Beverley & Callard, "Distinctive functional charcteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti–T cell antibody" *European Journal of Immunology* 11:329–334 (1981).
Bird et al., "Single–chain antigen–binding proteins" *Science* 242:423–426 (Oct. 1988).
Brennan et al., "Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G_1 fragments" *Science* 229:81–83 (Jul. 1985).
Bruccoleri et al., "Structure of antibody hypervariable loops reproduced by a conformational search algorithm" *Nature* 335:564–568 (Oct. 1988).
Caron et al., "Biological and Immunological Features of Humanized M195 (Anti–CD33) Monoclonal Antibodies" *Cancer Research* 52:6761–6767 (Dec. 1992).
Chothia & Lesk, "The relation between the divergence of sequence and structure in proteins" *EMBO Journal* 5(4):823–826 (1986).
Co & Queen, "Humanized antibodies for therapy" *Nature* 351:501–502 (Jun. 1991).
Co et al., "Chimeric and Humanized Antibodies with Specificity for the CD33 Antigen" *J. of Immunology* 148(4):1149–1154 (Feb. 1992).
Co et al., "Humanized Anti–Lewis Y Antibodies: In Vitro Properties and Pharmacokinetics in Rhesus Monkeys" *Cancer Research* 56:1118–1125 (Mar. 1996).
Colman et al., "Crystal and Molecular Structure of the Dimer of Variable Domains of the Bence–Jones Protein ROY" *J. Mol. Biol.* 116:73–79 (1977).
Colman et al., "Three–dimensional structure of a complex of antibody with influenza virus neuraminidase" *Nature* 326:358–363 (Mar. 1987).
Cook et al., "A map of the human immunoglbulin V_H locus completed by analysis of the telometric region of chromosome 14q" *Nature Genetics* 7:162–168 (Jun. 1994).
Darsley & Rees, "Nucleotide sequences of five anti–lysozyme monoclonal antibodies" *EMBO Journal* 4(2):393–398 (1985).
Davies & Metzger, "Structural Basis of Antibody Function" *Ann. Rev. Immunol.* 1:87–117 (1983).

Samsung Bioepis Exhibit No. 1002
Page 7190

Davies et al., "Antibody–Antigen Complexes" *Journal of Biological Chemistry* 263(22):10541–10544 (Aug. 1988).

Eigenbrot et al., "X–Ray Structures of Fragments From Binding and Nonbinding Versions of a Humanized Anti–CD18 Antibody: Structural Indications of the Key Role of V_H Residues 59 to 65" *Proteins* 18:49–62 (1994).

Eigenbrot et al., "X–ray structures of the antigen–binding domains from three variants of humanized anti–p185HER2 antibody 4D5 and comparison with molecular modeling" *J. Mol. Biol.* 229:969–995 (1993).

Ellison et al., "The nucleotide sequence of a human immunoglobulin $C_{\gamma 1}$ gene" *Nucleic Acids Research* 10(13):4071–4079 (1982).

Emery & Adair, "Humanised monoclonal antibodies for therapeutic applications" *Exp. Opin. Invest. Drugs* 3(3):241–251 (1994).

Epp et al., "Crystal and Molecular Structure of a Dimer Composed of the Variable Portions of the Bence–Jones Protein REI" *European Journal of Biochemistry* 45:513–524 (1974).

Fanger et al., "Bispecific antibodies and targeted cellular cytotoxicity" *Immunology Today* 12(2):51–54 (1991).

Fanger et al., "Cytotoxicity mediated by human Fc receptors for IgG" *Immunology Today* 10(3):92–99 (1989).

Feldmann et al., "A Hypothetical Space–Filling Model of the V–Regions of the Galactan–Binding Myeloma Immunoglobulin J539" *Molecular Immunology* 18(8):683–698 (1981).

Fendley et al., "The Extracellular Domain of HER2/neu Is a Potential Immunogen for Active Specific Immunotherapy of Breast Cancer" *J. Biol. Resp. Mod.* 9:449–455 (1990).

Glennie et al., "Preparation and Performance of Bispecific $F(ab'\gamma)_2$ Antibody Containing Thioether–Linked $Fab'\gamma$ Fragments" *J. Immunol.* 139(7):2367–2375 (Oct. 1, 1987).

Gonzalez et al., "Humanization of Murine 6G425:An Anti–IL8 Monoclonal Antibody Which Blocks Binding of IL8 to Human Neutrophils" *1996 Keystone Symposia on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites* (Poster) pp. 1–21 (Feb. 1996).

Gussow & Seemann, "Humanization of Monoclonal Antibodies" *Meth. Enzymology,* Academic Press, Inc. vol. 203:99–121 (1991).

Hieter et al., "Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments" *Cell* 22 (Part 1):197–207 (1980).

Houghton, A., "Building a better monoclonal antibody" *Immunology Today* 9(9):265–267 (1988).

Huston et al., "Protein engineering of antibody binding sites: Recovery of specific activity in an anti–digoxin single–chain Fv analogue produced in *Escherichia coli*" *Proc. Natl. Acad. Sci. USA* 85:5879–5883 (Aug. 1988).

Isaacs et al., "Humanised Monoclonal Antibody Therapy for Rheumatoid Arthritis" *Lancet* 340:748–752 (Sep. 26, 1992).

Johnson et al., "Biological and Molecular Modeling Studies Comparing Murine Monoclonal Antibodies with Their Engineered Chimeric and Humanized Counterparts" *J. Cell. Biochem. Suppl 0 (13 Part A)* (18th Ann. UCLA Symp on Mol. & Cell. Biol., Park City, UT Jan. 17–22, 1989) pp. 87 (1989).

Kabat E., "Origins of Antibody Complementarity and Specificity—Hypervariable Regions and the Minigenen Hypothesis" *J. of Immunology* 125(3):961–969 (Sep. 1980).

Kabat et al. *Sequences of Proteins of Immunological Interest,* U.S. Dept. of Health and Human Services, NIH, 5th edition vol. 1:103–108, 324–331 (1991).

Kindt & Capra *The Antibody Enigma,* New York:Plenum Press pp. 79–86 (1984).

Lesk & Chothia, "Evolution of Proteins Formed by β–Sheets" *J. Mol. Biol.* 160:325–342 (1982).

Lesk & Chothia, "The response of protein structures to amino–acid sequence changes" *Phil. Trans. R. Soc. Lond.* A 317:345–356 (1986).

Mariuzza et al., "The Structure Basis of Antigen–Antibody Recognition" *Ann. Rev. Biophys. Biophys. Chem.* 16:139–159 (1987).

Nadler et al., "Immunogenicity of Humanized and Human Monoclonal Antibodies" *Clin. Pharmacology & Therapeutics* pp. 180 (Feb. 1994).

Nelson, H., "Targeted Cellular Immunotherapy with Bifunctional Antibodies" *Cancer Cells* 3:163–172 (1991).

Neuberger et al., "Antibody Engineering" *Proceedings 8th Intl. Biotech. Symp.,* Paris II:792–799 (1988).

Newmark, P., "Making Chimeric Antibodies Even More Human" *Bio/Technology* 6:468 (May 1988).

Nishimura et al., "Human c–erbB–2 Proto–Oncogene Product as a Target for Bispecific–Antibody–Directed Adoptive Tumor Immunotherapy" *Int. J. Cancer* 50:800–804 (1992).

Nitta et al., "Preliminary trial of specific targeting therapy against malignant glioma" *Lancet* 335(8686):368–371 (Feb. 17, 1990).

Nitta, T. et al., "Bispecific $F(ab')_2$ monomer prepared with anti–CD3 and anti–tumor monoclonal antibodies is most potent in induction of cytolysis of human T cells" *European Journal of Immunology* 19:1437–1441 (1989).

Nolan et al., "Bifunctional antibodies: concept, production and applications" *Biochimica et Biophysica Acta* 1040:1–11 (1990).

O'Connor et al., "Calcium Dependence of an Anti–Protein C Humanized Antibody Involves Framework Residues" (manuscript).

Orlandi et al., "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction" *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (May 1989).

Orlandi et al., "Cloning of cDNA Corresponding to Heavy and Light Chain Immunoglobulin Variable Domains" *Protein and Pharmaceutical Engineering* pp. 90 (1989).

Ostberg & Queen, "Human and humanized monoclonal antibodies: preclinical studies and clinical experience" *Biochem. Soc. Transactions* pp. 1038–1043 (1995).

Pedlan et al., "Model–building Studies of Antigen–binding Sites:The Hapten–binding Site of MOPC–315" *Cold Springs Harbor Symposia On Quantitative Biology* XLI:627–637 (1977).

Padlan, E., "Anatomy of the Antibody Molecule" *Molecular Immunology* 31(3):169–217 (1994).

Padlan, E., "Evaluation of the Structural Variation Among Light Chain Variable Domains" *Molecular Immunology* 16:287–296 (1979).

Palm & Hilschmann, "Primary structure of a crystalline monoclonal immunoglobulin K–type L–chain, subgroup I (Bence–Jones preotin Rei); isolation & characterization of the tryptic peptides: . . . " *Hoppes–Seyler's Z. Physiol. Chem.* 356:167–191 (Feb. 1975).

Samsung Bioepis Exhibit No. 1002
Page 7191

Palm & Hilschmann, "The primary structure of a crystalline, monoclonal immunoglobulin–L–chain of the x–type, subgroup I (Bence–Jones Protein Rei): a contribution to the elucidation of the three–dimensional structure of the immunoglobulins" *Hoppe–Seyler's Z. Physiol. Chem.* 354:1651–1654 (Dec. 1973).

Panka et al., "Variable region framework differences result in decreased or increased affinity of variant anti–digoxin antibodies" *Proc. Natl. Acad. Sci. USA* 85:3080–3084 (May 1988).

Presta et al., "Humanization of an Antibody Directed Against IgE" *J. Immunol.* 151(5):2623–2632 (Sep. 1, 1993).

Preval & Fougereau, "Specific Interaction between V_H and V_L Regions of Human Monoclonal Immunoglobulins" *J. Mol. Biol.* 102:657–678 (1976).

Queen et al., "Construction of Humanized Antibodies and Testing in Primates" *J. Cell. Biochem. Suppl. 15 (Part E)* (20th Ann. Mtg. Keystone Symp. Denver, CO Mar. 10–16, 1991) pp. 137 (1991).

Queen et al., "Humanised antibodies to the IL–2 receptor" *Protein Eng. Antibody Mol. Prophyl. Ther. Appl. Man,* Clark, M., Nottingham, UK:Academic Titles pp. 159–170 (1993).

Rhodes & Birch, "Large–Scale Production of Proteins from Mammalian Cells" *Bio/Technology* 6:518, 521, 523 (May 1988).

Riechmann, "Humanizing of Recombinant Antibodies" (Intl. Symp. on Clin. Appl. of Monoclonal Antibodies, Guildford, England) pp. 33–34 (Sep. 1987).

Riechmann & Winter, "Recombinant Antibodies" (U. of London Royal Postgraduate Medical School, Wolfson Institute, Abstract) (May 1987).

Riechmann et al. *Alignment of VL Sequences* (1988).

Roberts & Rees, "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering" *Nature* 328:731–734 (Aug. 1987).

Rostapshov et al., "Effective method for obtaining long nucleotide chains on partially complementary templates" *FEBS Letters* 249(2):379–382 (Jun. 1989).

Schneider et al., "The Anti–Idiotypic Response by Cynomolgus Mondkeys to Humanized Anti–Tac Is Primarily Directed to Complementarity–Determining Regions H1, H2, and L3" *J. of Immunology* 150:3086–3090 (Apr. 1993).

Sedlacek et al., "Monoclonal Antibodies in Tumor Therapy", Karger pp. 119–126, 133–179 (1988).

Shields et al., "Inhibition of Allergic Reactions with Antibodies to IgE" *International Archives of Allergy and Immunology* 107(1–3):308–312 (May 1995).

Sims et al., "A Humanized CD18 Antibody Can Block Function Without Cell Destruction" *The Journal of Immunology* 151(4):2296–2308 (Aug. 1993).

Smith–Gill et al., "A Three–dimensional Model of an Anti–lysozyme Antibody" *Mol. Biol.* 194:713–724 (1987).

Songsivilai et al., "Bispecific antibody: a tool for diagnosis and treatment of disease" *Clin. Exp. Immunol.* 79:315–321 (1990).

Stanford, "A Predictive Method for Determining Possible Three–dimensional Foldings of Immunoglobulin Backbones Around Antibody Combining Sites" *Theor. Biol.* 88:421–439 (1981).

Stickney et al., "Bifunctional Antibody: ZCE/CHA ^{III}Indium BLEDTA–IV Clinical Imaging in Colorectal Carcinoma" *Antibody, Immuno Radiopharm* 2:1–13 (1989).

Tighe et al., "Delayed Allograft Rejection in Primates Treated with Anti–IL–2 Receptor Monoclonal antibody Campath–6" *Transplantation* 45(1):226–228 (Jan. 1988).

Verhoeyen & Riechmann, "Engineering of Antibodies" *BioEssays* 8(2):74–78 (Feb./Mar. 1988).

Verhoeyen et al., "Grafting Hypervariable Regions in Antibodies" *Protein Structure, Folding, and Design 2* (Proc. DuPont–UCLA Symp. Streamboat Springs, CO, Apr. 4–11, 1987), Dale L. Oxender, New York:Alan R. Liss, Inc. pp. 501–502 (1987).

Verhoeyen et al., "Humanising Mouse Antibodies: A Protein Engineering Approach" *Soc. for Analytical Cytology* (XIIth Intl. Mtg. for the Soc. for Analytical Cytology, Cambridge, UK) pp. 22 and slide presented at mtg.

Verhoeyen et al., "Re–shaped human anti–PLAP antibodies" *Monoclonal Antibodies Applications in clinical oncology,* Epenetos, 1st edition, Chapman & Hall Medical pp. 37–43 (1991).

Ward et al., "Expression and Secretion of Repertoires of VH Domains in *Escherichia coli:* Isolation of Antigen Binding Activites" *Progress in Immunology* (7th Intl. Congress Immunol. Berlin, W. Germany), F. Melchers vol. VII:1144–1151 (1989).

Ward, E.S. et al., "Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli" Nature* 341:544–546 (1989).

Werther et al., "Humanization of an Anti–Lymphocyte Function–Associated Antigen (LFA)–1 Monoclonal Antibody and Reengineering of the Humanized Antibody for Binding to Rhesus LFA–1" *J. of Immunology* pp. 4986–4995 (1996).

Whittle et al., "Construction and Expression of A CDR–Grafted Anti–TNF Antibody" *J. Cell Biochem. Suppl. 0* (Symp. on Protein and Pharm. Eng. Mol. and Cell. Biol. Park City, Utah)13 Part A:96 (1989).

Winter & Neuberger, "Restructuring Enzymes and Antibodies" *Investigation and Exploitation of Antibody Combining Sites,* Eric Reid, Plenum Press pp. 139–140 (1985).

Winter et al., "Protein Engineering by Site Directed Mutagenesis" *Chemical Synthesis in Molecular Biology,* H. Blocker et al., VCH pp. 189–197 (1987).

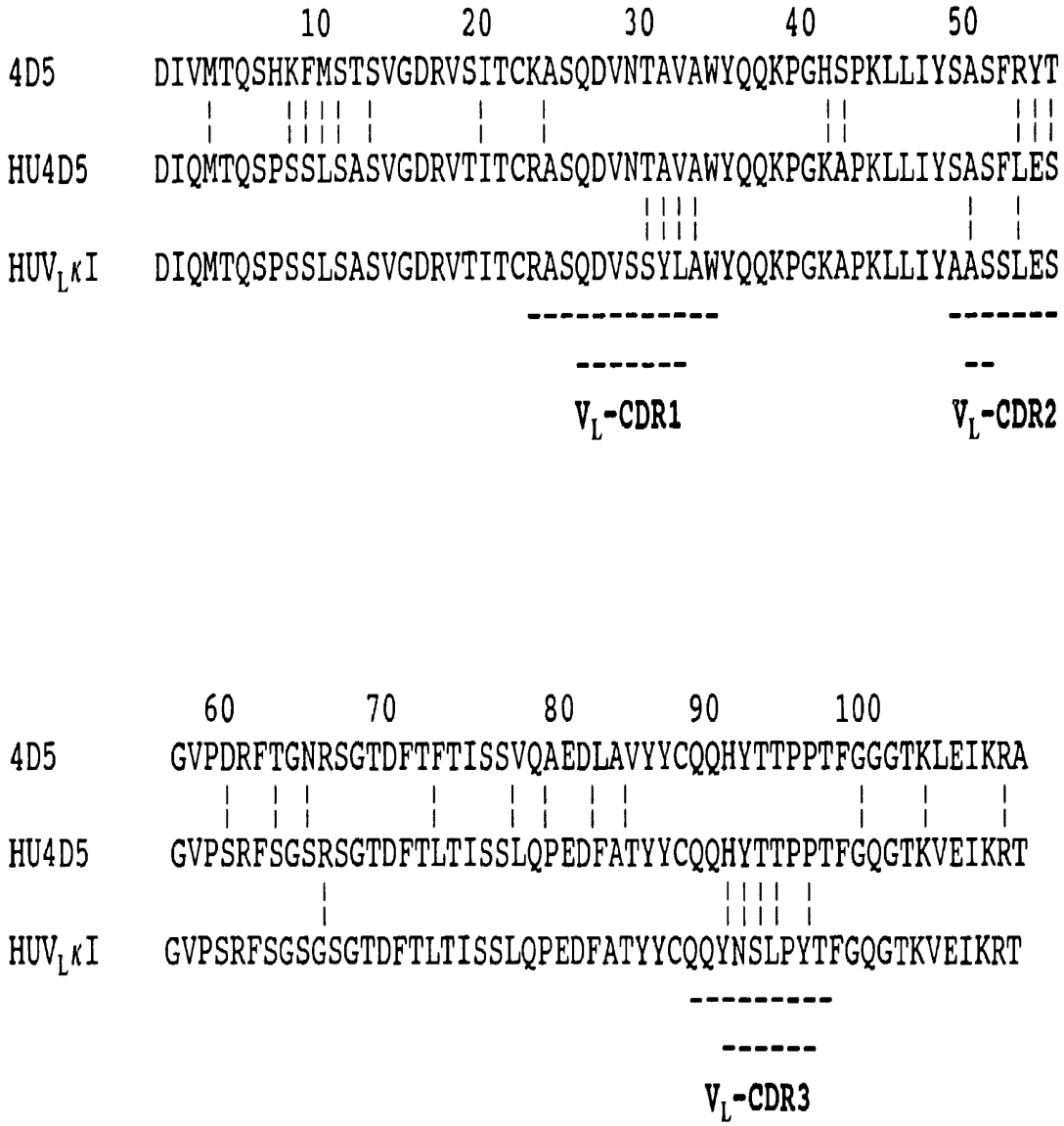
Winter G., "Antibody Engineering" *Phil. Trans. R. Soc. Lond. B* 324:99–109 (1989).

Woodle et al., "Humanized OKT3 Antibodies: Successful Transfer of Immune Modulating Properties and Idiotype Expression" *J. of Immunology* 148(9):2756–2763 (May 1992).

* cited by examiner

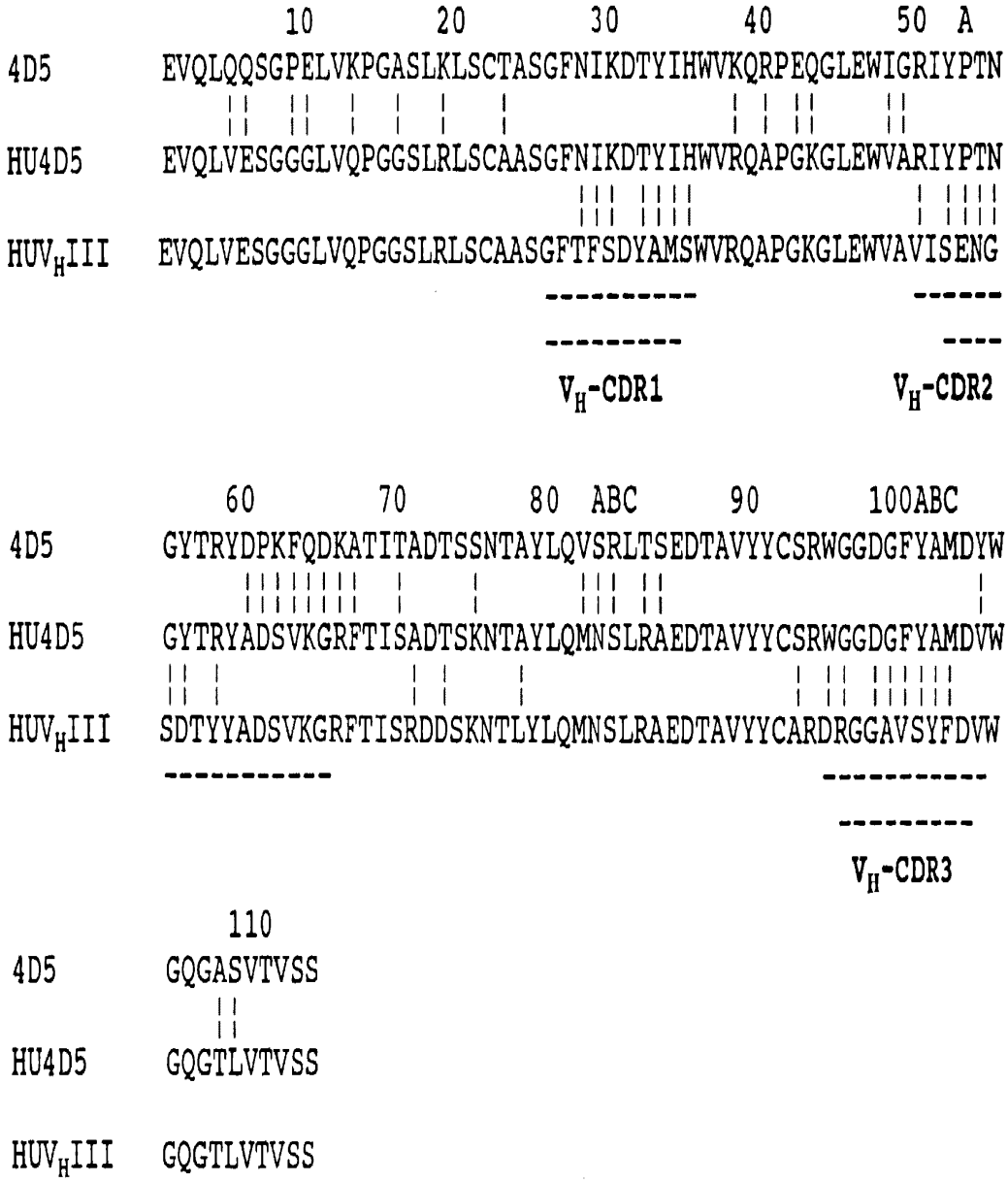
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FIG. 1A



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FIG. 1B



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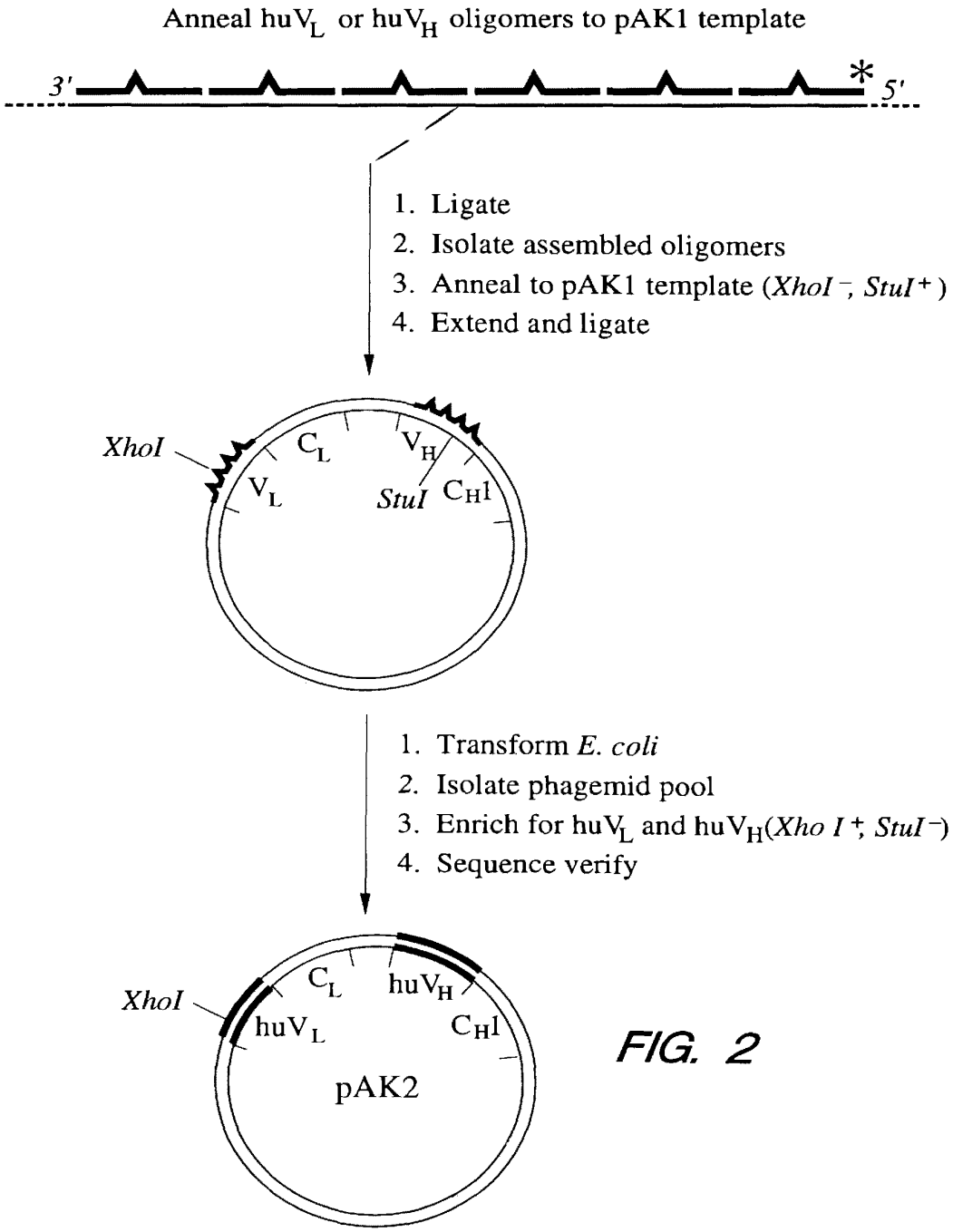


FIG. 2

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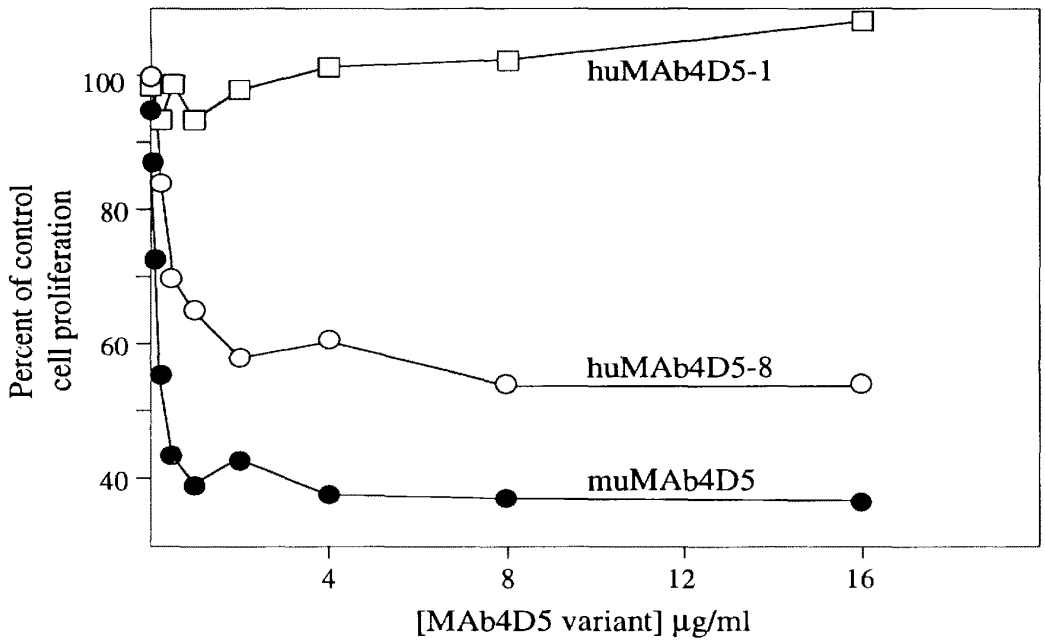


FIG. 3

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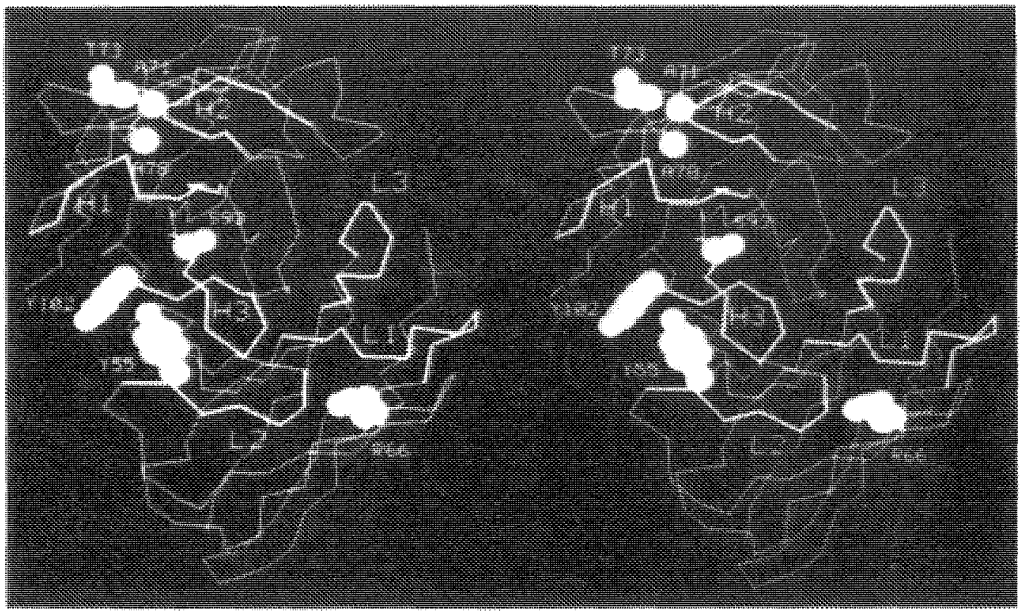


FIG. 4

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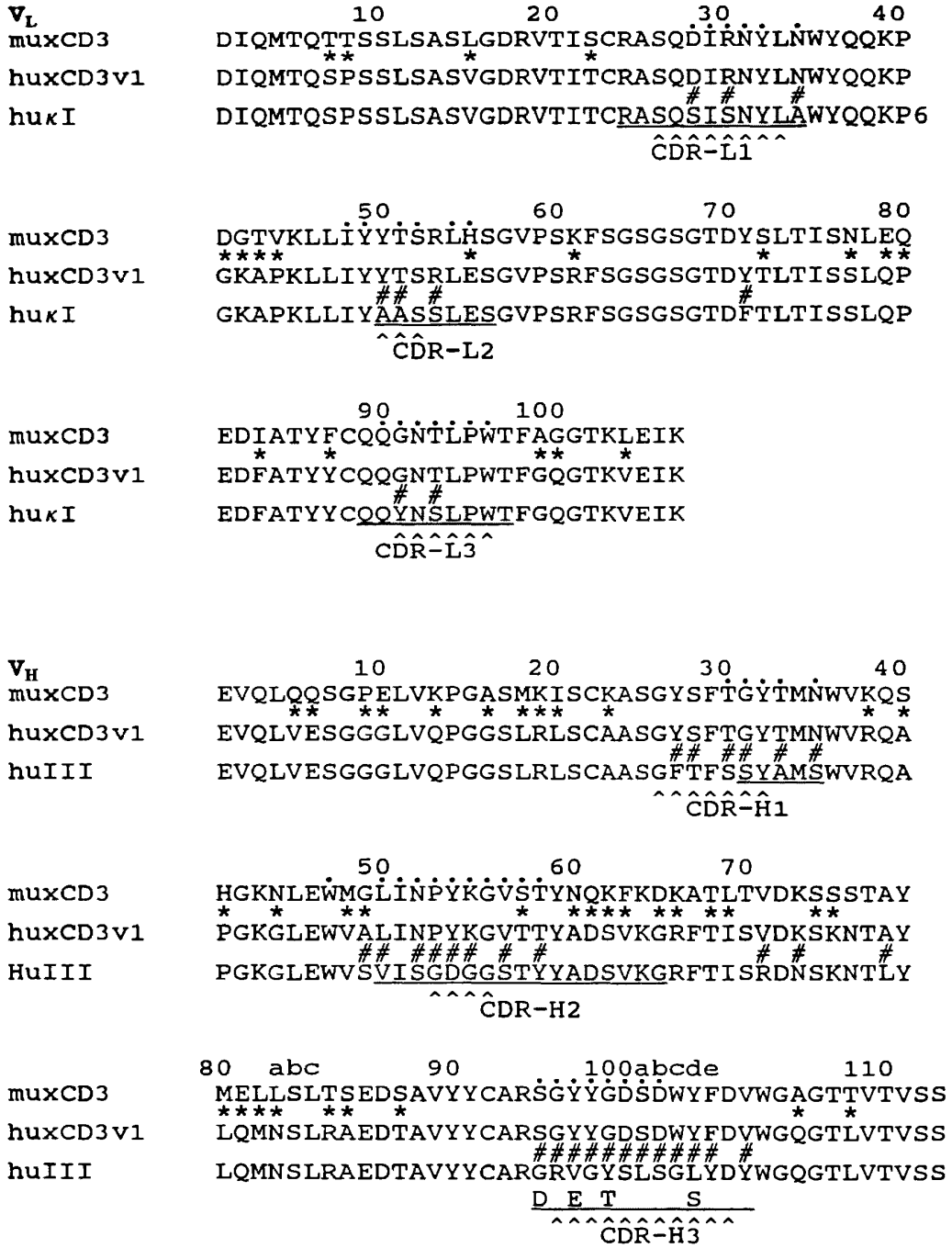


FIG. 5

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FIG. 6A-1

```
                                          10        20        30
H52H4-160                          QVQLQQSGPELVKPGASVKISCKTSGYTFTE
                                   .*** .**  **.**.*...** ********
pH52-8.0    MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTE
              10        20        30        40        50

                    40        50        60        70        80
H52H4-160   YTMHWMKQSHGKSLEWIGGFNPKNGGSSHNQRFMDKATLAVDKSTSTAYM
            ******.*. **.***..*.******.********. *..**********
pH52-8.0    YTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISVDKSTSTAYM
              60        70        80        90        100

                   90       100       110       120       130
H52H4-160   ELRSLTSEDSGIYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPS
            .. ** .**...********************** ** ************
pH52-8.0    QMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPS
                  110       120       130       140       150

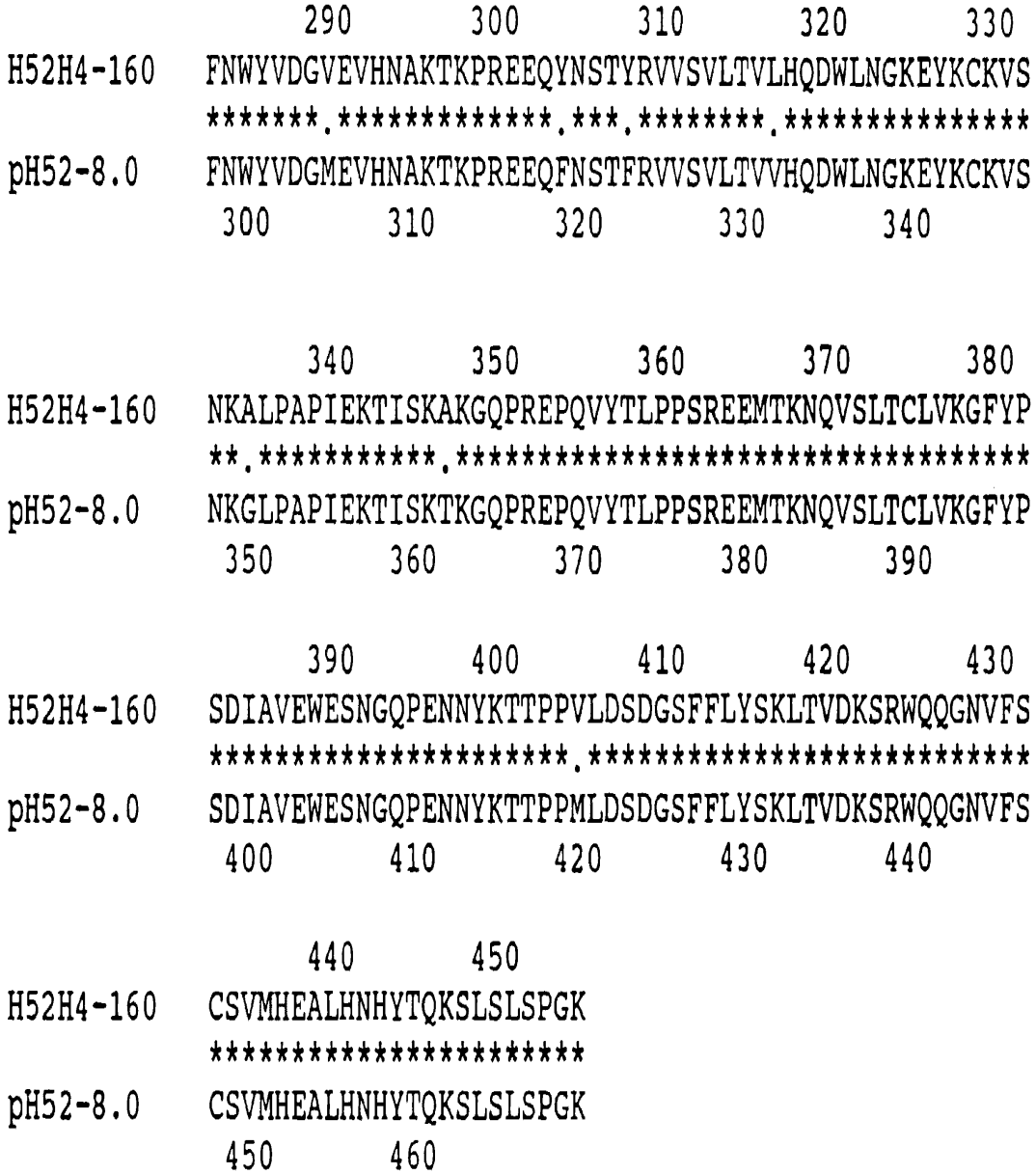
                  140       150       160       170       180
H52H4-160   VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
            ****** *.*** .**********************************
pH52-8.0    VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
                  160       170       180       190       200

                  190       200       210       220       230
H52H4-160   QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
            ************** **..***** ***.********** ** * *
pH52-8.0    QSSGLYSLSSVVTVTSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC---V
                  210       220       230       240

                  240       250       260       270       280
H52H4-160   TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
             *******  ..**********************************.
pH52-8.0    ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
            250       260       270       280       290
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FIG. 6A-2



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FIG. 6B

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                                        10        20        30
H52L6-158                       DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                *.****. ******.****** *********
pH52-9.0    MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                    10        20        30        40        50

                   40        50        60        70        80
H52L6-158   YLNWYQQKPNGTVKLLIYYTSTLHSGVPSRFSGSGSGTDYSLTISNLDQE
            ********* . **************************.****.*. *
pH52-9.0    YLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYTLTISSLQPE
                    60        70        80        90       100

                  90       100       110       120       130
H52L6-158   DIATYFCQQGNTLPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
            *.***.************ ******************************
pH52-9.0    DFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
                    110       120       130       140       150

                  140       150       160       170       180
H52L6-158   VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
            ************************************************
pH52-9.0    VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
                    160       170       180       190       200

                  190       200       210
H52L6-158    SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
             ********************************
pH52-9.0     SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
                    210       220       230
```


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METHOD FOR MAKING HUMANIZED ANTIBODIES

CROSS REFERENCES

This application is a continuation-in-part of U.S. application Ser. No. 07/715,272 filed Jun. 14, 1991 (abandoned) which application is incorporated herein by reference and to which application priority is claimed under 35 USC §120.

FIELD OF THE INVENTION

This invention relates to methods for the preparation and use of variant antibodies and finds application particularly in the fields of immunology and cancer diagnosis and therapy.

BACKGROUND OF THE INVENTION

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end, the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia et al., *J. Mol. Biol.* 186:651–663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592–4596 (1985).

The constant domains are not involved directly in binding the antibody to an antigen, but are involved in various effector functions, such as participation of the antibody in antibody-dependent cellular cytotoxicity. The variable domains of each pair of light and heavy chains are involved directly in binding the antibody to the antigen. The domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. et a., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda, Md., (1987)). The four framework regions largely adopt a β-sheet conformation and the CORs form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site.

Widespread use has been made of monoclonal antibodies, particularly those derived from rodents including mice, however they are frequently antigenic in human clinical use. For example, a major limitation in the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller, R. A. et al., *Blood* 62:988–995 (1983); Schroff, R. W. et al., *Cancer Res.* 45:879–885 (1985)).

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (Cabilly et al., U.S. Pat. No. 4,816,567; Morrison, S. L. et al., *Proc. Natl. Acad. Sci. USA* 81:6851–6855 (1984); Boulianne, G. L. et al., *Nature* 312:643–646 (1984); Neuberger, M. S. et al., *Nature* 314:268–270 (1985)). The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

The isotype of the human constant domain may be selected to tailor the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (see e.g. Brüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987); Riechmann, L. et al., *Nature* 332:323–327 (1988); Love et al., *Methods in Enzymology* 178:515–527 (1989); Bindon et al., *J. Exp. Med.* 168:127–142 (1988).

In the typical embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody, OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers, G. J. et a., *Transplantation* 41:572–578 (1986)).

In a further effort to resolve the antigen binding functions of antibodies and to minimize the use of heterologous sequences in human antibodies, Winter and colleagues (Jones, P. T. et al., *Nature* 321:522–525 (1986); Riechmann, L. et al., *Nature* 332:323–327 (1988); Verhoeyen, M. et al., *Science* 239:1534–1536 (1988)) have substituted rodent CDRs or CDR sequences for the corresponding segments of a human antibody. As used herein, the term "humanized" antibody is an embodiment of chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. et al., *Nature* 332:323–327 (1988); Hale, G. et al., *Lancet i:*1394–1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co et al., *Proc. Natl. Acad. Sci. USA* 88:2869–2873 (1991); Gorman et al., *Proc. Natl. Acad. Sci. USA* 88:4181–4185 (1991); Daugherty et al., *Nucleic Acids Research* 19(9):2471–2476 (1991); Brown et al., *Proc. Natl. Acad. Sci. USA* 88:2663–2667 (1991); Junghans et al., *Cancer Research* 50:1495–1502 (1990).

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones, P. T. et al., *Nature* 321:522–525 (1986); Verhoeyen, M. et al., *Science* 239:1534–1536 (1988)), whereas in other cases it has been necessary to additionally replace one (Riechmann, L. et al., *Nature* 332:323–327 (1988)) or several (Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)) framework region (FR) residues. See also Co et al., supra.

For a given antibody a small number of FR residues are anticipated to be important for antigen binding. Firstly for example, certain antibodies have been shown to contain a few FR residues which directly contact antigen in crystal structures of antibody-antigen complexes (e.g., reviewed in Davies, D. R. et al., *Ann. Rev. Biochem.* 59:439–473 (1990)).

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Secondly, a number of FR residues have been proposed by Chothia, Lesk and colleagues (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987); Chothia, C. et al., *Nature* 342:877–883 (1989); Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding. See also Margolies et al., *Proc. Natl. Acad. Sci. USA* 72:2180–2184 (1975).

It is also known that, in a few instances, an antibody variable domain (either V_H or V_L) may contain glycosylation sites, and that this glycosylation may improve or abolish antigen binding, Pluckthun, *Biotechnology* 9:545–51 (1991); Spiegelberg et al., *Biochemistry* 9:4217–4223 (1970); Wallic et al., *J. Exp. Med.* 168:1099–1109 (1988); Sox et al., *Proc. Natl. Acad. Sci. USA* 66:975–982 (1970); Margni et al., *Ann. Rev. Immunol* 6:535–554 (1988). Ordinarily, however, glycosylation has no influence on the antigen-binding properties of an antibody, Pluckthun, supra, (1991).

The three-dimensional structure of immunoglobulin chains has been studied, and crystal structures for intact immunoglobulins, for a variety of immunoglobulin fragments, and for antibody-antigen complexes have been published (see e.g., Saul et al., *Journal of Biological Chemistry* 25:585–97 (1978); Sheriff et al., *Proc. Natl. Acad. Sci. USA* 84:8075–79 (1987); Segal et al., *Proc. Natl. Acad. Sci. USA* 71:4298–4302 (1974); Epp et al., *Biochemistry* 14(22) :4943–4952 (1975); Marquart et al., *J. Mol. Biol.* 141:369–391 (1980); Furey et al., *J. Mol. Biol.* 167:661–692 (1983); Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267–279, Alan R. Liss, Inc. pubs. (1986); Chothia and Lesk, *J. Mol. Bol.* 196:901–917 (1987); Chothia et al., *Nature* 342:877–883 (1989); Chothia et al., *Science* 233:755–58 (1986); Huber et al., *Nature* 264:415–420 (1976); Bruccoleri et al., *Nature* 335:564–568 (1988) and *Nature* 336:266 (1988); Sherman et al., *Journal of Biological Chemistry* 263:4064–4074 (1988); Amzel and Poljak, *Ann. Rev. Biochem.* 48:961–67 (1979); Silverton et al., *Proc. Natl. Acad. Sci. USA* 74:5140–5144 (1977); and Gregory et al., *Molecular Immunology* 24:821–829 (1987). It is known that the function of an antibody is dependent on its three dimensional structure, and that amino acid substitutions can change the three-dimensional structure of an antibody, Snow and Amzel, supra. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. et al., *Nature* 332:323–327 (1988); Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)).

Humanizing an antibody with retention of high affinity for antigen and other desired biological activities is at present difficult to achieve using currently available procedures. Methods are needed for rationalizing the selection of sites for substitution in preparing such antibodies and thereby increasing the efficiency of antibody humanization.

The proto-oncogene HER2 (human epidermal growth factor receptor 2) encodes a protein tyrosine kinase ($p185^{HER2}$) that is related to and somewhat homologous to the human epidermal growth factor receptor (see Coussens, L. et al., *Science* 230:1132–1139 (1985); Yamamoto, T. et al., *Nature* 319:230–234 (1986); King, C. R. et al., *Science* 229:974–976 (1985)). HER2 is also known in the field as c-erbB-2, and sometimes by the name of the rat homolog, neu. Amplification and/or overexpression of HER2 is associated with multiple human malignancies and appears to be integrally involved in progression of 25–30% of human breast and ovarian cancers (Slamon, D. J. et al., *Science* 235:177–182 (1987), Slamon, D. J. et al., *Science* 244:707–712 (1989)). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (Slamon, supra, Science 1989).

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)), directed against the extracellular domain (ECD) of $p185^{HER2}$, specifically inhibits the growth of tumor cell lines overexpressing $p185^{HER2}$ in monolayer culture or in soft agar (Hudziak, R. M. et al., *Molec. Cell. Biol* 9:1165–1172 (1989); Lupu, R. et al., *Science* 249:1552–1555 (1990)). MuMAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor, an important effector molecule in macrophage-mediated tumor cell cytotoxicity (Hudziak, supra, 1989; Shepard, H. M. and Lewis, G. D. *J. Clinical Immunology* 8:333–395 (1988)). Thus muMAb4D5 has potential for clinical intervention in and imaging of carcinomas in which $p185^{HER2}$ is overexpressed. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published Jul. 27, 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. However, this antibody may be immunogenic in humans.

It is therefore an object of this invention to provide methods for the preparation of antibodies which are less antigenic in humans than non-human antibodies but have desired antigen binding and other characteristics and activities.

It is a further object of this invention to provide methods for the efficient humanization is of antibodies, i.e. selecting non-human amino acid residues for importation into a human antibody background sequence in such a fashion as to retain or improve the affinity of the non-human donor antibody for a given antigen.

It is another object of this invention to provide humanized antibodies capable of binding $p185^{HER2}$.

Other objects, features, and characteristics of the present invention will become apparent upon consideration of the following description and the appended claims.

SUMMARY OF THE INVENTION

The objects of this invention are accomplished by a method for making a humanized antibody comprising amino acid sequence of an import, non-human antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

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g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, the method of this invention comprises the additional steps of determining if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), retaining the consensus residue.

Additionally, in certain embodiments the method of this invention comprises the feature wherein the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71 L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)).

In certain embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another embodiment of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

Certain alternate embodiments of the methods of this invention comprise obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 78H, 91H, 92H, 93H, and 103H.

In preferred embodiments, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody.

Optionally, this just-recited embodiment comprises the additional steps of following the method steps appearing at the beginning of this summary and determining whether a particular amino acid residue can reasonably be expected to have undesirable effects.

This invention also relates to a humanized antibody comprising the CDR sequence of an import, non-human antibody and the FR sequence of a human antibody, wherein an amino acid residue within the human FR sequence located at any one of the sites 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H has been substituted by another residue. In preferred embodiments, the residue substituted at the human FR site is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained. In other embodiments, no human FR residue other than those set forth in this group has been substituted.

This invention also encompasses specific humanized antibody variable domains, and isolated polypeptides having homology with the following sequences.

1. SEQ. ID NO. 1, which is the light chain variable domain of a humanized version of muMAb4D5:
DIQMTQSPSSLSASVGDRVTITCRASQD-
VNTAVAWYQQKPGKAPKLLIYSASFLES-
GVPSRFSGSRSGTDFTLTISSLQPEDFA-
TYYCQQHYTTPPTFGQGTKVEIKRT

2. SEQ. ID NO. 2, which is the heavy chain variable domain of a humanized version of muMAb4D5):
EVQLVESGGGLVQPGGSLRLSCAASGFNIK
DTYIHWVRQAPGKGLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLQMNSLRAED
TAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

In another aspect, this invention provides a consensus antibody variable domain amino acid sequence for use in the preparation of humanized antibodies, methods for obtaining, using, and storing a computer representation of such a consensus sequence, and computers comprising the sequence data of such a sequence. In one embodiment, the following consensus antibody variable domain amino acid sequences are provided:

SEQ. ID NO. 3 (light chain): DDIQMTQSPSSLSAS-
VGDRVTITCRASQDVSSYLAWYQQKPGKAPKLL
IYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCQQYNSLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain): EVQLVESGGGLVQPG
GSLRLSCAASGFTFSDYAMSWVRQAPGKGL
EWVAVISENGGYTRYADSVKGRFTISADTSKNT
AYLQMNSLRAEDTAWYCSRWGGDGFYAMD
VWGQGTLVTVSS

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus sequence (FIG. 1A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIG. 1B shows the comparison between the V_H domain amino acid residues of the muMAb4D5, huMAb4D5, and a consensus sequence (FIG. 1B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both FIGS. 1A and 1B use the generally accepted numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987)). In both FIG. 1A

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and FIG. 1B, the CDR residues determined according to a standard sequence definition (as in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are indicated by the first underlining beneath the sequences, and the CDR residues determined according to a structural definition (as in Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)) are indicated by the second, lower underlines. The mismatches between genes are shown by the vertical lines.

FIG. 2 shows a scheme for humanization of muMAb4D5 V_L and V_H by gene conversion mutagenesis.

FIG. 3 shows the inhibition of SK-BR-3 proliferation by MAb4D5 variants. Relative cell proliferation was determined as described (Hudziak, R. M. et al., *Molec. Cell. Biol.* 9:1165–1172 (1989)) and data (average of triplicate determinations) are presented as a percentage of results with untreated cultures for muMAb4D5 (●), huMAb4D5-8 (○) and huMAb4D5-1 (□).

FIG. 4 shows a stereo view of α-carbon tracing for a model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 3) are shown.

FIG. 5 shows an amino acid sequence comparison of V_L (top panel) and V_H (lower panel) domains of the murine anti-CD3 monoclonal Ab UCHT1 (muxCD3, Shalaby et al., *J. Exp. Med.* 175, 217–225 (1992) with a humanized variant of this antibody (huxCD3v1). Also shown are consensus sequences (most commonly occurring residue or pair of residues) of the most abundant human subgroups, namely V_L κ 1 and V_H III upon which the humanized sequences are based (Kabat, E. A. et al., *Sequences of Proteins of immunological Interest,* 5th edition, National Institutes of Health, Bethesda, Md., USA (1991)). The light chain sequences—muxCD3, huxCD3v1 and huKI—correspond to SEQ.ID. NOs 16, 17, and 18, respectively. The heavy chain sequences—muxCD3, huxCD3v1 and huxI—correspond to SEQ.ID.NOs 19, 26, and 21, respectively. Residues which differ between muxCD3 and huxCD3v1 are identified by an asterisk (*), whereas those which differ between humanized and consensus sequences are identified by a sharp sign (#). A bullet (•) denotes that a residue at this position has been found to contact antigen in one or more crystallographic structures of antibody/antigen complexes (Kabat et al., 1991; Mian, I. S. et al., *J. Mol. Biol* 217, 133–151 (1991)). The location of CDR residues according to a sequence definition (Kabat et al., 1991) and a structural definition (Chothia and Lesk, supra 1987) are shown by a line and carats (^) beneath the sequences, respectively.

FIG. 6A compares murine and humanized amino acid sequences for the heavy chain of an anti-CD18 antibody. H52H4-160 (SEQ. ID. NO. 22) is the murine sequence, and pH52–8.0 (SEQ. ID. NO. 23) is the humanized heavy chain sequence. pH52–8.0 residue 143S is the final amino acid in the variable heavy chain domain V_H, and residue 144A is the first amino acid in the constant heavy chain domain C_{H1}.

FIG. 6B compares murine and humanized amino acid sequences for the light chain of an anti-CD18 antibody. H52L6-158 (SEQ. ID. NO. 24) is the murine sequence, and pH52–9.0 (SEQ. ID. NO. 25) is the humanized light chain sequence. pH52–9.0 residue 128T is the final amino acid in the light chain variable domain V_L, and residue 129V is the first amino acid in the light chain constant domain C_L.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

In general, the following words or phrases have the indicated definitions when used in the description, examples, and claims:

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 and its uses are described in PCT application WO 89/06692 published Jul. 27, 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. In this description and claims, the terms muMAb4D5, chMAb4D5 and huMAb4D5 represent murine, chimerized and humanized versions of the monoclonal antibody 4D5, respectively.

A humanized antibody for the purposes herein is an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a FR region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are referred to herein as "import" residues, which are typically taken from an "import" antibody domain, particularly a variable domain. An import residue, sequence, or antibody has a desired affinity and/or specificity, or other desirable antibody biological activity as discussed herein.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and lgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%.

In general, humanized antibodies prepared by the method of this invention are produced by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those

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skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen.

Residues that influence antigen binding are defined to be residues that are substantially responsible for the antigen affinity or antigen specificity of a candidate immunoglobulin, in a positive or a negative sense. The invention is directed to the selection and combination of FR residues from the consensus and import sequence so that the desired immunoglobulin characteristic is achieved. Such desired characteristics include increases in affinity and greater specificity for the target antigen, although it is conceivable that in some circumstances the opposite effects might be desired. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (although not all CDR residues are so involved and therefore need not be substituted into the consensus sequence). However, FR residues also have a significant effect and can exert their influence in at least three ways: They may noncovalently directly bind to antigen, they may interact with CDR residues and they may affect the interface between the heavy and light chains.

A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which have atoms which are separated from antigen spatially by 3.2 Angstroms or less may also non-covalently interact with antigen. Such residues typically are the relatively larger amino acids having the side chains with the greatest bulk, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

A residue that interacts with a CDR generally is a residue that either affects the conformation of the CDR polypeptide backbone or forms a noncovalent bond with a CDR residue side chain. Conformation-affecting residues ordinarily are those that change the spatial position of any CDR backbone atom (N, Ca, C, O, Cβ) by more than about 0.2 Angstroms. Backbone atoms of CDR sequences are displaced for example by residues that interrupt or modify organized structures such as beta sheets, helices or loops. Residues that can exert a profound affect on the conformation of neighboring sequences include proline and glycine, both of which are capable of introducing bends into the backbone. Other residues that can displace backbone atoms are those that are capable of participating in salt bridges and hydrogen bonds.

A residue that interacts with a CDR side chain is one that is reasonably expected to form a noncovalent bond with a CDR side chain, generally either a salt bridge or hydrogen bond. Such residues are identified by three dimensional positioning of their side chains. A salt or ion bridge could be expected to form between two side chains positioned within about 2.5–3.2 Angstroms of one another that bear opposite charges, for example a lysinyl and a glutamyl pairing. A hydrogen bond could be expected to form between the side chains of residue pairs such as seryl or threonyl with aspartyl or glutamyl (or other hydrogen accepting residues). Such pairings are well known in the protein chemistry art and will be apparent to the artisan upon three dimensional modeling of the candidate immunoglobulin.

Immunoglobulin residues that affect the interface between heavy and light chain variable regions ("the V_L–V_H interface") are those that affect the proximity or orientation of the two chains with respect to one another. Certain residues involved in interchain interactions are already known and include V_L residues 34, 36, 38, 44, 46, 87, 89, 91, 96, and 98 and V_H residues 35, 37, 39, 45, 47, 91, 93, 95, 100, and 103 (utilizing the nomenclature setforth in Kabat et al., *Sequences of Proteins of immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)). Additional residues are newly identified by the inventors herein, and include 43L, 85L, 43H and 60H. While these residues are indicated for IgG only, they are applicable across species. In the practice of this invention, import antibody residues that are reasonably expected to be involved in interchain interactions are selected for substitution into the consensus sequence. It is believed that heretofore no humanized antibody has been prepared with an intrachain-affecting residue selected from an import antibody sequence.

Since it is not entirely possible to predict in advance what the exact impact of a given substitution will be it may be necessary to make the substitution and assay the candidate antibody for the desired characteristic. These steps, however, are per se routine and well within the ordinary skill of the art.

CDR and FR residues are determined according to a standard sequence definition (Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda Md. (1987), and a structural definition (as in Chothia and Lesk, *J. Mol. Biol.* 196:901–917 (1987). Where these two methods result in slightly different identifications of a CDR, the structural definition is preferred, but the residues identified by the sequence definition method are considered important FR residues for determination of which framework residues to import into a consensus sequence.

Throughout this description, reference is made to the numbering scheme from Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987) and (1991). In these compendiums, Kabat lists many amino acid sequences for antibodies for each subclass, and lists the most commonly occurring amino acid for each residue position in that subclass. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. The Kabat numbering scheme is followed in this description.

For purposes of this invention, to assign residue numbers to a candidate antibody amino acid sequence which is not included in the Kabat compendium, one follows the following steps. Generally, the candidate sequence is aligned with any immunoglobulin sequence or any consensus sequence in Kabat. Alignment may be done by hand, or by computer using commonly accepted computer programs; an example of such a program is the Align 2 program discussed in this description. Alignment may be facilitated by using some amino acid residues which are common to most Fab

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sequences. For example, the light and heavy chains each typically have two cysteines which have the same residue numbers; in V_L domain the two cysteines are typically at residue numbers 23 and 88, and in the V_H domain the two cysteine residues are typically numbered 22 and 92. Framework residues generally, but not always, have approximately the same number of residues, however the CDRs will vary in size. For example, in the case of a CDR from a candidate sequence which is longer than the CDR in the sequence in Kabat to which it is aligned, typically suffixes are added to the residue number to indicate the insertion of additional residues (see, e.g. residues 100abcde in FIG. **5**). For candidate sequences which, for example, align with a Kabat sequence for residues 34 and 36 but have no residue between them to align with residue 35, the number 35 is simply not assigned to a residue.

Thus, in humanization of an import variable sequence, where one cuts out an entire human or consensus CDR and replaces it with an import CDR sequence, (a) the exact number of residues may be swapped, leaving the numbering the same, (b) fewer import amino acid residues may be introduced than are cut, in which case there will be a gap in the residue numbers, or (c) a larger number of amino acid residues may be introduced then were cut, in which case the numbering will involve the use of suffixes such as 100abcde.

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all immunoglobulins of any particular subclass or subunit structure. The consensus sequence may be based on immunoglobulins of a particular species or of many species. A "consensus" sequence, structure, or antibody is understood to encompass a consensus human sequence as described in certain embodiments of this invention, and to refer to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass or subunit structure. This invention provides consensus human structures and consensus structures which consider other species in addition to human.

The subunit structures of the live immunoglobulin classes in humans are as follows:

Class	Heavy Chain Subclasses		Light Chain	Molecular Formula
IgG	γ	$\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$	κ or λ	$(\gamma_2\kappa_2)$, $(\gamma_2\lambda_2)$
IgA	α	$\alpha 1$, $\alpha 2$	κ or λ	$(\alpha_2\kappa_2)_n^{8}$, $(\alpha_2\lambda_2)_n^{8}$
IgM	μ	none	κ or λ	$(\mu_2\kappa_2)_5$, $(\mu_2\lambda_2)_5$
IgD	δ	none	κ or λ	$(\delta_2\kappa_2)$, $(\delta_2\lambda_2)$
IgE	ϵ	none	κ or λ	$(\epsilon_2\kappa_2)$, $(\epsilon_2\lambda_2)$

($^{8}_{n}$ may equal 1, 2, or 3)

In preferred embodiments of an IgGγl human consensus sequence, the consensus variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda Md. (1987), namely V_L κ subgroup I and V_H group III. In such preferred embodiments, the V_L consensus domain has the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQD-VSSYLAWYQQKPGKAPKLLIYAASSLES-GVPSRFSGSGSGTDFTLTISSLQPEDFA-TYYCQQYNSLPYTFGQGTKVEIKRT (SEQ. ID NO. 3);

the V_H consensus domain has the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSW VRQAPGKGLEWVAVISENGGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 4).

These sequences include consensus CDRs as well as consensus FR residues (see for example in FIG. **1**).

While not wishing to be limited to any particular theories, it may be that these preferred embodiments are less likely to be immunogenic in an individual than less abundant subclasses. However, in other embodiments, the consensus sequence is derived from other subclasses of human immunoglobulin variable domains. In yet other embodiments, the consensus sequence is derived from human constant domains.

Identity or homology with respect to a specified amino acid sequence of this invention is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments. While such alignments may be done by hand using conventional methods, a suitable computer program is the "Align 2" program for which protection is being sought from the U.S. Register of Copyrights (Align 2, by Genentech, Inc., application filed Dec. 9, 1991).

"Non-homologous" import antibody residues are those residues which are not identical to the amino acid residue at the analogous or corresponding location in a consensus sequence, after the import and consensus sequences are aligned.

The term "computer representation" refers to information which is in a form that can be manipulated by a computer. The act of storing a computer representation refers to the act of placing the information in a form suitable for manipulation by a computer.

This invention is also directed to novel polypeptides, and in certain aspects, isolated novel humanized anti-p185^{HER2} antibodies are provided. These novel anti-p185^{HER2} antibodies are sometimes collectively referred to herein as huMAb4D5, and also sometimes as the light or heavy chain variable domains of huMAb4D5, and are defined herein to be any polypeptide sequence which possesses a biological property of a polypeptide comprising the following polypeptide sequence:

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWY QQKPGKAPKLLIYSASFLESGVPSRFSGSRSGT DFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTK VEIKRT (SEQ. ID NO. 1, which is the light chain variable domain of huMAb4D5); or

EVQLVESGGGLVOPGGSLRLSCAASGFNIKDTYIHW VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFT ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD GFYAMDVWGQGTLVTVSS (SEQ. ID NO. 2, which is the heavy chain variable domain of huMAb4D5).

"Biological property", as relates for example to anti-p185^{HER2}, for the purposes herein means an in vivo effector or antigen-binding function or activity that is directly or indirectly performed by huMAb4D5 (whether in its native or denatured conformation). Effector functions include p185^{HER2} binding, any hormonal or hormonal antagonist activity, any mitogenic or agonist or antagonist activity, any

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cytotoxic activity. An antigenic function means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of huMAb4D5.

Biologically active huMAb4D5 is defined herein as a polypeptide that shares an effector function of huMAb4D5. A principal known effector function of huMAb4D5 is its ability to bind to $p185^{HER2}$.

Thus, the biologically active and antigenically active huMAb4D5 polypeptides that are the subject of certain embodiments of this invention include the sequence of the entire translated nucleotide sequence of huMAb4D5; mature huMAb4D5; fragments thereof having a consecutive sequence of at least 5, 10, 15, 20, 25, 30 or 40 amino acid residues comprising sequences from muMAb4D5 plus residues from the human FR of huMAb4D5; amino acid sequence variants of huMAb4D5 wherein an amino acid residue has been inserted N- or C-terminal to, or within, huMAb4D5 or its fragment as defined above; amino acid sequence variants of huMAb4D5 or its fragment as defined above wherein an amino acid residue of huMAb4D5 or its fragment as defined above has been substituted by another residue, including predetermined mutations by, e.g., site-directed or PCR mutagenesis; derivatives of huMAb4D5 or its fragments as defined above wherein huMAb4D5 or its fragments have been covalent modified, by substitution, chemical, enzymatic, or other appropriate means, with a moiety other than a naturally occurring amino acid; and glycosylation variants of huMAb4D5 (insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of suitable residues). Such fragments and variants exclude any polypeptide heretofore identified, including muMAb4D5 or any known polypeptide fragment, which are anticipatory order 35 U.S.C. 102 as well as polypeptides obvious thereover under 35 U.S.C. 103.

An "isolated" polypeptide means polypeptide which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, for example, a polypeptide product comprising huMAb4D5 will be purified from a cell culture or other synthetic environment (1) to greater than 95% by weight of protein as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a gas- or liquid-phase sequenator (such as a commercially available Applied Biosystems sequenator Model 470, 477, or 473), or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated huMAb4D5 includes huMAb4D5 in situ within recombinant cells since at least one component of the huMAb4D5 natural environment will not be present. Ordinarily, however, isolated huMAb4D5 will be prepared by at least one purification step.

In accordance with this invention, huMAb4D5 nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active huMAb4D5, is complementary to nucleic acid sequence encoding such huMAb4D5, or hybridizes to nucleic acid sequence encoding such huMAb4D5 and remains stably bound to it under stringent conditions, and comprises nucleic acid from a muMAb4D5 CDR and a human FR region.

Preferably, the huMAb4D5 nucleic acid encodes a polypeptide sharing at least 75% sequence identity, more preferably at least 80%, still more preferably at least 85%, even more preferably at 90%, and most preferably 95%, with the huMAb4D5 amino acid sequence. Preferably, a nucleic acid molecule that hybridizes to the huMAb4D5 nucleic acid contains at least 20, more preferably 40, and most preferably 90 bases. Such hybridizing or complementary nucleic acid, however, is further defined as being novel under 35 U.S.C. 102 and unobvious under 35 U.S.C. 103 over any prior art nucleic acid.

Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0/1% $NaDodSO_4$ at 50° C.; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serumalbumin/0/1% Ficoll/0/1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42° C.; or (3) employ 50% formamide, 5×SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5×Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C., with washes at 42 C. in 0.2×SSC and 0.1% SDS.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032 published May

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4, 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al., *Nucl. Acids Res.,* 14: 5399–5407 [1986]). They are then purified on polyacrylamide gels.

The technique of "polymerase chain reaction," or "PCR," as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued Jul. 28, 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.,* 51: 263 (1987); Erlich, ed., *PCR Technology,* (Stockton Press, N.Y., 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

Suitable Methods for Practicing the Invention

Some aspects of this invention include obtaining an import, non-human antibody variable domain, producing a desired humanized antibody sequence and for humanizing an antibody gene sequence are described below. A particularly preferred method of changing a gene sequence, such as gene conversion from a non-human or consensus sequence into a humanized nucleic acid sequence, is the cassette mutagenesis procedure described in Example 1. Additionally, methods are given for obtaining and producing antibodies generally, which apply equally to native non-human antibodies as well as to humanized antibodies.

Generally, the antibodies and antibody variable domains of this invention are conventionally prepared in recombinant cell culture, as described in more detail below. Recombinant synthesis is preferred for reasons of safety and economy, but it is known to prepare peptides by chemical synthesis and to purify them from natural sources; such preparations are included within the definition of antibodies herein.

Molecular Modeling

An integral step in our approach to antibody humanization is construction of computer graphics models of the import and humanized antibodies. These models are used to determine if the six complementarity-determining regions (CDRs) can be successfully transplanted from the import framework to a human one and to determine which framework residues from the import antibody, if any, need to be incorporated into the humanized antibody in order to maintain CDR conformation. In addition, analysis of the sequences of the import and humanized antibodies and reference to the models can help to discern which framework residues are unusual and thereby might be involved in antigen binding or maintenance of proper antibody structure.

All of the humanized antibody models of this invention are based on a single three-dimensional computer graphics structure hereafter referred to as the consensus structure. This consensus structure is a key distinction from the approach of previous workers in the field, who typically begin by selecting a human antibody structure which has an amino acid sequence which is similar to the sequence of their import antibody.

The consensus structure of one embodiment of this invention was built in five steps as described below.

Step 1

Seven Fab X-ray crystal structures from the Brookhaven Protein Data Bank were used (entries 2FB4, 2RHE, 3FAB, and 1 REI which are human structures, and 2MCP, 1 FBJ, and 2HFL which are murine structures). For each structure, protein mainchain geometry and hydrogen bonding patterns were used to assign each residue to one of three secondary structure types: alpha-helix, beta-strand or other (i.e. non-helix and non-strand). The immunoglobulin residues used in superpositioning and those included in the consensus structure are shown in Table 1.

TABLE I

	Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure							
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
				$V_L\kappa$ domain				
								2–11
	18–24	18–24	19–25	18–24	19–25	19–25	19–25	16–27
	32–37	34–39	39–44	32–37	32–37	32–37	33–38	33–39
								41–49
	60–66	62–68	67–72	53–66	60–65	60–65	61–66	59–77
	69–74	71–76	76–81	69–74	69–74	69–74	70–75	
	84–88	86–90	91–95	84–88	84–88	84–88	85–89	82–91
								101–105
RMS[c]		0.40	0.60	0.53	0.54	0.48	0.50	
				V_H domain				
								3–8
	18–25		18–25	18–25	18–25	18–25		17–23
	34–39		34–39	34–39	34–39	34–39		33–41
	46–52		46–52	46–52	46–52	46–52		45–51
	57–61		59–63	56–60	57–61	57–61		57–61
	68–71		70–73	67–70	68–71	68–71		66–71
	78–84		80–86	77–83	78–84	78–84		75–82
	92–99		94–101	91–98	92–99	92–99		88–94
								102–108

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TABLE I-continued

	Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure							
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
RMS[c]			0.43	0.85	0.62	0.91		
RMS[d]	0.91		0.73	0.77	0.92			

[a]Four-letter code for Protein Data Bank file.
[b]Residue numbers for the crystal structures are taken from the Protein Data Bank files. Residue numbers for the consensus structure are according to Kabat et al.
[c]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2FB4.
[d]Root-mean-square deviation in Å for (N, Cα, C) atoms superimposed on 2HFL.

Step 2

Having identified the alpha-helices and beta-strands in each of the seven structures, the structures were superimposed on one another using the INSIGHT computer program (Biosym Technologies, San Diego, Calif.) as follows: The 2FB4 structure was arbitrarily chosen as the template (or reference) structure. The 2FB4 was held fixed in space and the other six structures rotated and translated in space so that their common secondary structural elements (i.e. alpha-helices and beta-strands) were oriented such that these common elements were as close in position to one another as possible. (This superpositioning was performed using accepted mathematical formulae rather than actually physically moving the structures by hand.)

Step 3

With the seven structures thus superimposed, for each residue in the template (2FB4) Fab one calculates the distance from the template alpha-carbon atom (Cα) to the analogous Cα atom in each of the other six superimposed structures. This results in a table of Cα-Cα distances for each residue position in the sequence. Such a table is necessary in order to determine which residue positions will be included in the consensus model. Generally, is if all Cα-Cα distances for a given residue position were ≦1.0 Å, that position was included in the consensus structure. If for a given position only one Fab crystal structure was >1.0 Å, the position was included but the outlying crystal structure was not included in the next step (for this position only). In general, the seven β-strands were included in the consensus structure while some of the loops connecting the β-strands, e.g. complementarity-determining regions (CDRs), were not included in view of Cα divergence.

Step 4

For each residue which was included in the consensus structure after step 3, the average of the coordinates for individual mainchain N, Cα, C, O and Cβ atoms were calculated. Due to the averaging procedure, as well as variation in bond length, bond angle and dihedral angle among the crystal structures, this "average" structure contained some bond lengths and angles which deviated from standard geometry. For purposes of this invention, "standard geometry" is understood to include geometries commonly accepted as typical, such as the compilation of bond lengths and angles from small molecule structures in Weiner, S. J. et. al., *J. Amer. Chem. Soc.,* 106: 765–784 (1984).

Step 5

In order to correct these deviations, the final step was to subject the "average" structure to 50 cycles of energy minimization (DISCOVER program, Biosym Technologies) using the AMBER (Weiner, S. J. et. al., *J. Amer. Chem. Soc.,* 106: 765–784 (1984)) parameter set with only the Ca coordinates fixed (i.e. all other atoms are allowed to move) (energy minimization is described below). This allowed any deviant bond lengths and angles to assume a standard (chemically acceptable) geometry. See Table II.

TABLE II

Average Bond Lengths and Angles for "Average" (Before) and Energy-Minimized Consensus (After 50 Cycles) Structures

	V_LK before (Å)	V_LK after (Å)	V_H before (Å)	V_H after (Å)	Standard Geometry (Å)
N—Cα	1.459(0.012)	1.451(0.004)	1.451(0.023)	1.452(0.004)	1.449
Cα-C	1.515(0.012)	1.523(0.005)	1.507(0.033)	1.542(0.005)	1.522
O=C	1.208(0.062)	1.229(0.003)	1.160(0.177)	1.231(0.003)	1.229
C—N	1.288(0.049)	1.337(0.002)	1.282(0.065)	1.335(0.004)	1.335
Cα-Cβ	1.508(0.026)	1.530(0.002)	1.499(0.039)	1.530(0.002)	1.526
	(*)	(*)	(*)	(*)	(*)
C—N—Cα	123.5(4.2)	123.8(1.1)	125.3(4.6)	124.0(1.1)	121.9
N—Cα-C	110.0(4.0)	109.5(1.9)	110.3(2.8)	109.5(1.6)	110.1
Cα-C—N	116.6(4.0)	116.6(1.2)	117.6(5.2)	116.6(0.8)	116.6
O=C—N	123.1(4.1)	123.4(0.6)	122.2(4.9)	123.3(0.4)	122.9
N—Cα-Cβ	110.3(2.1)	109.8(0.7)	110.6(2.5)	109.8(0.6)	109.5
Cβ-Cα-C	111.4(2.4)	111.1(0.7)	111.2(2.2)	111.1(0.6)	111.1

Values in parentheses are standard deviations. Note that while some bond length and angle averages did not change appreciably after energy-minimization, the corresponding standard deviations are reduced due to deviant geometries assuming standard values after energy-minimization. Standard geometry values are from the AMBER forcefield as implemented in DISCOVER (Biosym Technologies).

The consensus structure might conceivably be dependent upon which crystal structure was chosen as the template on which the others were superimposed. As a test, the entire procedure was repeated using the crystal structure with the worst superposition versus 2FB4, i.e. the 2HFL Fab structure, as the new template (reference). The two consensus structures compare favorably (root-mean-squared deviation of 0.11 Å for all N, Cα and C atoms).

Note that the consensus structure only includes mainchain (N, Cα, C, O, Cβ atoms) coordinates for only those residues which are part of a conformation common to all seven X-ray crystal structures. For the Fab structures, these include the common β-strands (which comprise two β-sheets) and a few non-CDR loops which connect these β-strands. The consensus structure does not include CDRs or sidechains, both of which vary in their conformation among the seven structures. Also, note that the consensus structure includes only the V_L and V_H domains.

This consensus structure is used as the archetype. It is not particular to any species, and has only the basic shape without side chains. Starting with this consensus structure

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the model of any import, human, or humanized Fab can be constructed as follows. Using the amino acid sequence of the particular antibody V_L and V_H domains of interest, a computer graphics program (such as INSIGHT, Biosym Technologies) is used to add sidechains and CDRs to the consensus structure. When a sidechain is added, its conformation is chosen on the basis of known Fab crystal structures (see the Background section for publications of such crystal structures) and rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193: 775–791 (1987)). The model also is constructed so that the atoms of the sidechain are positioned so as to not collide with other atoms in the Fab.

CDRs are added to the model (now having the backbone plus side chains) as follows. The size (i.e. number of amino acids) of each import CDR is compared to canonical CDR structures tabulated by Chothia et al., *Nature,* 342:877–883 (1989)) and which were derived from Fab crystals. Each CDR sequence is also reviewed for the presence or absence of certain specific amino acid residues which are identified by Chothia as structurally important: e.g. light chain residues 29 (CDR1) and 95 (CDR3), and heavy chain residues 26, 27, 29 (CDR1) and 55 (CDR2). For light chain CDR2, and heavy chain CDR3, only the size of the CDR is compared to the Chothia canonical structure. If the size and sequence (i.e. inclusion of the specific, structurally important residues as denoted by Chothia et al.) of the import CDR agrees in size and has the same structurally important residues as those of a canonical CDR, then the mainchain conformation of the import CDR in the model is taken to be the same as that of the canonical CDR. This means that the import sequence is assigned the structural configuration of the canonical CDR, which is then incorporated in the evolving model.

However, if no matching canonical CDR can be assigned for the import CDR, then one of two options can be exercised. First, using a program such as INSIGHT (Biosym Technologies), the Brookhaven Protein Data Bank can be searched for loops with a similar size to that of the import CDR and these loops can be evaluated as possible conformations for the import CDR in the model. Minimally, such loops must exhibit a conformation in which no loop atom overlaps with other protein atoms. Second, one can use available programs which calculate possible loop conformations, assuming a given loop size, using methods such as described by Bruccoleri et al., *Nature* 335: 564–568 (1988).

When all CDRs and sidechains have been added to the consensus structure to give the final model (import, human or humanized), the model is preferably subjected to energy minimization using programs which are available commercially (e.g. DISCOVER, Biosym Technologies). This technique uses complex mathematical formulae to refine the model by performing such tasks as checking that all atoms are within appropriate distances from one another and checking that bond lengths and angles are within chemically acceptable limits.

Models of a humanized, import or human antibody sequence are used in the practice of this invention to understand the impact of selected amino acid residues of the activity of the sequence being modeled. For example, such a model can show residues which may be important in antigen binding, or for maintaining the conformation of the antibody, as discussed in more detail below. Modeling can also be used to explore the potential impact of changing any amino acid residue in the antibody sequence.

Methods for Obtaining a Humanized Antibody Sequence

In the practice of this invention, the first step in humanizing an import antibody is deriving a consensus amino acid sequence into which to incorporate the import sequences. Next a model is generated for these sequences using the methods described above. In certain embodiments of this invention, the consensus human sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)), namely V_L κ subgroup I and V_H group III, and have the sequences indicated in the definitions above.

While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the at least one CDR from the non-human, import sequence into the consensus human structure, after the entire corresponding human CDR has been removed. The humanized antibody may contain human replacements of the non-human import residues at positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) or as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)). For example, huMAb4D5 contains human replacements of the muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol Bol.* 196:901–917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S.

Differences between the non-human import and the human consensus framework residues are individually investigated to determine their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is desirably performed through modeling, by examination of the characteristics of the amino acids at particular locations, or determined experimentally through evaluating the effects of substitution or mutagenesis of particular amino acids.

In certain preferred embodiments of this invention, a humanized antibody is made comprising amino acid sequence of an import, non-human antibody and a human antibody, utilizing the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L–V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least

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one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, one determines if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), one may retain the consensus residue.

Additionally, in certain embodiments the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71 L, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)).

In preferred embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). if the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another preferred embodiment of the methods of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

In certain alternate embodiments, one need not utilize the modeling and evaluation steps described above, and may instead proceed with the steps of obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. fin the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

Preferably, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody. If desired, one may utilize the other method steps described above for determining whether a particular amino acid residue can reasonably be expected to have undesirable effects, and remedying those effects.

If after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one preferably reexamines the potential effects of the amino acids at the specific locations recited above. Additionally, it is desirable to reinvestigate any buried residues which are reasonably expected to affect the V_L–V_H interface but may not directly affect CDR conformation. It is also desirable to reevaluate the humanized antibody utilizing the steps of the methods claimed herein.

In certain embodiments of this invention, amino acid residues in the consensus human sequence are substituted for by other amino acid residues. In preferred embodiments, residues from a particular non-human import sequence are substituted, however there are circumstances where it is desired to evaluate the effects of other amino acids. For example, if after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one may compare the sequences of other classes or subgroups of human antibodies, or classes or subgroups of antibodies from the particular non-human species, and determine which other amino acid side chains and amino acid residues are found at particular locations and substituting such other residues.

Antibodies

Certain aspects of this invention are directed to natural antibodies and to monoclonal antibodies, as illustrated in the Examples below and by antibody hybridomas deposited with the ATCC (as described below). Thus, the references throughout this description to the use of monoclonal antibodies are intended to include the use of natural or native antibodies as well as humanized and chimeric antibodies. As used herein, the term "antibody" includes the antibody variable domain and other separable antibody domains unless specifically excluded.

In accordance with certain aspects of this invention, antibodies to be humanized (import antibodies) are isolated from continuous hybrid cell lines formed by the fusion of antigen-primed immune lymphocytes with myeloma cells. in certain embodiments, the antibodies of this invention are obtained by routine screening. Polyclonal antibodies to an antigen generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. It may be useful to conjugate the antigen or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic is anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

The route and schedule of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. While mice are frequently employed as the test model, it is contemplated that any mammalian subject including human subjects or antibody-producing cells obtained therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

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Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅕ to 1/10 the original amount of conjugate in Freund's complete adjuvant (or other suitable adjuvant) by subcutaneous injection at multiple sites. 7 to 14 days later animals are bled and the serum is assayed for antigen titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

After immunization, monoclonal antibodiesare prepared by recovering immune lymphoid cells—typically spleen cells or lymphocytes from lymph node tissue—from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, *Eur. J. Immunol.* 6:511 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

It is possible to fuse cells of one species with another. However, it is preferable that the source of the immunized antibody producing cells and the myeloma be from the same species.

The hybrid cell lines can be maintained in culture in vitro in cell culture media. The cell lines of this invention can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored. and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, Ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM as the case may be that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g. ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile filtered, and optionally are conjugated to a detectable marker such as an enzyme or spin label for use in diagnostic assays of the antigen in test samples.

While routinely rodent monoclonal antibodies are used as the source of the import antibody, the invention is not limited to any species. Additionally, techniques developed for the production of chimeric antibodies (Morrison et al., *Proc. Natl. Acad. Sci.,* 81:6851 (1984); Neuberger et al., *Nature* 312:604 (1984); Takeda et al., *Nature* 314:452 (1985)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate ADCC) can be used; such antibodies are within the scope of this invention.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. One extracts antibody-specific messenger ANA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the CDNA into a bacterial expressions system. One example of such a technique suitable for the practice of this invention was developed by researchers at Scripps/Stratagene, and incorporates a proprietary bacteriophage lambda vector system which contains a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional FAb fragments for those which bind the antigen. Such FAb fragments with specificity for the antigen are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

Amino Acid Sequence Variants

Amino acid sequence variants of the antibodies and polypeptides of this invention (referred to in herein as the target polypeptide) are prepared by introducing appropriate nucleotide changes into the DNA encoding the target polypeptide, or by in vitro synthesis of the desired target polypeptide. Such variants include, for example, humanized variants of non-human antibodies, as well as deletions from, or insertions or substitutions of, residues within particular amino acid sequences. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the target polypeptide, such as changing the number or position of glycosylation sites, altering any membrane anchoring characteristics, and/or altering the intra-cellular location of the target polypeptide by inserting, deleting, or otherwise affecting any leader sequence of the native target polypeptide.

In designing amino acid sequence variants of target polypeptides, the location of the mutation site and the nature of the mutation will depend on the target polypeptide characteristics) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1–3. In certain embodiments, these choices are guided by the methods for creating humanized sequences set forth above.

A useful method for identification of certain residues or regions of the target polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesism" as described by Cunningham and Wells (*Science,* 244: 1081–1085 [1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, ala scanning or random mutagenesis may be conducted at the target codon or region and the

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expressed target polypeptide variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants: the location of the mutation site and the nature of the mutation. In general, the location and nature of the mutation chosen will depend upon the target polypeptide characteristic to be modified.

Amino acid sequence deletions of antibodies are generally not preferred, as maintaining the generally configuration of an antibody is believed to be necessary for its activity. Any deletions will be selected so as to preserve the structure of the target antibody.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the target polypeptide sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5, most preferably 1 to 3. Examples of terminal insertions include the target polypeptide with an N-terminal methionyl residue, an artifact of the direct expression of target polypeptide in bacterial recombinant cell culture, and fusion of a heterologous N-terminal signal sequence to the N-terminus of the target polypeptide molecule to facilitate the secretion of the mature target polypeptide from recombinant host cells. Such signal sequences generally will be obtained from, and thus homologous to, the intended host cell species. Suitable sequences include STII or Ipp for *E. coli,* alpha factor for yeast, and viral signals such as herpes gD for mammalian cells.

Other insertional variants of the target polypeptide include the fusion to the N- or C-terminus of the target polypeptide of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli* trp locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922 published Apr. 6, 1989.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the target polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s) of the target polypeptide, and sites where the amino acids found in the target polypeptide from various species are substantially different in terms of side-chain bulk, charge, and/or hydrophobicity. Other sites for substitution are described infra, considering the effect of the substitution of the antigen binding, affinity and other characteristics of a particular target antibody.

Other sites of interest are those in which particular residues of the target polypeptides obtained from various species are identical. These positions may be important for the biological activity of the target polypeptide. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. If such substitutions result in a change in biological activity, then other changes are introduced and the products screened until the desired effect is obtained.

Substantial modifications in function or immunological identity of the target polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, lb) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gin, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another. Such substituted residues may be introduced into regions of the target polypeptide that are homologous with other antibodies of the same class or subclass, or, more preferably, into the non-homologous regions of the molecule.

Any cysteine residues not involved in maintaining the proper conformation of target polypeptide also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

DNA encoding amino acid sequence variants of the target polypeptide is prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the target polypeptide. A particularly preferred method of gene conversion mutagenesis is described below in Example 1. These techniques may utilized target polypeptide nucleic acid (DNA or RNA), or nucleic acid complementary to the target polypeptide nucleic acid.

Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion, and insertion variants of target polypeptide DNA. This technique is well known in the art as described by Adelman et al., *DNA,* 2: 183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the target polypeptide. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the target polypeptide DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci. USA,* 75: 5765 [1978]).

Single-stranded DNA template may also be generated by denaturing double-stranded plasmid (or other) DNA using standard techniques.

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase 1, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for

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synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the target polypeptide, and the other strand (the original template) encodes the native, unaltered sequence of the target polypeptide. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thio-deoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham Corporation). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion.

After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell such as *E. coli* JM101, as described above.

DNA encoding target polypeptide variants with more than one amino acid to be substituted may be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they may be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If, however, the amino acids are located some distance from each other (separated by more than about ten amino acids), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

PCR mutagenesis is also suitable for making amino acid variants of target polypeptide. While the following discussion refers to DNA, it is understood that the technique also finds application with RNA. The PCR technique generally refers to the following procedure (see Erlich, supra, the chapter by R. Higuchi, p. 61–70): When small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, one of the primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a stretch of sequence of the opposite strand of the plasmid, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone.

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

In a specific example of PCR mutagenesis, template plasmid DNA (1 μg) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide triphosphates and is included in the GeneAmp® kits (obtained from Perkin-Elmer Cetus, Norwalk, Conn. and Emeryville, Calif.), and 25 pmole of each oligonucleotide primer, to a final volume of 50μl. The reaction mixture is overlayed with 35 μl mineral oil. The reaction is denatured for 5 minutes at 100° C., placed briefly on ice, and then 1 μl *Thermus aquaticus* (Taq) DNA polymerase (5 units/μl, purchased from Perkin-Elmer Cetus, Norwalk, Conn. and Emeryville, Calif.) is added below the mineral oil layer. The reaction mixture is then inserted into a DNA Thermal Cycler (purchased from Perkin-Elmer Cetus) programmed as follows: 2 min. at 55° C., then 30 sec. at 72° C., then 19 cycles of the following: 30 sec. at 94° C., 30 sec. at 55° C., and 30 sec. at 72° C.

At the end of the program, the reaction vial is removed from the thermal cycler and the aqueous phase transferred to a new vial, extracted with phenol/chloroform (50:50:vol), and ethanol precipitated, and the DNA is recovered by standard procedures. This material is subsequently subjected to the appropriate treatments for insertion into a vector.

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Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al. (*Gene,* 34: 315 [1985]). The starting material is the plasmid (or other vector) comprising the target polypeptide DNA to be mutated. The codon(s) in the target polypeptide DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the target polypeptide DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated target polypeptide DNA sequence.

Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding the target polypeptide is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on 1) whether it is to be used for DNA amplification or for DNA expression, 2) the size of the DNA to be inserted into the vector, and 3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(a) Signal Sequence Component

In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector.

The target polypeptides of this invention may be expressed not only directly, but also as a fusion with a heterologous polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. Included within the scope of this invention are target polypeptides with any native signal sequence deleted and replaced with a heterologous signal sequence. The heterologous signal sequence selected should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native target polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin 11 leaders. For yeast secretion the native target polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

(b) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using Bacillus species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in Bacillus genomic DNA. Transfection of Bacillus with this vector results in homologous recombination with the genome and insertion of the target polypeptide DNA. However, the recovery of genomic DNA encoding the target polypeptide is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the target polypeptide DNA.

(c) Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for Bacilli.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et al., *J. Molec. Appl. Genet.,* 1: 327 [1982]), mycophenolic acid (Mulligan et al., *Science=:* 1422 [1980]) or hygromycin (Sugden et al., *Mol. Cell. Biol.,* 5: 410–413 [1985]). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the target polypeptide nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants

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under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the target polypeptide. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the target polypeptide are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA,* 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the target polypeptide. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the target polypeptide, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature,* 22: 39 [1979]; Kingsman et al., *Gene,* 7: 141 [1979]; or Tschemper et al., *Gene,* 10: 157 [1980]). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, *Genetics,* 5: 12 [1977]). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

(d) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the target polypeptide nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as that encoding the target polypeptide, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to DNA encoding the target polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native target polypeptide promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target polypeptide DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed target polypeptide as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems (Chang et al., *Nature,* 275: 615 [1978]; and Goeddel et al., *Nature,* 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.,* 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the tao promoter (deBoer et al., *Proc. Natl. Acad. Sci, USA,* 80: 21–25 [1983]). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding the target polypeptide (Siebenlist et al., *Cell,* 20: 269 [1980]) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the target polypeptide.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.,* 255: 2073 [1980]) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.,* 2: 149 [1968]; and Holland, *Biochemistry,* 17: 4900 [1978]), such asenolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman et al., EP 73,657A. Yeast enhancers also ate advantageously used with yeast promoters.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Target polypeptide transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published Jul. 5, 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the target polypeptide sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that

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also contains the SV40 viral origin of replication. Fiers et al., *Nature,* 273:113 (1978); Mulligan and Berg, *Science,* 209: 1422–1427 (1980); Pavlakis et al., *Proc. Natl. Acad. Sci. USA,* 78: 7398–7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway et al., *Gene,* 18: 355–360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Gray et al., *Nature,* 29: 503–508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes et al., *Nature,* 297: 598–601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, *Proc. Natl. Acad. Sci. USA,* 79: 5166–5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman et al., *Proc. Natl. Aced. Sci. USA,* 79: 6777–6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

(e) Enhancer Element Component

Transcription of DNA encoding the target polypeptide of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10–300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins et al., *Proc. Natl. Acad. Sci. USA,* 78: 993 [1981]) and 3' (Lusky et al., *Mol. Cell Bio.* 3: 1108 [1983]) to the transcription unit, within an intron (Banerji et al., *Cell,* 33: 729 [1983]) as well as within the coding sequence itself (Osborne et al., *Mol. Cell Bio.,* 4: 1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100–270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature,* 297: 17–18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the target polypeptide DNA, but is preferably located at a site 5' from the promoter.

(f) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the target polypeptide. The 3' untranslated regions also include transcription termination sites.

Construction of suitable vectors containing one or more of the above listed components the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced is by the method of Messing et al., *Nucleic Acids Res.,* 9: 309 (1981) or by the method of Maxam et al., *Methods in Enzymology* 65: 499 (1980).

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the target polypeptide. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of the target polypeptide that have target polypeptide-like activity.

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the target polypeptide in recombinant vertebrate cell culture are described in Gething et al., *Nature,* 293: 620–625 [1981]; Mantei et al., *Nature,* 281: 40–46 [1979]; Levinson et al.,; EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the target polypeptide is pRK5 (EP pub. no. 307,247) or pSVI6B.

Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *E. coli* , Bacilli such as *B. subtilis*, Pseudomonas species such as *P. aeruginosa, Salmonella typhimurium,* or *Serratia marcescans*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. Alternatively, in vitro methods of cloning, e.g. PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for target polypeptide-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* [Beach and Nurse, *Nature,* 290: 140 (1981); EP 139,383 published May 2, 1985], Kluyveromyces hosts (U.S. Pat. No. 4,943,529) such as, e.g., *K. lactis* [Louvencourt et al., *J. Bacteriol.,* 737 (1983)], *K. fragilis, K. bulgaricus, K. thermotolerans,* and *K. marxianus,* yarrowia [EP 402,226], *Pichia pastoris* [EP 183,070; Sreekrishna et al., *J. Basic Microbiol.,* 28: 265–278 (1988)], Candida, *Trichoderma reesia* [EP 244,2341], *Neurospora crassa* [Case et al., *Proc. Natl. Acad. Sci. USA,* 76: 5259–5263 (1979)], and filamentous fungi such as, e.g, Neurospora, Penicillium, Tolypocladium [WO 91/00357 published Jan. 10, 1991], and Aspergillus hosts such as *A. nidulans* [Ballance et al., *Biochem. Biophys. Res. Commun.* 112: 284–289 (1983); Tilburn et al., *Gene,* 26: 205–221 (1983); Yelton et al., *Proc. Natl. Acad. Sci. USA,* 81: 1470–1474 (1984)] and *A. niger* [Kelly and Hynes, *EMBO J.,* 4: 475–479 (1985)].

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Suitable host cells for the expression of glycosylated target polypeptide are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow et al., *Bio/Technologvy* 6: 47–55 (1988); Miller et al., in *Genetic Engineering* Setlow, J. K. et a., eds., Vol. 8 (Plenum Publishing, 1986), pp. 277–279; and Maeda et al., *Nature,* 315: 592–594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cutures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens,* which has been previously manipulated to contain the target polypeptide DNA. During incubation of the plant cell culture with *A. tumefaciens,* the DNA encoding target polypeptide is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the target polypeptide DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker et al., *J. Mol. Appl. Gen.,* 1: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published Jun. 21, 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [*Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.,* 36: 59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA,* 77: 4216 [1980]); mouse sertoli cells (TM4, Mather, *Biol. Reprod.,* 23: 243–251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HS 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.,* 383: 44–68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook et al., supra, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., *Gene,* 23: 315 (1983) and WO 89/05859 published Jun. 29, 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30–16.37 of Sambrook et al., supra, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. Pat. No. 4,399,216 issued Aug. 16, 1983. Transformations into yeast are typically carried out according to the method of Van Solingen et al., *J. Bact.,* 130: 946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA),* 76: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or protoplast fusion may also be used.

Culturina the Host Cells

Prokaryotic cells used to produce the target polypeptide of this invention are cultured in suitable media as described generally in Sambrook et al., supra.

The mammalian host cells used to produce the target polypeptide of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, *Meth. Enz.,* 58: 44 (1979), Barnes and Sato, *Anal. Biochem.* 102: 255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Pat. No. Re. 30,985, may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in in vitro culture as well as cells that are within a host animal.

It is further envisioned that the target polypeptides of this invention may be produced by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already containing DNA encoding the target polypeptide currently in use in the field.

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For example, a powerful promoter/enhancer element, a suppressor, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired target polypeptide. The control element does not encode the target polypeptide of this invention, but the DNA is present in the host cell genome. One next screens for cells making the target polypeptide of this invention, or increased or decreased levels of expression, as desired.

Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA,* 77: 5201–5205 [1980]), dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu et al., *Am. J. Clin. Path.,* 75: 734–738 (1980).

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native target polypeptide or against a synthetic peptide based on the DNA sequences provided herein as described further in Section 4 below.

Purification of the Target Polypeptide

The target polypeptide preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal.

When the target polypeptide is expressed in a recombinant cell other than one of human origin, the target polypeptide is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the target polypeptide from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the target polypeptide. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The membrane and soluble protein fractions are then separated. The target polypeptide may then be purified from the soluble protein fraction and from the membrane fraction of the culture lysate, depending on whether the target polypeptide is membrane bound. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG.

Target polypeptide variants in which residues have been deleted, inserted or substituted are recovered in the same fashion, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of a target polypeptide fusion with another protein or polypeptide, e.g. a bacterial or viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen (or containing antigen, where the target polypeptide is an antibody) can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-target polypeptide column can be employed to absorb the target polypeptide variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native target polypeptide may require modification to account for changes in the character of the target polypeptide or its variants upon expression in recombinant cell culture.

Covalent Modifications of Target Polypeptides

Covalent modifications of target polypeptides are included within the scope of this invention. One type of covalent modification included within the scope of this invention is a target polypeptide fragment. Target polypeptide fragments having up to about 40 amino acid residues may be conveniently prepared by chemical synthesis, or by enzymatic or chemical cleavage of the full-length target polypeptide or variant target polypeptide. Other types of covalent modifications of the target polypeptide or fragments thereof are introduced into the molecule by reacting specific amino acid residues of the target polypeptide or fragments thereof with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides,3-nitro-2-pyridyl disulfide, methyl2-pyridyldisulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5–7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal;

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chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in so introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N=C=N—R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking target polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-target polypeptide antibodies, and vice versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis (succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modification include hydroxylation of proline and lysine, phophorylation of hydroxyl groups of seryl or threonyl resides, methylation of the α-amino groups of lysine, arginine, and histidine side chains, (T. E. Creighton, *Protein: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco, pp. 79–86 [1983]), acetylation of the N-terminal amine, and amidaatioon of any C-terminal carboxyl group.

Another type of covalent modification of the target polypeptide included within the scope of this invention comprises altering the native glucosylatuion pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in the native target polypeptide, and/or adding one or more glycosylation sites that are not present in the native target polypeptide.

Gylcosylation of polypeptides is typically either N-linked or O-linked refers to the attachment of the carbonhydrate moiety to the side chain of an asparagine reisdue. The tri-peptide sequences asparagine-X-resine and asparagine-X-threonine, where X is any aminoe acid except proline, are the recoginition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the target polypeptide is conveniently accomplished by altering the amino acid sequence such that it contains one or more of hte above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or theonine resides to the native target polypeptide sequence (for O-linked glycosylation sites). For ease, the target polypeptide amino acid sequences is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that condons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above under the heading of "Amino Acid Sequence Variants of Target Polypeptide".

Another means of increasing the number of carbohydrate moieties on the target polypeptide is by chemical or enzymatic coupling glycosides to the polypeptides. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the couple mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston (*CRC Crit. Rev. Biochem., pp.* 259–306 [1981]).

Removal of carbohydrate moieties present on the native target polypeptide may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al. (*Arch. Biochem. Biophys.*, 259:52 [1987]) and by Edge et al. (*Anal. Biochem.*, 118:131 [1981]). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. (*Meth. Enzymol.* 138:350 [1987]).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al. (*J. Biol. Chem.*, 257:3105 [1982]). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of the target polypeptide comprises linking the target polypeptide to various nonproteinaceous polymers, e.g. polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the

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manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The target polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacylate]microcapsules, respectively), in colloidal drug deliverysystems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Reminaton's Pharmaceutical Sciences,* 16th edition, Osol, A., Ed., (1980).

Target polypeptide preparations are also useful in generating antibodies, for screening for binding partners, as standards in assays for the target polypeptide (e.g. by labeling the target polypeptide for use as a standard in a radioimmunoassay, enzyme-linked immunoassay, or radioreceptor assay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with radioiodine, enzymes, fluorophores, spin labels, and the like.

Since it is often difficult to predict in advance the characteristics of a variant target polypeptide, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. For example, a change in the immunological character of the target polypeptide molecule, such as affinity for a given antigen or antibody, is measured by a competitive-type immunoassay. The variant is assayed for changes in the suppression or enhancement of its activity by comparison to the activity observed for the target polypeptide in the same assay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, stability in recombinant cell culture or in plasma, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

Diagnostic and Related Uses of the Antibodies

The antibodies of this invention are useful in diagnostic assays for antigen expression in specific cells or tissues. The antibodies are detectably labeled and/or are immobilized on an insoluble matrix.

The antibodies of this invention find further use for the affinity purification of the antigen from recombinant cell culture or natural sources. Suitable diagnostic assays for the antigen and its antibodies depend on the particular antigen or antibody. Generally, such assays include competitive and sandwich assays, and steric inhibition assays. Competitive and sandwich methods employ a phase-separation step as an integral part of the method while steric inhibition assays are conducted in a single reaction mixture. Fundamentally, the same procedures are used for the assay of the antigen and for substances that bind the antigen, although certain methods will be favored depending upon the molecular weight of the substance being assayed. Therefore, the substance to be tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or antibody, and proteins that bind to the analyte are denominated binding partners, whether they be antibodies, cell surface receptors, or antigens.

Analytical methods for the antigen or its antibodies all use one or more of the following reagents: labeled analyte analogue, immobilized analyte analogue, labeled binding partner, immobilized binding partner and steric conjugates. The labeled reagents also are known as "tracers."

The label used (and this is also useful to label antigen nucleic acid for use as a probe) is any detectable functionality that does not interfere with the binding of analyte and its binding partner. Numerous labels are known for use in immunoassay, examples including moieties that may be detected directly, such as fluorochrome, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels include the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotintavidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Conventional methods are available to bind these labels covalenily to proteins or polypeptides. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like may be used to tag the antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels. See, for example, U.S. Pat. No. 3,940,475 (fluorimetry) and U.S. Pat. No. 3,645,090 (enzymes); Hunter et al., *Nature,* 144: 945 (1962); David et al., *Biochemistry,* 13: 1014–1021 (1974); Pain et al., *J. Immunol. Methods,* 40: 219–230 (1981); and Nygren, *J. Histochem. and Cytochem.,* 30: 407–412 (1982). Preferred labels herein are enzymes such as horseradish peroxidase and alkaline phosphatase.

The conjugation of such label, including the enzymes, to the antibody is a standard manipulative procedure for one of ordinary skill in immunoassay techniques. See, for example, O'Sullivan et al., "Methods for the Preparation of Enzyme-antibody Conjugates for Use in Enzyme Immunoassay," in *Methods in in Enzymology,* ed. J. J. Langone and H. Van Vunakis, Vol. 73 (Academic Press, New York, N.Y., 1981), pp. 147–166. Such bonding methods are suitable for use with the antibodies and polypeptides of this invention.

Immobilization of reagents is required for certain assay methods. Immobilization entails separating the binding partner from any analyte that remains free in solution. This conventionally is accomplished by either insolubilizing the binding partner or analyte analogue before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al., U.S. Pat. No. 3,720,760), by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the partner or analogue afterward, e.g., by immunoprecipitation.

Other assay methods, known as competitive or sandwich assays, are well established and widely used in the commercial diagnostics industry.

Competitive assays rely on the ability of a tracer analogue to compete with the test sample analyte for a limited number of binding sites on a common binding partner. The binding partner generally is insolubilized before or after the competition and then the tracer and analyte bound to the binding partner are separated from the unbound tracer and analyte. This separation is accomplished by decanting (where the binding partner was preinsolubilized) or by centrifuging (where the binding partner was precipitated after the competitive reaction). The amount of test sample analyte is inversely proportional to the amount of bound tracer as measured by the amount of marker substance. Dose-

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response curves with known amounts of analyte are prepared and compared with the test results to quantitatively determine the amount of analyte present in the test sample. These assays are called ELISA systems when enzymes are used as the detectable markers.

Another species of competitive assay, called a "homogeneous" assay, does not require a phase separation. Here, a conjugate of an enzyme with the analyte is prepared and used such that when anti-analyte binds to the analyte the presence of the anti-analyte modifies the enzyme activity. In this case, the antigen or its immunologically active fragments are conjugated with a bifunctional organic bridge to an enzyme such as peroxidase. Conjugates are selected for use with antibody so that binding of the antibody inhibits or potentiates the enzyme activity of the label. This method per se is widely practiced under the name of EMIT.

Steric conjugates are used in steric hindrance methods for homogeneous assay. These conjugates are synthesized by covalently linking a low-molecular-weight hapten to a small analyte so that antibody to hapten substantially is unable to bind the conjugate at the same time as anti-analyte. Under this assay procedure the analyte present in the test sample will bind anti-analyte, thereby allowing anti-hapten to bind the conjugate, resulting in a change in the character of the conjugate hapten, e.g., a change in fluorescence when the hapten is a fluorophore.

Sandwich assays particularly are useful for the determination of antigen or antibodies. In sequential sandwich assays an immobilized binding partner is used to adsorb test sample analyte, the test sample is removed as by washing, the bound analyte is used to adsorb labeled binding partner, and bound material is then separated from residual tracer. The amount of bound tracer is directly proportional to test sample analyte. In "simultaneous" sandwich assays the test sample is not separated before adding the labeled binding partner. A sequential sandwich assay using an anti-antigen monoclonal antibody as one antibody and a polyclonal anti-antigen antibody as the other is useful in testing samples for particular antigen activity.

The foregoing are merely exemplary diagnostic assays for the import and humanized antibodies of this invention. Other methods now or hereafter developed for the determination of these analytes are included within the scope hereof, including the bioassays described above.

Immunotoxins

This invention is also directed to immunochemical derivatives of the antibodies of this invention such as immunotoxins (conjugates of the antibody and a cytotoxic moiety). Antibodies which carry the appropriate effector functions, such as with their constant domains, are also used to induce lysis through the natural complement process, and to interact with antibody dependent cytotoxic cells normally present.

For example, purified, sterile filtered antibodies are optionally conjugated to a cytotoxin such as ricin for use in AIDS therapy. U.S. patent application Ser. No. 07/350,895 illustrates methods for making and using immunotoxins for the treatment of HIV infection. The methods of this invention, for example, are suitable for obtaining humanized antibodies for use as immunotoxins for use in AIDS therapy.

The cytotoxic moiety of the immunotoxin may be a cytotoxic drug or an enzymatically active toxin of bacterial, fungal, plant or animal origin, or an enzymatically active fragment of such a toxin. Enzymatically active toxins and fragments thereof used are diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. In another embodiment, the antibodies are conjugated to small molecule anticancer drugs such as cis-platin or 5FU. Conjugates of the monoclonal antibody and such cytotoxic moieties are made using a variety of bifunctional protein coupling agents. Examples of such reagents are SPDP, IT, bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis (p-azidobenzoyl) hexanediamine, bis-diazonium derivatives such as bis-(p-diazoniumbenzoyl)-ethylenediamine, diisocyanates such as tolylene 2,6-diisocyanate and bis-active fluorine compounds such as 1,5-difluoro-2,4-dinitrobenzene. The lysing portion of a toxin may be joined to the Fab fragment of the antibodies.

Immunotoxins can be made in a variety of ways, as discussed herein. Commonly known crosslinking reagents can be used to yield stable conjugates.

Advantageously, monoclonal antibodies specifically binding the domain of the antigen which is exposed on the infected cell surface, are conjugated to ricin A chain. Most advantageously the ricin A chain is deglycosylated and produced through recombinant means. An advantageous method of making the ricin immunotoxin is described in Vitetta et al., *Science* 238:1098 (1987).

When used to kill infected human cells in vitro for diagnostic purposes, the conjugates will typically be added to the cell culture medium at a concentration of at least about 10 nM. The formulation and mode of administration for in vitro use are not critical. Aqueous formulations that are compatible with the culture or perfusion medium will normally be used. Cytotoxicity may be read by conventional techniques.

Cytotoxic radiopharmaceuticals for treating infected cells may be made by conjugating radioactive isotopes (e.g. I, Y, Pr) to the antibodies. Advantageously alpha particle-emitting isotopes are used. The term "cytotoxic moiety" as used herein is intended to include such isotopes.

In a preferred embodiment, ricin A chain is deglycosylated or produced without oligosaccharides, to decrease its clearance by irrelevant clearance mechanisms (e.g., the liver). In another embodiment, whole ricin (A chain plus B chain) is conjugated to antibody if the galactose binding property of B-chain can be blocked ("blocked ricin").

In a further embodiment toxin-conjugates are made with Fab or $F(ab')_2$ fragments. Because of their relatively small size these fragments can better penetrate tissue to reach infected cells.

In another embodiment, fusogenic liposomes are filled with a cytotoxic drug and the liposomes are coated with antibodies specifically binding the particular antigen.

Antibody Dependent Cellular Cytotoxicity

Certain aspects of this invention involve antibodies which are (a) directed against a particular antigen and (b) belong to a subclass or isotype that is capable of mediating the lysis of cells to which the antibody molecule binds. More specifically, these antibodies should belong to a subclass or isotype that, upon complexing with cell surface proteins, activates serum complement and/or mediates antibody dependent cellular cytotoxicity (ADCC) by activating effector cells such as natural killer cells or macrophages.

Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananue and Benacerraf,

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Textbook of Immunology, 2nd Edition, Williams & Wilkins, p. 218 (1984)). This includes their ability to activate complement and to mediate antibody-dependent cellular cytotoxicity (ADCC) as effected by leukocytes. Antibodies of different classes and subclasses differ in this respect, as do antibodies from the same subclass but different species; according to the present invention, antibodies of those classes having the desired biological activity are prepared. Preparation of these antibodies involves the selection of antibody constant domains are their incorporation in the humanized antibody by known technique. For example, mouse immunoglobulins of the IgG3 and lgG2a class are capable of activating serum complement upon binding to the target cells which express the cognate antigen, and therefore humanized antibodies which incorporate IgG3 and lgG2a effector functions are desirable for certain therapeutic applications.

In general, mouse antibodies of the IgG2a and IgG3 subclass and occasionally IgG1 can mediate ADCC, and antibodies of the IgG3, IgG2a, and IgM subclasses bind and activate serum complement. Complement activation generally requires the binding of at least two IgG molecules in close proximity on the target cell. However, the binding of only one IgM molecule activates serum complement.

The ability of any particular antibody to mediate lysis of the target cell by complement activation and/or AOCC can be assayed. The cells of interest are grown and labeled in vitro; the antibody is added to the cell culture in combination with either serum complement or immune cells which may be activated by the antigen antibody complexes. Cytolysis of the target cells is detected by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the in vitro test can then be used therapeutically in that particular patient.

This invention specifically encompasses consensus Fc antibody domains prepared and used according to the teachings of this invention.

Therapeutic and Other Uses of the Antibodies

When used in vivo for therapy, the antibodies of the subject invention are administered to the patient in therapeutically effective amounts (i.e. amounts that have desired therapeutic effect). They will normally be administered parenterally. The dose and dosage regimen will depend upon the degree of the infection, the characteristics of the particular antibody or immunotoxin used, e.g., its therapeutic index, the patient, and the patient's history. Advantageously the antibody or immunotoxin is administered continuously over a period of 1–2 weeks, intravenously to treat cells in the vasculature and subcutaneously and intraperitoneally to treat regional lymph nodes. Optionally, the administration is made during the course of adjunct therapy such as combined cycles of radiation, chemotherapeutic treatment, or administration of tumor necrosis factor, interferon or other cytoprotective or immunomodulatory agent.

For parenteral administration the antibodies will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic, and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate can also be used. Liposomes may be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The antibodies will typically be formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

Use of IgM antibodies may be preferred for certain applications, however IgG molecules by being smaller may be more able than IgM molecules to localize to certain types of infected cells.

There is evidence that complement activation in vivo-leads to a variety of biological effects, including the induction of an inflammatory response and the activation of macrophages (Uananue and Benecerraf, *Textbook of Immunology,* 2nd Edition, Williams & Wilkins, p. 218 (1984)). The increased vasodilation accompanying inflammation may increase the ability of various agents to localize in infected cells. Therefore, antigen-antibody combinations of the type specified by this invention can be used therapeutically in many ways. Additionally, purified antigens (Hakomori, *Ann. Rev. Immunol.* 2:103 (1984)) or anti-idiotypic antibodies (Nepom et al., *Proc. Natl. Acad. Sci.* 81:2864 (1985); Koprowski et al., *Proc. Natl. Acad. Sci.* 81:216 (1984)) relating to such antigens could be used to induce an active immune response in human patients. Such a response includes the formation of antibodies capable of activating human complement and mediating ADCC and by such mechanisms cause infected cell destruction.

Optionally, the antibodies of this invention are useful in passively immunizing patients, as exemplified by the administration of humanized anti-HIV antibodies.

The antibody compositions used in therapy are formulated and dosages established in a fashion consistent with good medical practice taking into account the disorder to be treated, the condition of the individual patient, the site of delivery of the composition, the method of administration and other factors known to practitioners. The antibody compositions are prepared for administration according to the description of preparation of polypeptides for administration, infra.

Deposit of Materials

As described above, cultures of the muMAb4D5 have been deposited with the American Type Culture Collection, 10801 University Blvd., Mauassas, Va., USA (ATCC).

This deposit was made under the provisions of the Budapest Treaty on the international Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures' availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed

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when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention.

EXAMPLES

Example 1

Humanization of muMAb4D5

Here we report the chimerization of muMAb4D5 (chMAb4D5) and the rapid and simultaneous humanization of heavy (V_H) and light (V_L) chain variable region genes using a novel "gene conversion mutagenesis" strategy. Eight humanized variants (huMAb4D5) were constructed to probe the importance of several FR residues identified by our molecular modeling or previously proposed to be critical to the conformation of particular CDRs (see Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987); Chothia, C. et al., *Nature* 342:877–883 (1989); Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)). Efficient transient expression of humanized variants in non-myeloma cells allowed us to rapidly investigate the relationship between binding affinity for $p185^{HER2}$ ECD and anti-proliferative activity against $p185^{HER2}$ overexpressing carcinoma cells.

Materials and Methods

Cloning of Variable Region Genes. The muMAb4D5 V_H and V_L genes were isolated by polymerase chain reaction (PCR) amplification of mRNA from the corresponding hybridoma (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) as described by Orlandi et al. (Orlandi, R. et al., *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (1989)). Amino terminal sequencing of muMAb4D5 V_L and V_H was used to design the sense strand PCR primers, whereas the anti-sense PCR primers were based upon consensus sequences of murine framework residues (Orlandi, R. et al., *Proc. Natl. Acad. Sci. USA* 86:3833–3837 (1989); Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences: V_L sense, 5'-TCC GATATCCAGCTGACCCAGTCTCCA-3' (SEQ. ID NO. 7), EcoRV; V_L anti-sense, 5'-GTTTGATCTCCAGCTT GGTACCHSCDCCGAA-3' (SEQ. ID NO. 8), Asp718; V_H sense, 5'-AGGTSMARCTGCAGSAGTCWGG-3' (SEQ. ID NO. 9), PstI and V_H anti-sense, 5'-TGAGGAGAC GGTGACCGTGGTCCCTTGGCCCCAG-3' (SEQ. ID. NO. 10), BstEII; where H=A or C or T, S=C or G, D=A or G or T, M=A or C, R=A or G and W=A or T. The PCR products were cloned into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* 153:3–11 (1987)) and five clones for each variable domain sequenced by the dideoxy method (Sanger, F. et al., *Proc. Natl. Acad. Sci. USA* 74:5463–5467 (1977)).

Molecular Modelling. Models for muMAb4D5 V_H and V_L domains were constructed separately from consensus coordinates based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. et al., *J. Mol. Biol.* 141:369–391 (1980)) was first chosen as a template for V_L and V_H domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Siosym Technologies). The distance from the template Cα to the analogous Cα in each of the superimposed structures was calculated for each residue position. If all (or nearly all) Cα—Cα distances for a given residue were ≦1 Å, then that position was included in the consensus structure. In most cases the β-sheet framework residues satisfied these criteria whereas the CDR loops did not. For each of these selected residues the average coordinates for individual N, Cα, C, O and Cβ atoms were calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. et al., *J. Amer. Chem. Soc.* 106:765–784 (1984)) and Cα coordinates fixed. The side chains of highly conserved residues, such as the disulfide-bridged cysteine residues, were then incorporated into the resultant consensus structure. Next the sequences of muMAb4D5 V_L and V_H were incorporated starting with the CDR residues and using the tabulations of CDR conformations from Chothia et al. (Chothia, C. et al., *Nature* 342:877–883 (1989)) as a guide. Side-chain conformations were chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193:775–791 (1987)) and packing considerations. Since V_H-COR3 could not be assigned a definite backbone conformation from these criteria, two models were created from a search of similar sized loops using the INSIGHT program. A third model was derived using packing and solvent exposure considerations. Each model was then subjected to 5000 cycles of energy minimization.

In humanizing muMAb4D5, consensus human sequences were first derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)), namely V_L κ subgroup I and V_H group III, and a molecular model generated for these sequences using the methods described above. A structure for huMAb4D5 was created by transferring the CDRs from the muMAb4D5 model into the consensus human structure. All huMAb4D5 variants contain human replacements of muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but notas defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901–917 (1987)):

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V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S. Differences between muMAb4D5 and the human consensus framework residues (FIG. **1**) were individually modeled to investigate their possible influence on CDR conformation and/or binding to the $p185^{HER2}$ ECD.

Construction of Chimeric Genes. Genes encoding chMAb4D5 light and heavy chains were separately assembled in previously described phagemid vectors containing ihe human cytomegalovirus enhancer and promoter, a 5' intron and SV40 polyadenylation signal (Gorman, C. M. et al., *DNA & Prot. Engin. Tech.* 2:3–10 (1990)). Briefly, gene segments encoding muMAb4D5 V_L (FIG. **1A**) and REI human κ_1 light chain C_L (Palm, W. & Hilschmann, N., *Z. Physiol. Chem.* 356:167–191 (1975)) were precisely joined as were genes for muMAb4D5 V_H (FIG. **1B**) and human γ1 constant region (Capon, D. J. et al., *Nature* 337:525–531 (1989)) by simple subcloning (Boyle, A., in *Current Protocols in Molecular Biology,* Chapter 3 (F. A. Ausubel et al., eds., Greene Publishing & Wiley-Interscience, New York, 1990)) and site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The γ1 isotype was chosen as it has been found to be the preferred human isotype for supporting ADCC and complement dependent cytotoxicity using matched sets of chimeric (Brüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987)) or humanized antibodies (Riechmann, L. et al., *Nature* 332:323–327 (1988)). The PCR-generated V_L and V_H fragments (FIG. **1**) were subsequently mutagenized so that they faithfully represent the sequence of muMAb4D5 determined at the protein level: V_H Q1E, V_L V_{104}L and T109A (variants are denoted by the amino acid residue and number followed by the replacement amino acid). The human γ1 constant regions are identical to those reported by Ellison et al. (Ellison, J. W. et al., *Nucleic Acids Res.* 13:4071–4079 (1982)) except for the mutations E359D and M361L (Eu numbering, as in Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) which we installed to convert the antibody from the naturally rare A allotype to the much more common non-A allotype (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)). This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.

Construction of Humanized Genes. Genes encoding chMAb4D5 light chain and heavy chain Fd fragment (V_H and C_H1 domains) were subcloned together into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* 153:3–11 (1987)) to create pAK1 and simultaneously humanized in a single step (FIG. **2**). Briefly, sets of 6 contiguous oligonucleotides were designed to humanize V_H and V_L (FIG. **1**). These oligonucleotides are 28 to 83 nucleotides in length, contain zero to 19 mismatches to the murine antibody template and are constrained to have 8 or 9 perfectly matched residues at each end to promote efficient annealing and ligation of adjacent oligonucleotides. The sets of V_H and V_L humanization oligonucleotides (5 pmol each) were phosphorylated with either ATP or γ-^{32}P-ATP (Carter, P. *Methods Enzymol.* 154: 382–403 (1987)) and separately annealed with 3.7 pmol of pAK1 template in 40 μl 10 mM Tris-HCl (pH 8.0) and 10 mM $MgCl_2$ by cooling from 100° C. to room temperature over ~30 min. The annealed oligonucleotides were joined by incubation with T4 DNA ligase (12 units; New England Biolabs) in the presence of 2 μl 5 mM ATP and 2 μl 0.1 M DTT for 10 min at 14° C. After electrophoresis on a 6% acrylamide sequencing gel the assembled oligonucleotides were located by autoradiography and recovered by electroelution. The assembled oligonucleotides (~0.3 pmol each) were simultaneously annealed to 0.15 pmol single-stranded deoxyuridine-containing pAK1 prepared according to Kunkel et al. (Kunkel, T. A. et al., *Methods Enzymol.* 154:367–382 (1987)) in 10 μl 40 mM Tris-HCl (pH 7.5) and 16 mM $MgCl_2$ as above. Heteroduplex DNA was constructed by extending the primers with T7 DNA polymerase and transformed into *E. coli* BMH 71–18 mutL as previously described (Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991)). The resultant phagemid DNA pool was enriched first for huV_L by restriction purification using XhoI and then for huV_H by restriction selection using StuI as described in Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991); and in Wells, J. A. et al., *Phil. Trans. R. Soc. Lond., A* 317:415–423 (1986). Resultant clones containing both huV_L and huV_H genes were identified by nucleotide sequencing (Sanger, F. et al., *Proc. Natl. Acad. Sci. USA* 74:5463–5467 (1977)) and designated pAK2. Additional humanized variants were generated by site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach,* Chapter 1 (IRL Press, Oxford, UK 1991)). The muMAb4D5 V_L and V_H gene segments in the transient expression vectors described above were then precisely replaced with their humanized versions.

Expression and Purification of MAb4D5 Variants. Appropriate MAb4D5 light and heavy chain cDNA expression vectors were co-transfected into an adenovirus transformed human embryonic kidney cell line, 293 (Graham, F. L. et al., *J. Gen. Virol.* 36:59–72 (1977)) using a high efficiency procedure (Gorman, C. M. et al., *DNA & Prot. Engin. Tech.* 2:3–10 (1990); Gorman, C., in *DNA Cloning,* vol II, pp 143–190 (D. M. Glover, ed., IRL Press, Oxford, UK 1985)). Media were harvested daily for up to 5 days and the cells re-fed with serum free media. Antibodies were recovered from the media and affinity purified on protein A sepharose CL-4B (Pharmacia) as described by the manufacturer. The eluted antibody was buffer-exchanged into phosphate-buffered saline by G25 gel filtration, concentrated by ultrafiltration (Centriprep-30 or Centricon-100, Amicon), sterile-filtered (Millex-GV, Millipore) and stored at 4° C. The concentration of antibody was determined by using both total immunoglobulin and antigen binding ELISAs. The standard used was huMAb4D5-5, whose concentration had been determined by amino acid composition analysis.

Cell Proliferation Assay. The effect of MAb4D5 variants upon proliferation of the human mammary adenocarcinoma cell line, SK-BR-3, was investigated as previously described (Fendly, B. M. et al., *Cancer Res.* 50:1550–1558 (1990)) using saturating MAb4D5 concentrations.

Affinity Measurements. The antigen binding affinity of MAb4D5 variants was determined using a secreted form of the $p185^{HER2}$ ECD prepared as described in Fendly, B. M. et al., *J. Biol. Resp. Mod.* 9:449–455 (1990). Briefly, antibody and $p185^{HER2}$ ECD were incubated in solution until equilibrium was found to be reached. The concentration of free antibody was then determined by ELISA using immobilized $p185^{HER2}$ ECD and used to calculate affinity (K_d) according to Friguet et al. (Friguet, B. et al., *J. Immunol. Methods* 77:305–319 (1985)).

Results

Humanization of muMAb4D5. The muMAb4D5 V_L and V_H gene segments were first cloned by PCR and sequenced (FIG. **1**). The variable genes were then simultaneously humanized by gene conversion mutagenesis using preassembled oligonucleotides (FIG. **2**). A 311-mer oligonucle-

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otide containing 39 mismatches to the template directed 24 simultaneous amino acid changes required to humanize muMAb4D5 V_L. Humanization of muMAb4D5 V_H required 32 amino acid changes which were installed with a 361-mer containing 59 mismatches to the muMAb4D5 template. Two out of 8 clones sequenced precisely encode huMAb4D5-5, although one of these clones contained a single nucleotide imperfection. The 6 other clones were essentially humanized but contained a small number of errors: <3 nucleotide changes and <1 single nucleotide deletion per kilobase. Additional humanized variants (Table 3) were constructed by site-directed mutagenesis of huMAb4D5-5.

Expression levels of huMAb4D5 variants were in the range of 7 to 15 μg/ml as judged by ELISA using immobilized $p185^{HER2}$ ECD. Successive harvests of five 10 cm plates allowed 200 μg to 500 mg of each variant to be produced in a week. Antibodies affinity purified on protein A gave a single band on a Coomassie blue stained SDS polyacrylamide gel of mobility consistent with the expected M_r of ~150 kDa. Electrophoresis under reducing conditions gave 2 bands consistent with the expected M_r of free heavy (48 kDa) and light (23 kDa) chains (not shown). Amino terminal sequence analysis (10-cycles) gave the mixed sequence expected (see FIG. **1**) from an equimolar combination of light and heavy chains (not shown).

huMAb4D5 Variants. In general, the FR residues were chosen from consensus human sequences (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) and CDR residues from muMAb4D5. Additional variants were constructed by replacing selected human residues in huMAb4D5-1 with their muMAb4D5 counterparts. These are V_H residues 71, 73, 78, 93 plus 102 and V_L residues 55 plus 66 identified by our molecular modeling. V_H residue 71 has previously been proposed by others (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) to be critical to the conformation of V_H-CDR2. Amino acid sequence differences between huMAb4D5 variant molecules are shown in Table 3, together with their $p185^{HER2}$ ECD binding affinity and maximal anti-proliferative activities against SK-BR-3 cells. Very similar K_d values were obtained for binding of MAb4D5 variants to either SK-BR-3 cells or to $p185^{HER2}$ ECD (Table 3). However, K_d estimates derived from binding of MAb4D5 variants to $p185^{HER2}$ ECD were more reproducible with smaller standard errors and consumed much smaller quantities of antibody than binding measurements with whole cells.

The most potent humanized variant designed by molecular modeling, huMAb4D5-8, contains 5 FR residues from muMAb4D5. This antibody binds the $p185^{HER2}$ ECD 3-fold more tightly than does muMAb4D5 itself (Table 3) and has comparable anti-proliferative activity with SK-BR-3 cells (FIG. **3**). In contrast, huMAb4D5-1 is the most humanized but least potent muMAb4D5 variant, created by simply installing the muMAb4D5 CDRs into the consensus human sequences. huMAb4D5-1 binds the $p185^{HER2}$ ECD 80-fold less tightly than does the murine antibody and has no detectable anti-proliferative activity at the highest antibody concentration investigated (16 μg/ml).

The anti-proliferative activity of huMAb4D5 variants against $p185^{HER2}$ overexpressing SK-BR-3 cells is not simply correlated with their binding affinity for the $p185^{HER2}$ ECD. For example, installation of three murine residues into the V_H domain of huMAb4D5-2 (D73T, L78A and A93S) to create huMAb4D5-3 does not change the antigen binding affinity but does confer significant anti-proliferative activity (Table 3).

The importance of V_H residue 71 (Tramontano, A. et al., *J. Mol. Biol.* 215:175–182 (1990)) is supported by the observed 5-fold increase in affinity for $p185^{HER2}$ ECD on replacement of R71 in huMAb4D5-1 with the corresponding murine residue, alanine (huMAb4D5-2). In contrast, replacing V_H L78 in huMAb4D5-4 with the murine residue, alanine (huMAb4D5-5), does not significantly change the affinity for the $p185^{HER2}$ ECD or change anti-proliferative activity, suggesting that residue 78 is not of critical functional significance to huMAb4D5 and its ability to interact properly with the extracellular domain of $p185^{HER2}$.

V_L residue 66 is usually a glycine in human and murine κ chain sequences (Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987)) but an arginine occupies this position in the muMAb4D5 κ light chain. The side chain of residue 66 is likely to affect the conformation of V_L-CDR1 and V_L-CDR2 and the hairpin turn at 68–69 (FIG. **4**). Consistent with the importance of this residue, the mutation V_L G66R (huMAb4D5-3→huMAb4D5-5) increases the affinity for the $p185^{HER2}$ ECD by 4-fold with a concomitant increase in anti-proliferative activity.

From molecular modeling it appears that the tyrosyl side chain of muMAb4D5 V_L residue 55 may either stabilize the conformation of V_H-CDR3 or provide an interaction at the V_L–V_H interface. The latter function may be dependent upon the presence of V_H Y102. In the context of huMAb4D5-5 the mutations V_L E55Y (huMAb4D5-6) and V_H V102Y (huMAb4D5-7) individually increase the affinity for $p185^{HER2}$ ECD by 5-fold and 2-fold respectively, whereas together (huMAb4D5-8) they increase the affinity by 11-fold. This is consistent with either proposed role of V_L Y55 and V_H Y102.

Secondary Immune Function of huMAb4D5-8. MuMAb4D5 inhibits the growth of human breast tumor cells which overexpress $p185^{HER2}$ (Hudziak, R. M. et al., *Molec. Cell. Biol.* 9:1165–1172 (1989)). The antibody, however, does not offer the possibility of direct tumor cytotoxic effects. This possibility does arise in huMAb4D5-8 as a result of its high affinity (Kd_d=0.1 μM) and its human IgG_1 subtype. Table 4 compares the ADCC mediated by huMAb4D5-8 with muMAb4D5 on a normal lung epithelial cell line, WI-38, which expresses a low level of $p185^{HER2}$ and on SK-BR-3, which expresses a high level of $p185^{HER2}$. The results demonstrate that: (1) huMAb4D5 has a greatly enhanced ability to carry out ADCC as compared with its murine parent; and (2) that this activity may be selective for cell types which overexpress $p185^{HER2}$.

Discussion

MuMAb4D5 is potentially useful for human therapy since it is cytostatic towards human breast and ovarian tumor lines overexpressing the HER2-encoded $p185^{HER2}$ receptor-like tyrosine kinase. Since both breast and ovarian carcinomas are chronic diseases it is anticipated that the optimal MAb4D5 variant molecule for therapy will have low immunogenicity and will be cytotoxic rather than solely cytostatic in effect. Humanization of muMAb4D5 should accomplish these goals. We have identified 5 different huMAb4D5 variants which bind tightly to $p185^{HER2}$ ECD ($K_d \leqq 1$ nM) and which have significant anti-proliferative activity (Table 3). Furthermore huMAb4D5-8 but not muMAb4D5 mediates ADCC against human tumor cell lines overexpressing $p185^{HER2}$ in the presence of human effector cells (Table 4) as anticipated for a human γ1 isotype (Brcüggemann, M. et al., *J. Exp. Med.* 166:1351–1361 (1987); Riechmann, L. et al., *Nature* 332:323–327 (1988)).

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Rapid humanization of huMAb4D5 was facilitated by the gene conversion mutagenesis strategy developed here using long preassembled oligonucleotides. This method requires less than half the amount of synthetic DNA as does total gene synthesis and does not require convenient restriction sites in the target DNA. Our method appears to be simpler and more reliable than a variant protocol recently reported (Rostapshov, V. M. et al., *FEBS Lett.* 249: 379–382 (1989)). Transient expression of huMAb4D5 in human embryonic kidney 293 cells permitted the isolation of a few hundred micrograms of huMAb4D5 variants for rapid characterization by growth inhibition and antigen binding affinity assays. Furthermore, different combinations of light and heavy chain were readily tested by co-transfection of corresponding cDNA expression vectors.

The crucial role of molecular modeling in the humanization of muMAb4D5 is illustrated by the designed variant huMAb4D5-8 which binds the $p185^{HER2}$ ECD 250-fold more tightly than the simple CDR loop swap variant, huMAb4D5-1. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. et al., *Nature* 332:323–327 (1988); Queen, C. et al., *Proc. Natl. Acad. Sci. USA* 86:10029–10033 (1989)). Here we have extended this earlier work by others with a designed humanized antibody which binds its antigen 3-fold more tightly than the parent rodent antibody. While this result is gratifying, assessment of the success of the molecular modeling must await the outcome of X-ray structure determination. From analysis of huMAb4D5 variants (Table 3) it is apparent that their anti-proliferative activity is not a simple function of their binding affinity for $p185^{HER2}$ ECD. For example the huMAb4D5–8 variant binds $p185^{HER2}$ 3-fold more tightly than muMAb4D5 but the humanized variant is slightly less potent in blocking the proliferation of SK-SR-3 cells. Additional huMAb4D5 variants are currently being constructed in an attempt to identify residues triggering the anti-proliferative activity and in an attempt to enhance this activity.

In addition to retaining tight receptor binding and the ability to inhibit cell growth, the huMAb4D5-8 also confers a secondary immune function (ADCC). This allows for direct cytotoxic activity of the humanized molecule in the presence of human effector cells. The apparent selectivity of the cytotoxic activity for cell types which overexpress $p185^{HER2}$ allows for the evolution of a straightforward clinic approach to those human cancers characterized by overexpression of the HER2 protooncogene.

TABLE 3

$p185^{HER2}$ ECD binding affinity and anti-proliferative activities of MAb4D5 variants

	V_H Residue*					V_L Residue*			
MAb4D5 cell Variant proliferation‡	71 FR3	73 FR3	78 FR3	93 FR3	102 CDR3	55 CDR2	56 FR3	K_d† nM	Relative
huMAb4D5-1	R	D	L	A	V	E	G	25	102
huMAb4D5-2	Ala	D	L	A	V	E	G	4.7	101
huMAb4D5-3	Ala	Thr	Ala	Ser	V	E	G	4.4	66
huMAb4D5-4	Ala	Thr	L	Ser	V	E	Arg	0.82	56
huMAb4D5-5	Ala	Thre	Ala	Ser	V	E	Arg	1.1	48
huMAb4D5-6	Ala	Thr	Ala	Ser	V	Tyr	Arg	0.22	51
huMAb4D5-7	Ala	Thr	Ala	Ser	Tyr	E	Arg	0.62	53
huMAb4D5-8	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.10	54
muMAb4D5	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.30	37

*Human and murine residues are shown in one letter and three letter amino acid code respectively.
†K_d values for the $p185^{HER2}$ ECD were determined using the method of Friguet et al. (43) and the standard error of each estimate is ≦ ± 10%.
‡Proliferation of SK-BR-3 cells incubated for 96 hr with MAb4D5 variants shown as a percentage of the untreated control as described (Hudziak, R. M. et al., Molec. Cell. Biol. 9: 1165–1172 (1989)). Data represent the maximal anti-proliferative effect for each variant (see FIG. 3A) calculated as the mean of triplicate determinations at a MAb4D5 concentration of 8 μg/ml. Data are all taken from the same experiment with an estimated standard error of ≦ ± 15%.

TABLE 4

Selectivity of antibody dependent tumor cell cytotoxicity mediated by huMAb4D5-8

Effector:Target ratio†	WI-38* muMAb4D5	WI-38* huMAb4D5-8	SK-BR-3 muMAb4D5	SK-BR-3 huMAb4D5-8
A.‡				
25:1	<1.0	9.3	7.5	40.6
12.5:1	<1.0	11.1	4.7	36.8
6.25:1	<1.0	8.9	0.9	35.2
3.13:1	<1.0	8.5	4.6	19.6
B.				
25:1	<1.0	3.1	6.1	33.4
12.5:1	<1.0	1.7	5.5	26.2
6.25:1	1.3	2.2	2.0	21.0
3.13:1	<1.0	0.8	2.4	13.4

*Sensitivity to ADCC of two human cell lines (WI-38, normal lung epithelium; and SK-BR-3, human breast tumor cell line) are compared. WI-38 expresses a low level of $p185^{HER2}$ (0.6 pg per μg cell protein) and SK-BR-3 expresses a high level of $p185^{HER2}$ (64 pg $p185^{HER2}$ per μg cell protein), as determined by ELISA (Fendly et al., J. Biol. Resp. Mod. 9:449–455 (1990)).
†ADCC assays were carried out as described in Brüggemann et al., J. Exp. Med. 166:1351–1361 (1987). Effector to target ratios were of IL-2 activated human peripheral blood lymphocytes to either WI-38 fibroblasts or SK-BR-3 tumor cells in 96-well microtiter plates for 4 hours at 37° C. Values given represent percent specific cell lysis as determined by ^{51}Cr release. Estimated standard error in these quadruplicate determinations was ≦±10%.
‡Monoclonal antibody concentrations used were 0.1 μg/ml (A) and 0.1 μg/ml (B).

Example 2
Schematic Method for Humanizing an Antibody Sequence

This example illustrates one stepwise elaboration of the methods for creating a humanized sequence described

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above. It will be understood that not all of these steps are essential to the claimed invention, and that steps may be taken in different order.

1. ascertain a consensus human variable domain amino acid sequence and prepare from it a consensus structural model.
2. prepare model of import (the non-human domain to be humanized) variable domain sequences and note structural differences with respect to consensus human model.
3. identify CDR sequences in human and in import, both by using Kabat (supra, 1987) and crystal structure criteria. If there is any difference in CDR identity from the different criteria, use of crystal structure definition of the CDR, but retain the Kabat residues as important framework residues to import.
4. substitute import CDR sequences for human CDR sequences to obtain initial "humanized" sequence.
5. compare import non-CDR variable domain sequence to the humanized sequence and note divergences.
6. Proceed through the following analysis for each amino acid residue where the import diverges from the humanized.
   a. If the humanized residue represents a residue which is generally highly conserved across all species, use the residue in the humanized sequence. If the residue is not conserved across all species, proceed with the analysis described in 6b.
   b. If the residue is not generally conserved across all species, ask if the residue is generally conserved in humans.
      i. If the residue is generally conserved in humans but the import residue differs, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs by considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, leave the humanized residue unchanged.
      ii. If the residue is also not generally conserved in humans, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs be considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, proceed to the next step.
         a) Examine the structural models of the import and human sequences and determine if the residue is exposed on the surface of the domain or is buried within. If the residue is exposed, use the residue in the humanized sequence. If the residue is buried, proceed to the next step.
            (i) Examine the structural models of the import and human sequences and determine if the residue is likely to affect the V_L–V_H interface. Residues involved with the interface include: 34L, 36L, 38L, 43L, 33L, 36L, 85L, 87L, 89L, 91L, 96L, 98L, 35H, 37H, 39H, 43H, 45H, 47H, 60H, 91H, 93H, 95H, 100H, and 103H. If no effect is likely, use the residue in the humanized sequence. If some affect is likely, substitute the import residue.
7. Search the import sequence, the consensus sequence and the humanized sequence for glycosylation sites outside the CDRs, and determine if this glycosylation site is likely to have any affect on antigen binding and/or biological activity. If no effect is likely, use the human sequence at that site; if some affect is likely, eliminate the glycosylation site or use the import sequence at that site.
8. After completing the above analysis, determine the planned humanized sequence and prepare and test a sample. If the sample does not bind well to the target antigen, examine the particular residues listed below, regardless of the question of residue identity between the import and humanized residues.
   a. Examine particular peripheral (non-CDR) variable domain residues that may, due to their position, possibly interact directly with a macromolecular antigen, including the following residues (where the * indicates residues which have been found to interact with antigen based on crystal structures):
      i. Variable light domain: 36, 46, 49*, 63–70
      ii. Variable heavy domain: 2, 47*, 68, 70, 73–76.
   b. Examine particular variable domain residues which could interact with, or otherwise affect, the conformation of variable domain CDRs, including the following (not including CDR residues themselves, since it is assumed that, because the CDRs interact with one another, any residue in one CDR could potentially affect the conformation of another CDR residue) (L=LIGHT, H=HEAVY, residues appearing in bold are indicated to be structurally important according the Chothia et al., Nature 342:877 (1989), and residues appearing in italic were altered during humanization by Queen et al. (PDL), Proc. Natl. Acad. Sci. USA 86:10029 (1989) and Proc. Natl. Acad. Sci. USA 88:2869 (1991).):
      i. Variable light domain:
         a) CDR-1 (residues 24L–34L): 2L, 4L, 66L-69L, 71L
         b) CDR-2 (residues 50L–56L): 35L, 46L, 47L, 48L, 49L, 58L, 62L, 64L–66L, 71L, 73L
         c) CDR-3 (residues 89L–97L): 2L, 4L, 36L, 98L, 37H, 45H, 47H, 58H, 60H
      ii. Variable heavy domain:
         a) CDR-1 (residues 26H–35H): 2H, 4H, 24H, 36H, 71H, 73H, 76H, 78H, 92H, 94H
         b) CDR-2 (residues 50H–55H): 49H, 69H, 69H, 71H, 73H, 78H
         c) CDR-3 (residues 95H–102H): examine all residues as possible interaction partners with this loop, because this loop varies in size and conformation much more than the other CDRs.
9. If after step 8 the humanized variable domain still is lacking in desired binding, repeat step 8. In addition, re-investigate any buried residues which might affect the V_L–V_H interface (but which would not directly affect CDR conformation). Additionally, evaluate the accessibility of non-CDR residues to solvent.

Example 3

Engineering a Humanized Bisnecific $F(ab')_2$ Fragment

This example demonstrates the construction of a humanized bispecific antibody ($BsF(ab')_2v1$ by separate *E. coli* expression of each Fab' arm followed by directed chemical coupling in vitro. $BsF(ab')_2v1$ (anti-CD3/anti-$p185^{HER2}$) was demonstrated to retarget the cytotoxic activity of human

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$CD3^+$CTL in vitro against the human breast tumor cell line, SK-BR-3, which overexpresses the $p185^{HER2}$ product of the protooncogene HER2. This example demonstrates the minimalistic humanization strategy of installing as few murine residues as possible into a human antibody in order to recruit antigen-binding affinity and biological properties comparable to that of the murine parent antibody. This strategy proved very successful for the anti-$p185^{HER2}$arm of BsF $(ab')_2$v1. In contrast $BsF(ab')_2$ v1 binds to T cells via its anti-CD3 arm much less efficiently than does the chimeric $BsF(ab')_2$ which contains the variable domains of the murine parent anti-CD3 antibody. Here we have constructed additional $BsF(ab')_2$ fragments containing variant anti-CD3 arms with selected murine residues restored in an attempt to improve antibody binding to T cells. One such variant, Ss $F(ab')_2$v9, was created by replacing six residues in the second hypervariable loop of the anti-CD3 heavy chain variable domain of $BsF(ab')_2$v1 with their counterparts from the murine parent anti-CD3 antibody. $BsF(ab')_2$v9 binds to T cells (Jurkat) much more efficiently than does $BsF(ab')_2$v1 and almost as efficiently as the chimeric $BsF(ab')_2$. This improvement in the efficiency of T cell binding of the humanized $BsF(ab')_2$ is an important step in its development as a potential therapeutic agent for the treatment of $p185^{HER2}$-overexpressing cancers.

Bispecific antibodies (BsAbs) with specificities for tumor-associated antigens and surface markers on immune effector cells have proved effective for retargeting effector cells to kill tumor targets both in vitro and in vivo (reviewed by Fanger, M. W. et al., *Immunol. Today* 10: 92–99 (1989); Fanger, M. W. et al., *Immunol. Today* 12: 51–54 (1991); and Nelson, H., *Cancer Cells* 3: 163–172 (1991)). $BsF(ab')_2$ fragments have often been used in preference to intact BsAbs in retargeted cellular cytotoxicity to avoid the risk of killing innocent bystander cells binding to the Fc region of the antibody. An additional advantage of $BsF(ab')_2$ over intact BsAbs is that they are generally much simpler to prepare free of contaminating monospecific molecules (reviewed by Songsivilai, S. and Lachmann, P. J., *Clin. Exp. Immunol.* 79: 315–321 (1990) and Nolan, O. and O'Kennedy, R., *Biochim. Biophys. Acta* 1040: 1–11 (1990)).

$BsF(ab')_2$ fragments are traditionally constructed by directed chemical coupling of Fab' fragments obtained by limited proteolysis plus mild reduction of the parent rodent monoclonal Ab (Brennan, M. et al., *Science* 229, 81–83 (1985) and Glennie, M. J. et al., *J. Immunol.* 139: 2367–2375 (1987)). One such $BsF(ab')_2$ fragment (anti-glioma associated antigen/anti-CD3) was found to have clinical efficacy in glioma patients (Nitta, T. et al., *Lancet* 335: 368–371 (1990) and another $BsF(ab')_2$ (anti-indium chelate/anti-carcinoembryonic antigen) allowed clinical imaging of colorectal carcinoma (Stickney, D. R. et al., *Antibody, Immunoconj. Radiopharm.* 2: 1–13 (1989)). Future $SsF(ab')_2$ destined for clinical applications are likely to be constructed from antibodies which are either human or at least "humanized" (Riechmann, L. et al., *Nature* 332: 323–327 (1988) to reduce their immunogenicity (Hale, G. et al., *Lancet i:* 1394–1399 (1988)).

Recently a facile route to a fully humanized $BsF(ab')_2$ fragment designed for tumor immunotherapy has been demonstrated (Shalaby, M. R. et al., *J. Exp. Med.* 175: 217–225 (1992)). This approach involves separate *E. coli* expression of each Fab' arm followed by traditional directed chemical coupling in vitro to form the $BsF(ab')_2$. One arm of the $BsF(ab')_2$ was a humanized version (Carter, P. et al., *Proc. Natl. Aced. Sci. USA* (1992a) and Carter, P., et al., *Bio/Technology* 10: 163–167 (1992b)) of the murine monoclonal Ab 4D5 which is directed against the $p185^{HER2}$ product of the protooncogene HER2 (c-erbB-2) (Fendly, B. M. et al. *Cancer Res.* 50: 1550–1558 (1989)). The humanization of the antibody 4D5 is shown in Example 1 of this application. The second arm was a minimalistically humanized anti-CD3 antibody (Shalaby et al. supra) which was created by installing the CDR loops from the variable domains of the murine parent monoclonal Ab UCHT1 (Beverley, P. C. L. and Callard, R. E., *Eur. J. Immunol.* 11: 329–334 (1981)) into the humanized anti-$p185^{HER2}$ antibody. The $BsF(ab')_2$ fragment containing the most potent humanized anti-CD3 variant (v1) was demonstrated by flow cytometry to bind specifically to a tumor target overexpressing $p185^{HER2}$ and to human peripheral blood mononuclear cells carrying CD3. In addition, $BsF(ab')_2$v1 enhanced the cytotoxic effects of activated human CTL 4-fold against SK-SR-3 tumor cells overexpressing $p185^{HER2}$. The example descries efforts to improve the antigen binding affinity of the humanized anti-CD3 arm by the judicious recruitment of a small number of additional murine residues into the minimalistically humanized anti-CD3 variable domains.

Materials and Methods

Construction of Mutations in the Anti-CD3 Variable Region Genes

The construction of genes encoding humanized anti-CD3 variant 1 (v1) variable light (V_l) and heavy (V_H) chain domains in phagemid pUC119 has been described (Shalaby et al. supra). Additional anti-CD3 variants were generated using an efficient site-directed mutagenesis method (Carter, P., *Mutagenesis: a practical approach,* (M. J. McPherson, Ed.), Chapter 1, IRL Press, Oxford, UK (1991)) using mismatched oligonucleotides which either install or remove unique restriction sites. Oligonucleotides used are listed below using lowercase to indicate the targeted mutations. Corresponding coding changes are denoted by the starting amino acid in one letter code followed by the residue numbered according to Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest,* 5th edition, National Institutes of Health, Bethesda, Md., USA (1991), then the replacement amino acid and finally the identity of the anti-CD3 variant:

HX11, 5' GTAGATAAATCCtctAACACAGC-CTAtCTGCAAATG 3' (SEQ.ID. NO. 11) V_HK75S, v6;

HX12, 5' GTAGATAAATCCAAAtctACAGC-CTAtCTGCAAATG 3' (SEQ.ID. NO. 12) V_H N76S, v7;

HX13, 5' GTAGATAAATCCtcttctACAGC-CTAtCTGCAAATG 3' (SEQ.ID. NO. 13) V_H K75S:N76S, v8;

X14, 5' CTTATAAAGGTGTTtCcACCTATaaCcA-gAaatTCAAGGatCGTTTCACgATAtc-CGTAGATAAATCC 3' (SEO.ID.NO. 14) V_H T57S:A60N:D61Q:S62K:V63F:G65D, v9;

LX6, 5' CTATACCTCCCGTCTgcatTCTGGAGTCCC 3' (SEQ.ID. NO. 15) V_L E55H, v11.

Oliconucleotides HX11, HX12 and HX13 each remove a site for BspMI, whereas LX6 removes a site for XhoI and HX14 installs a site for EcoRV (bold). Anti-CD3 variant v10 was constructed from v9 by site-directed mutagenesis using oligonucleotide HX13. Mutants were verified by dideoxynucleotide sequencing (Sanger, F. et al., *Proc. Natl. Acad, Sci. USA* 74: 5463–5467 (1977)).

E. coli Expression of Fab' Fragments

The expression plasmid, pAK19, for the co-secretion of light chain and heavy chain Fd' fragment of the most preferred humanized anti-$p185^{HER2}$ variant, HuMAb4D5-8, is described in Carter et al., 1992b, supre. Briefly, the Fab' expression unit is bicistronic with both chains under the

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transcriptional control of the ohoA promoter. Genes encoding humanized V_L and V_H domains are precisely fused on their 5' side to a gene segment encoding the heat-stable enterotoxin II signal sequence and on their 3' side to human k_1 C_L and IgG1C_H1 constant domain genes, respectively. The C_H1gene is immediately followed by a sequence encoding the hinge sequence CysAlaAla and followed by a bacteriophage λ t_o transcriptional terminator. Fab' expression plasmids for chimeric and humanized anti-CD3 variants (v1 to v4, Shalaby et al.,supra; v6 to v12, this study) were created from pAK19 by precisely replacing anti-p185^{HER2} V_L and V_H gene segments with those encoding murine and corresponding humanized variants of the anti-CD3 antibody, respectively, by sub-cloning and site-directed mutagenesis. The Fab' expression plasmid for the most potent humanized anti-CD3 variant identified in this study (v9) is designated pAK22. The anti-p185^{HER2} Fab' fragment was secreted from *E. coli* K12 strain 25F2 containing plasmid pAK19 grown for 32 to 40 hr at 37° C. in an aerated 10 liter fermentor. The final cell density was 120–150 OD_{550} and the titer of soluble and functional anti-p185^{HER2} Fab' was 1–2 g/liter as judged by antigen binding ELISA (Carter et al., 1992b, suora). Anti-CD3 Fab' variants were secreted from *E. coli* containing corresponding expression plasmids using very similar fermentation protocols. The highest expression titers of chimeric and humanized anti-CD3 variants were 200 mgaliter and 700 mgaliter, respectively, as judged by total immunoglobulin ELISA.

Construction of BsF(ab')$_2$ Fragments

Fab' fragments were directly recovered from *E. coli* fermentation pastes in the free thiol form (Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter et al., 1992b supra). Thioether linked BsF(ab')$_2$ fragments (anti-p185^{HER2}/anti-CD3) were constructed by the procedure of Glennie et al. supra with the following modifications. Anti-p185^{HER2} Fab'-SH in 100 mM Tris acetate, 5 mM EDTA (pH 5.0) was reacted with 0.1 vol of 40 mM N,N'-1,2-phenylenedimalemide (o-PDM) in dimethyl formamide for ~1.5 hr at 20° C. Excess o-PDM was removed by protein G purification of the Fab' maleimide derivative (Fab'-mal) followed by buffer exchange into 20 mM sodium acetate, 5 mM EDTA (pH 5.3) (coupling buffer) using centriprep-30 concentrators (Amicon). The total concentration of Fab' variants was estimated from the measured absorbance at 280 nm (HuMAb4D5-8 Fab' $e^{0.1\%}$=1.56, Carter et al., 1992b, supra). The free thiol content of Fab' preparations was estimated by reaction with 5,5' -dithiobis (2-nitrobenzoic acid) as described by Creighton, T. E., *Protein structure: a practical approach,* (T. E. Creighton, Ed.), Chapter 7, IRL Press, Oxford, UK (1990). Equimolar amounts of anti-p185^{HER2} Fab'-mal (assuming quantitative reaction of Fab'-SH with o-PDM) and each anti-CD3 Fab'-SH variant were coupled together at a combined concentration of 1 to 2.5 mg/ml in the coupling buffer for 14 to 48 hr at 4° C. The coupling reaction was adjusted to 4 mM cysteine at pH 7.0 and incubated for 15 min at 20 ° C. to reduce any unwanted disulfide-linked F(ab')$_2$ formed. These reduction conditions are sufficient to reduce inter-heavy chain disulfide bonds with virtually no reduction of the disulfide between light and heavy chains. Any free thiols generated were then blocked with 50 mM iodoacetamide. BsF(ab')$_2$ was isolated from the coupling reaction by S100-HR (Pharmacia) size exclusion chromatography (2.5 cm×100 cm) in the presence of PBS. The BsF(ab')$_2$ samples were passed through a 0.2 mm filter flash frozen in liquid nitrogen and stored at −70° C.

Flow Cytometric Analysis of F(ab')$_2$Binding to Jurkat Cells

The Jurkat human acute T cell leukemia cell line was purchased from the American Type Culture Collection (Manassas Va.) (ATCC TIB 152) and grown as. recommended by the ATCC. Aliquots of 10^6 Jurkat cells were incubated with appropriate concentrations of BsF(ab')$_2$ (anti-p185^{HER2}/anti-CD3 variant) or control mono-specific anti-p185^{HER2} F(ab')$_2$ in PBS plus 0.1% (w/v) bovine serum albumin and 10 mM sodium azide for 45 min at 4° C. The cells were washed and then incubated with fluorescein-conjugated goat anti-human F(ab')$_2$ (Organon Teknika, West Chester, Pa.) for 45 min at 4° C. Cells were washed and analyzed on a FACScan® (Becton Dickinson and Co., Mountain View, Calif.). Cells (8×10^3) were acquired by list mode and gated by forward light scatter versus side light scatter excluding dead cells and debris.

Results

Design of Humanized anti-CD3 Variants

The most potent humanized anti-CD3 variant previously identified, v1, differs from the murine parent antibody, UCHT1 at 19 out of 107 amino acid residues within V_L and at 37 out of 122 positions within V_H (Shalaby et al.,supra) 1992). Here we recruited back additional murine residues into anti-CD3 v1 in an attempt to improve the binding affinity for CD3. The strategy chosen was a compromise between minimizing both the number of additional murine residues recruited and the number of anti-CD3 variants to be analyzed. We focused our attentions on a few CDR residues which were originally kept as human sequences in our minimalistic humanization regime. Thus human residues in V_H CDR2 of anti-CD3 v1 were replaced en bloc with their murine counterparts to give anti-CD3 v9: T57S:A60N:D61Q:S62K:V63F:G65D (SEQ ID NO:20). Similarly, the human residue E55 in V_L CDR2 of anti-CD3 v_1 was replaced with histidine from the murine anti-CD3 antibody to generate anti-CD3 v11. In addition, V_H framework region (FR) residues 75 and 76 in anti-CD3 v1 were also replaced with their murine counterparts to create anti-CD3 v8: K75S:N76S. V_H residues 75 and 76 are located in a loop close to V_H CDR1 and CDR2 and therefore might influence antigen binding. Additional variants created by combining mutations at these three sites are described below.

Preparation of BsF(ab')$_2$ Fragments

Soluble and functional anti-p185^{HER2} and anti-CD3 Fab' fragments were recovered directly from corresponding *E. coli* fermentation pastes with the single hinge cysteine predominantly in the free thiol form (75–100% Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter et al., 1992b, supra). Thioether-linked BsF(ab')$_2$ fragments were then constructed by directed coupling using o-PDM as described by Glennie et al., supra. One arm was always the most potent humanized anti-p185^{HER2} variant, HuMAb4D5-8 (Carter et al., 1992a, supra) and the other either a chimeric or humanized variant of the anti-CD3 antibody. Anti-p185^{HER2} Fab'-SH was reacted with o-PDM to form the maleimide derivative (Fab'-mal) and then coupled to the Fab'-SH for each anti-CD3 variant. F(ab')$_2$ was then purified away from unreacted Fab' by size exclusion chromatography as shown for a representative preparation (BsF(ab')$_2$ v8) in data not shown. The F(ab')$_2$ fragment represents ~54% of the total amount of antibody fragments (by mass) as judged by integration of the chromatograph peaks.

SDS-PAGE analysis of this BsF(ab')$_2$v8 preparation under non-reducing conditions gave one major band with the expected mobility (M, ~96 kD) as well as several very minor

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bands (data not shown). Amino-terminal sequence analysis of the major band after electroblotting on to polyvinylidene difluoride 76 are located in a loop close to V_H CDR1 and CDR2 and therefore might membrane Matsudaira, P., *J. Biol. Chem.* 262: 10035–10038 (1987) gave the expected mixed sequence from a stoichiometric 1:1 mixture of light and heavy chains (V_L/V_H: D/E, I/V, Q/D, M/L, T/V, D/E, S/S) expected for BsF(ab')$_2$. The amino terminal region of both light chains are identical as are both heavy chains and correspond to consensus human FR sequences. We have previously demonstrated that F(ab')$_2$ constructed by directed chemical coupling carry both anti-p185^{HER2} and anti-CD3 antigen specificities (Shalaby et al., supra). The level of contamination of the BsF(ab')$_2$ with monospecific F(ab')$_2$ iS likely to be very low since mock coupling reactions with either anti-p185^{HER2} w Fab'-mal or anti-CD3 Fab'-SH alone did not yield detectable quantities of F(ab')$_2$. Furthermore the coupling reaction was subjected to a mild reduction step followed by alkylation to remove trace amounts of disulfide-linked F(ab')$_2$ that might be present. SDS-PAGE of the purified F(ab')$_2$ under reducing conditions gave two major bands with electrophoretic mobility and amino terminal sequence anticipated for free light chain and thioether-linked heavy chain dimers.

Scanning LASER densitometry of a o-PDM coupled F(ab')$_2$ preparation suggest that the minor species together represent ~10% of the protein. These minor contaminants were characterized by amino terminal sequence analysis and were tentatively identified on the basis of stoichiometry of light and heavy chain sequences and their electrophoretic mobility (data not shown). These data are consistent with the minor contaminants including imperfect F(ab')$_2$ in which the disulfide bond between light and heavy chains is missing in one or both arms, trace amounts of Fab' and heavy chain thioether-linked to light chain.

Binding of BsF(ab')$_2$ to Jurkat Cells

Binding of BsF(ab')$_2$ containing different anti-CD3 variants to Jurkat cells (human acute T cell leukemia) was investigated by flow cytometry (data not shown). BsF(ab')$_2$v9 binds much more efficiently to Jurkat cells than does our starting molecule, BsF(ab')$_2$vl, and almost as efficiently as the chimeric BsF(ab')$_2$. Installation of additional murine residues into anti-CD3 v9 to create v10 (V_HK75S:N76S) and v12 (V_HK75S:N76S plus V_L E55H) did not further improve binding of corresponding BsF(ab')$_2$to Jurkat cells. Nor did recruitment of these murine residues into anti-CD3 v1 improve Jurkat binding: V_HK75S (v6), V_HN76S (v7), V_HK75S:N76S (V8), V_LE55H (v11) (not shown). BsF(ab')$_2$v9 was chosen for future study since it is amongst the most efficient variants in binding to Jurkat cells and contains fewest murine residues in the humanized anti-CD3 arm. A monospecific anti-p185^{HEE2} F(ab')$_2$ did not show significant binding to Jurkat cells consistent with the interaction being mediated through the anti-CD3 arm.

Discussion

A minimalistic strategy was chosen to humanize the anti-p185^{HER2} (Carter et al., 1992a, supra) and anti-CD3 arms (Shalaby et al., supra) of the BsF(ab')$_2$ in this study in an attempt to minimize the potential immunogenicity of the resulting humanized antibody in the clinic. Thus we tried to install the minimum number of murine CDR and FR residues into the context of consensus human variable domain sequences as required to recruit antigen-binding affinity and biological properties comparable to the murine parent antibody. Molecular modeling was used firstly to predict the murine FR residues which might be important to antigen binding and secondly to predict the murine CDR residues that might not be required. A small number of humanized variants were then constructed to test these predictions.

Our humanization strategy was very successful for the anti-p185^{HER2} antibody where one out of eight humanized variants (HuMAb4D5-8, IgG1) was identified that bound the p185^{HER2} antigen ~3-fold more tightly than the parent murine antibody (Carter et al., 1992a, supra). HuMAb4D5-8 contains a total of five murine FR residues and nine murine CDR residues, including V_H CDR2 residues 60–65, were discarded in favor of human counterparts. In contrast, BsF(ab')$_2$v1 containing the most potent humanized anti-CD3 variant out of four originally constructed (Shalaby et al., supra) binds J6 cells with an affinity (K_d) of 140 nM which is ~70-fold weaker than that of the corresponding chimeric BsF(ab')$_2$.

Here we have restored T cell binding of the humanized anti-CD3 close to that of the chimeric variant by replacing six human residues in V_H CDR2 with their murine counterparts: T57S:A60N:D61Q:S62K:V63F:G65D (anti-CD3 v9, FIG. **5**). It appears more likely that these murine residues enhance antigen binding indirectly by influencing the conformation of residues in the N-terminal part of V_H CDR2 rather than by directly contacting antigen. Firstly, only N-terminal residues in V_H CDR2 (50–58) have been found to contact antigen in one or more of eight crystallographic structures of antibody/antigen complexes (Kabat et al., supra; and Mian, I. S. et al., *J. Mol. Biol* 217: 133–151 (1991), FIG. **5**). Secondly, molecular modeling suggests that residues in the C-terminal part of V_H CDR2 are at least partially buried (FIG. **5**). BsF(ab')$_2$v9 binds to SK-BR-3 breast tumor cells with equal efficiency to BsF(ab')$_2$v1 and chimeric BsF(ab')$_2$ as anticipated since the anti-p185^{HER2} arm is identical in all of these molecules (Shalaby et al., supra, not shown).

Our novel approach to the construction of BsF(ab')$_2$ fragments exploits an *E. coli* expression system which secretes humanized Fab' fragments at gram per liter titers and permits their direct recovery as Fab'-SH (Carter et al., 1992b, supra). Traditional directed chemical coupling of Fab'-SH fragments is then used to form BsF(ab')$_2$ in vitro (Brennan et al.,supra; and Glennie et al., supra). This route to Fab'-SH obviates problems which are inherent in their generation from intact antibodies: differences in susceptibility to proteolysis and nonspecific cleavage resulting in heterogeneity, low yield as well as partial reduction that is not completely selective for the hinge disulfide bonds. The strategy of using *E. coli*-derived Fab'-SH containing a single hinge cysteine abolishes some sources of heterogeneity in BsF(ab')$_2$ preparation such as intra-hinge disulfide formation and contamination with intact parent antibody whilst greatly diminishes others, eg. formation of F(ab')$_3$ fragments.

BsF(ab')2 fragments constructed here were thioether-linked as originally described by Glennie et al., supra with future in vivo testing of these molecules in mind. Thioether bonds, unlike disulfide bonds, are not susceptible to cleavage by trace amounts of thiol, which led to the proposal that thioether-linked F(ab')$_2$ may be more stable than disulfide-linked F(ab')$_2$ in vivo (Glennie et al., supra). This hypothesis is supported by our preliminary pharmacokinetic experiments in normal mice which suggest that thioether-linked BsF(ab')$_2$ v1 has a 3-fold longer plasma residence time than BsF(ab')$_2$ v1 linked by a single disulfide bond. Disulfide and thioether-linked chimeric BsF(ab')$_2$ were found to be indistinguishable in their efficiency of cell binding and in their retargeting of CTL cytotoxicity, which suggests that o-PDM directed coupling does not compromise binding of the

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$BsF(ab')_2$ to either antigen (not shown). Nevertheless the nature of the linkage appears not to be critical since a disulfide-linked $BsF(ab')_2$ (murine anti-$p185^{HER2}$/murine anti-CD3) was recently shown by others (Nishimura et al., Int. *J. Cancer* 50: 800–804 (1992) to have potent anti-tumor activity in nude mice. Our previous study (Shalaby et al., supra) together with this one and that of Nishimura, T. et al., supra improve the potential for using $BsF(ab')_2$ in targeted immunotherapy of $p185^{HER2}$-overexpressing cancers in humans.

Example 4

Humanization of an anti-CD18 Antibody

A murine antibody directed against the leukocyte adhesion receptor β-chain (known as the H52 antibody) was humanized following the methods described above. FIGS. **6A** and **6B** provide amino acid sequence comparisons for the murine and humanized antibody light chains and heavy chains.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 26

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
                 20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
                 20                  25                  30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75
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-continued

```
Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 1 00                 1 05

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                  120
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
                 20                  25                  30

Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                 20                  25                  30

Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Ser Asp Thr Tyr Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
                 65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Gly Ala Val Ser
                 95                 1 00                 1 05

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                  120
```


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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
1 5 10 15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
50 55 60

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
65 70 75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
95 1 00 1 05

Ile Lys Arg Ala
109

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
65 70 75

Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
95 1 00 1 05

Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGATATCC AGCTGACCCA GTCTCCA 27

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTGATCTC CAGCTTGGTA CCHSCDCCGA A 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTAGATAAAT CCTCTAACAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTAGATAAAT CCAAATCTAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTAGATAAAT CCTCTTCTAC AGCCTATCTG CAAATG 36

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(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTATAAAGG TGTTTCCACC TATAACCAGA AATTCAAGGA TCGTTTCACG 50

ATATCCGTAG ATAAATCC 68

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTATACCTCC CGTCTGCATT CTGGAGTCCC 30

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
1 5 10 15

Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Arg
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
35 40 45

Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser
50 55 60

Lys Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
65 70 75

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
80 85 90

Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly Gly Thr Lys Leu Glu
95 1 00 1 05

Ile Lys
107

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Arg
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys

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```
                35                  40                  45

Leu Leu Ile Tyr Tyr Thr Ser Arg Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

Gly Asn Thr Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 1 00                 1 05

Ile Lys
    107
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
                20                  25                  30

Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 1 00                 1 05

Ile Lys
    107
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
  1               5                  10                  15

Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu
                35                  40                  45

Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
                50                  55                  60

Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser
                65                  70                  75

Ser Ser Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp
                80                  85                  90
```


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```
Ser Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                 95                  1 00                 1 05

Asp Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val
                110                  115                  120

Ser Ser
    122


(2) INFORMATION FOR SEQ ID NO:20:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

Gly Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
                50                  55                  60

Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
                65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                95                  1 00                 1 05

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
               110                  115                  120

Ser Ser
    122


(2) INFORMATION FOR SEQ ID NO:21:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                20                  25                  30

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ser Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
                65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
                95                  1 00                 1 05

Ser Gly Leu Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
               110                  115                  120
```


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Ser Ser
122

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Lys Gln Ser His Gly Lys Ser Leu
35 40 45

Glu Trp Ile Gly Gly Phe Asn Pro Lys Asn Gly Gly Ser Ser His
50 55 60

Asn Gln Arg Phe Met Asp Lys Ala Thr Leu Ala Val Asp Lys Ser
65 70 75

Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp
80 85 90

Ser Gly Ile Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 1 00 1 05

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
275 280 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
290 295 300

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
305 310 315

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
320 325 330

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```
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                335                 340                 345

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                350                 355                 360

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                365                 370                 375

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                380                 385                 390

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                395                 400                 405

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                410                 415                 420

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                425                 430                 435

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                440                 445                 450

Ser Pro Gly Lys
            454
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 469 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  1                 5                  10                  15

Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
                 20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly
                 35                  40                  45

Tyr Thr Phe Thr Glu Tyr Thr Met His Trp Met Arg Gln Ala Pro
                 50                  55                  60

Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Asn Pro Lys Asn Gly
                 65                  70                  75

Gly Thr Ser His Asn Gln Arg Phe Met Asp Arg Phe Thr Ile Ser
                 80                  85                  90

Val Asp Lys Ser Thr Ser Thr Ala Tyr Met Gln Met Asn Ser Leu
                 95                 1 00                 1 05

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Trp Arg Gly
                110                 115                 120

Leu Asn Tyr Gly Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Gln
                125                 130                 135

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
                140                 145                 150

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
                155                 160                 165

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
                170                 175                 180

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
                185                 190                 195

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
                200                 205                 210
```


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```
Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr
                215                 220                 225

Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr
                230                 235                 240

Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                260                 265                 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                275                 280                 285

Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
                290                 295                 300

Val Asp Gly Met Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                305                 310                 315

Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val
                320                 325                 330

Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
                335                 340                 345

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                350                 355                 360

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                365                 370                 375

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                380                 385                 390

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                395                 400                 405

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
                410                 415                 420

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                425                 430                 435

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                440                 445                 450

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                455                 460                 465

Ser Pro Gly Lys
            469
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Asp Val Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Asn Cys Arg Ala Ser Gln Asp Ile Asn
                 20                  25                  30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asn Gly Thr Val Lys
                 35                  40                  45

Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
                 65                  70                  75
```


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```
Ser Asn Leu Asp Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln
                 80                  85                  90

Gly Asn Thr Leu Pro Pro Thr Phe Gly Gly Gly Thr Lys Val Glu
                 95                 1 00                 1 05

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
                110                 115                  120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
                125                 130                  135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
                140                 145                  150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
                155                 160                  165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
                170                 175                  180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
                185                 190                  195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
                200                 205                  210

Arg Gly Glu Cys
            214
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
  1                  5                  10                  15

Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
                 20                  25                  30

Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
                 35                  40                  45

Gln Asp Ile Asn Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly
                 50                  55                  60

Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser
                 65                  70                  75

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
                 80                  85                  90

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
                 95                 1 00                 1 05

Tyr Cys Gln Gln Gly Asn Thr Leu Pro Pro Thr Phe Gly Gln Gly
                110                 115                  120

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
                125                 130                  135

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
                140                 145                  150

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
                155                 160                  165

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
                170                 175                  180

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                185                 190                  195
```


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```
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
                200                 205                 210

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
                215                 220                 225

Lys Ser Phe Asn Arg Gly Glu Cys
                230          233


(2) INFORMATION FOR SEQ ID NO:26:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 122 amino acids
          (B) TYPE: Amino Acid
          (D) TOPOLOGY: Linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1                5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr
                20                  25                  30

Gly Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Leu Ile Asn Pro Tyr Lys Gly Val Thr Thr Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser
                65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Ser Gly Tyr Tyr Gly Asp Ser
                95                 1 00                 1 05

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
               110                 115                  120

Ser Ser
    122
```

We claim:

1. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat.

2. The humanized variable domain of claim **1** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

3. The humanized variable domain of claim **1** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

4. The humanized variable domain of claim **1** wherein the human antibody variable domain is a consensus human variable domain.

5. The humanized variable domain of claim **1** wherein the residue at site 4L has been substituted.

6. The humanized variable domain of claim **1** wherein the residue at site 38L has been substituted.

7. The humanized variable domain of claim **1** wherein the residue at site 43L has been substituted.

8. The humanized variable domain of claim **1** wherein the residue at site 44L has been substituted.

9. The humanized variable domain of claim **1** wherein the residue at site 58L has been substituted.

10. The humanized variable domain of claim **1** wherein the residue at site 62L has been substituted.

11. The humanized variable domain of claim **1** wherein the residue at site 65L has been substituted.

12. The humanized variable domain of claim **1** wherein the residue at site 66L has been substituted.

13. The humanized variable domain of claim **1** wherein the residue at site 67L has been substituted.

14. The humanized variable domain of claim **1** wherein the residue at site 68L has been substituted.

15. The humanized variable domain of claim **1** wherein the residue at site 69L has been substituted.

16. The humanized variable domain of claim **1** wherein the residue at site 73L has been substituted.

17. The humanized variable domain of claim **1** wherein the residue at site 85L has been substituted.

18. The humanized variable domain of claim **1** wherein the residue at site 98L has been substituted.

19. The humanized variable domain of claim **1** wherein the residue at site 2H has been substituted.

20. The humanized variable domain of claim **1** wherein the residue at site 4H has been substituted.

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21. The humanized variable domain of claim 1 wherein the residue at site 36H has been substituted.

22. The humanized variable domain of claim 1 wherein the residue at site 39H has been substituted.

23. The humanized variable domain of claim 1 wherein the residue at site 43H has been substituted.

24. The humanized variable domain of claim 1 wherein the residue at site 45H has been substituted.

25. The humanized variable domain of claim 1 wherein the residue at site 69H has been substituted.

26. The humanized variable domain of claim 1 wherein the residue at site 70H has been substituted.

27. The humanized variable domain of claim 1 wherein the residue at site 74H has been substituted.

28. The humanized variable domain of claim 1 wherein the residue at site 92H has been substituted.

29. An antibody comprising the humanized variable domain of claim 1.

30. An antibody which binds $p185^{HER2}$ and comprises a humanized antibody variable domain, wherein the humanized antibody variable domain comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind $p185^{HER2}$ incorporated into a human antibody variable domain, and further comprises a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

31. The antibody of claim 30 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

32. The antibody of claim 30 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

33. The antibody of claim 30 wherein the human antibody variable domain is a consensus human variable domain.

34. The antibody of claim 30 wherein the residue at site 4L has been substituted.

35. The antibody of claim 30 wherein the residue at site 38L has been substituted.

36. The antibody of claim 30 wherein the residue at site 43L has been substituted.

37. The antibody of claim 30 wherein the residue at site 44L has been substituted.

38. The antibody of claim 30 wherein the residue at site 46L has been substituted.

39. The antibody of claim 30 wherein the residue at site 58L has been substituted.

40. The antibody of claim 30 wherein the residue at site 62L has been substituted.

41. The antibody of claim 30 wherein the residue at site 65L has been substituted.

42. The antibody of claim 30 wherein the residue at site 66L has been substituted.

43. The antibody of claim 30 wherein the residue at site 67L has been substituted.

44. The antibody of claim 30 wherein the residue at site 68L has been substituted.

45. The antibody of claim 30 wherein the residue at site 69L has been substituted.

46. The antibody of claim 30 wherein the residue at site 73L has been substituted.

47. The antibody of claim 30 wherein the residue at site 85L has been substituted.

48. The antibody of claim 30 wherein the residue at site 98L has been substituted.

49. The antibody of claim 30 wherein the residue at site 2H has been substituted.

50. The antibody of claim 30 wherein the residue at site 4H has been substituted.

51. The antibody of claim 30 wherein the residue at site 36H has been substituted.

52. The antibody of claim 30 wherein the residue at site 39H has been substituted.

53. The antibody of claim 30 wherein the residue at site 43H has been substituted.

54. The antibody of claim 30 wherein the residue at site 45H has been substituted.

55. The antibody of claim 30 wherein the residue at site 69H has been substituted.

56. The antibody of claim 30 wherein the residue at site 70H has been substituted.

57. The antibody of claim 30 wherein the residue at site 74H has been substituted.

58. The antibody of claim 30 wherein the residue at site 75H has been substituted.

59. The antibody of claim 30 wherein the residue at site 76H has been substituted.

60. The antibody of claim 30 wherein the residue at site 78H has been substituted.

61. The antibody of claim 30 wherein the residue at site 92H has been substituted.

62. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a consensus human variable domain, and further comprising an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

63. A humanized antibody which lacks immunogenicity compared to a non-human parent antibody upon repeated administration to a human patient in order to treat a chronic disease in that patient, wherein the humanized antibody comprises non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprises an amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, 75H, 76H, 78H and 92H, utilizing the numbering system set forth in Kabat.

64. A humanized variant of a non-human parent antibody which binds an antigen and comprises a human variable domain comprising the most frequently occurring amino acid residues at each location in all human immunoglobulins of a human heavy chain immunoglobulin subgroup wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) introduces a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the V_L–V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another.

65. The humanized variant of claim 63 which binds the antigen up to 3-fold more in the binding affinity than the parent antibody binds antigen.

66. A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining

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Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 24H, 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

67. The humanized variable domain of claim **66** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

68. The humanized variable domain of claim **66** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

69. The humanized variable domain of claim **66** wherein the human antibody variable domain is a consensus human variable domain.

70. The humanized variable domain of claim **66** wherein the residue at site 24H has been substituted.

71. The humanized variable domain of claim **66** wherein the residue at site 73H has been substituted.

72. The humanized variable domain of claim **66** wherein the residue at site 76H has been substituted.

73. The humanized variable domain of claim **66** wherein the residue at site 78H has been substituted.

74. The humanized variable domain of claim **66** wherein the residue at site 93H has been substituted.

75. The humanized variable domain of claim **66** which further comprises an amino acid substitution at site 71H.

76. The humanized variable domain of claim **66** which further comprises amino acid substitutions at sites 71H and 73H.

77. The humanized variable domain of claim **66** which further comprises amino acid substitutions at sites 71H, 73H and 78H.

78. An antibody comprising the humanized variable domain of claim **66**.

79. A humanized variant of a non-human parent antibody which binds an antigen, wherein the humanized variant comprises Complementarity Determining Region (CDR) amino acid residues of the non-human parent antibody incorporated into a human antibody variable domain, and further comprises Framework Region (FR) substitutions at heavy chain positions 71H, 73H, 78H and 93H, utilizing the numbering system set forth in Kabat.

80. A humanized antibody variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution where the substituted FR residue:

(a) noncovalently binds antigen directly;

(b) interacts with a CDR; or

(c) participates in the V_L–V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another, and wherein the substituted FR residue is at a site selected from the group consisting of: 4L, 38L, 43L, 44L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 24H, 36H, 39H, 43H, 45H, 69H, 70H, 73H, 74H, 76H, 78H, 92H and 93H, utilizing the numbering system set forth in Kabat.

81. The humanized variable domain of claim **80** wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

82. The humanized variable domain of claim **80** wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,407,213 B1
DATED : June 18, 2002
INVENTOR(S) : Carter et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 88,
Line 63, please delete "63" and insert therefor -- 79 --.

Signed and Sealed this

Third Day of December, 2002



JAMES E. ROGAN
Director of the United States Patent and Trademark Office

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(54) Title: RATIONALLY DESIGNED ANTIBODIES

(57) Abstract: Antibodies or fragments thereof having at least two CDR regions replaced or fused with biologically active peptides are described. Compositions containing such antibodies or fragments thereof are useful in therapeutic and diagnostic modalities.

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RATIONALLY DESIGNED ANTIBODIES

Related Applications

This application is a continuation-in-part of U.S. Application Serial No. 10/006,593 filed December 5, 2001 which claims priority to U.S. Provisional Patent Application No. 60/251,448 filed December 5, 2000, and to U.S. Provisional Patent Application No. 60/288,889 filed May 4, 2001, and to U.S. Provisional Patent Application No. 60/294,068 filed May 29, 2001. The disclosures of these three provisional applications are incorporated herein in their entirety by this reference.

TECHNICAL FIELD

The present invention relates to antibody molecules and biologically active peptides as diagnostic and therapeutic reagents.

BACKGROUND OF RELATED ART

Antibodies are produced by B lymphocytes and defend against infection. Antibodies are produced in millions of forms, each with a different amino acid sequence. Antibody molecules are composed of two identical light chains and two identical heavy chains. When digested by the enzyme papain, two identical Fab fragments are produced along with one Fc fragment. When digested with the enzyme pepsin one $F(ab')_2$ fragment is produced. Light and heavy chains consist of constant and variable regions. Within the variable regions are hypervariable regions (aka complementarity determining regions (CDRs)) which form the antigen binding site. The remaining parts of the variable regions are referred to as framework regions.

Important biological functions, such as receptor binding, activation and enzymatic activity, are often attributable to discrete regions of larger protein molecules, comprising a limited number of amino acid residues. Peptides displaying binding, activation or enzymatic activity have also been discovered by screening libraries of peptides generated by the random linking of amino acid residues. These peptides may not correspond to a linear arrangement of amino acids in a larger protein

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molecule exhibiting similar biological activity and are referred to as discontinuous peptide epitopes or mimotopes. Certain peptide mimetics have been described and cloned. See, e.g., U.S. Pat. No. 6,083,913 (thrombopoietin (TPO) mimetic), U.S. Pat. No. 5,835,382 (erythropoietin (EPO) mimetic), U.S. Pat. No. 5,830,851 (EPO mimetic) and Wrighton et al, Science, (1996) 273:458-63. Peptide epitopes and mimotopes due to their small size are potentially advantageous over large protein molecules for use as therapeutic reagents. However, the results with these peptides as therapeutics may often be unsatisfactory. One drawback to the use of peptides as therapeutic reagents is that they are generally unstable *in vivo*, i.e., their clearance rates from serum may be quite rapid. In addition, it is difficult to predict the activity, therapeutic or otherwise, of a peptide if it is fused into a larger molecule since conformational changes and other molecular forces may interfere with or totally negate the activity of the peptide. Attempts have been made to introduce certain polypeptides into CDR regions of antibodies. See, e.g., PCT Appln. WO 94/18221. However, as mentioned previously, due to conformational changes which may be caused by surrounding amino acids, the biological activity of active polypeptides may be diminished or negated. Therefore, it is an object herein to provide rationally designed antibodies or fragments thereof which include biologically active peptides for use as diagnostic and therapeutic reagents.

SUMMARY

Provided herein are biologically active recombinant antibodies and fragments thereof that mimic the activity of biologically active peptides, methods of making such antibodies and methods for their use in therapy and diagnosis. These antibodies and fragments thereof do not suffer from some of the disadvantages of isolated peptides, as antibodies naturally have long serum half-lives and are highly specific in binding their target. It has surprisingly been found that incorporation of particular amino acids surrounding a target peptide that has been combined into an antibody molecule actually increases the biological activity of the peptide.

Immunoglobulins or fragments thereof have a peptide of interest inserted into a complementarity determining region (CDR) of an antibody molecule. The antibody molecule serves as a scaffold for presentation of the peptide and confers upon the

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peptide enhanced stability. The peptide optionally replaces all the amino acids of a CDR region, or may be added to an existing CDR, whereby the original antigen specificity is disrupted, wherein the CDR region is defined by either of the two accepted schemes (See, Kabat et al., Sequences of Proteins of Immunologics Interest, 5th ed (1991), NIH Publication 91-3242 and Chothia et al. J.Mol. Bio (1992) (227)776-98.) Furthermore, additional amino acids may be randomly introduced which flank the peptide and allow for the screening of optimum peptide presentation in the antibody framework. It has been surprisingly found that in certain cases a proline flanking the peptide provides an increase in biological activity.

In particular embodiments an immunoglobulin molecule or fragment has amino acids residues corresponding to one complementarity determining region (CDR) replaced with amino acid residues comprising a biologically active hemopoietic or thrombopoietic peptide. In another particular embodiment, amino acid residues corresponding to at least two complementarity determining regions (CDRs) are each replaced by amino acid residues comprising such a biologically active peptide. In a single immunoglobulin molecule or fragment thereof, one or more complementarity determining regions can be replaced with a peptide; for example, CDR3 of a heavy chain, CDR3 of a light chain, CDR3 of both a heavy and light chain, CDR2 and CDR3 of a heavy chain, or CDR2 and CDR3 of a light chain. Other combinations of replaced CDR regions are possible, including the replacement of CDR1. In addition, instead of replacement of a CDR, one could add the peptide to a native CDR without actual replacement of amino acid residues while still disrupting the original antigen specificity.

Thus, in one aspect, a biologically active peptide is provided with enhanced activity by adding a proline to its carboxy terminus to form a proline-extended biologically active peptide which is used to replace or add to at least a portion of at least one CDR region in an immunoglobulin molecule or fragment thereof. In another aspect, an immunoglobulin molecule or fragment thereof is provided which has either a TPO mimetic peptide or EPO mimetic peptide as a replacement for at least one native CDR region. In this aspect, the TPO mimetic peptide or EPO mimetic peptides may optionally be proline-extended as described herein.

In further particular embodiments the immunoglobulin molecule or fragment thereof is an Fab, a ScFv, a heavy chain variable region, a light chain or a full IgG molecule. The immunoglobulin molecule or fragment thereof can also have a dimerization domain, so as to enable immunoglobulin molecules which have only one CDR replaced with a peptide to dimerize and thus activate receptors that require dimerization for activation.

In certain embodiments, the biologically active peptide can be a linear peptide epitope or a discontinuous peptide epitope. Furthermore, the biologically active peptide, when substituted for a CDR region, can have in addition to proline, one, two or more additional flanking amino acid residues proximate to the amino and/or the carboxyl termini of the peptide, which are positioned between the peptide and immunoglobulin framework region residues (i.e., at what was the junction between a CDR and the adjoining framework). The flanking amino acid residues are not typically present in the active peptide. If preferred flanking amino acid residues are already known, the flanking amino acid residues are encoded by codons which designate those specific amino acid residues. However, by initially utilizing codons, such as NNK, NNY, NNR, NNS and the like, which designate multiple amino acid residues, a collection of peptides that differ from one another merely by the flanking residues is generated. The flanking amino acid residues may determine the presentation of the peptide in the immunoglobulin molecule or fragment thereof and thus may influence the binding and/or biological activity exhibited by the peptide. This random collection of flanking amino acids allows for the selection of the best context to display the peptide sequence within the antibody framework that results in specific binding to the target molecule and the exhibition of optimal biological activity. Screening of libraries of immunoglobulins having a common peptide but different flanking amino acid residues can be carried out using binding, growth and activation assays known by those skilled in the art and as described herein.

The peptide replacing the amino acid residues comprising a CDR can be any peptide which specifically binds a target molecule and whose utility could be altered by incorporation in an antibody framework. The peptide could also exhibit a specific activity (e.g., agonist, antagonist, enzymatic, etc.). In a particular embodiment the

peptide is an agonist or an antagonist for a cell surface receptor. For example, the cell surface receptor can be for a cytokine, a growth factor, or a growth inhibitor.

In particularly useful embodiments, replacement of at least a portion of a CDR with a peptide provides an antibody that acts as an agonist. The peptide used to replace at least a portion of a CDR may itself have agonist properties. Alternatively, the peptide (although specifically binding to a receptor) may not exhibit agonist activity. Rather, agonist activity might be exhibited only when the peptide is substituted for at least a portion of a CDR and is thus present in the engineered antibody. In such embodiments, the presence or absence of proline flanking the peptide is not critical, but can, in some instances, be preferred.

Thus, in one aspect the present disclosure provides for an agonist antibody comprising an antibody framework engineered to contain at least one biologically active peptide inserted at, or in place of at least a portion of, one or more CDRs. The biologically active peptide may or may not exhibit agonist activity prior to insertion into the antibody framework. In certain embodiments the antibody framework is engineered to contain two peptides capable of dimerizing with each other.

In yet another aspect, the present disclosure provides for an immunoglobulin molecule or fragment thereof comprising a region where amino acid residues corresponding to at least a portion of a complementary determining region (CDR) are replaced with a biologically active peptide, whereby the immunoglobulin molecule or fragment thereof exhibits agonist activity. The biologically active peptide may or may not exhibit agonist activity prior to insertion into the antibody framework. In particularly useful embodiments the immunoglobulin molecule or fragment thereof exhibits c-mpl agonist activity.

In yet another aspect, the present disclosure provides for an immunoglobulin molecule or fragment thereof comprising a biologically active peptide inserted at a complementary determining region (CDR), whereby the immunoglobulin molecule or fragment thereof exhibits agonist activity.

In yet another aspect, the present disclosure provides for an immunoglobulin molecule or fragment thereof comprising a region where amino acid residues corresponding to at least a portion of a complementary determining region (CDR) are

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replaced with a biologically active peptide, whereby the immunoglobulin molecule or fragment thereof exhibits c-mpl agonist activity.

In further particular embodiments, the peptide replacing the amino acids of a CDR is an agonist TPO mimetic peptide. One such agonist peptide has at least the sequence IEGPTLRQWLAARA (SEQ. ID. NO. 1). Other sequences are possible for TPO agonist mimetic peptides, which can be found using binding, growth and activation assays known by those skilled in the art and as described herein. Agonist TPO mimetic peptides when positioned in CDR regions can have one or more additional amino acid residues at the amino and/or carboxyl termini of the peptide which become covalently bonded to immunoglobulin framework residues. One such TPO mimetic peptide has an additional proline residue added to the carboxyl terminus; IEGPTLRQWLAARAP (SEQ. ID. NO: 2). Other immunoglobulin molecules or fragments thereof have a CDR region replaced by the TPO mimetic peptides comprising the amino acid sequence of SEQ. ID. NOs: 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, and 49 (see Fig. 5).

Another biologically active peptide that can replace the amino acid residues of a CDR is an agonist EPO mimetic peptide. One such EPO agonist peptide has as its amino acid sequence DYHCRMGPLTWVCKPLGG (SEQ. ID. NO: 3). Other amino acid sequences are possible for EPO agonist mimetic peptides, which can be found using binding, growth and activation assays known by those skilled in the art and as described herein. Agonist EPO mimetic peptides when located in CDR regions can also have one or more additional amino acid residues at the amino and/or carboxyl termini of the peptide which become covalently bonded to immunoglobulin residues. Thus, in particular embodiments provided herein are immunoglobulin molecules (IgG) or fragments (e.g., Fab, scFv, heavy or light chains) that have a CDR region replaced with a TPO or EPO mimetic peptide. For example, the TPO peptide can include at least the sequence IEGPTLRQWLAARA (SEQ. ID. NO:1) and may further optionally have an additional proline at the immediate downstream position. The EPO mimetic encompasses at least the sequence DYHCRMGPLTWVCKPLGG (SEQ. ID. NO: 3). Likewise, it may optionally have an additional proline at the immediate downstream position.

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Any immunoglobulin molecule (antibody) or fragment thereof could potentially provide the framework and have a CDR replaced with a peptide according to the present disclosure. For therapeutic or *in vivo* diagnostic use it is preferable that the antibody is of human origin or humanized, such as an anti-tetanus toxoid immunoglobulin. Furthermore, independent of or in conjunction with the presence of additional flanking amino acids bound to the peptide, one or more amino acid residues in other regions of the immununoglobulin, other CDR region(s) and/or framework regions, can be altered to modify the binding, activity and/or expression displayed by the peptide in the context of the immunoglobulin molecule.

It is contemplated that after construction of biologically active recombinant antibodies and/or fragments thereof, such recombinants can be subjected to randomization methods known in the art to introduce mutations at one or more points in the sequence to alter the biological activity of the antibodies. After generation of such mutants using randomization methods such as those described herein, the resulting recombinants may be assayed for activity using binding, growth, expression and activation assays.

Further provided are nucleic acid molecules encoding immunoglobulin molecules or fragments thereof which have the amino acids of one or more CDR regions replaced by a biologically active peptide. These nucleic acid molecules can be present in an expression vector, which can be introduced (transfected) into a recombinant host cell for expression of these molecules. Also provided are methods of producing an immunoglobulin molecule or fragment thereof containing a biologically active peptide, comprising culturing a recombinant host cell under conditions such that the nucleic acid contained within the cell is expressed.

Also provided are compositions, comprising an immunoglobulin molecule or fragment thereof which has amino acid residues corresponding to a CDR replaced with amino acid residues comprising a TPO or EPO mimetic peptide and a pharmaceutically acceptable carrier.

Further provided are EPO mimetic peptides with additional flanking residues which are suitable for replacement of CDRs. Also provided are nucleic acid molecules encoding these peptides.

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Further provided are methods of engineering immunoglobulin molecules or fragments thereof to exhibit an agonist activity in which a biologically active peptide replaces at least a portion of one or more CDR regions of light and/or heavy chains. The methods encompass inserting a nucleic acid molecule encoding a biologically active peptide in place of at least a CDR region of a nucleic acid molecule encoding an immunoglobulin heavy or light chain or adding the molecule to the native CDR sequence and then expressing the nucleic acid molecule encoding the immunoglobulin heavy or light chain variable domain along with its complementary variable region domain, such that the two domains associate.

Further provided are methods of engineering immunoglobulin molecules or fragments thereof to exhibit an activity (property) of a biologically active TPO or EPO peptide in which a biologically active peptide replaces one or more CDR regions of light and/or heavy chains. The methods encompass inserting a nucleic acid molecule encoding a biologically active peptide in place of at least a portion of a CDR region of a nucleic acid molecule encoding an immunoglobulin heavy or light chain or adding the molecule to the native CDR sequence; and expressing the nucleic acid molecule encoding the immunoglobulin heavy or light chain variable domain along with its complementarity variable region domain, such that the two chains associate.

In another aspect, this disclosure provides a method for producing in a polypeptide a binding site capable of binding a preselected agent, the method including the steps of introducing a nucleotide sequence that codes for an amino acid residue sequence defining said binding site into a CDR region of a nucleic acid comprising an immunoglobulin heavy or light chain gene by amplifying the CDR region of the immunoglobulin gene, the introduced nucleotide sequence having the formula – X_a-Y-X_b wherein X is the same or different at each occurrence and represents a randomizing trinucleotide, the sum of a and b is 4 or less and Y is a nucleotide sequence that encodes a minimum recognition domain of said binding site. In particularly useful embodiments, amplification is achieved using overlap PCR, however, any known amplification technique could be employed, such as, for example, the methods disclosed in WO94/18221, the disclosure of which is incorporated herein by reference.

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In yet another aspect, this disclosure provides methods for creation of a library of monoclonal antibodies that can be screened for a desired activity. These methods of making a library include the steps of inserting a nucleic acid molecule encoding a biologically active peptide into, or in place of at least a portion of, one or more CDR regions of a nucleic acid molecule encoding an immunoglobulin heavy or light chain, providing up to a pair of randomizing trinucleotides on either side of the inserted nucleic acid molecule, and expressing a library of monoclonal antibodies. In particularly useful embodiments, a pair of randomizing trinucleotides is provided on both sides of the inserted nucleic acid molecules. The library of monoclonal antibodies thus produced can then be screened for a desired activity.

In a specific embodiment, antibodies and fragments thereof have different amino acids flanking the peptide at the amino and the carboxyl termini where the peptide becomes bound to the antibody scaffold. This, results in a population of antibody molecules or fragments thereof that may differ in the presentation of the peptide. The population is screened for those antibodies that exhibit the biological activity of the peptide. In a preferred embodiment, the amino acid immediately adjacent the peptide is a proline.

If the activity of the biologically active peptide is to activate a target molecule, this may require dimerization of two target molecules (e.g. receptors in the hematopoietic superfamilies). For dimerization to occur, two peptides must be positioned to each bind a target molecule such that the two bound target molecules can then properly associate. This can be accomplished by having two peptides present on the same antibody or fragment thereof or by causing two antibody molecules each containing one peptide to bind together. Thus, for example, a single peptide can be inserted into or substituted for at least a portion of a CDR and then expressed as an immunoglobulin or a $F(ab')_2$ fragment. As another example, two peptides can be inserted into or substituted for at least a portion of one or more CDRs and expresses as any antibody or antibody fragment.

The screening of antibodies or fragments thereof can be accomplished by panning with cells that have surface molecules to which the peptide specifically binds. Solid phase binding using purified target molecules or fragments thereof can also be

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used. Binding can also be carried out in solution using labeled target molecules. In addition, antibodies or fragments thereof can be screened by the use of biological assays for agonist or antagonist activity of the peptide.

Also provided are libraries of different immunoglobulin molecules or fragments thereof wherein amino acid residues corresponding to a complementarity determining region (CDR) are replaced with amino acid residues comprising a biologically active peptide which has at least one additional amino acid residue at the amino or the carboxyl terminus and the immunoglobulin molecules or fragments thereof differ by the additional amino acid residue of the peptide.

In specific embodiments the biologically active peptide is a TPO mimetic or an EPO mimetic. The antibodies of the library are displayed on phage.

Further provided are methods of stimulating proliferation, differentiation or growth of cells, which include contacting the cells with an effective amount of an immunoglobulin molecule or fragment thereof having one or more CDRs replaced with a biologically active peptide which binds to a receptor on the cells surface. In specific embodiments the biologically active peptide is a TPO mimetic or an EPO mimetic.

In other specific embodiments, is provided a method of stimulating proliferation, differentiation or growth of megakaryocytes by contacting megakaryocytes with an effective amount of an immunoglobulin molecule or fragment thereof having one or more CDRs replaced with a TPO mimetic peptide. Also provided is a method of increasing platelet production, which involves contacting megakaryocytes with an effective amount of an immunoglobulin molecule or fragment thereof having one or more CDR regions replaced with a TPO mimetic peptide. Also provided is a method of stimulating megakaryocytes and/or increasing platelet production in a patient, in which an effective amount of an immunoglobulin molecule or fragment thereof having one or more CDRs replaced with a TPO mimetic peptide is administered to a patient in need thereof. The immunoglobulin molecule and the megagakarocytes can also be contacted in vitro and the resultant cells can be introduced into the patient. In addition, an antibody or fragment thereof having at least one TPO mimetic peptide incorporated therein can be administered to a subject who intends to donate platelets, thus

increasing the capacity of a donor to generate platelets to provide a more robust source of such platelets.

Also provided herein is a method of stimulating proliferation, differentiation or growth of hematopoietic cells, comprising contacting the cells with an effective amount of an immunoglobulin molecule or fragment thereof having one or more CDRs replaced with a EPO mimetic peptide.

Also embodied herein is a method of activating a homodimeric receptor protein, by contacting the receptor with an immunoglobulin molecule or fragment thereof having a CDR region replaced with a biologically active peptide that specifically binds the receptor and which has been dimerized. In a further embodiment the receptor is a thrombopoietin receptor.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagrammatic representation of the vector pRL4.

Figures 2A and B show the sequence of the human tetanus toxoid antibody framework, light and heavy chains, respectively.

Figure 3 is a diagram depicting the grafting of the TPO mimetic peptide AF12505 into the heavy chain CDR3 region of the tetanus toxoid framework antibody. XX represents flanking random amino acids.

Figure 4 is a diagram of the construction of a peptide cloned into the heavy chain CDR3 region.

Figure 5 represents the amino acid and nucleotide sequences of clones that encode TPO mimetic peptide AF1205 with different random flanking residues.

Figure 6A-C depicts the nucleic acid sequence of plasmid pRL8 (SEQ. ID. NO: 60). pRL8 is a modified version of pRL4 (pRL4 is also known as pComb 3X). The pRL4 was modified between the Spe I and neighboring Sfi I restriction sites (shown by underlining) to include a flexible linker (murine kappa hinge region) followed by a Jun leucine zipper dimerization domain.

Figure 7 is a schematic depiction of a portion of the plasmid pRL8.

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Figure 8 depicts the nucleic acid sequence of a portion of plasmid pRL8 (SEQ. ID. NO: 52) along with amino acid sequences corresponding to certain delineated nucleic acid sequences (SEQ. ID. NO: 53).

Figure 9 is a chart showing sequences of certain TPO positive clones herein.

Figure 10 is a bar graph showing activity of certain Fab clones containing 2 TPO mimetic peptides.

Figure 11 is a bar graph showing activity of certain Fab clones containing 2 or 3 TPO mimetic peptides.

Figure 12 graphically depicts the activity of Clone 59 as reflected by induction of luciferase activity.

Figure 13A depicts the amino acid sequence and nucleic acid sequence of the 5G1.1-TPO heavy chain (SEQ. ID. NOS: 67 and 68, respectively).

Figure 13B depicts the amino acid sequence and nucleic acid sequence of the 5G1.1 light chain (SEQ. ID. NOS: 69 and 70, respectively).

Figure 14 is a bar graph showing FACS analysis of cMpl receptor binding of purified 5G1.1+ TPO mimetic peptide compared to parental 5G1.1 antibody.

Figure 15 is a bar graph showing comparative activity of 5G1.1 antibody containing the TPO mimetic peptide in connection with cells transfected with a control vector containing no cMpl-R and cells transfected with a vector containing cMpl-R.

Figure 16 shows the sequence of clone 429/Xb4 (SEQ. ID. NO: 116)

Figure 17 is a flow chart showing the initial steps for making vector pRL5-Kappa.

Figure 18 is a flow chart showing additional steps for making vector pRL5-Kappa.

Figure 19 is a map of vector pRL5.

Figure 20 is a schematic of vector pRL5-Kappa.

Figure 21A-I show the nucleic acid sequence of vector pRL5-Kappa.

Figure 22 shows the amino acid sequences for the heavy and light chain of clone 116.

Figure 23 shows the activity of clone 116.

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Figure 24 shows the sequences of heavy chain clones in accordance with an alternative embodiment of the present disclosure.

DETAILED DESCRIPTION

As used herein, "immunoglobulin" refers to an entire immunoglobulin molecule or molecules that contain immunologically active portions of whole immunoglobulin molecules and includes Fab, F(abɔ)2, scFv, Fv, heavy chain variable regions and light chain variable regions. The terms immunoglobulin and antibody are used interchangeably herein.

Any peptide that exhibits a useful property is suitable for insertion in an antibody framework. Peptide activities and uses include, but are not limited to, binding a receptor, binding a membrane bound surface molecule, binding a ligand, binding an enzyme or structural protein, activating or inhibiting a receptor, targeted drug delivery, or any enzymatic activity. Those peptides whose utility can be increased from the enhanced stability conferred upon them when presented in the context of an immunoglobulin molecule are usually selected. It should be understood that "biological activity" as used herein includes any activity associated with a molecule having activity in a biological system, including, but not limited to, the stimulatory or inhibitory activity triggered by protein-protein interactions as well as the kinetics surrounding such interactions including the stability of a protein-porotein complex. Enhancing or increasing "biological activity" herein is meant to include an increase in overall activity or an increase in any component of overall activity. It should be understood that a peptide may exhibit one biological activity (such as, e.g., simply binding to a target) before insertion into the antibody framework, and a different or enhanced biological activity (such as, e.g., agonist activity) after insertion into the antibody framework.

Many peptides which could benefit from display in the context of an immunoglobulin have been identified and are known to those who practice the art, e.g., EPO and TPO mimetic peptides. Other examples include peptides that bind to receptors which are activated by ligand-induced homo-dimerization including active fragments displaying G-CSF activity, GHR activity and prolactin activity as described in

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Whitty and Borysenko, *Chem Biol.*, (1999) Apr 6(4):R107-18; other examples of suitable peptides include a nerve growth factor mimetic from the CD loop as described in Zaccaro et al., *Med. Chem.* (2000) 43(19); 3530-40; an IL-2 mimetic as described in Eckenberg, et al., *J. Immunol.* (2000) 165(8):4312-8; glucogon-like peptide-1 as described in Evans et al., *Drugs R.D.* (1999) 2(2): 75-94; tetrapeptide I (D-lysine-L-asparaginyl-L-prolyl-L-tyrosine) which stimulates mitogen activated B cell proliferation as described in Gagnon et al., *Vaccine* (2000) 18(18):1886-92. Peptides which exhibit receptor antagonistic activity are also contemplated. For example, N-terminal peptide of vMIP-II as an antagonist of CXCR4 for HIV therapy as described in Luo et al., *Biochemistry* (2000) 39(44):13545-50; antagonist peptide ligand (AFLARAA) of the thrombin receptor for antithrombotic therapy as described in Pakala et al., *Thromb. Res.* (2000) 100(1): 89-96; peptide CGRP receptor antagonist CGRP (8-37) for attenuating tolerance to narcotics as described in Powell et al., *Br. J. Pharmacol.* (2000) 131(5): 875-84; parathyroid hormone (PTH)-1 receptor antagonist known as tuberoinfundibular peptide (7-39) as described in Hoare et al., *J. Pharmacol. Exp. Ther.* (2000) 295(2):761-70; opioid growth factor as described in Zagon et al., *Int. J. Oncol.* (2000) 17(5): 1053-61; high affinity type I interleukin 1 receptor antagonists as disclosed in Yanofsky, et al., *Proc. Natl. Acad. Sci. USA*, Vol. 93, pp. 7381-7386, July 1996 and Vigers, et al., *J. Biol. Chem.*, Vol 275, No 47, pages 36927-36933, 2000; and acid fibroblast growth factor binding peptide as described in Fan et al., *IUBMB Life* (2000) 49 (6) 545-48. Peptides can also be discovered using methods familiar to those skilled in the art. In order to identify a region of a protein that is involved in a specific biological function, a survey of the shorter peptide fragments making up that protein may reveal the linear peptide epitope responsible. Alternatively by surveying libraries of random peptides, a peptide that represents an optimal linear epitope or a discontinuous epitope may be discovered that mimics the activity of the natural protein. One method for selection is termed peptide phage-display. In this approach, a random peptide epitope library is generated so that peptides are present on the surface of a bacteriophage particle. These collections, or libraries, of peptides can then be surveyed for those able to bind to a specific immobilized target protein. (Pasqualini, R. et al., *j. Cell Biol.*, 130, 1995, 1189-1196; Wrighton, N.C., et al.,

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Science, 273, 1996, pages 458-463; Cwirla, S.E., et al., *Science*, 276, 1997, pages 1696-1699; Koivunen et al, *J. Biol, Chem.*, 268, 1993, pages 20205-20210; Koivunen et al., *Bio/Technol.*, 13, 1995, pages 265-270; Healy et al., *Biochem.*, 34, 1995, pages 3948-3955; Pasqualini et al., *J. Cell Biol.*, 130, 1995, pages 1189-1196). Alternative peptide selection systems are also possible including cell surface display and ribosomal display.

Peptide mimetics used in accordance with this description are generally less than or equal to the number of amino acid residues that make up a CDR region, although they could be longer.

Any antibody can serve as a scaffold sequence, however typically human antibodies are chosen as human therapeutics is one of the ultimate objectives. Human or humanized antibodies are less likely to cause an adverse immune response in a human patient. The major criteria in selecting an antibody to serve as a framework for insertion of a peptide, is that the replacement of one or more CDRs of the antibody with the peptide must change the antigen specificity. The antibody can be a complete antibody or an Fab, scFv or $F(ab')_2$ fragment or portion thereof.

Alternatively, a library of antibodies can have one or more heavy and/or light chain CDRs replaced with a desired peptide. The resulting library can then be screened to identify antibodies having a desired activity. It should be understood that randomization with in the substituted peptide can also be provided to generate an antibody library.

A useful antibody is the anti-tetanus toxoid (TT) Fab, as it is human and because modification of the HCDR3 is sufficient to change the antigen specificity of the antibody (Barbas et al., *J. Am. Chem. Soc.*, 116, 1994, pages 2161-2162 and Barbas et al., *Proc. Natl. Acad. Sci. USA*, 92, 1995, pages 2529-2533).

Grafting of the DNA sequence of the peptide of choice into an antibody so as to replace the CDR(s) of an antibody with the peptide sequence is carried out using recombinant DNA techniques known to those skilled in the art.

Examples of methods which can be utilized to graft a desired peptide having biological activity in place of a CDR region include, but are not limited to, PCR overlap, restriction enzyme site cloning, site specific mutagenesis and completely synthetic

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means. For a description of techniques involving overlap PCR, see, e.g., Example 1 herein. Site specific mutagenesis can be accomplished in several ways. One is based on dut/ung Kunkel mutagenesis (Kunkel, T.A., *Proc. Natl. Acad. Sci.* (1985) vol. 82, pp. 488-92). The Muta-Gene *in Vitro* Mutagenesis kit is available from BioRad based on this methodology (cat. # 170-3581 or 170-3580). Several PCR amplification based mutagenesis approaches are also commercially available such as Stratagene's QuickChange Site-Directed Mutagenesis Kit and the ExSite PCR-based Site-Directed Mutagenesis Kit. Another non-PCR method is available from Promega as the GeneEditor *in vitro* Site-Directed Mutagenesis System. Completely synthetic means are also well-known and described, e.g., in Deng, et al., *Methods Mol. Biol.* (1995) 51:329-42; Kutemeler et al., *Biotechniques*, (1994) 17(2): 242-246; Shi et al., *PCR Methods Appl.*, (1993) 3(1): 46-53 and Knuppik et al., *J. Mol. Biol.*, (2000) 11:296(1): 571-86 each incorporated herein by reference. In addition, the above methods used for replacing all or a portion of at least one CDR sequence can be utilized to graft a desired peptide into or adjacent to at least one native CDR sequence without replacing the original CDR sequence. In this manner, a CDR/biologically active peptide mimetic fusion construct is formed.

It is contemplated that flanking sequences may be added to the carboxyl and/or amino terminal ends of the biologically active peptide. Flanking sequences can be useful to reduce structural constraints on the grafted peptide to allow it to more easily adopt a conformation necessary for biological activity. In a preferred embodiment, a flanking region including a proline is covalently attached to the carboxy terminus of the biologically active peptide to create a proline extended biologically active peptide.

In one embodiment, a flanking region can be generated by randomizing two amino acid positions on each side of the peptide graft in order to determine the best sequence. In this manner, a library having members with multiple varied sequences can be generated. The resulting constructs are then tested for biological activity as described below by, e.g., panning techniques. Recombinant proteins can be generated that have random amino acids at specific positions. This can be accomplished by modifying the encoding DNA. When introducing randomization at a specific amino acid's codon position, a preferable deoxyribonucleotide "doping

strategy" is $(NNK)_x$ in order to cover all 20 amino acids and to minimize the number of encoded stop codons. Accordingly, N may be A, C, G, or T (nominally equimolar), K is G or T (nominally equimolar), and x is typically up to about 5, 6, 7, or 8 or more, thereby producing libraries of mono-, di-, tri-, quadra-, penta-, hexa-, hepta-, and octa-peptides or more. The third position may also be G or C, designated "S". Thus, NNK or NNS (i) code for all the amino acids, (ii) code for only one stop codon, and (iii) reduce the range of codon bias from 6:1 to 3:1. There are 32 possible codons resulting from the NNK motif: 1 for each of 12 amino acids, 2 for each of 5 amino acids, 3 for each of 3 amino acids, and only one of the three stop codons. Other alternatives include, but are not limited to:

$(NNN)_x$ which would provide all possible amino acids and all stops;

$(NNY)_x$ eliminates all stops and still cover 14 of 20 amino acids;

$(NNR)_x$ covers 14 of 20 amino acids; and

$(NNS)_x$ covers all 20 amino acids and only one stop.

The third nucleotide position in the codon can be custom engineered using any of the known degenerate mixtures. However, the group NNK, NNN, NNY, NNR, NNS cover the most commonly used doping strategies and the ones used herein.

The collection of engineered antibodies that are created during this process can be surveyed for those that exhibit properties of the peptide as, e.g., phage displayed antibodies, essentially as has been described in Barbas, C.F., III, Kang, A.S., Lerner R.A., and Benkovic, S.J., Assembly of combinatorial antibody libraries on phage surfaces: the gene III site, *Proc. Natl. Acad. Sci. USA*, 88, 1991, pages 7978-7982 incorporated herein by reference. This technology allows recombinant antibodies (as complete antibodies, Fab $F(ab')_2$, or scFv) to be expressed on the surface of a filamentous bacteriophage. That same phage will have within it the genes encoding that specific antibody.

It is contemplated that any other known method of introducing randomization into a sequence may be utilized herein. For example, error prone PCR can introduce random mutations into nucleic acid sequences (See, e.g., Hawkins et al., J. Mol. Biol, (1992) 226(3): 889-96). Briefly, PCR is run under conditions which compromise the fidelity of replication, thus introducing random mutations in sequences as those skilled

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in the art would accomplish. After generation of such random mutants, they can be placed into phage display formats, panned and thus evaluated for activity. Likewise, particular bacteria known to provide random mutations of genes, such as Epicurian Coli® XL1-Red Competent cells (commercially available from Stratagen, La Jolla, CA.), which do so during plasmid replication can be utilized to provide random mutants which are then screened for biological activity in accordance with the present disclosure.

It is also contemplated that randomization may be introduced at any point in the nucleotide sequence after incorporation of an active peptide into the antibody or fragment thereof to alter the overall biological activity of the antibody. In this manner, not only can alterations be made in the biological activity of a peptide mimetic by causing mutations within the peptide's sequence, but mutations in the surrounding scaffold can be incorporated with the resulting constructs being assayed for alterations in biological activity or expression. Indeed, it is contemplated that libraries having repertoires of multiple constructs resulting from such randomization can be generated and assayed.

Single chain libraries can be utilized in accordance with the present disclosure because an entire binding domain is contained on one polypeptide. The light chain variable region is separated from heavy chain variable region by a linker region. The use of short linkers (< 11 amino acids) favors a dimeric complex where V_H of one ScFv associates with V_L of another ScFv molecule and visa versa, these molecules are termed diabodies (Kortt, A.A., Malky, R.L., Caldwell, J.B., Gruen, L.C., Ivanci, N., Lawrence, M.G. et al. *Eur. J. Biochem.* 221:151-157, 1994). This is because folding of monomeric ScFv is impaired with linkers < 11 amino acids (Alfthan, K., Takkinen, K., Sizman, D., Soderlund, H., and Teeri, T.T. *Protein-Eng.* 8:725-731, 1995). Longer linkers (> 11 amino acids) favors folding of monomeric ScFv into a single antigen binding domain, thus precluding dimer formation.

One useful phage display vector is pRL4 which is also known as pComb 3X (see Fig. 1). This vector enables display of chimeric expression products on the surface of packaged phagemid particles. pRL4 is a modified version of pComb3H (Barbas, C.F. III and Burton, D.R. 1994. Monoclonal Antibodies from Combinatorial

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Libraries. Cold Spring Harbor Laboratory Course Manual, Cold Spring Harbor, N.Y.; Burton, D.R.; Barbas, C.F. III. *Advances in Immunology* 57:191-280, 1994; Lang, I.M., Chuang, T.L., Barbas, C.F. 3rd, Schleef, R.R. *J. Biol. Chem.* 271: 30126-30135, 1996; Rader and Barbas, Phage Display, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000). The design of pRL4 allows for dimerization of scFv antigen binding domains on the phage surface and in soluble form as detailed below. When the plasmid is transformed into a *supE* bacterial host such as ER2537 (F' *Sup E*, New England Biolabs, Beverly, MA), the amber mutation is suppressed approximately fifty percent of the time. In this way half of the expressed scFvs are fused with the filamentous phage gene III protein (amino acids 230-406) and the other half will be terminated just prior to gene III to produce soluble scFv. Both the scFv-pIII fusion and soluble scFv products have the *Omp A* signal sequence and will be transported to the periplasm where they will be able to form dimeric scFv complexes, termed diabodies (Kortt, A.A., Malby, R.L., Caldwell, J.B., Gruen, L.C., Ivanci, N., Lawrence, M.C. et al. *Eur. J. Biochem.* 221: 151-157, 1994). Diabodies are expected to fold such that the V_H of one scFv will pair with the V_L of a second scFv-pIII resulting in divalent antibody fragments. In a non-*sup E* host, such as TOP1OF' (InVitrogen, Carlsbad, CA), the amber stop codon is recognized yielding soluble scFv diabodies.

In the final single chain expression construct in pRL4, the single chain antibody fragments are cloned downstream of the *E. coli* lacZ promoter, ribosome binding site, and *omp* A leader sequence. These elements allow induction of expression by IPTG, and the secretion out of the cell via the *omp* A leader sequence when expressed in the suppressor strain ER2537. The single chain fragments are fused in frame with filamentous phage gene III (gIII) sequences (amino acids 230-406). The gIII protein product, pIII, is a minor coat protein necessary for infectivity. Upon promoter induction by IPTG, the single chain antibody-pIII fusion is synthesized and transported to the bacterial periplasmic space. In the periplasmic space, the scFv-gene III fusion proteins are inserted into the membrane. Upon superinfection with helper phage, these fragments are exported out of the cell on the surface of phage as pIII-antibody

fragments. Other possible proteins to be used for fusion on the surface of phagemids include filamentous coat protein pVIII and other coat proteins.

Fab fragment libraries, that maintain the native antigen recognition site, are useful to ensure that affinity is maintained.

In the final hybrid Fab expression construct in pRL4, the light and heavy chains are cloned as a single SfiI fragment. In this way, the light chain fragments are cloned downstream of the *E. coli* lacZ promoter, ribosome binding site, and *omp* A leader sequence. These elements allow induction of expression by IPTG, and secretion out of the cell via the *omp* A leader sequence. The light chain fragments are followed by a stop codon, a second ribosome binding site, the *E. coli pel* B leader sequence and heavy chain. Hybrid heavy chain genes are fused in frame with filamentous phage gene III (gIII) sequences (amino acids 230-406). An amber stop codon is present at the fusion junction. In a *sup E* bacterial host such as ER2357 (New England Biolabs, Beverly, MA), the amber mutation is suppressed. Upon promoter induction, a single polycistronic message is transcribed and translated as two polypeptides, a light chain and a heavy chain-gene III fusion protein. Following synthesis the polypeptides are transported to the bacterial periplasmic space as directed by the leader sequences. In the periplasmic space the heavy chain-pIII fusion proteins are inserted into the membrane, and the light and heavy chains are associated covalently through disulfide bonds, forming the antigen binding sites. The human constant region CH1 and C_L sequences include the cysteines that form the disulfide bond between heavy and light chains. Upon superinfection with helper phage, these fragments are exported out of the cell on the surface of phage as Fab-cpIII fusion. In a non-*sup E* host, such as TOP1OF' (Invitrogen, Carlsbad, CA), the amber stop codon is recognized yielding soluble Fab fragments. Important features of the pRL4 phage display system used include a purification His 6 tag, an HA epitope tag following the heavy chain, as well as a suppressible amber stop codon which is located between the heavy chain and the phage gene III. The HA tag is recognized by HA.11 antibody (Babco, Berkeley, CA) and 12CA5 antibody (Roche Molecular Biochemical, Indianapolis, Ind.). The His6 tag allows affinity purification of antibody fragments by Nickel-chelate chromatograph (Qiagen, Valencia, CA). The amber stop allows for quick conversion from a fusion

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Fab-cpIII product (for incorporation on the phage coat) when the stop is suppressed, to the soluble Fab which is made in a non-suppressor bacterial host.

Selection involves isolating from the library the best candidates that specifically bind to the peptides target molecule and display biological activity.

The phage expressing antibody fragments on their surface can be produced and concentrated so that all members of a library can be allowed to bind to the target molecule. The target molecule can be immobilized on a microtiter dish, on whole cells, the membranes of whole cells, or present in solution. Non-specific Ab-phage are washed away, and bound phage particles are released from the antigen, often by the use of low pH. The recovered Ab-phage are infectious and so can be amplified in a bacterial host. Typically, multiple rounds of this sort of selection are performed. Individual antibody fragment clones can then be analyzed as soluble Fabs or scFvs for identification of those that specifically recognize the target molecule.

Prior to any selection strategy, initial libraries are electroporated into host cells, such as ER2537. Library cultures are grown to log phase and superinfected with helper phage, such as VCSM13, a commercially available helper phage (Stratagene, La Jolla, CA). Superinfection provides the remaining phage components needed for packaging plasmids into phagemid particles. Alternatively, phage display without the use of helper phage may be utilized. Following overnight growth, phagemids in the culture supernate are precipitated with polyethylene glycol (PEG). PEG precipitated phage are used in panning (solid phase cell surface, internalization and membrane), FACS sorting, or magnetic sorting to purify specific binding antibodies from non specific binders.

In cell based panning, antibody-phage libraries are incubated with target cells, and the non-adherent phage are removed with multiple washes. A typical panning protocol is as follows:

1. Block phage particles with PBS + 1%BSA or 10% FBS + 4% milk powder + NaN_3, (except when internalized antibodies are assayed).

2. Add target cells to blocked phages (approximately 5×10^6 cells).

3. Mix and rotate slowly at 4° C or 37 ° C.

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4. Wash cells twice with 1 ml ice cold PBS/1%BSA/NaN_3 or room temperature PBS/1%BSA/NaN_3.

5. Specific antibody-phage bound to cells can be eluted by low pH, for example with 76 mM citric acid ph 2.5 in PBS for 5 to 10 minutes at room temperature.

6. Neutralize eluted phage with 1M Tris-HCl pH 7.4.

7. After neutralization, antibody-phage can be used to infect ER2537 bacteria and amplify during overnight growth for the next round of panning.

Generally, 3-4 rounds of panning are performed on each library. Phage ELISAs using commercially available secondary antibody (sheep anti-M13 antibody-HRP) or soluble antibody ELISAs using a commercially available HA. 11 antibody (Babco, Berkeley, CA) that recognizes the HA tag incorporated into each antibody from PRL4 sequences, can be performed following each round of panning to allow estimation of the enrichment of binding antibodies over non-binders. Following the last round of panning, the antibody-phage can be picked as single colonies from agar plates, grown as monoclonal antibody-phage and screened by ELISA for identification of specific binders. FACS analysis may also be utilized. Specifically the antibody-phage are infected into Top10F' bacteria and plated for single colonies. Single colonies are picked form agar plates, grown and induced with IPTG. Soluble antibody is screened by ELISA for identification of specific binders. Screening can be done against live cells, against intact, mildly fixed target cells, or recombinant protein(s).

Methods for whole cell panning have been described previously (Siegel, D.L., Chang, T.Y., Russell, S.L., and Bunya, V.Y. 1997. *J. Immunol. Methods* 206:73-85 incorporated herein by reference). Other techniques for selection which can be applied include fluorescent activated cell sorting (FACs). Alternative methods for selection using libraries include, but are not limited to, ribosome display and plaque hybridization to a labeled antigen.

Following panning to isolate high affinity antibody binders, bioassays for functional screens of agonist antibodies can be carried out. Dimerization is often a prerequisite for activation of many receptors and thus bioassays focus on agonist antibodies that stimulate receptors via promotion of dimerization. As previously described, single chain multivalency is approached in linker design. Fab fragment

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multivalency can be approached in a number of ways. A number of recent reports in the literature have shown success in dimeric antibody fragment formation which is applicable to phage display (DeKruif, J., and Logtenberg, T. 1996. *J. Biol. Chem.* 271:7630-7634, Pack, P., and Pluckthun, A. 1992. *Biochemistry* 31:1579-1584, and Holliger, P., and Winter, G. 1993. *Current Opin. Biotech.* 4:446-449). Divalent Fabs can be created in at least two ways. In one approach dimerization is achieved by addition of a dimerization domain to pRL4, forming pRL8 (See Figs. 6A-C, 7 and 8). There are a number of dimerization domains (lexA, Zn fingers, fos, jun etc.) that can be utilized in these vectors to obtain multivalency of Fab fragments. Dimerization domains are selected from, but not limited to, the following: jun (DeKruif, J. and Logtenberg, T. *J. Biol. Chem.* 271:7630-7634, 1996; Kostelny, S.A., Cole, M.S., and Tso, J.Y. *J.Immunol.* 148:1547-1553, 1992) the LexA dimerization region (Kim, B. and Little, J.W. *Science* 255:203-206, 1992), the yeast GCN4 dimerization domain (van Heeckeren, W.J., Sellers, J.W., Struhl, K. *Nucleic Acids Res.* 20:3721-3724,1992), Gin invertase from the bacteriophage Mu (Spaeny-Dekking, L., Schlicher, E., Franken, K., van de Putte, P., Goosen, N. *J. Bacteriol.* 34:1779-1786, 1995), *E. coli* NTRC protein dimerization domain (Klose, K.E., North, A.K., Stedman, K.M., Kustu, S. *J. Mol. Biol.* 241:233-245, 1994), and HSV-1 ICP4 dimerization domain (Gallinari, P., Wiebauer, K., Nardi, M.C., Jiricny, J. *J. Virol.* 68:3809-3820, 1994) all incorporated by reference. Also, a high temperature dimer domain from thermus organisms can be utilized (MacBeath, G., Kast, P., Hilvert, D., *Biochemistry* 37:100062-73, 1998 and MacBeath, G., Kast, P., Hilvert, D., *Science* 279:1958-61, 1998). These are functional domains that when incorporated into a molecule allow for dimerization to occur. In addition, dimerization can be achieved in cells through the use of full IgG vectors, or dimerization domains such as CH3 dimerization domains. Those of ordinary skill in the art are familiar with these and other dimerization domains and their use to dimerize proteins.

Additional methods that may be utilized to generate antibody constructs which contain at least two binding sites are known. The antibody or antibody fragments created by each of these approaches could be utilized for testing agonistic antibody activity as described in Example 1 below for whole IgG produced in

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mammalian cells. These methods include chemical dimerization of Fab, pegylation of Fab, production of Fab'2, generation of whole IgG in bacterial cells, and use of diabodies (scFvs). Importantly, any of the antibody forms generated for analysis of agonistic activity could be used as the final therapeutic product.

Chemical dimerization may be also achieved using a variety of chemical crosslinking reagents. For example, SMCC (Succinimidyl trans-4 (maleimidylmethyl) cyclohexane-1-carboxylate) from Molecular Probes (Eugene, Oregon), Cat # S-1534. This reagent will modify primary amino groups in the antibody. After incubating the antibody with the SMCC at room temperature, the reaction is run over a PD-10 column. This maleimide derivitized Fab can be added to either a second Fab or a separate batch of the same Fab that has been treated with TCEP [(Tris (2-carboxyethyl) phosphine, hydrochloride): Molecular Probes Cat #T-2556] to reduce the thiol groups to SH. The reduction reaction is carried out in the dark for 15 minutes. The conjugation of the maleimide Fab and the thiol reduced Fab occurs at a 1:1 ratio. Dimers are isolated by passing the reaction over a sephadex 200 gel filtration column. Other chemical linkers known to those skilled in the art may be used for dimerization. Production of an Fab' that has an extra cysteine residue engineered into the hinge region has been described, e.g., in U.S. Patent 5,677,425 and Carter, et al.,*BioTechnology*, Vol 10, Feb. 1992, pages 163-167 the disclosures of which are hereby incorporated by reference. That thiol site can be used for conjugation to moieties such as polyethyleneglycol (PEG). Pegylation technology is known, for example, see Koumenis et al, Int. J. Pharm. (2000) 198(1): 83-95, incorporated herein by reference, which makes it possible to link two Fab' molecules together using PEG coupling.

Technology for bacterially producing Fab'2 involves cloning the human IgG hinge region, and optionally part of the CH2, as part of the Fd which includes additional cysteines and is described, e.g., in Better, et al., PNAS USA (1993) 90(2): 457-61, incorporated herein by reference. The additional thiol groups on the Fd hinge can interact and cause two Fab' molecules to dimerize, creating a Fab'2. Fab'2 can be purified directly from the bacterial cells. Additionally, undimerized Fab' from the bacteria can be isolated and chemically converted to Fab'2.

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As described earlier, the variable regions of the antibody can be cloned as a single chain wherein the variable light (VL) is connected to the variable heavy (VH) by a linker region. If that linker region is short (for example 5-7 amino acids), the folding of the scFv will favor association of two scFvs where the VL of one scFv paris with the VH of the second scFv. In this manner, two antigen binding sites are presented on the diabody.

Antibody constructs which contain two binding sites may be generated using any of these methods in order to test agonist activity and/or be used as the final therapeutic product.

Following the panning or sorting steps of Fab libraries, the library of panned molecules are restricted with Sac I and *Spe* I and cloned into pRL8. Subcloning to pRL8 vector individually or *en masse* following FACS sorting or panning allows expression of dimeric soluble binding Fabs for analysis in bioassays. In pRL8, the antibody fragments are transported to the periplasmic space and form dimers there. The advantage of this approach is that it permits panning of monomeric Fab fragments, favoring high affinity Fabs.

Another approach uses a secondary antibody. pRL4 has the hemagglutinin decapeptide tag recognized by the commercially available HA. 11 antibody (Babco, Berkeley, CA). Fabs identified in FACS sorting or panning to be tested in bioassay are preincubated with HA. 11 which will promote dimerization, prior to addition to bioassays.

Once binding scFv's or Fabs are identified by panning or another selection method, the individual clones, each expressing a unique dimerized antibody fragment on the phage surface are tested for proliferation, differentiation, activation or survival effects on target cells. In addition, soluble dimerized antibody are examined in bioassays.

Biological Assays for Screening for TPO-like Activity

1. Colony formation assays - Megakaryocytic colonies from bone marrow (Megacult C Kit from Stem Cell Technologies Inc., Vancouver BC, Canada).

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2. Proliferation assays - proliferation of Ba/F3 cells (Cwirla et al. 1997, Science, Vol. 276 pages 1696-1699). The Ba/F3-mpl cell line was established (F. de Sauvage *et al.*, Nature, 369:533 (1994)) by introduction of the cDNA encoding the entire cMpl receptor into the IL-3 dependent murine lymphobiastoid cell line Ba/F3. Stimulation of proliferation of Ba/F3-mpl cells in response to various concentrations of antibodies or TPO was measured by the amount of incorporation of ^{3}H-thymidine as previously described (F. de Sauvage *et al., supra).*

3. Phosphorylation assays - phosphorylation of JAK2 (Drachman et al., J. Biol. Chem., (1999), Vol. 274, pages 13480-13484).

4. Transcriptional based assays - Transiently co-transfect full length cMpl receptor with c-Fos promoter luciferase reporter construct. 24 hour post transfection starve the cells in 0.5% FCS for 24 hours. Stimulate the cells, harvest after 6 hours and take luciferase readings (see also Example 1, Biological Assays section).

Biological Assays for Screening for EPO-like Activity

1. Bone marrow erthroid colony formation in Methylcellulose (Wrighton et al., Science, 1996, Vol. 273 pages 458-463).

2. TF-1 cell (Human erythroleukemia cell line) proliferation. TF-1 cells express both full length and a truncated form of the Epo-R. (J.Cell Physiol., 1989, Vol 140, pages 323-334).

3. The EPO receptor couples directly to JAK2 kinase to induce tyrosine phosphorylation. Epo induces cFos in TF-1 cells. c-Fos transcriptional activation. (Witthuhn et al., Cell, (1993), Vol. 74, pages 227-236).

A number of bioassays can be used in high-throughput screening. Those of ordinary skill in the art are familiar with these and other suitable bioassays. Several non-radioactive assays have been developed in which either DNA synthesis or enzyme activity can be analyzed. For example, an MTT cell proliferation assay (Promega Corporation, Madison, WI) that is based on an assay described by Mosmann (Mossman, T. 1983. *J. Immunol. Methods* 65:55-57 incorporated herein by reference) can be used. This protocol is fast and easy. In the assay, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide), a tetrazolium salt, is converted

into a blue formazan product by mitochondrial dehydrogenase activity in living cells. The dehydrogenase content, and therefore the amount of colored product produced, is proportional to cell number. The colored product is detectable in an ELISA plate reader at 570nm. Assays are performed in triplicate, *en masse* in 96 well microtiter plates. Briefly, target cells are plated in 100 µl aliquots in culture medium in 96-well plates. Following addition of various concentrations of antibodies, cells are incubated for 48-72 hours at 37°C and 5% CO_2 in a fully humidified atmosphere. MTT is added to each well, and proliferation monitored via ELISA plate reader.

For example, in proliferation assays using TF-1 cells, bacterial cells containing phagemids expressing antibodies are grown overnight at 37°C in 96 well deep well plates in 1 ml of a media that is a mixture of mammalian cell media and bacterial media (in the case of TF-1 cells: RPMI 2.7/SB 0.3/Carb 100ug/ml). TF-1 cells are a human bone marrow erythroleukemia cell line that responds to multiple cytokines (Kitamura, T., Tange, T., Terasawa, T., Chiba, S., Kuwaki, T., Miyagawa, K., Piao, Y.F., Miyazono, K., Urabe, A., Takaku, F., *Cell Physiol.* 140:323-334, 1989; Kitamura, T., Tojo, A., Kuwaki, T., Chiba, S., Miyazono, K., Urabe, A., Takaku, F., *Blood* 73:375-380, 1989; Kitamura, T., Takaku, F., Miyajima, A., *Int. Immunol.* 3:571-577, 1991) On the following day, the overnight cultures are subcultured 1/10 to fresh trays, and placed at 37°C for 2 hours. Following induction with IPTG at 37°C for 4 hours, the plates are centrifuged at 2000 rpm/15' at room temperature. 50 ul each culture supernate are filtered in 96 well filter trays (Millipore) to sterile 96 well assay plates. Mammalian cells are prewashed to remove growth factor and resuspended at a concentration of 1 X 10^5 cells/ml. 50 ul cells are added to each well. Assay plates are incubated in 37°C/5% CO_2 incubator for 72 hours. At 72 hours, the trays are developed by adding 40 ul media/MTS/PMS per well. MTS is an improved more soluble version of MTT. Both assays are based on the cellular conversion of tetrazolium salt. A MTS proliferation assay kit (catalogue number G5421) can be purchased from Promega, Inc. (Madison, WI). Plates are kept at 37°/CO_2 incubator and read at OD_{490} at 1 hour, 4 hours, 8 hours with microplate reader.

The activities of cytokines are often synergistic. Synergy could be manifested through the binding of ligands to two different receptors which then sends the correct

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signal, or via a priming effect whereby interaction of ligand/receptor primes the cell to respond to a second cytokine. Furthermore, cytokines that act early in lineage development are more often synergistic than cytokines that act at later stages in a developmental pathway. Therefore, suboptimal concentrations of growth factors can be used in these bioassays to examine synergism. Conditions for suboptimal concentrations are determined for each assay. This is done by adding serial dilutions of growth factors, individually and as a mixture, to the assays and determining the levels below which a single factor does not promote a response compared to the mixture, and the level below which the mixture does not promote a response in the bioassay. Bone marrow stromal cells can also be added in bioassays to provide other necessary factors that may play a role in a synergistic response.

In addition, cell proliferation can be examined by monitoring DNA synthesis. A non-radioactive, colorimetric assay that examines 5-bromo-2'-deoxy-uridine (BrdU) incorporation (Roche Molecular Biochemicals, Indianapolis, IN) can be performed in microtiter plate format. Here, cells are cultured in 96-well plates and incubated with BrdU and sub-optimal concentrations of cytokines. The amount of BrdU is determined after labeling with a peroxidase labeled anti-BrdU antibody. Final results are analyzed by ELISA plate reader at 405nm.

A radioactive mitogenesis assay that measures the rate of DNA synthesis as an indication of proliferation (Raines and Ross, *Methods of Enzymol.* 109: 749-773, 1985) can also be used. In these assays, changes in rate of incorporation of [^{3}H]-thymidine in target cells is examined. Again, these assays permit concurrent and rapid screening of many antibody fragments. They have been widely used as a convenient method of assessing the stimulatory and inhibitory effects on the growth of many different cells. Cells are cultured in suspension until they reach exponential growth rate. Cells are then washed free of the medium in which they were cultured, and replated in fresh medium. Cells are aliquoted into 96 well plates in a total volume of 100 ul at a concentration of about 1-2 x 10^5 cells/ml. Dilutions of phage supernatant, soluble dimerized Fab or ScFv antibodies are added and cells are incubated for 18-48 hours in a gassed CO_2 incubator at a temp of 37°C. Following incubation, [^{3}H]thymidine (937kBq) is added to each well and incubated for a further 4 hours. The

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cells are then removed from the incubator and counted directly in a bench top microplate scintillation counter such as Packard Top Count NXT Instrument (Packard, Meriden, CT). Alternatively cells can be serially transferred to GF/C filters on a Millipore cell harvester (Millipore, Bedford, MA) or similar apparatus. Radioactivity associated with acid-insoluble material retained on the filter is then determined. Dilutions of commercially available growth factors are applied to positive control wells. Negative controls would include supernatants from cells carrying non-insert containing plasmids or irrelevant antibodies treated similarly. The relative growth promoting activities of the standard and the diluents of the phage supernatants under test are compared to quantify the growth promoting activity in the sample.

Activation can be tested for by assaying second messengers or by transcriptional readout assays.

Survival can be assayed, for example, by monitoring apoptosis using assays such as tunnel assays or by other methods known to those who practice the art.

Other useful assays to analyze cellular signal transduction, the activity of kinases and phosphatases and ultimately cellular activities as a result of agonist activity include measurement of the generation of second messengers, e.g. cAMP, Ca+ +, diacylglycerol (DAG), and inositol 1,4,5-triphosphate (IP3). Measurement of spikes in intracellular calcium concentration, intracellular pH and membrane potential in high throughput screening assays can be performed using instruments such as the FLIPR Fluormetric Imaging Plate Reader System (Molecular Devices, Sunnyvale, CA). A number of fluorescent probes are available for examination of second messenger concentrations (Molecular Probes, Eugene OR). Measurement of concentrations of second messengers can also be done on the single cell level (DeBernardi, M.A. and Brooker, G. *Proc. Natl. Acad. Sci USA* 93:4577-4582, 1996). In addition, assays that examine other signaling events such as phosphorylation, apoptosis or levels of RNA or protein of specific genes would be useful. For example. most cytokines have been shown to activate the enzyme PI 3-K (reviewed in Silvennoinen, O., Ihle, J.N. Signaling by the Hematopoietic Cytokine Receptors, R.G. Landes company, Austin, TX 1996). Furthermore, the Jak family of tyrosine kinases have been shown to be central mediators for cytokine receptor signaling (Ihle, J.N., Witthuhn, B.A., Quelle, *F. W.*

Annu. Rev. Immunol. 13:369-398, 1995). In addition, several other tyrosine kinases, e.g., members of the Src family, are activated in response to certain cytokine stimulations. In the case of RNA or proteins, e.g., c-Jun and c-Fos are rapidly and transiently upregulated upon cytokine stimulation, while c-Myc induction is slower. These proteins are required for G1 transition and proliferation (reviewed in Silvennoinen, O., Ihle, J.N. Signaling by Hematopoietic Cytokine Receptors, R.G. Landes Company, Austin, TX 1996). High throughput screens that detect increases in these transcripts could be utilized.

In transcriptional read out assays, changes in the transcription of specific genes are observed following exposure of cells to a growth factor ' or growth factor mimetic (agonist or inhibitory antibody). For example, in a myc read-out assay, cells such as IL-3 dependent FDCP-mix cell line is starved of IL-3 growth factor for 8 hours, followed by exposure to growth factor mimetics, or native growth factors for 2 hours at 37°C. At this time, the cells are harvested, RNA is isolated, and reverse transcriptase-polymerase chain reactions (RT-PCR) are performed with primers specific for the myc gene. The RT-PCR reactions are electrophoresed in horizontal agarose gels for quantitation of PCR product. In this case expression of a single gene is being monitored.

Alternatively assay for changes in expression of genes can be monitored using CHIP technology, agonist antibodies could be identified under conditions of high probe sensitivity and a dynamic range. In this way, up to 10,000 or more could be analyzed for changes in expression. Desired genes that could be monitored could include c-myc, c-jun, NF-κB, among others. These genes are downstream of various signal transduction pathways and their expression should change upon a mitogenic response. In one type of commercially available CHIP (Affymetrix, Santa Clara, CA), oligonucleotides from desired test genes can be printed out onto glass surface. Target cells are exposed to test agonist antibodies. RNA is isolated from the cells exposed to test agonist antibodies, copied to cDNA, and *in vitro* transcribed in the presence of biotin. Hybridization of *in vitro* transcribed, biotinylated mRNA is used as probe in the arrays. Chips are then scanned to determine genes that show increases in transcription upon exposure to test agonist antibodies. In another version of CHIP

technology (Incyte, Palo Alto, CA), the amount of DNA is not normalized on the glass, therefore, one would set up a competitive hybridization. RNA is isolated from the cells before and after exposure to agonist. cDNA is made from each sample whereby one cDNA reaction has one label incorporated, for example, Cy-3, and the other cDNA population has a different label incorporated, for example Cy-5. Signals are detected and compared on a dual laser scan to collect images.

Visual assays can also be used such as traditional methylcellulose colony forming assays (Stem Cell Technologies, Vancouver BC, Canada). In these assays, colony growth, and morphological changes are scored via light microscope. Visual examination for proliferation or differentiation effects in semi-solid agar cultures or methylcellulose can be performed using the appropriate cell line. Williams Hematology 5 (eds. E. Beutler, M.A. Lichtman, B.S. Coller L T.J. Kipps), McGraw-Hill, Inc., pp L22-L26, 1995). Addition of methylcellulose allows clonal progeny of a single progenitor cell to stay together and facilitates the recognition and enumeration of distinct colonies. All necessary components are added to a basic methylcellulose medium (such as Iscove's MDM, BSA, (-mercaptoethanol, L-glutamine) except colony-stimulating factor supplements and test antibodies (phage supernatants, soluble antibodies) are added to see if they can substitute for growth factors. Cells in methylcellulose culture are incubated for 10-12 days following the addition of antibodies in a 37°C humidified atmosphere of 5% CO_2 in air. After 10-12 days of incubation, colonies are counted using an inverted microscope. After another 8-10 days, colonies are counted again. Comparisons are made between media containing antibodies and controls with and without growth factors. In addition, colonies can be picked from methylcellulose and individual cells examined cytologically by staining with Wright's stain (see Atlas of Hematological Cytology, F.G.J. Hayhoe and R.J. Flemans, Wiley-InterScience 1970).

The receptor-binding affinities of antibody fragments can be calculated (Lfas & Johnson, 1990) from association and dissociation rate constants measured using a BIACORE surface plasmon resonance system (Pharmacia Biosensor). A biosensor chip is activated for covalent coupling of gD-mpl receptor using N-ethyl-N'''-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide

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(NHS) according to the supplier's (Pharmacia Biosensor) instructions. gD-mpl is buffer-exchanged into 10 mM sodium acetate buffer (pH 4.5) and diluted to approximately 30 µg/mL. An aliquot (5 µL) is injected at a flow rate of 1 µL/min to achieve approximately 400 response units (RU) of coupled protein. Finally, 1M ethanolamine is injected as a blocking agent. For kinetics measurements, 1.5 serial dilutions of antibody are injected in PBS/Tween buffer (0.05% Tween-20 in phosphate buffered saline) at 25°C using a flow rate of 20 µL/min. Equilibrium dissociation constants, K_d s, from SPR measurements are calculated as k_{off}/k_{on}. Standard deviations, s_{on} for k_{on} and s_{off} for k_{off}, are typically obtained from measurements with > 4 protein concentrations (k_{on}) or with > 7 protein concentrations (k_{off}). Dissociation data are fit to a simple AB->A+B model to obtain koff +/- s.d. (standard deviation of measurements). Pseudo-first order rate constant (ks) are calculated for each association curve, and plotted as a function of protein concentration to obtain kon +/- s.e. (standard error of fit).

For conversion of antibody clones into full IgGs, the coding regions for both the light and heavy chains, or fragments thereof, can be separately cloned out of a bacterial vector and into mammalian vector(s). A single vector system, such as pDR1 or its derivatives, can be used to clone both light and heavy chain cassettes into the same plasmid. Alternatively, dual expression vectors where heavy and light chains are produced by separate plasmids can be used. Mammalian signal sequences need to be either already present in the final vector(s) or appended to the 5' end of the light and heavy chain DNA inserts. This can be accomplished by initial transfer of the chains into a shuttle vector(s) containing the proper mammalian leader sequences. Following restriction enzyme digestion, the light chain and heavy chain regions, or fragments thereof, are introduced into final vector(s) where the remaining constant regions for IgG1 are provided either with or without introns. In some cases where introns are used, primer design for PCR amplifying the light and heavy chain variable regions out of pRL4 may need to include exon splice donor sites in order to get proper splicing and production of the antibodies in mammalian cells.

With either vector expression system (single or dual plasmid), the production of antibody heavy and light chains can be driven by promoters that work in mammalian cells such as, but not limited to, CMV, SV40, or IgG promoters. Additionally, the

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vector(s) will contain a selectable marker for growth in bacteria (such as, but not limited to, ampicillin, chloramphenicol, kanamycin, or zeocin resistance). Selectable markers for mammalian cells (such as, but not limited to, DHFR, GS, gpt, Neomyocin, or hygromyocin resistance) may also be present in the IgG vector(s), or could be provided on a separate plasmid by co-transfection.

Those of ordinary skill in the art using known techniques would be able to synthesize antibodies in other organisms such as yeast, mammalian, insect, and plants (Carlson, J.R. and Weissman, I.L., *Mol. Cell. Biol.,* 8:2647-2650, 1988; Trill, J.J., Shatzman, A.R., Ganguly, S. *Curr. Opin. Biotechnol.* 6:553-560, 1995; Hiatt, A., Cafferkey, R. Bowdish, K. *Nature* 342: 76-78, 1989).

As stated previously, antibodies made in accordance with the disclosure herein provide increased half-life (duration of action) to the activity of small peptides or peptide mimetics such as the TPO mimetic described herein. In another aspect, the serum half-life of an antibody can itself be prolonged by making derivatives that are pegylated. See, e.g., Lee, et al., Bioconjug. Chem (1999) 10(6): 973-81, incorporated herein by reference. Another advantage, e.g., of the TPO mimetic antibody described herein is that normal TPO treatment may result in generation of TPO neutralizing antibodies in patients which interfere with the activity of a patient's naturally occurring TPO. The present TPO mimetic antibody substantially reduces the likelihood that a detrimental immune response will be produced toward native TPO because it has a different amino acid sequence.

The molecules encompassed by the claims can be used in diagnostics where the antibodies or fragments thereof are conjugated to detectable markers or used as primary antibodies with secondary antibodies that are conjugated to detectable markers. Detectable markers, include radioactive and non-radioactive labels and are well-known to those with skill in the art. Common non-radioactive labels include detectable enzymes such as horseradish peroxidase, alkaline phosphatase and fluorescent molecules. Fluorescent molecules absorb light at one wavelength and emit it at another, thus allowing visualization with, e.g., a fluorescent microscope. Spectrophotometers, fluorescence microscopes, fluorescent plate readers and flow sorters are well-known and are often used to detect specific molecules which have

been made fluorescent by coupling them covalently to a fluorescent dye. Fluorochromes such as green flurorescent protein, red shifted mutants of green fluorescent protein, amino coumarin acetic acid (AMCA), fluorescein isothiocyanate (FITC), tetramethylchodamine isothiocyanate (TRITC), Texas Red, Cy3.0 and Cy5.0 are examples of useful labels.

The molecules can be used in cell isolation strategies such as fluorescence-activated cell sorting (FACS) if fluorescent markers are used, In fluorescence-activated cell sorting, cells tagged with fluorescent molecules are sorted electronically on a flow cytometer such as a Becton-Dickinson (San Jose, California) FACS IV cytometer or equivalent instrument. The fluorescent molecules are antibodies that recognize specific cell surface antigens. The antibodies are conjugated to fluorescent markers such as fluorescein isothiocyanate (FITC) or Phycoerythrin (PE).

Magnetic sorting is also possible. In magnetic sorting procedures, the antibody is linked directly or indirectly to magnetic microbeads. Cells are precoated with antibodies that recognize cell surface molecules, e.g., receptors involved in proliferation, differentiation, activation or survival. The antibodies are attached to magnetic beads conjugated with a secondary immunoglobulin that binds to the primary antibody displaying the peptide, such as to the HA molecular tag engineered into each antibody. The cells are then removed with a magnet. Magnetic sorting can be positive selection where cells of interest are bound by the antibody and hence the magnet, or negative selection where undesired cells are isolated onto the magnet.

Alternatively, radiolabeled antibodies can be used for diagnostic purposes.

Antibodies and fragments thereof disclosed herein are useful for the amplification of a variety of clinically relevant cell types. Treatment can be *in vivo* or *ex vivo*. For example, agonist antibodies are useful to treat patients suffering from a deficiency in a cell population caused by disease, disorder or treatment related to for example suppression of hematopoiesis where less than the normal number of cells of a given lineage or lineages are present in a patient. The following represent only some examples of the conditions that can be treated with the antibodies containing biologically active peptides disclosed herein, those who practice the art would be able to identify other diseases and conditions that would benefit from such treatment. For

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example, HIV-infected patients, patients undergoing chemotherapy, bone marrow transplant patients, stem cell transplant patients, and patients suffering from myeloproliferative disorders show subnormal levels of specific hematopoietic lineages.

Thrombocytopenia can be a result of chemotherapy, bone marrow transplantation or chronic disease such as idiopathic thrombocytopenia (ITP) which all result in low platelet levels. The present TPO mimetic antibodies can be used to treat such patients.

Patients undergoing renal dialysis often suffer from treatment related anemia with subnormal levels of red blood cells. In aplastic anemia, bone marrow suppression can cause pancytopenia or may affect only the red blood cells, the white cells, or the platelets. The disclosed antibodies will augment the armamentarium of therapeutic agents for these and other diseases and disorders characterized by deficiencies in specific cell populations, such as hematopoietic cells.

The molecules encompassed by the claimed invention can also be used for *ex vivo* proliferation and differentiation of cells. This is useful for gene therapy purposes, for example for traditional viral vector approaches, and for autologous bone marrow transplants.

In addition, certain antibodies in accordance with the present disclosure can be radiolabeled for radioimmunotherapy or conjugated to toxins to deliver such toxins to specific cell types and result in the killing of those cells.

A biologically active *c-mpl* agonist antibody capable of stimulating proliferation, differentiation and maturation of hematopoietic cells may be used in a sterile pharmaceutical preparation or formulation to stimulate megakaryocytopoietic or thrombopoietic activity in patients suffering from thrombocytopenia due to impaired production, sequestration, or increased destruction of platelets. Thrombocytopenia-associated bone marrow hypoplasia *(e.g.,* aplastic anemia following chemotherapy or bone marrow transplant) may be effectively treated with the disclosed antibodies as well as disorders such as disseminated intravascular coagulation (DIC), immune thrombocytopenia (including HIV-induced ITP and non HIV-induced ITP), chronic idiopathic thrombocytopenia, congenital thrombocytopenia, myelodysplasia, and thrombotic thrombocytopenia.

The biologically active *c-mpl* agonist antibodies disclosed herein containing the TPO mimetic peptide may be used in the same way and for the same indications as thrombopoietin (TPO). Thrombopoietin (TPO) stimulates megakaryocytopoiesis and platelet production. These antibodies are expected to have a longer half-life than native or pegylated TPO and thus are used in indications where a longer half-life are indicated.

An example of an assay useful for determining activity of TPO mimetics is the rebound thrombocytosis assay which involves administering to mice a single injection of goat anti-mouse platelet serum to induce acute thrombocytopenia (day 0). On days 5 and 6 mice are injected with test samples. On day 8 platelet counts are determined (^{35}S incorporation into platelets).

EPO mimetic antibodies herein stimulate hematopoiesis in a manner similar to naturally occurring EPO. Such therapy is useful in treating conditions where red blood cell production is compromised such as in chronic renal failure. The biological activity of EPO mimetic antibodies may be determined using *in vitro* or *in vivo* assays.

One *in vitro* assay measures the effect of erythropoietin mimetic antibodies on erythropoiesis in intact mouse spleen cells according to the procedure of Krystal, G., *Exp. Hematol.* 11:649-660 (1983). To screen various embodiments of the EPO mimetic antibodies for activity, for example, *in vitro* or *in vivo*, the EPO mimetic antibodies can be evaluated for the extent of erythropoiesis or receptor binding. Tests to determine biological activity are well-known to those of skill in the art. For example, the biological activity of erythropoietin can be measured as described in, e.g., U.S. Pat. No. 5,614,184 and U.S. Pat. No. 5,580,853 herein incorporated by reference.

The route of antibody administration is in accord with known methods, *e.g.*, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, subcutaneous, intraocular, intraarterial, intrathecal, inhalation or intralesional routes, topical or by sustained release systems as noted below. The antibody is preferably administered continuously by infusion or by bolus injection. One may administer the antibodies in a local or systemic manner.

The antibodies of the invention may be prepared in a mixture with a pharmaceutically acceptable carrier. Techniques for formulation and administration of

the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. This therapeutic composition can be administered intravenously or through the nose or lung, preferably as a liquid or powder aerosol (lyophilized). The composition may also be administered parenterally or subcutaneously as desired. When administered systematically, the therapeutic composition should be sterile, pyrogen-free and in a parenterally acceptable solution having due regard for pH, isotonicity, and stability. These conditions are known to those skilled in the art.

Briefly, dosage formulations of the compounds of the present invention are prepared for storage or administration by mixing the compound having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to the recipients at the dosages and concentrations employed, and may include buffers such as TRIS HCl, phosphate, citrate, acetate and other organic acid salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidinone; amino acids such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium and/or nonionic surfactants such as TWEEN, PLURONICS or polyethyleneglycol.

When used for *in vivo* administration, the antibody formulation must be sterile and can be formulated according to conventional pharmaceutical practice. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The antibody ordinarily will be stored in lyophilized form or in solution. Other vehicles such as naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be desired. Buffers, preservatives, antioxidants and the like can be incorporated according to accepted pharmaceutical practice.

Pharmaceutical compositions suitable for use include compositions wherein one or more rationally designed antibodies are contained in an amount effective to achieve

their intended purpose. More specifically, a therapeutically effective amount means an amount of antibody effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Therapeutically effective dosages may be determined by using *in vitro* and *in vivo* methods.

An effective amount of antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. In addition, the attending physician takes into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

For any antibody containing a peptide, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the EC_{50} as determined in cell culture *(e.g.,* the concentration of the test molecule which promotes or inhibits cellular proliferation or differentiation). Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the antibody molecules described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.,* for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Molecules which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such molecules lies preferably within a range of circulating

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concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See *e.g.*, Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the antibody which are sufficient to promote or inhibit cellular proliferation or differentiation or minimal effective concentration (MEC). The MEC will vary for each antibody, but can be estimated from *in vitro* data using described assays. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Antibody molecules should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the effective local concentration of the antibody may not be related to plasma concentration.

A typical daily dosage might range from about 1μ/kg to up to 1000mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer the molecule until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

Depending on the type and severity of the disease, from about 0.001 mg/kg to abut 1000 mg/kg, more preferably about 0.01 mg to 100 mg/kg, more preferably about 0.010 to 20 mg/kg of the agonist antibody might be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs or the desired improvement in the patient's condition is achieved. However, other dosage regimes may also be useful.

EXAMPLES

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

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EXAMPLE 1

Library Construction Of TPO Mimetic Sequences Grafted Into A Human Antibody Framework

An agonist TPO mimetic-peptide IEGPTLRQWLAARA (SEQ. ID. NO: 1) was grafted into the anti-tetanus toxoid (TT) Fab heavy chain CDR3 (HCDR3), replacing the entire HCDR3 sequence GDTIFGVTMGYYAMDV (SEQ. ID. NO: 4). Figure 2A shows the sequence for the human tetanus toxoid antibody employed. Two grafting approaches were taken. In the first approach the agonist peptide was inserted into the H-CDR3 region with two glycines flanking each side. This was to reduce structural constraints on the grafted peptide so that it could more easily adopt the needed conformation. In the second approach, two amino acid positions on each side of the peptide graft were randomized in order that the best presentation of the peptide could be achieved (Figure 3). These two approaches were taken in order to determine whether the peptide alone was sufficient or if specific residues were required for proper presentation of the agonist peptide on the antibody scaffold, thereby conferring its activity to the antibody.

Figure 4 outlines the library construction process. Briefly, the anti-tetanus toxoid Fab was amplified as two fragments. Fragment A was amplified using a forward primer (N-Omp: 5' TAT CGC GAT TGC AGT GGC ACT GGC 3') (SEQ. ID. NO: 5) that annealed to the Omp A leader for the light chain in combination with a backward primer (TPOCDR3-B: 5' GC CAG CCA TTG CCG CAG CGT CGG CCC TTC AAT YNN YNN TCT CGC ACA ATA ATA TAT GGC 3') (SEQ. ID. NO: 6) that annealed at the end of the heavy chain framework region (FR) 3. The reverse primer contained a tail encoding the new CDR3. Fragment B was generated using a forward primer (TPOCDR3-F: 5' CCG ACG CTG CGG CAA TGG CTG GCG GCG CGC GCG NNY NNY TGG GGC CAA GGG ACC ACC GT 3')(SEQ. ID. NO:7) that annealed at the FR4 and the reverse primer Seq-G3Rev (5' TCA AAA TCA CCG GAA CCA GAG C 3') (SEQ. ID. NO: 8) which annealed in the gene III region of the plasmid, downstream of the heavy chain stop signal. The TPOCDR3-F primer also had a tail of bases that encoded the new CDR3 region. TAQ DNA Polymerase (Perkin Elmer) was used in the following PCR program: 94° 30 seconds, then 30 cycles of 94° for 15 sec, 55° for 15

seconds, and 72° for 90 seconds, followed by an extension period at 72° for 10 minutes, and a hold at 4°. After the fragments were generated by PCR and gel purified, they were combined for an overlap extension PCR. The new CDR3 primer encoded regions were complementary and provided 23 bases of overlap. Primers N-Omp and SeqG3Rev were used in the overlap PCR protocol to generate the full Fab DNA product. Taq DNA Polymerase (Perkin Elmer) was used in the following PCR program: 94° 30", then 20 cycles of 94° 30", 56° 30", and 72° 3'15", then an extension period of 72° for 15' followed by a 4° hold. After gel purification of the Fab product, an Sfi 1 digest was performed at 50° for 5 hours. Inserts were ligated into Sfi 1digested pRL4 vector overnight. Ligation products were ethanol precipitated, resuspended in H_2O, and then electroporated into competent ER2537 bacteria (suppressor strain, New England Biolabs). Following one hour of shaking in 5 mls SOC, an equal volume of SB was added. Carbenicillin was added to 20ug/ml and the culture shaken for one hour at 37°, followed by one hour at 37° in 50ug/ml carbenicillin. The library culture was transferred into a flask containing 100 mls fresh SB, 50 ug/ml Carbenicillin, and 10^{12} VCS M13 helper phage. After two hours at 37°, kanamycin was added to select for those bacteria that had been infected with helper phage. The following day, the overnight cultures were spun down and the phage in the supernate were precipitated on ice using 4% PEG/0.5 M NaCl. After spinning down the phage, the pellet was resuspended in 1%BSA/PBS, filtered and dialyzed against PBS. Library phage were stored at 4°.

The construction of Fabs containing the non-randomly linked peptide was performed as described above by substituting primers TPOCDR3-B and TPOCDR3-F with alternate specific primers. For PP-(IEGPTLRQWLAARA)-GG (SEQ. ID. NO: 25) grafted antibody, primers used were TPOCDR3g-B (5' GC CAG CCA TTG CCG CAG CGT CGG CCC TTC AAT NGG NGG TCT CGC ACA ATA ATA TAT GGC 3') (SEQ. ID. NO: 9) and TPOCDR3g-F (5' CCG ACG CTG CGG CAA TGG CTG GCG GCG CGC GCG GGN GGN TGG GGC CAA GGG ACC ACC GT 3')(SEQ. ID. NO: 10). For GG-(IEGPTLRQWLAARA)-GG (SEQ. ID. NO: 29) grafted antibody, primers used were TPO-CDR3-ggB (5' GC CAG CCA TTG CCG CAG CGT CGG CCC TTC AAT NCC NCC TCT CGC ACA ATA ATA TAT GGC 3')(SEQ. ID. NO: 11) and TPOCDR3g-F (5'

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CCG ACG CTG CGG CAA TGG CTG GCG GCG CGC GCG GGN GGN TGG GGC CAA GGG ACC ACC GT 3') (SEQ. ID. NO: 12).

Selection of the TPO Mimetic Peptide Heavy Chain CDR3 Library

In order to select for the optimal peptide display, panning was performed on human platelets. Because platelets express approximately 1800 TPO receptors per cell on their surface (cMpl receptors), they represented a good cell target. In addition, platelets are readily available from a local Blood Bank. To 1ml of concentrated indated human platelets from the Blood Bank, 50 uls of freshly prepared Fab-phage were added in a 15ml conical tube with 0.1% NaN_3. The tube was mixed at room temperature for 1-2 hours. Typically, 10 mls of 50% human serum (taken off the remaining platelets) + 50%{IMDM/10% FBS/0.1% azide/2mM EDTA} was added to the phage/cells. Platelets were pelleted at 5500xg for 5 minutes at room temperature. Supernatant was drained and the pellet was left resting under ~500 uls of the wash for 20 minutes. The platelets were very gently resuspended and then 10mls of 25% human serum (taken off the remaining platelets) +75%{IMDM/10% FBS/0.1% azide/2mM EDTA} was added to the phage/cells. The centrifugation, pellet rest, and resuspension of the platelets was repeated. In panning rounds 3 and 4, a third wash was performed. The washed phage/cells were transferred to an eppindorf tube and spun at 5200xg. Phage were eluted from the platelets 10 minutes at room temperature using acid elution buffer (0.1M HCl, 1mg/ml BSA, and glycine to pH 2.2). Platelets were pelleted at max speed and the eluted phage transferred to a 50 ml conical tube, neutralized with 2M Tris Base. Phage were then allowed to infect fresh ER2537 bacteria for 15 minutes at room temperature and amplified overnight as described above. Four rounds of platelet panning were performed.

After the fourth round of panning, pools of 3 Fab clones expressed as soluble proteins in nonsuppressor bacterial strain TOP10F' (Invitrogen, Carlsbad, CA) were tested by Facs for binding to platelets by utilizing the Fabs' HA epitope tag with rat high affinity anti-HA followed by anti-Rat-FITC (Sigma, St. Louis, Missouri). 25 uls indated concentrated human platelets (washed once with PBS / 5mM EDTA / 2% FBS) were incubated with 100 uls bacterial supernate (60 uls bacterial supernate from three

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pooled Fab clones were pre-incubated with 40 uls 5% Milk / PSS at 4° for 15 minutes) at room temperature for 20-30 minutes. 1 ml of FACS buffer (PBS / 2% FBS / 5mM EDTA) was added and cells spun down at 5200xg for 5 minutes. Pelleted cells were resuspended in 50 uls of l:10 diluted (in PBS / 1% BSA / 0.1% NaN_3) 2° anti-HA antibody [Rat IgG anti-HA High Affinity clone 3F10 (Roche Molecular Biochemicals)] was added. After 30 minutes at room temperature, the cells were washed with 1 ml FACS buffer as above. Following centrifugation, cells were resuspended in 100 uls of 1:160 diluted (in PBS /1% BSA /0.1% NaN_3) 3° anti-Rat IgG-FITC antibody (Sigma) and incubated 20 minutes at room temperature in the dark. Cells were washed with 1 ml FACS buffer then resuspended in 200 uls FACS buffer for analysis. As a positive control a commercially available sheep anti-IIb/IIIa Ab followed by anti-sheep FITC was used. Many pools of Fabs were clearly positive for binding to platelets by Facs. Follow up Facs analysis was then performed to identify individual clones that bound to the platelets.

Examination Of Individual Candidates By Binding Activity

Several Fabs, as bacterial supernatants, were tested for reactivity to the original antigen tetanus toxoid in order to determine if that binding specificity was retained. The antibody scaffold anti-TT Fab does bind to its antigen TT, but not to BSA. However, four TPO-mimetic peptide grafted Fab clones did not show significant binding to TT or BSA. As seen in previous experiments, the replacement of anti-TT Fab HCDR3 was sufficient to change the specificity of the antibody.

To further examine the binding capabilities of Fabs, Facs analysis was performed on CMK cells, a Megakaryocytic cell line (from German Collection of Microorganisms and Cell Cultures) which also expresses the cMpl receptor. Fab clones that bound CMK cells were then analyzed to verify that the platelet and CMK cell binding was occurring via the cMpl receptor. For that experiment, 293 EBNA cells were transfected with or without the cMpl-R, which had been cloned from Tf-1 cells by RT-PCR. 1 X 10^6 transfected cells were incubated with bacterial supernate from each Fab clone (pre-blocked as described above) for 20-30 minutes at room temperature. Cells were spun down at 2000 rpm for 5 minutes. Pelleted cells were resuspended in

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90 uls FACS buffer (PBS / 2% FBS / 1mM EDTA) then 10 uls of 2° anti-HA antibody [Rat IgG anti-HA High Affinity clone 3F10 (Boehringer Mannheim Biochemicals) was added for a final 1:10 dilution. After 20 minutes at room temperature, the cells were washed with 1 ml FACS buffer. Following centrifugation, cells were resuspended in 100 uls of 1:50 diluted (in PBS /1% BSA /0.1% NaN_3) 3° anti-Rat IgG-PE antibody (Research Diagnostics Incorporated, RDI) and incubated 20 minutes at room temperature in the dark. Cells were washed with 1 ml FACS buffer then resuspended in 200 uls FACS buffer for analysis. Fabs selected during panning demonstrated strong binding to cells transfected with the cMpl-R but not to control vector transfected cells lacking the cMpl-R. This indicates that cell surface binding was occurring specifically through the cMpl receptor. Anti-TT Fab does not bind to control vector or cMpl-R transfected 293 cells. However, Fab clone X1c shows a shift from 3% binding of control (non-cMpl receptor) transfected cells to 95% binding of cells expressing the cMpl-R.

Examination Of Individual Candidates By Sequence

Sequence analysis of Fab clones which specifically bound to the cMpl receptor (see Fig. 5), revealed the selection of preferred amino acids at the downstream linkage site. The DNA sequence data was analyzed and the amino acid and DNA sequences are as follows:

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Clone	Binding Properties	SEQ. ID. NO	Sequence
X1a	weak	25	Pro Pro (14 aa peptide) Gly Gly
X1a-11	weak	27	Gly Gly (14 aa peptide) Gly Gly
X1a-13	weak	29	Gly Gly (14 aa peptide) Gly Gly
X1c	strong	31	Trp Leu (14 aa peptide) Pro Val
X2c	weak	33	Met Ile (14 aa peptide*) Val Gly
X3a	strong	35	Val Val (14 aa peptide) Pro Val
X3b	strong	37	Gly Pro (14 aa peptide) Pro Asp
X4b	strong	39	Leu Pro (14 aa peptide) Pro Val
X4c	strong	41	Ser Leu (14 aa peptide) Pro Ile
X5a	strong	43	Thr Met (14 aa peptide) Pro Val
X5c	strong	45	Trp Leu (14 aa peptide) Pro-Val
X7a	weak	47	Thr Arg (14 aa peptide*) Cys Ser
X7b	weak		deletion mutant this clone has lost the peptide
X7c	strong	49	Gln Thr (14 aa peptide) Pro Asp

All clones which demonstrated strong binding, were found to contain a proline just downstream of the 14 amino acid TPO mimetic peptide. Selection by panning of a proline in the downstream linker position represents determination of a surprising amino acid choice which confers improved binding characteristics to the grafted TPO mimetic peptide. Weak binders did not contain this proline although they still contained the TPO mimetic peptide. It should be noted that clone X7a had a silent mutation in this peptide (GCG to GCA retaining the Ala at position 11 in the peptide) and that

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clone X2c had a mutation in this peptide switching a Thr for Ala at position 11 of the peptide.

Biological Assays

Clones were tested for agonist activity using a transcriptional based assay measuring luciferase activity driven by the c-Fos promoter. Dimerization of the cMpl receptor activates Jak which stimulates the MAP kinase pathway. Thus activation can be measured by assaying luciferase production and activity stimulated by MAP kinase via the cFos promoter. Since dimerization of the cMpl receptor is required for activation, either full IgG or dimerized Fab fragments capable of dimerizing the receptor, could be used to stimulate cMpl receptor activity. Fabs produced in bacteria were dimerized via the HA tag utilizing the 12CA5 anti-HA antibody. Increasing amounts of 12CA5 was added to the bacterial Fabs to dimerize the Fab clones in order that they might in turn dimerize and activate the cMpl receptor. For measurement of agonist activities Fab containing bacterial supernatants (2mls) mixed with 12CA5 were applied to NIH3T3 cells which had been co-transfected with either a control vector or the cMpl receptor and the Fos promoter/luciferase reporter construct. Co-transfections of 3T3 cells were performed by plating NIH 3T3 cells at 3 x 10^5 cells per 6 cm dish and then transfecting the following day. NIH 3T3 cells were transfected using the Effectine lipofection reagent (Qiagen), transfecting each plate with 0.1 ug pEGFP (a tracer to measure transfection efficiency), 0.2 ug of the Fos promoter/luciferase construct and 0.7 ug of either the empty control vector or the plasmid expressing the cMpl receptor. 3T3 cells were placed in 0.5% serum 24 hours post transfection and incubated for an additional 24 hours in this low serum media to reduce the background activation of the Fos promoter. Antibody supernatants were then applied to these cells for 6 hours. Cells were harvested and luciferase assays performed using 50 ug of cell lysate. No activation was stimulated by the antibodies in the absence of cMpl receptor expression. However, agonist activity was observed in cMpl receptor co-transfected 3T3 cells. This allowed us to demonstrate that the agonist activity observed was through the cMpl receptor and its interaction with the antibody. The data is as follows:

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Bacterial Supernatants with 12CA5		
CELL Treatment	Relative Fold Luciferase activity	
	CMpl-R transfected	Control transfected
Untreated:	1.0	1.0
+ 10% FCS	3.43	3.0
+ TPA	2.3	2.42
+ TPO	2.32	0.76
+ X4c Sup (alone)	1.03	0.94
+ X4c + 60ul α-HA	1.97	0.84
+ X4c + 30ul α-HA	1.52	0.87
+ X4c + 10ul α-HA	1.22	0.86
+ X4c + 3ul α-HA	0.94	0.68
+ X4c + 1ul α-HA	0.91	1.05
+ X4c + 0.3ul α-HA	1.01	0.95

Activation of cMpl receptor can be tested in a similar manner using full IgGs (converted from Fab as described herein) produced by transient or stable transfection of mammalian cells rather than bacterially produced Fabs dimerized by anti-HA 12CA5. Experimentally transient transfection can be performed essentially as described here. For transfections 2 x 10^6 cells (such as 293 EBNA) would be plated in 6cm dishes for each test sample. The following day each plate would be transfected with 2.5 ug of total DNA (2 ug total of the light chain and heavy chain plasmid(s), 0.25 ug of pAdVAntage (Promega, Madison, Wisconsin), and 0.25 ug of pEGFP) using the

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Effectine reagent (Qiagen). The 293 cells would be placed in 0.5% serum 24 hours post transfection and incubated for an additional 24 hours in this low serum media to obtain full IgG. Residual growth factors are negligible in this media in stimulating receptors as seen in controls experiments. After 24 hours supernatants would be collected and spun for 5 minutes at 3000rpm to remove any residual cells. For measurement of agonist activities of the full IgGs, 3mls of the conditioned 293 cell supernatants would be applied to NIH3T3 cells as described above.

EXAMPLE 2

Additional Libraries Containing TPO Mimetic Sequences Grafted Into A Human Antibody Framework

Another approach to linking two agonistic peptides together in an antibody framework is to insert the agonist peptide in more than one position within a single Fab fragment. In order to do that, additional libraries containing TPO mimetic sequences grafted into a human antibody framework were constructed. Following selection of peptides properly presented in the context of CDR1, CDR2 or CDR3 of the light chain, or CDR2 of the heavy chain, the binding sequences were combined into a single Fab molecule, for example as listed in Table 1 below, and analyzed for enhanced activity.

TABLE 1

Peptide 1	Peptide 2
H-CDR3	H-CDR2
H-CDR3	L-CDR1
H-CDR3	L-CDR2
H-CDR3	L-CDR3
H-CDR2	L-CDR2
H-CDR2	L-CDR3
L-CDR3	L-CDR2

Four additional libraries have been constructed separately replacing the heavy chain CDR2 as well as the light chain CDR1, CDR2 and CDR3 with the TPO mimetic peptide flanked by 2 random amino acids using an NNK doping strategy. The generation of the libraries was similar to that described for the heavy chain CDR3 TPO

peptide library, except that only the chain (heavy or light) being modified to form a library was amplified by PCR and used as the insert. PCR was performed using Expand High Fidelity PCR System (Roche) which contains a mixture of Taq and Pwo Polymerases. The first round of PCR was performed using the program: 94° 30", then 30 cycles of 94° 15", 56° 30", and 72° 2', followed by elongation for 10' at 72° and a 4° hold. Overlap PCR was performed for 10 cycles without primers using the program listed above to allow the full DNA template to be generated by the polymerases. Primers were then added to the PCR reaction tubes for 20 cycles of the same program for amplification.

For the HCDR2 library, the fragment A was created using the forward primer lead VH (5' GCT GCC CAA CCA GCC ATG GCC 3')(SEQ. ID. NO: 13), which annealed at the pel B leader signal located in front of the heavy chain, and the reverse primer HR2 CMPL ANTI (5' AGC CAG CCA CTG GCG CAG GGT TGG GCC TTC GAT MNN MNN TCC CAT CCA CTC AAG CCC TTG 3')(SEQ. ID. NO: 51) that annealed at the end of the heavy chain FR2. The reverse primer contained a tail encoding the new CDR2. Fragment B was created using forward primer HR2 cMpl CODE (5' CCA ACC CTG CGC CAG TGG CTG GCT GCT CGC GCT NNK NNK AGA GTC ACC ATT ACC GCG GAC 3')(SEQ. ID. NO: 14) which annealed at FR3 of the heavy chain and reverse primer N-dp (5' AGC GTA GTC CGG AAC GTC GTA CGG 3')(SEQ. ID. NO: 15) which annealed in the HA epitope tag region of the plasmid, downstream of the heavy chain constant region. The HR2 cMpl CODE primer also had a tail of bases that encoded the new CDR2 region. After the fragments were generated by PCR and gel purified, they were combined for an overlap extension PCR. The new CDR2 primer encoded regions were complementary and provided 24 bases of overlap. Primers leadVH and N-dp were used in the overlap PCR protocol to generate the full heavy chain DNA product. Following gel purification of the heavy chain product, a Xho I / Spe I digest was performed at 37° for 3 hours. Inserts were gel purified and then ligated into Xho I / Spe I digested pRL4 vector containing the anti-TT light chain. Ligation products were precipitated and electroporated into ER2537 bacteria as described above for the generation of the Fab-phage library.

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The light chain CDR3 library was similarly made using primers for Fragment A of forward primer N-omp and reverse primer LR3 cMpl ANTI (5' AGC CAG CCA CTG GCG CAG GGT TGG GCC TTC GAT MNN MNN ACA GTA GTA CAC TGC AAA ATC 3') (SEQ. ID. NO: 16) and for Fragment B of forward primer LR3 cMpl CODE (5' CCA ACC CTG CGC CAG TGG CTG GCT GCT CGC GCT NNK NNK TTC GGC CAA GGG ACC AAG GTG 3')(SEQ. ID. NO: 17) and reverse primer leadB (5' GGC CAT GGC TGG TTG GGC AGC 3') (SEQ. ID. NO: 18). Primer leadB annealed to the pelB leader sequence located before the VH. The LR3 cMpl ANTI reverse and LR3 cMpl CODE forward primers annealed to the FR3 and FR4 of anti-TT light chain respectively. Both LR3 cMpl primers contain a tail of nucleotides encoding the new CDR3 peptide library, which provides the 24 basepair overlap region for the fusion PCR of Fragment A and Fragment B. Following purification of the light chain PCR products, a Sac I / Xba I digest was performed at 37° for 3 hours. The light chain fragments were then ligated into Sac I / Xba I digested pRL4 containing the anti-TT heavy chain overnight at room temperature. Ligation products were precipitated and electroporated into ER2537 bacteria as described above for the generation of the Fab-phage library.

The construction of the light chain CDR2 library was carried out as described above for the light chain CDR3 library with the exception that specific primers LR2 cMpl ANTI (5' AGC CAG CCA CTG GCG CAG GGT TGG GCC TTC GAT MNN MNN ATA GAT GAG GAG.CCT GGG AGC 3')(SEQ. ID. NO: 19) which annealed at the end of light chain FR2 and primer LR2 cMpl CODE (5' CCA ACC CTG CGC CAG TGG CTG GCT GCT CGC GCT NNK NNK GGC ATC CCA GAC AGG TTC AGT 3')(SEQ. ID. NO: 20) which annealed at the beginning of light chain FR3 were used in place of the LR3 cMpl primers.

The construction of the light chain CDR1 library was also carried out as previously described for the light chain CDR3 library with the exception that specific primers TPOLR1CODE (5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTNNKNNKTGGTACCAGCAGA AACCTGGC 3') (SEQ. ID. NO: 58) which annealed at the beginning of the light chain

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FR2 and the primer TPOLR1ANTI (5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATMNNMNNGCAGGAGAGGGT GGCTCTTTC 3') (SEQ. ID. NO: 59) which annealed to the end of the light chain FR1 were used in place of the LR3 cMpl primers.

The three additional libraries, which separately replace the heavy chain CDR2 and the light chain CDR2 and CDR3 with the TPO mimetic peptide flanked by 2 random amino acids using the NNK doping strategy, were separately panned on platelets, as was previously described in Example I for the heavy chain CDR3 replacement library. Four rounds of panning were performed and clones were screened by FACS on platelets and cMpl receptor transfected 293 cells as previously described. Two positive clones were obtained from these screens. These clones had the TPO mimetic peptide in the heavy chain CDR2. Unlike with the heavy chain CDR3 clones neither of the heavy chain CDR2 clones had a proline in downstream position. Instead both were found to contain a tyrosine in the upstream position (See Figure 9-clones HC-CDR2 No. 24 and No. 39).

The libraries, including LCDR1, were separately subjected to another panning experiment using cMpl receptor transfected 293 cells instead of platelets during the panning. The 293 cells were observed to reproducibly transfect at a high efficiency and express very high levels of the functional cMpl-receptor on their surface. Thus these cells represented a good cell target for use in panning. For these experiments different groups of plates of 293 cells were separately and sequentially transfected four days in a row. Each group of plates was then sequentially used for the four separate rounds of panning. Each round of harvesting of the cells and panning occurred two days after transfection. For harvesting, cells were removed from the plates using cell disassociation buffer, spun down at 1500 rpm for 5 minutes and re-suspended in IMDM supplemented with 10% FCS, 0.1% sodium azide and 5mM EDTA at a concentration of 1 x 10^6 cell per ml (3x10^6 for LC-CDR1). In the round one pan, 3 x 10^{11} phage from each library were separately applied to 2 ml of cells (6x10^6 for LC-CDR1 and 2 x 10^6 cells for all others) and rotated in a 15 ml conical tube for two hours at room temperature. Cells were washed twice using 10 mls of the IMDM/10%

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FCS/0.1% sodium azide/5mM EDTA buffer. Phage were eluted in acid and amplified as previously described in Example 1. In round two 4 x 10^6 cells (6x10^6 for LC-CDR1) were used in 2 ml of buffer and 3 x 10^{11} phage from the amplified round one eluted phage was combined with 3 x 10^{11} phage from the un-panned library and added to the cells. Washing, elution and amplification proceeded similar to round one. In round three 4 x 10^6 cells (6x10^6 for LC-CDR1) were used in 2 ml of buffer and 3 x 10^{11} phage from the amplified round two eluted phage were used. Cells were washed three times prior to elution. In round four, 4 x 10^6 cells (6x10^6 for LC-CDR1) were again used in 2 ml of buffer and 3 x 10^{11} phage from the amplified round three eluted and amplified phage were used. Cells were again washed three times prior to elution. At least thirty individual clones were screened by FACS on cMpl receptor transfected 293 cells as previously described. 12 positive clones were obtained from the heavy chain CDR2 library and 25 positive clones were obtained from the light chain CDR2 library, and 14 positive clones were obtained from the light chain CDR1 library. Clones were further analyzed by DNA sequence. The selected flanking amino acid residues for the positive clones are depicted in the attached Figure 9. It is of interest to note that the light chain CDR2 grafted Fabs have a strong selection for a proline (Pro) upstream of the TPO mimetic peptide.

EXAMPLE 3

Combinations of the TPO mimetic peptide grafted Fab clones from Figure 9 have been generated. Thus a single antibody might contain multiple copies of the TPO mimetic peptide within a single light or heavy chain. Alternatively, both the light and heavy chains might contain peptide grafts giving multiple copies within a single Fab. Positive clones selected from the same CDR library were pooled. New libraries were constructed by combining the pool of one TPO mimetic peptide containing CDR with the pool of another. Combinations where one of the TPO mimetic peptides is in the light chain and the other is in the heavy chain are made using simple cloning techniques using the pooled plasmid DNAs, and the unique restriction sites flanking the heavy (Xho I-SpeI) and light chains (Sac I-Xba I). For example, the plasmid DNA for the H-CDR3 peptide grafted heavy chains were combined and digested by Xho I

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and Spe I. The purified heavy chain inserts were ligated into the Xho I/Spe I digested plasmid containing the L-CDR2 grafts. The resulting library contained heavy chains with CDR3 peptide grafts and light chains with CDR2 peptide grafts. It should be understood that individual clones could also be combined rather that using pools of clones for the pairing of two peptide containing CDRs. For example, a single heavy chain clone with a CDR3 peptide graft was paired with several individual light chain CDR1 clones to create Fabs with multiple copies of TPO mimetic peptides.

Combinations where two TPO mimetic peptides were combined within a given heavy chain was performed using overlap PCR to generate the fragment for cloning. Two overlapping primers which bind between CDR2 and CDR3, and flanking primers, such as "N omp" and "lead B" primers from the light chain and "Lead VH" and "Ndp" primers for the heavy chain were used. For example, to combine H-CDR2 and H-CDR3 the first PCR was performed using lead VH (a primer that anneals in the vector at the heavy chain pelB leader signal) and a reverse primer annealing at FR3 using the H-CDR2 pooled plasmid DNA as the template. The sequence of that primer was 5' CCA TGT AGG CTG TGC CCG TGG ATT 3' (SEQ. ID. NO: 63). In a separate reaction, the pooled plasmids containing the H-CDR3 grafts underwent PCR with a forward primer annealing in FR3 (which is complementary to the above FR3- reverse primer) and N-dp (which anneals in the vector at an epitope tag sequence). The sequence of that primer was 5' CCA CGG GCA CAG CCT ACA TGG AGC 3' (SEQ. ID. NO: 64). The first PCR products were purified then combined in an overlap PCR reaction, where fusion of the two fragments occurred through the complementary FR2 sequences. The full heavy chain product was cloned by Xho I/Spe I into a plasmid containing the anti-tetanus toxoid light chain. In all, five classes of Fabs bearing multiple copies of TPO mimetic peptides were created as detailed in Table 2 below.

TABLE 2

Peptide 1	Peptide 2	Peptide 3
H-CDR3	H-CDR2	
H-CDR3	L-CDR2	
H-CDR2	L-CDR2	
H-CDR3	H-CDR2	L-CDR2
H-CDR3	L-CDR1	

20 clones from each combination library and four individual clones of H-CDR3 plus L-CDR1 were tested for biological activity in a luciferase reporter assay as previously described. See Figures 10 and 11. In both experiments, the negative controls can include uninduced cells, cells treated with an irrelevant Fab (anti-tetanus toxoid), cells treated with a Fab clone that only weakly binds cMpl receptor, and X4b and/or X1c Fabs which do bind the cMpl receptor but only have a single binding domain and so can not activate the receptor. The positive control was the addition of TPO. All remaining samples were from the newly formed combination libraries. As can be observed, several clones have significant activity as Fabs. This indicates that incorporation of multiple TPO mimetic peptides into a single Fab molecule is able to bind two receptors and cause receptor activation.

In fact, using Fab 59 the agonistic activity obtained can be as high as native TPO activity. Clone 59 containing two TPO mimetic peptides (HC CDR3 sample x4c and LC CDR2 sample 19 as identified in Fig. 9) and a His6 tag was partially purified from a bacterial periplasmic prep by FPLC using nickel column chromatography. The activity of this Fab was measured and found to be approximately equivalent to that of TPO (see Figure 12), as estimated by cMpl-R specific induction of luciferase activity in direct comparison to known concentrations of TPO. A quantitative western blot was performed in order to determine the Fab 59 antibody concentrations.

These Fabs, or various other two CDR combinations, could be used as a therapeutic product. Alternatively, these clones could be converted to framework germline sequences (either with or without codon optimization) for use as a therapeutic agent so long as activity was maintained.

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EXAMPLE 4

The TPO mimetic peptide graft in Fab clone X4b has been transplanted into the heavy chain CDR3 of another antibody framework, 5G1.1. Construction of 5G1.1 is described in U.S. Appln. Ser. No. 08/487,283, incorporated herein by reference. The sequence was cloned into 5G1.1 in such a fashion as to replace the native CDR3 with 5' ttg cca ATT GAA GGG CCG ACG CTG CGG CAA TGG CTG GCG GCG CGC GCG cct gtt 3' (SEQ. ID. NO: 65). The peptide graft translated into amino acids is Leu Pro Ile Glu Gly Pro Thr Leu Arg Gln Trp Leu Ala Ala Arg Ala Pro Val (SEQ. ID. NO: 66). The 5G1+peptide was produced as a whole IgG antibody (See Figures 13A and 13B).

Purified 5G1.1+peptide antibody as well as the parental 5G1.1 were analyzed for their ability to bind to cMpl receptor by FACS analysis. Binding to receptor expressing and non-receptor expressing 293 cells was compared. See Figure 14. The FACS staining was performed essentially as described previously herein, with the exception that the detection was done using PE conjugated F(ab')2 fragment of goat anti-human IgG (H+L). The negative controls of 3° only anti-tetanus toxoid irrelevant Fab, and Fab X1a which binds weakly to cMpl receptor all showed very little staining. However, binding Fabs X1c and X4b showed strong staining as did the 5G1.1+peptide. None of those clones demonstrated binding to the non-receptor expressing cells indicating that the cell staining is occurring through specific recognition of the cMpl receptor. The parental 5G1.1 without the TPO mimetic peptide did not show staining to any of the cells tested.

The ability of the 5G1.1+ peptide whole IgG to activate the cMpl receptor using the luciferase reporter assay has been determined (see Figure 15). The results herein indicate that the configuration of a whole IgG causes steric limitations in its ability to productively bring the two cMpl receptors together for activation. The activity of the 5G1.1 full IgG construct containing the TPO mimetic peptide in the heavy chain CDR3 positions, was only weakly activating and required approximately 100-200 fold higher molar concentrations as compared to TPO, to stimulate equivalent activity. As was previously observed with the binding experiments, activation by the 5G1.1 containing the peptide was observed only when the cMpl-R was expressed on the cell surface.

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No receptor specific binding or activity was observed with the parental 5G1.1 not containing the peptide. These results demonstrate that binding and activity of the TPO mimetic peptide and selected amino acid flanking sequences is not limited to or specific for the Tetanus Toxoid antibody framework, but can be applied to other antibody frameworks. Thus the flanking amino acid sequences that were selected during panning are specific for presentation of the TPO mimetic peptide within a given CDR position, but not for amino acid sequence of the antibody framework.

EXAMPLE 5

Library Construction Of Epo Mimetic Sequences Grafted Into A Human Antibody Framework

An agonist EPO mimetic-peptide DYHCRMGPLTWVCKPLGG (SEQ. ID. NO: 3) (designated EMP2 in Wrighton et al. 1996) was grafted separately into the anti-tetanus toxoid Fab heavy and light chain CDR3 region creating two antibody libraries as XXDYHXRMGPLTWVXKPLGGXX (SEQ. ID. NO: 71). Randomized positions were generated using an NNK doping strategy. As with the TPO mimetic peptide, two amino acids flanking the EPO mimetic peptide were randomized in order to select for the optimum presentation of the peptide. In addition the cysteine residues, which formed a disulfide bridge in the original cyclic peptide, were randomized. This was done not only because the CDRs already form loop structures and so the disulfide bridge was not necessary to constrain the peptide, but also because the cysteines might in fact disrupt the normal disulfide bonds of the antibody.

The CDR 3 of the anti-TT antibody heavy chain was completely replaced by the EPO peptide library graft. The generation of the library was essentially as described for the TPO heavy chain CDR2 library. Two alternate primers were used for the HCDR3 library: the reverse primer HR3 EPO ANTI (5' CAC CCA GGT CAG TGG GCC CAT GCG MNN ATG ATA GTC MNN MNN TCT CGC ACA ATA ATA TAT GGC 3')(SEQ. ID. NO: 21) that annealed at the end of the heavy chain FR3, and forward primer HR3 EPO CODE (5' CGC ATG GGC CCA CTG ACC TGG GTG NNK AAA CCA CTG NNK NNK TGG GGC CAA GGG ACC ACG GTC 3')(SEQ. ID. NO: 22) which annealed at FR4 of the heavy chain.

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The light chain CDR3 EPO peptide library was constructed essentially as described above for the light chain CDR3 TPO peptide library using reverse primer LR3 EPO ANTI (5' CAC CCA GGT CAG TGG GCC CAT GCG MNN ATG ATA GTC MNN MNN ACA GTA GTA CAC TGC AAA ATC 3') (SEQ. ID. NO: 23) that annealed at the end of light chain FR3 and forward primer LR3 EPO CODE (5' CGC ATG GGC CCA CTG ACC TGG GTG NNK AAA CCA CTG NNK NNK TTC GGC CAA GGG ACC AAG GTG 3') (SEQ. ID. NO: 24) which annealed to FR4 of the light chain.

Selection Of The Epo Mimetic Peptide Heavy Chain Cdr3 Library And Light Chain Cdr3 Library

Selection for peptide presentation was performed by solid phase panning on the soluble EPO receptor. In this method, 1ug of purified human EPO-soluble R (hEPO-sR from R&D Systems, Minneapolis, MN cat#307-ER-050) was immobilized on a microtiter dish overnight at 4°. After washing off free hEPO-sR, the plates were blocked with 1% BSA/PBS for one hour at 37°. Phage, prepared as described above, were added to the wells and incubated two hours at 37°. Washes were performed using PBS/0.5% Tween 20 for 5' at room temperature per wash. 1, 5, 10, and 10 washes were performed in the first, second, third and fourth panning rounds respectively. After the washing steps were completed, the bound Fab-phage were eluted with 30 uls of elution buffer for 10' at room temperature. Eluted phage were then neutralized and amplified as described in Example 1.

EXAMPLE 6

A library was generated by the insertion of a TPO mimetic peptide and previously selected flanking amino acids (NP-IEGPTLRQWLAARA-RG) (SEQ. ID. NO: 61) into a collection of human kappa gene fragments, in this case the CDR2 of the light chain. Stocks of human kappa light chains from multiple human peripheral blood lymphocyte (PBL) donors had been previously generated and cloned into pBluescript II SK+. Those constructs served as the source of antibody gene fragments.

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Antibody Gene Banks

Total RNA from human PBLs was isolated using TRI Reagent (Molecular Research Center, Cincinnati, OH) followed by mRNA purification with Oligotex mRNA purification System (QIAGEN, Valencia, CA) according to kit instructions. First strand cDNA was made using SuperScript RTase II cDNA Synthesis Kit (Life Technologies, Rockville, Maryland) with a modified oligo dT primer. The sequence of the primer was 5' TAGGATGCGGCCGCACAGGTC(T_{20}) 3' (SEQ. ID. NO: 62). Samples were cleaned up over a PCR purification Kit spin column (QIAGEN, Valencia, CA) according to kit directions. Light chain products were amplified using the reverse "Not I" primer and forward primers which annealed at the framework 1 (FR1) position of Kappa chains on the 1st strand cDNA. The "Not I" primer had sequence which was identical to the 5' end of the modified oligo dT primer (5' TAGGATGCGGCCGCACAGGTC 3')(SEQ. ID. NO: 72). The set of Kappa FR1 primers used were:

XVB Vk1a CACGCGCACAACACGTCTAGARACATCCAGATGACCCAG (SEQ. ID. NO: 73)

XVB Vk1b CACGCGCACAACACGTCTAGAGMCATCCAGTTGACCCAG (SEQ. ID. NO: 74)

XVB Vk1c CACGCGCACAACACGTCTAGAGCCATCCRGATGACCCAG (SEQ. ID. NO: 75)

XVB Vk1d CACGCGCACAACACGTCTAGAGTCATCTGGATGACCCAG (SEQ. ID. NO: 76)

XVB Vk2a CACGCGCACAACACGTCTAGAGATATTGTGATGACCCAG (SEQ. ID. NO: 77)

XVB Vk2b CACGCGCACAACACGTCTAGAGATRTTGTGATGACTCAG (SEQ. ID. NO: 78)

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XVB Vk3a CACGCGCACAACACGTCTAGAGAAATTGTGTTGACRCAG (SEQ. ID. NO: 79)

XVB Vk3b CACGCGCACAACACGTCTAGAGAAATAGTGATGACGCAG (SEQ. ID. NO: 80)

XVB Vk3c CACGCGCACAACACGTCTAGAGAAATTGTAATGACACAG (SEQ. ID. NO: 81)

XVB Vk4a CACGCGCACAACACGTCTAGAGACATCGTGATGACCCAG (SEQ. ID. NO: 82)

XVB Vk5a CACGCGCACAACACGTCTAGAGAAACGACACTCACGCAG (SEQ. ID. NO: 83)

XVB Vk6a CACGCGCACAACACGTCTAGAGAAATTGTGCTGACTCAG (SEQ. ID. NO: 84)

XVB Vk6b CACGCGCACAACACGTCTAGAGATGTTGTGATGACACAG (SEQ. ID. NO: 85)

A typical amplification reaction contained 2 µls cDNA reaction, dNTPs, "Not I" reverse primer, one of the XVB forward primers, Opti-prime buffer #5 (Stratagene, La Jolla, CA), and Expand High Fidelity polymerase mixture (Roche Molecular Biochemicals, Indianapolis, IN). Samples were heated to 94°C for 2 minutes, then carried through 10 cycles of 94°C for 15 seconds, 56°C for 30 seconds, and 72°C for 1 minute, followed by 20 cycles of 94°C for 15 seconds, 56°C for 30 seconds, and 72°C for (1 minute + 5 seconds/cycle). The cycles were followed by an extended incubation at 72°C (7 minutes) prior to 4°C hold. Products were ethanol precipitated and then gel purified. Fragments of approximately 850bp were isolated and then digested with Xba I and Sac I. The resulting kappa products were ligated into pBluescript II SK+ that had likewise been digested with Xba I and Sac I. The ligation products were electroporated into Top10F' (Invitrogen, Carlsbad, CA) and grown overnight. The bacterial pellet was used to isolate the Kappa library DNA with QIAGEN's MAXIprep DNA isolation kit.

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Construction Of Framework Library With TPO Mimetic Peptide

For construction of the TPO light chain framework library, equal amounts of four different kappa light chain libraries from four different patients were used as the starting template for the PCR reactions (25ng total per reaction). The TPO mimetic peptide and selected flanking amino acids were incorporated into the light chains by overlap PCR. In the first round of PCR a set of reverse primers (VK ANTI primers) which bound to the kappa light chain FR2 were separately combined with the forward T7 seq-F primer (5'-ATTAATACGACTCACTATAGGG-3') (SEQ. ID. NO: 86) to synthesize the N terminal piece of the light chain and part of the TPO mimetic peptide within the LC CDR2 position. A second set of forward primers (VK CODE primers), which bound to FR3, were combined separately with the T3 reverse primer (5'-AATTAACCCTCACTAAAGGG-3') (SEQ. ID. NO: 87) to synthesize the rest of the TPO mimetic peptide within the LC CDR2 position and the C terminal half of the light chain by PCR. Separate reactions were performed for each pair of primer combinations in duplicate.

VK6ANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTCCTGATGAGGAGC TTTGGRG-3' (SEQ. ID. NO: 88)

VK5ANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTTTGAATAATGAAAA TAGCAG-3' (SEQ. ID. NO: 89)

VK4ANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTGTAAATGAGCARCT TAGGAG-3' (SEQ. ID. NO: 90)

VK3ANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTATAGATGAGGAGC CTGGGMG-3' (SEQ. ID. NO: 91)

VK2AANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTATAAATTAGGCGCC TTGGAG-3' (SEQ. ID. NO: 92)

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VK2BANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTATAGATYAGGAGCT GTGGAG-3' (SEQ. ID. NO: 93)

VK1AANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTATAGATCAGGAGCT TAGGA-3' (SEQ. ID. NO: 94)

VK1BANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTRTAGATCAGGAGCT TAGGG-3' (SEQ. ID. NO: 95)

VK1CANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTATAGATCAGGGACT TAGGG-3' (SEQ. ID. NO: 96)

VK1DANTI
5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATCGGGTTATAGATCAGGYGCT TAGGG-3' (SEQ. ID. NO: 97)

VK6CODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGGGTCCCCTCGA GGTTCAG-3' (SEQ. ID. NO: 98)

VK5CODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGAATCCCACCTC GATTCAG-3' (SEQ. ID. NO: 99)

VK4CODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGGGTCCCTGACC GATTCAG-3' (SEQ. ID. NO: 100)

VK3ACODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGCATCCCAGMCA GGTTCAG-3' (SEQ. ID. NO: 101)

VK3BCODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGTATCCCAGCCA GGTTCAG-3' (SEQ. ID. NO: 102)

VK2ACODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGAGTSCCAGAYA GGTTCAG-3' (SEQ. ID. NO: 103)

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VK2BCODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGGGTCCCWGACA
GRTTCAG-3' (SEQ. ID. NO: 104)

VK1ACODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGGGTCCCATCAA
GGTTCAG-3' (SEQ. ID. NO: 105)

VK1BCODE
5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTCGTGGTGGGGTCCCATCTC
GGTTCAG-3' (SEQ. ID. NO: 106)

Fragments from the first rounds of PCR were gel purified. Those purified fragments were then combined, in an antibody family specific manner, in overlap PCR reactions to generate the full light chain. Reactions for each family were performed in triplicate using 40ng of both the N-terminal and C-terminal piece of the light chain in each reaction. The reactions were run for 10 cycles prior to the addition of the T3 and T7 Seq-F primers, followed by an additional 25 cycles after primer addition. The full length LC fusion PCR products were gel purified, digested with Sac I and Xba I, and then again gel purified. The light chain inserts were then ligated into an appropriate phage display vector, which had been similarly digested with Xba I and Sac I and gel purified. The pRL5-kappa vector used had restriction sites which were compatible with the LC fragments and contained the remaining Kappa constant region from the native Sac I site to the C-terminal Cys. In addition, the anti-tetanus toxoid heavy chain was inserted into the vector by Xho I and Spe I for Fab production.

The ligation mixture was transformed by electroporation into XL-1 Blue bacteria (Stratagene, La Jolla CA) and amplified. The library was panned four rounds on 293 EBNA cells transfected with the cMpl-R in a manner similar to that previously described. Clones obtained during panning were screened for binding by FACs analysis on 293 EBNA cells transfected with or without the cMpl-R as previously described. A number of clones, which specifically bound the cMpl-R, were obtained. DNA fingerprinting of the resulting light chains by digestion with Bst N1 indicated that the clones could be divided into 5 different groups. Partial sequencing of 8 of these clones showed that frameworks from at least two different kappa light chain families were selected during the panning (VK1 and VK3). In an initial test, 3 of the light chain

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framework clones were combined with the heavy chain of X4b to create a Fab with 2 TPO mimetic peptides. These clones induced activation of the cMpl-R in luciferase assays as previously described. The level of activation using bacterial supernatants of one such clone 429/X4b (see Figure 16) was approximately 10-20 fold lower than that observed with TPO, as estimated by comparing activity to known concentrations of TPO and using quantitative western blots to determine the concentration of the antibody in the supernatant. Additional clones can be screened in a similar fashion on order to identify clones with greater activity.

These Fabs, or various other LC, HC or intrachain CDR combinations, could be used as a therapeutic product. Alternatively, these clones could be converted to framework germline sequences (either with or without codon optimization) for use as a therapeutic agent so long as activity was maintained.

Modification of the Phage Display Vectors pAX131 and pRL5

The Not I site in pAX131 was removed by digesting the vector with Not I, using Klenow polymerase to fill in the Not I overhangs, and then re-ligating the vector. See Figure 17. Further modification was made by digesting pAX 131 with EcoR I and Xba I. An insert that replaced the elements removed by such digestion was generated using overlapping oligonucleotides with the following changes: conversion of the Sac I site to a new Xba I site (single underline in primer sequences below), conversion of the original Xba I site to a Not I site (double underline in the primer sequences below), and ending the insert with a Spe I overhang which is compatible with the vector's Xba I digest generated overhang. Ligation of the EcoR I / Spe I insert into the EcoR I / Xba I cut vector resulted in an Spe I / Xba I hybrid which will no longer cut with either Spe I or Xba I at that site. The sequence of the oligos used were: "EcoSpe" 5' AA TTC AAG GAG TTA ATT ATG AAA AAA ACC GCG ATT GCG ATT GCG GTG GCG CTG GCG GGC TTT GCG ACC GTG GCC CAG GCG GCC TCT AGA ATC TGC GGC CGC a 3'(SEQ. ID. NO: 107), and "SpeEco" 5' ct agt GCG GCC GCA GAT TCT AGA GGC CGC CTG GGC CAC GGT CGC AAA GCC CGC CAG CGC CAC CGC AAT CGC AAT CGC GGT TTT TTT CAT AAT TAA CTC CTT G 3' (SEQ. ID. NO: 108).

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The intermediate vector created was pAX131Xba/Not. The human kappa constant region was inserted between the Xba I and Not I sites generating pAX131-kappa (see Figure 18). The human kappa constant region was PCR amplified from human cDNA using primers that introduced the upstream Xba I site and in the downstream position a TAA stop codon followed by a Not I site. The primers used were CKXba I (5' GGA GTC TAG ATA ACT GTG GCT GCA CCA TCT GTC TTC 3') (SEQ. ID. NO: 109) and CKNotI (5' AGG AGC GGC CGC TTA ACA CTC TCC CCT GTT GAA GCT C 3') (SEQ. ID. NO: 110).

The light chain cloning modifications incorporated into pAX131-kappa were shuttled into the pRL5 vector (modified to have its Not I site removed as described above) by moving the EcoR I to Xho I fragment. See Figures 19 and 20. This vector was designated as pRL5-kappa. (See Figures 21A-I.)

EXAMPLE 7

(core H-CDR2 library)

An additional HC CDR2 library was constructed where a core sequence of the TPO mimetic peptide (GPTLRQWL) (SEQ. ID. NO: 112) flanked by a single random amino acid on each side was inserted into the heavy chain partially replacing the CDR2 (GXGPTLRQWLXYAQKFQG) (SEQ. ID. NO: 113). The construction of the heavy chain partial CDR2 replacement library was also carried out as previously described for the heavy chain CDR2 library with the exception that the specific primer 8HCR2anti0 (5'CAGCCACTGGCGCAGGGTTGGGCCMNNCCCTCCCATCCACTCAAGCCC-3') (SEQ. ID. NO: 114) which annealed at the end of the heavy chain FR2 and the primer 8HCR2code(5'GGCCCAACCCTGCGCCAGTGGCTGNNKTACGCACAGAAATTCCAG GGCAGAGTCACCATT-3') (SEQ. ID. NO: 115) which annealed to the beginning of the heavy chain FR3 were used in place of the previously described HR2 cMpl primers. The heavy chain partial CDR2 replacement library was panned four rounds on 293 EBNA cells transfected with the cMpl-R as described previously. These clones can now be screened for positive binding clones by FACs analysis as previously described.

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EXAMPLE 8

An individual clone was created by combining the heavy chain CDR3 clone X4b (See Figures 5 and 9), with the heavy chain CDR2 clone 19 (see Figure 9) to create a new Fab clone containing two TPO mimetic peptides. This clone was further modified by codon optimization and conversion to germline sequences. This new clone, which was named 116 (Figure 22) was purified to 95% homogeneity. The specific activity of the purified 116 Fab was then tested using the luciferase based assays as previously described. The specific activity of the purified 116 was found to be approximately 30 fold lower than TPO (Figure 23).

EXAMPLE 9

A third heavy chain CDR2 library was constructed replacing part of the tetanus toxoid (TT) heavy chain CDR2 with the TPO mimetic peptide flanked by two random amino acids on each side.

Computer modeling indicated that it might be beneficial to stability and folding of the heavy chain to retain four amino acids of the original TT HC CDR2. Specifically, the first three amino acids of the TT heavy chain CDR2 (GLY-ILE-PHE) and the last amino acid of the heavy chain CDR2 (GLY). Thus, the final product would contain these original four amino acids with the TPO mimetic peptide and random flanking amino acids positioned between the first three original amino acids and last original amino acid: GIFXXIEGPTLRQWLAARAXXG (SEQ. ID NO. 119).

The construction of the heavy chain partial CDR2 replacement library was also carried out as described for the previous 2 heavy chain CDR2 libraries with the exception that the specific primer HCR2+ANTI (5'AGCCAGCCACTGGCGCAGGGTTGGGCCTTCGATMNNMNNGAAGATCCCTCCC ATCCACTC-3') (SEQ. ID NO. 120) which annealed at the end of the heavy chain FR2 and the primer HCR2+CODE (5'CCAACCCTGCGCCAGTGGCTGGCTGCTCGCGCTNNKNNKGGCAGAGTCACCA TTACCGCG-3') (SEQ. ID NO. 121) which annealed to the beginning of the heavy chain FR3 were used in place of the previously described HR2 cMPL primers. The heavy chain partial CDR2 replacement library was panned four rounds on 293 EBNA

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cells transfected with the cMPL-R as described previously. A number of clones with strong binding characteristics were obtained and sequenced (Figure 24).

The clones of the round four pool from panning are used as the starting point for insertion of a second TPO mimetic peptide. For example, this library has been combined with the clone X4b containing a TPO mimetic peptide in the heavy chain CDR3 by sub-cloning, to create clones, which contain two TPO mimetic peptides (one in heavy chain CDR2 and one in heavy chain CDR3). These new clones are screened for TPO mimetic activity. In addition, the second peptide may be placed in light chain CDR1, CDR2 or CDR3, or heavy chain CDR1.

It will be understood that various modifications may be made to the embodiments disclosed herein. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of the claims appended herein.

WHAT IS CLAIMED IS:

1. An immunoglobulin molecule or fragment thereof comprising a region where amino acid residues corresponding to at least a portion of a two complementarity determining regions (CDRs) are replaced with a peptide mimetic selected from the group consisting of an EPO mimetic and a TPO mimetic.

2. An immunoglobulin molecule or fragment thereof according to claim 1 further comprising at least one flanking sequence including at least one amino acid covalently linked to at least one end of the peptide mimetic.

3. An immunoglobulin molecule or fragment thereof according to claim 2 wherein the at least one flanking sequence includes a flanking sequence having a proline that is covalently linked to the peptide mimetic.

4. An immunoglobulin molecule or fragment thereof according to claim 1 wherein the immunoglobulin molecule fragment is selected from the group consisting of Fab fragment, $F(ab')_2$ fragment and ScFv fragment.

5. An immunoglobulin molecule or fragment thereof according to claim 1 wherein the immunoglobulin molecule is a full IgG molecule.

6. An immunoglobulin molecule or fragment thereof according to claim 1 wherein the two CDRs are both located on a heavy chain.

7. An immunoglobulin molecule or fragment thereof according to claim 1 wherein the CDRs are a CDR3 of a heavy chain and a CDR2 of a heavy chain.

8. An immunoglobulin molecule or fragment thereof according to claim 1 wherein the TPO mimetic comprises SEQ. ID. NO. 1.

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9. An immunoglobulin molecule or fragment thereof according to claim 1 wherein a heavy chain CDR3 and CDR2 are replaced with a peptide and the heavy chain comprises a variable region of SEQ. ID. NO. 125.

10. An immunoglobulin molecule or fragment thereof according to claim 1 wherein the immunoglobulin molecule or fragment thereof is human.

11. An immunoglobulin molecule or fragment thereof according to claim 1 wherein the immunoglobulin molecule or fragment thereof is anti-tetanus toxoid.

12. Nucleic acid encoding an immunoglobulin molecule or fragment thereof according to claim 1.

13. An expression vector comprising nucleic acid according to claim 12.

14. A host cell transformed with an expression vector according to claim 13.

15. A method of producing an immunoglobulin molecule or fragment thereof comprising culturing a host cell according to claim 14 under conditions suitable for expression of the immunoglobulin or fragment thereof.

16. A composition comprising an immunoglobulin or fragment thereof according to claim 1 and a pharmaceutically acceptable carrier.

17. An immunoglobulin molecule or fragment thereof comprising a region where amino acid residues corresponding to at least a portion of a complementarity determining region (CDR) is replaced with a peptide mimetic including SEQ. ID NO. 126, wherein X at each occurrence represents any amino acid.

18. An immunoglobulin molecule or fragment thereof according to claim 17 wherein the immunoglobulin molecule or fragment thereof is human.

19. An immunoglobulin molecule or fragment thereof according to claim 17 wherein the immunoglobulin molecule or fragment thereof is anti-tetanus toxoid.

20. Nucleic acid encoding an immunoglobulin molecule or fragment thereof according to claim 17.

22. An expression vector comprising nucleic acid according to claim 20.

23. A host cell transformed with an expression vector according to claim 22.

24. A method of producing an immunoglobulin molecule or fragment thereof comprising culturing a host cell according to claim 23 under conditions suitable for expression of the immunoglobulin or fragment thereof.

25. A composition comprising an immunoglobulin or fragment thereof according to claim 17 and a pharmaceutically acceptable carrier.

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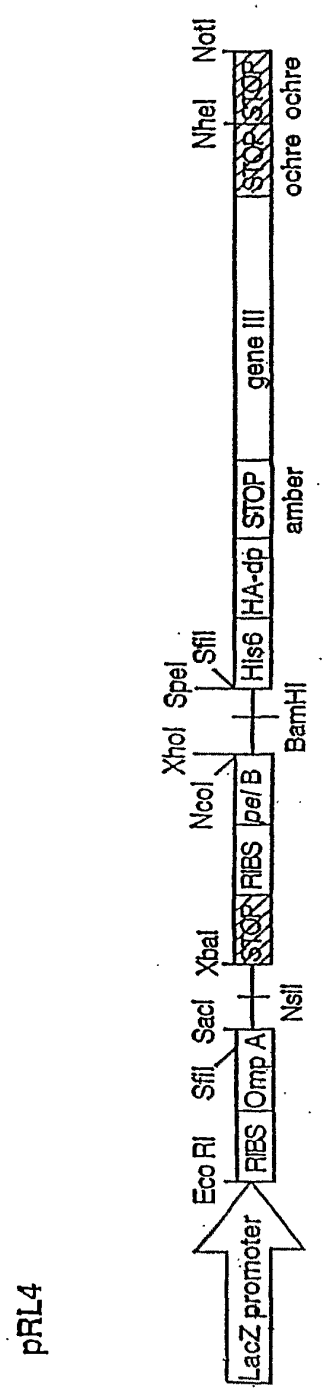


Fig. 1

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Human Antibody sequence (TT sequence) (SEQ ID NO: 54)

Heavy Chain: cloning sites Xho I and Spe I are underlined

```
1                                                   11
gag gtg cag ctg CTC GAG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG AAG
glu val gln leu leu glu gln ser gly ala glu val lys lys pro gly ser ser val lys
21                                                  31
GTC TCC TGC AGG GCT TCT GGA GGC ACC TTC AAC AAT TAT GCC ATC AGC TGG GTG CGA CAG
val ser cys arg ala ser gly gly thr phe asn asn tyr ala ile ser trp val arg gln
41                                                  51
GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC TTC CCT TTC CGT AAT ACA GCA AAG
ala pro gly gln gly leu glu trp met gly gly ile phe pro phe arg asn thr ala lys
61                                                  71
TAC GCA CAA CAC TTC CAG GGC AGA GTC ACC ATT ACC GCG GAC GAA TCC ACG GGC ACA GCC
tyr ala gln his phe gln gly arg val thr ile thr ala asp glu ser thr gly thr ala
81                                                  91
TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC ACG GCC ATA TAT TAT TGT GCG AGA GGG
tyr met glu leu ser ser leu arg ser glu asp thr ala ile tyr tyr cys ala arg gly
101                                                 111
GAT ACG ATT TTT GGA GTG ACC ATG GGA TAC TAC GCT ATG GAC GTC TGG GGC CAA GGG ACC
asp thr ile phe gly val thr met gly tyr tyr ala met asp val trp gly gln gly thr
121                                                 131
ACG GTC ACC GTC TCC GCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC
thr val thr val ser ala ala ser thr lys gly pro ser val phe pro leu ala pro ser
141                                                 151
TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
ser lys ser thr ser gly gly thr ala ala leu gly cys leu val lys asp tyr phe pro
161                                                 171
GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG
glu pro val thr val ser trp asn ser gly ala leu thr ser gly val his thr phe pro
181                                                 191
GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC
ala val leu gln ser ser gly leu tyr ser leu ser ser val val thr val pro ser ser
201                                                 211
AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG
ser leu gly thr gln thr tyr ile cys asn val asn his lys pro ser asn thr lys val
221                                                 231
GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA act agt
asp lys lys val glu pro lys ser cys asp lys thr ser
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Fig. 2A

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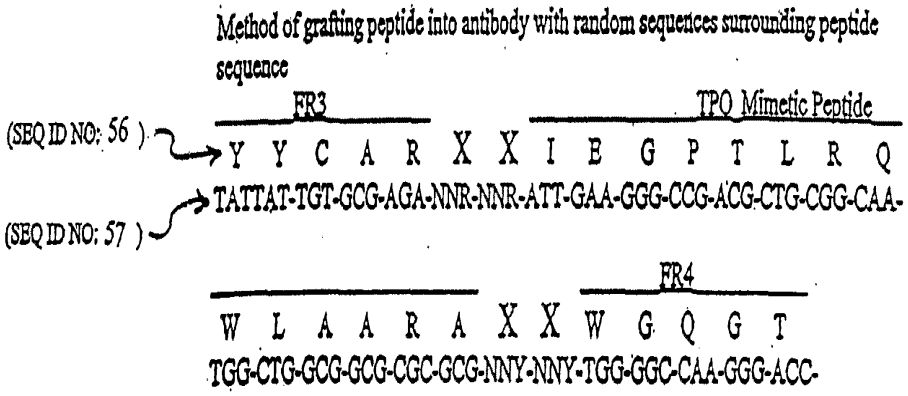
Human Antibody Sequence (TT sequence) (SEQ ID NO: 55)

Light Chain: cloning sites Sac I and Xba I are underlined

```
1                                                   11
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glu leu thr gln ser pro gly thr leu ser leu ser pro gly glu arg ala thr leu ser
21                                                  31
tgc agg gcc agt cac agt gtt agc agg gcc tac tta gcc tgg tac cag cag aaa cct ggc
cys arg ala ser his ser val ser arg ala tyr leu ala trp tyr gln gln lys pro gly
41                                                  51
cag gct ccc agg ctc ctc atc tat ggt aca tcc agc agg gcc act ggc atc cca gac agg
gln ala pro arg leu leu ile tyr gly thr ser ser arg ala thr gly ile pro asp arg
61                                                  71
ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga ctg gag cct gaa
phe ser gly ser gly ser gly thr asp phe thr leu thr ile ser arg leu glu pro glu
81                                                  91
gat ttt gca gtg tac tac tgt cag cag tat ggt ggc tca ccg tgg ttc ggc caa ggg acC
asp phe ala val tyr tyr cys gln gln tyr gly gly ser pro trp phe gly gln gly thr
101                                                 111
AAG GTG GAA CTC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT
lys val glu leu lys arg thr val ala ala pro ser val phe ile phe pro pro ser asp
121                                                 131
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA
glu gln leu lys ser gly thr ala ser val val cys leu leu asn asn phe tyr pro arg
141                                                 151
GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
glu ala lys val gln trp lys val asp asn ala leu gln ser gly asn ser gln glu ser
161                                                 171
GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC
val thr glu gln asp ser lys asp ser thr tyr ser leu ser ser thr leu thr leu ser
181                                                 191
AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
lys ala asp tyr glu lys his lys val tyr ala cys glu val thr his gln gly leu ser
201                                                 211
TTG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG Ttc tag a
leu pro val thr lys ser phe asn arg gly glu cys AMB
```

Fig. 2B

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The TPO mimetic peptide was grafted into the heavy chain CDR3 region of the tetanus toxoid antibody. The peptide was flanked on either side by two random amino acids, shown as "X"s in the figure.

Fig. 3

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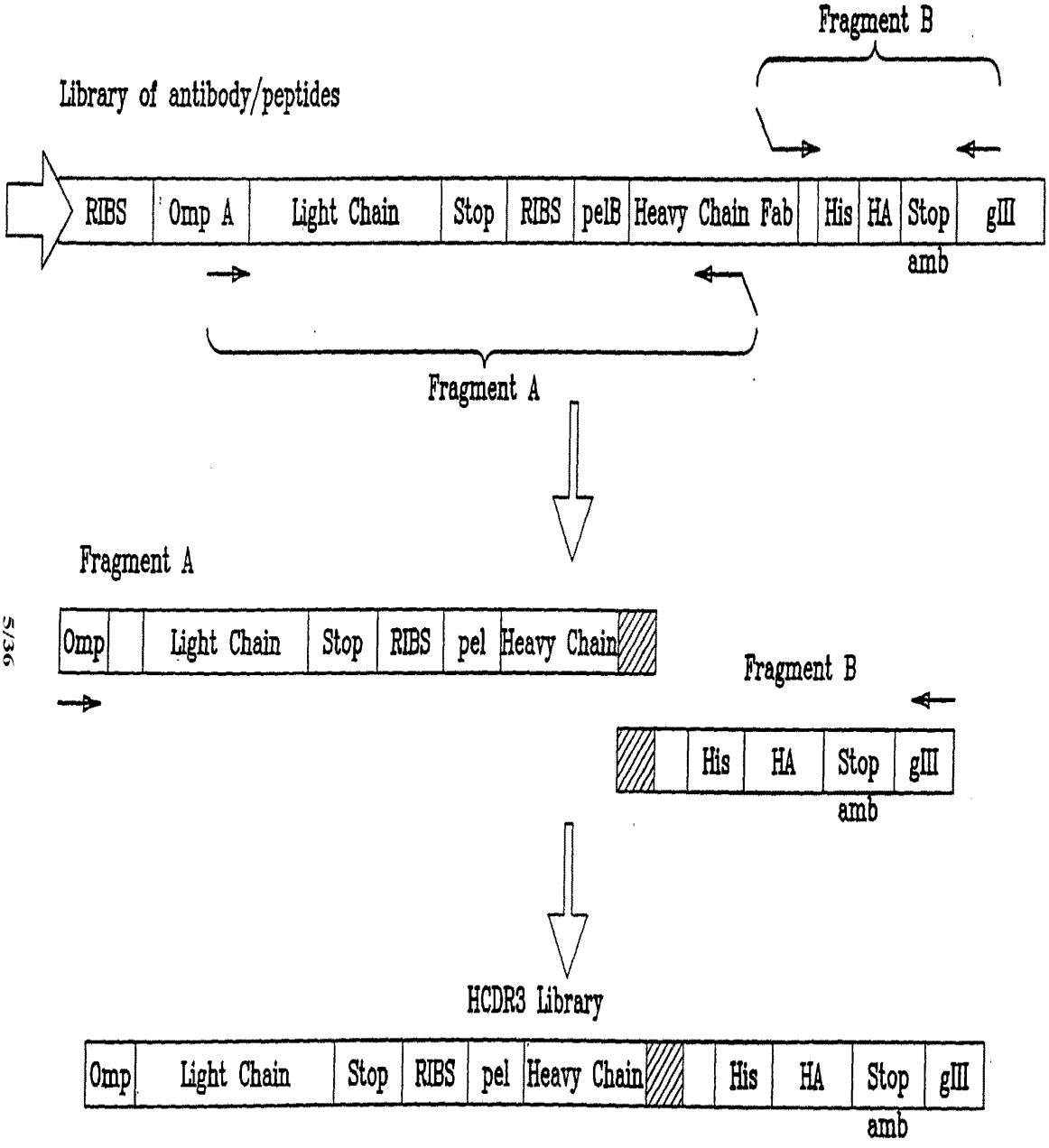


FIG. 4

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CLONE	AMINO ACID SEQUENCE	SEQ ID NO.
X1a	Pro-Prp-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly	25
	CCG-CCC-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGA-GGC	26
X1a-11	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly	27
	GGG-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGC-GGA	28
X1a-13	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly	29
	GGC-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGA-GGC	30
X1c	Trp-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	31
	TGG-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTC	32
X2c	Met-Ile-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Val-Gly	33
	ATG-ATA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GTT-GGC	34
X3a	Val-Val-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	35
	GTG-GTA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTT	36
X3b	Gly-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp	37
	GGG-CCG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-GAT	38
X4b	Leu-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	39
	TTG-CCA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTT	40
X4c	Ser-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Ile	41
	TCA-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-ATC	42
X5a	Thr-Met-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	43
	ACA-ATG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-GTT	44
X5c	Thr-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val	45
	ACG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTC	46
X7a	Thr-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Cys-Ser	47
	ACA-CGG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-TGC-AGC	48
X7b	no peptide deletion mutant	
X7c	Gln-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp	49
	CAG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GAC	50

Fig. 5

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pRL8

(SEQ ID NO: 60)

GGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAA
ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATC
AAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGT
CCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATC
AGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTC
GAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGA
GCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGT
AACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGC
ACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACA
TTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCC
TTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAA
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ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTG
GTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAA
GAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTT
ACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAAC
ATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAG
CCATACCAAACGACGAGCGTGTACACCACGATGCCTGTAGCAATGGCAACAAC
GTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAAT
TAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC
CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGT
CTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAA
GTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGG
ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCG
CTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGC
TCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTA
CCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGA

Fig. 6A

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ACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGG
AGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC
ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGT
TTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTG
CTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAA
CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACC
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAA
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GCGGCCGAGCTCGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCG
GCTGCCGTAGGCAATAGGTATTTCATTATGACTGTCTCCTTGGCGACTAGCTA
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TTCgGGGAAGTAGTCCTTGACCAGGCAGCCCAGGGCCGCTGTGCCCCCAGAG
GTGCTCTTGGAGGAGGGTGCCAGGGGGAAGACCGATGGGCCCTTGGTGGAG
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GCTCCTCATCTATGGTACATCCAGCAGGGCCACTGGCATCCCAGACAGGTTC
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CTGAAGATTTTGCAGTGTACTACTGTCAGCAGTATGGTGGCTCACCGTGGTTC
GGCCAAGGGACCAAGGTGGAACTCAAACGAACTGTGGCTGCACCATCTGTCT
TCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTG
TGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG
ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAG
CAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCacccatcagggcctgagttcgcccgtcac
aaagagcttcaacggaggagagtgttaatTCTAGATAATTAATTAGGAGGAATTTAAAATGAA
ATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAG
CCATGGCCGAGGTGCAGCTGCTCGAGATGAGCGATAAAATTATTCACCTGAC
TGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTC
GATTTCTGGGCAGAGTGGTGCGGTCCGTGCAAAATGATCGCCCCGATTCTGG
ATGAAATCGCTGACGAATATCAGGGCAAACTGACCGTTGCAAAACTGAACAT
CGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACT
CTGCTGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTTG
TCTAAAGGTCAGTTGAAAGAGTTCCTCGACGCTAACCTGGCGTACCCGTACG
ACGTTCCGGACTACGGTTCTACTAGTccgaaaccgtctaccccaccgggctcttcctgcggtggccgc
atcgcccgtctggaggaaaaagtgaaaaccctgaaagctcagaactccgagctggcgtccactgccaacatgctgcgcgaac

Fig. 6B

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aggtggcacagctgaaacagaaagttatgaaccatggcggttgtgctagtGGCCAGGCCGGCCAGCACCAT
CACCATCACCATGGCGCATACCCGTACGACGTTCCGGACTACGCTTCTTAGG
AGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGG
CGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAA
ACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTC
TGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCG
ATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGT
GATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTC
ACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGA
ATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCATATGAATTTTCTATTGATTG
TGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCAC
CTTTATGTATGTATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTA
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Fig. 6C

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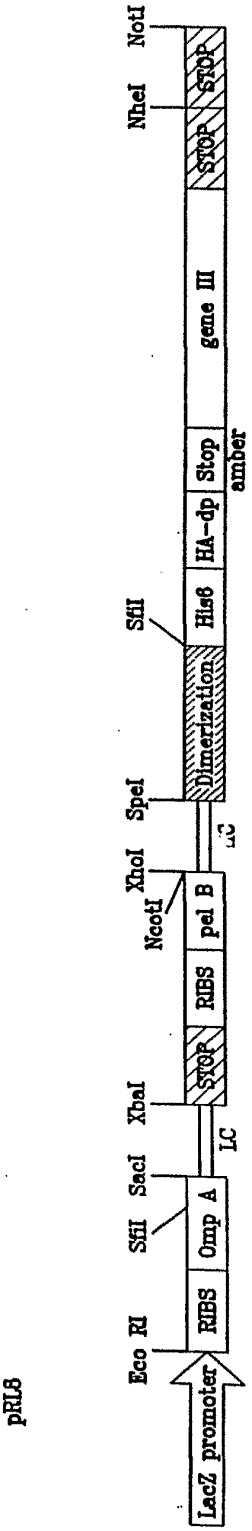


FIG. 7

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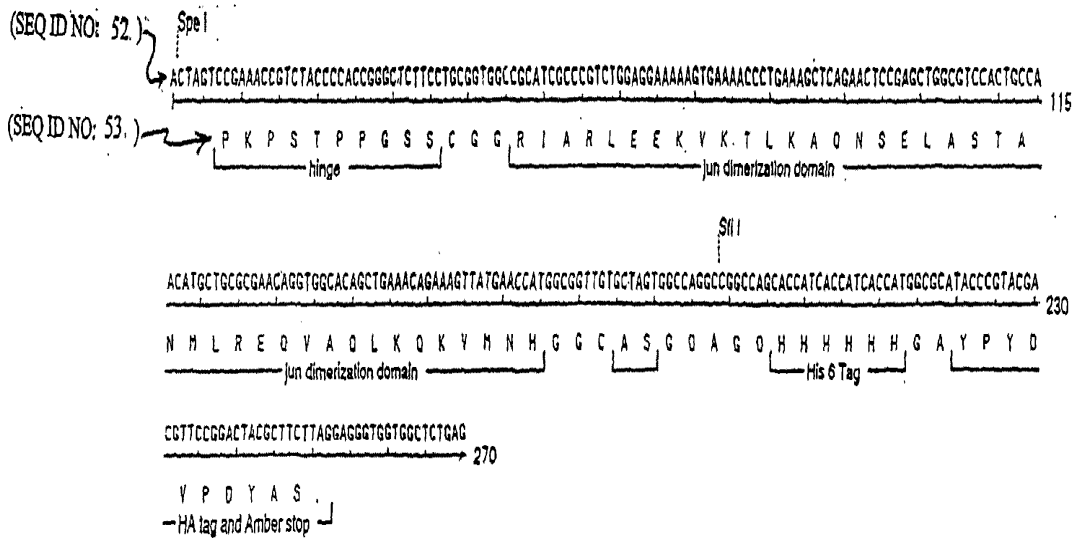


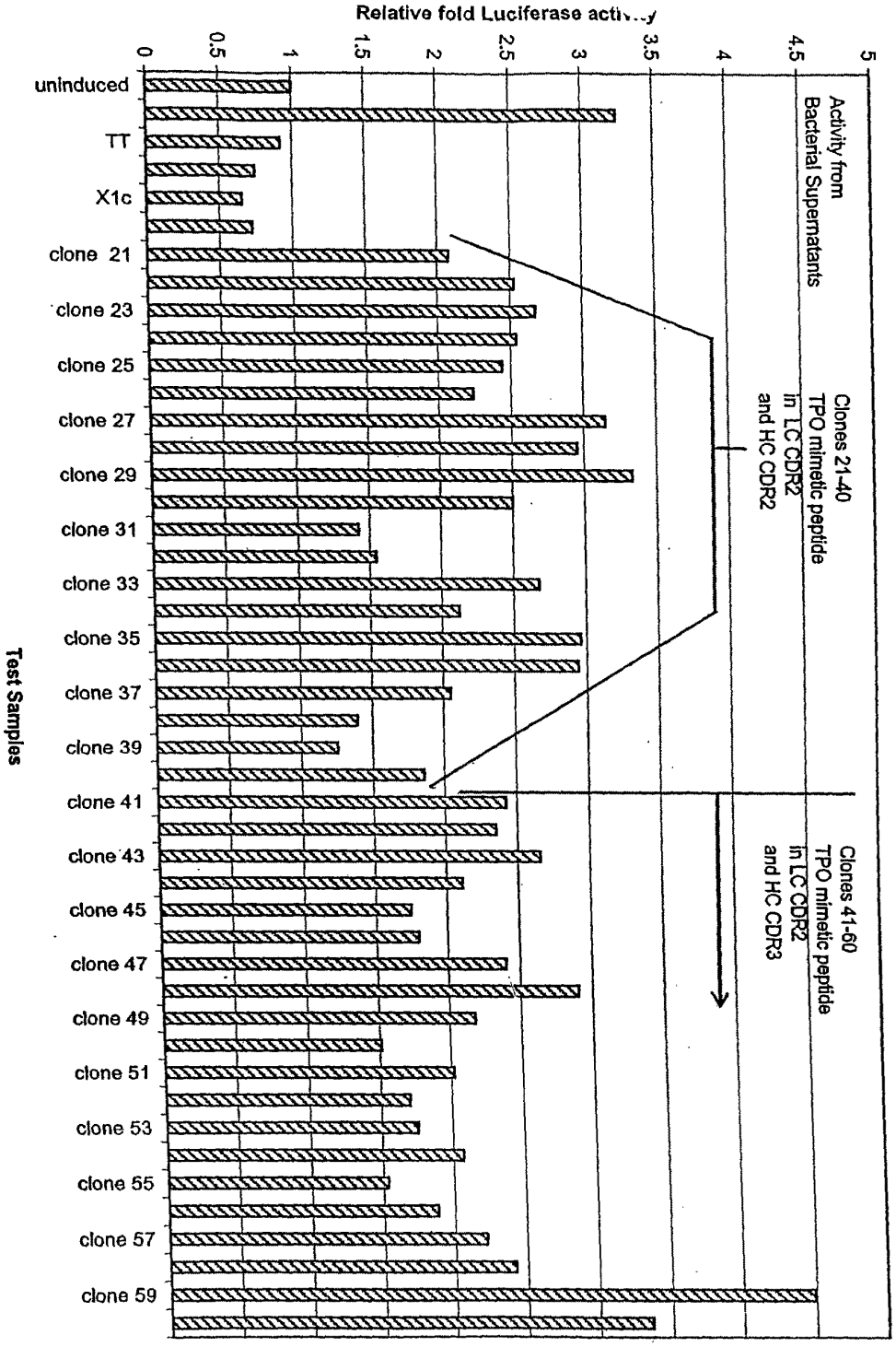
Fig. 8

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TPO Positive Clones nnk nnk nnk nnk
1 2 IEGPTLRQWLAARA 3 4

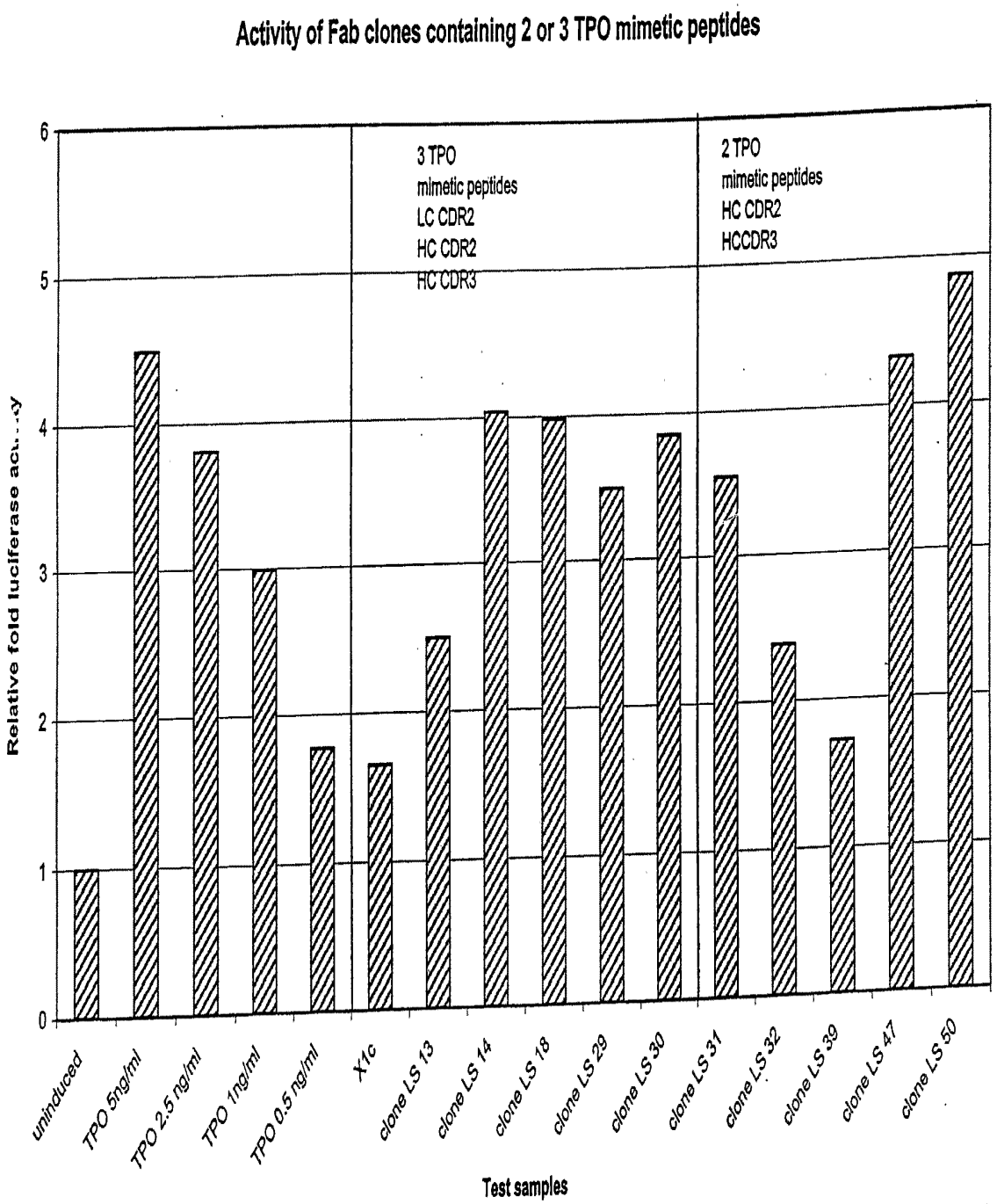
	Sample	nnk 1	nnk 2	nnk 3	nnk 4	Amino Acids 1	Amino Acids 2	Amino Acids 3	Amino Acids 4
HC CDR3	X1c	tgg	ctg	cct	gtc	Trp	Leu	Pro	Val
	X3a	gtg	gta	cct	gtt	Val	Val	Pro	Val
	X3b	ggg	ccg	ccc	gat	Gly	Pro	Pro	Asp
	X4b	ttg	cca	cct	gtt	Leu	Pro	Pro	Val
	X4c	tca	ctg	ccc	atc	Ser	Leu	Pro	Ile
	X5a	aca	atg	ccc	gtt	Thr	Met	Pro	Val
	X5c	acg	aca	cct	gtc	Trp	Leu	Pro	Val
	X7c	cag	aca	cct	cac	Gln	Thr	Pro	Asp
HC CDR2	24	ctt	tat	tct	aat	Leu	Tyr	Ser	Asn
	39	act	tac	ttg	cat	Thr	Tyr	Leu	His
	3	agg	atg	ctc	gag	Arg	Met	Leu	Glu
	7	aag	gaa	tct	aag	Lys	Glu	Ser	Lys
	8	gcg	cat	gtg	cag	Ala	His	Val	Gln
	10	cag	gag	att	agt	Gln	Glu	Ile	Ser
	11	cgg	aat	aat	ccg	Arg	Asn	Asn	Pro
	19	cag	cta	aat	tct	Gln	Leu	Asn	Ser
	25	agt	att	ttt	gtc	Ser	Ile	Phe	Val
	28	ggg	ccc	act	agt	Gly	Pro	Thr	Ser
LC CDR1	10	aag	ggt	gtt	agt	Lys	Gly	Val	Ser
	11	cat	ggg	gtg	gct	His	Gly	Val	Ala
	12a	cgt	acg	atg	gct	Arg	Thr	Met	Ala
	12b	cgt	ggt	gtt	aat	Arg	Gly	Val	Asn
	14	cgt	tcg	ctt	gcg	Arg	Ser	Leu	Ala
	16	cgg	ggt	gtt	gcg	Arg	Gly	Val	Ala
	18	agg	acg	gtg	tct	Arg	Thr	Val	Ser
	47	aag	ggg	gtg	gcg	Lys	Gly	Val	Ala
LC CDR2	1	aat	ccg	agg	ggt	Asn	Pro	Arg	Gly
	2	tcg	cct	cgg	agt	Ser	Pro	Arg	Ser
	3	tcg	cct	cgt	acg	Ser	Pro	Arg	Thr
	4	tcg	cct	tgg	cgt	Ser	Pro	Trp	Arg
	5	act	ccg	aat	tgg	Thr	Pro	Asn	Trp
	6	aat	cct	gcg	agg	Asn	Pro	Ala	Arg
	7	aat	ccg	tcg	ggg	Asn	Pro	Ser	Gly
	9	aat	cct	tat	tag	Asn	Pro	Tyr	Stop
	10	aat	ccg	cgg	tct	Asn	Pro	Arg	Ser
	11	aat	ccg	gat	gtg	Asn	Pro	Asp	Val
	12	tcg	ccg	tcg	cgg	Ser	Pro	Ser	Arg
	13	aat	cct	ctg	ttt	Asn	Pro	Leu	Phe
	14	aat	ctt	ggg	tat	Asn	Pro	Gly	Tyr
	15	aat	cct	att	agt	Asn	Pro	Ile	Ser
	16	aat	cct	cag	cgg	Asn	Pro	Gln	Arg
	18	aat	ccg	cgg	acg	Asn	Pro	Arg	Thr
	19	aat	ccg	cgt	ggg	Asn	Pro	Arg	Gly
	20	cat	ttg	aga	ctg	His	Leu	Arg	Leu
	21	aag	tag	att	tat	Lys	Stop	Ile	Tyr
	23	aat	cct	ggt	aag	Asn	Pro	Gly	Lys
	24	aat	cct	cgt	ggg	Asn	Pro	Arg	Gly
	26	aat	cct	aat	gtg	Asn	Pro	Asn	Val
	27	tct	ccg	cgg	gtt	Ser	Pro	Arg	Val
	29	acg	cct	cgg	ggt	Thr	Pro	Arg	Gly
	30	ct	tag	tgg	tgg	Pro	Stop	Trp	Trp

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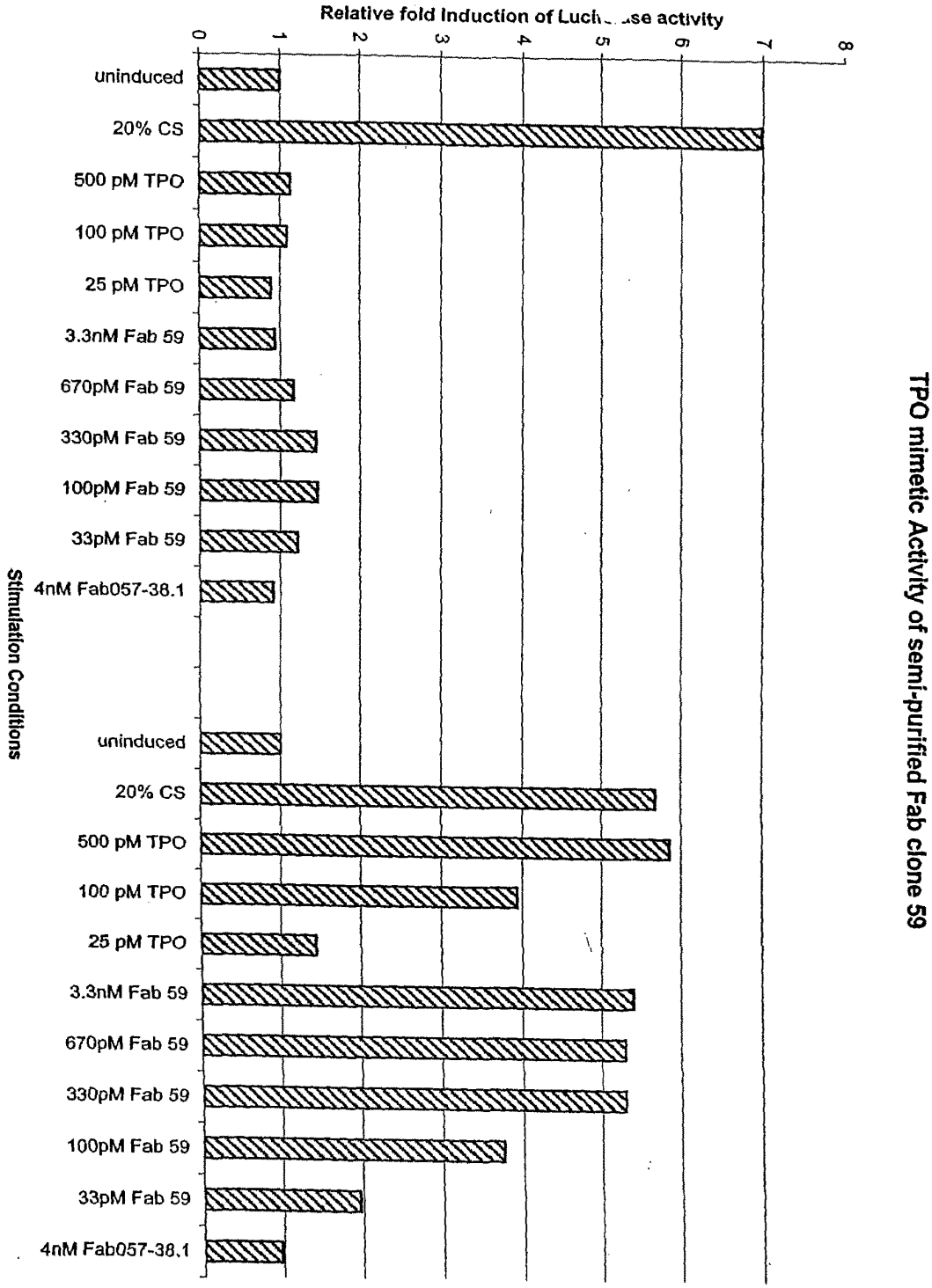


Activity of Fab clones containing 2 TPO mimetic peptides

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(SEQ ID NO: 67)

5G1.1 – TPO Heavy Chain (Bold denotes TPO mimetic) Amino acid sequence:

*MKIVSWVILFLLSVTAGVHS*QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQW
VRQAPGQGLEWMGEILPGSGSTEYTENFKDRVTMTRDTSTSTVYMELSSLRSED
TAVYYCARLPIEGPTLRQWLAARAPVWGQGTLVTVSSASTKGPSVFPLAPCSR
STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK.

(SEQ ID NO: 68)

5G1.1 – TPO Heavy Chain (Bold denotes TPO mimetic) Nucleic acid sequence:

ATGAAGTGGAGCTGGGTTATTCTCTTCCTCCTGTCAGTAACTGCCGGCGTCCA
CTCCCAAGTCCAACTGGTGCAATCCGGCGCCGAGGTCAAGAAGCCAGGGGCC
TCAGTCAAAGTGTCCTGTAAAGCTAGCGGCTATATTTTTTCTAATTATTGGAT
TCAATGGGTGCGTCAGGCCCCCGGGCAGGGCCTGGAATGGATGGGTGAGATC
TTACCGGGCTCTGGTAGCACCGAATATACCGAAAATTTTAAAGACCGTGTTA
CTATGACGCGTGACACTTCGACTAGTACAGTATACATGGAGCTCTCCAGCCTG
CGATCGGAGGACACGGCCGTCTATTATTGCGCGCGTTTGCCAATTGAAGGG
CCGACGCTGCGGCAATGGCTGGCGGCGCGCGCCTGTTTGGGGTCAAG
GAACCCTGGTCACTGTCTCGAGCGCCTCCACCAAGGGCCCATCCGTCTTCCCC
CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGC
CCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT
ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGAC
CTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAC
AGTTGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTG
GCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGAC
CCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCA
AGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAA
GGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCC
AAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAG
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACT
ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCT
GTCTCTGGGTAAATGA

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(SEQ ID NO: 69)

5G1.1 Light Chain Amino Acid Sequence

*MDMRVPAQLLGLLLLWLRGARC*DIQMTQSPSSLSASVGDRVTITCGASENIYGALN
WYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
NVLNTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC.

(SEQ ID NO: 70)

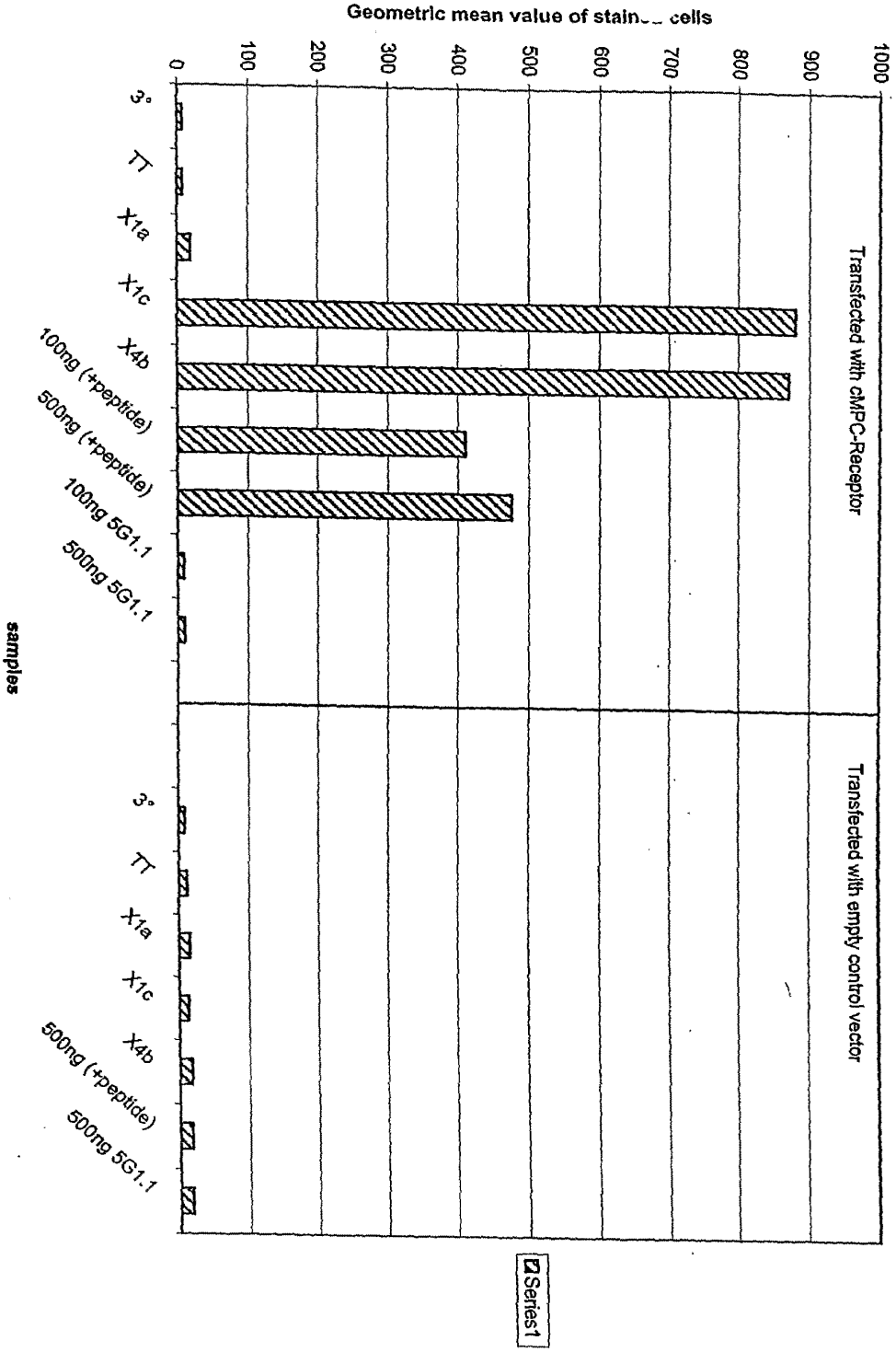
5G1.1 Light Chain Nucleic Acid Sequence

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCTGGCTCCG
AGGTGCCAGATGTGATATCCAGATGACCCAGTCCCCGTCCTCCCTGTCCGCCT
CTGTGGGCGATAGGGTCACCATCACCTGCGGCGCCAGCGAAAACATCTATGG
CGCGCTGAACTGGTATCAACAGAAACCCGGGAAAGCTCCGAAGCTTCTGATT
TACGGTGCGACGAACCTGGCAGATGGAGTCCCTTCTCGCTTCTCTGGATCCGG
CTCCGGAACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCTGAAGACTTC
GCTACGTATTACTGTCAGAACGTTTTAAATACTCCGTTGACTTTCGGACAGGG
TACCAAGGTGGAAATAAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGA
AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGTTAG

Note: Italics denotes leader sequence

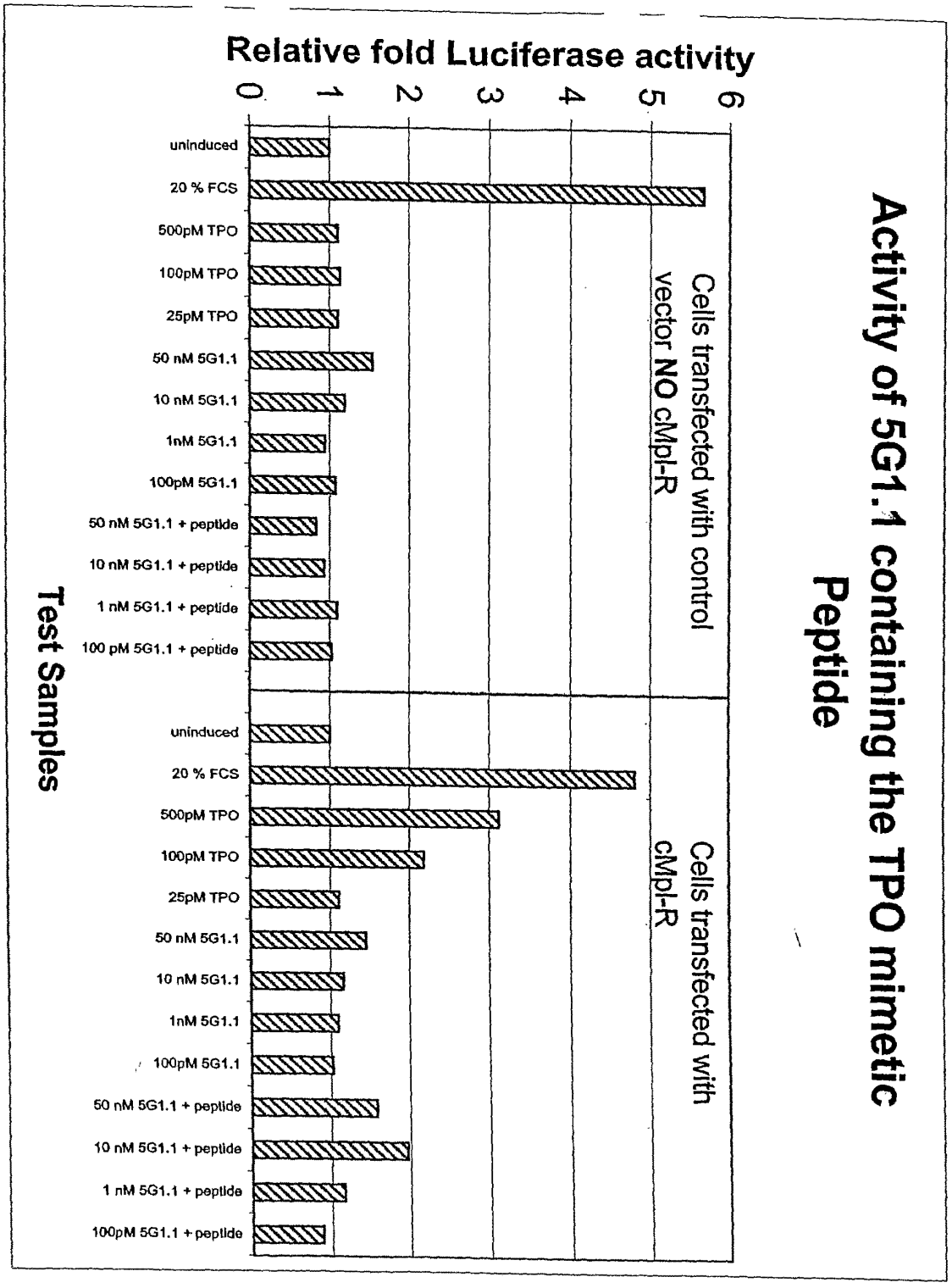
Fig. 13B

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FACS staining on transfected 293 cells

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VARIABLE REGION OF 4-29 LIGHT CHAIN

(SEQ ID NO: 116)

CDR1

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 27a 27b 27c 27d 27e 27f 28
GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CGG GCG AGT CAG ... ...AGT

CDR1 Grafted CDR2

29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 52a 52b 52c 52d 52e 52f 52g 52h 52i 52j
ATT AGT AGT TTG CTG GCC TGG TAT CAG CAG AAA CCA GGG AAA GCT CCT AAG CTC CTG ATC TAT AAC CCG ATC GAA GGC CCA ACC CTG CGC CAG TGG CTG GCT

Grafted CDR2

52k 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85
ACT CGC GCT CGT GGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT

CDR3

86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107
TAT TAC TGC CAA CAG TAT AAT AGT TAC CCT CCC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA

Fig. 16

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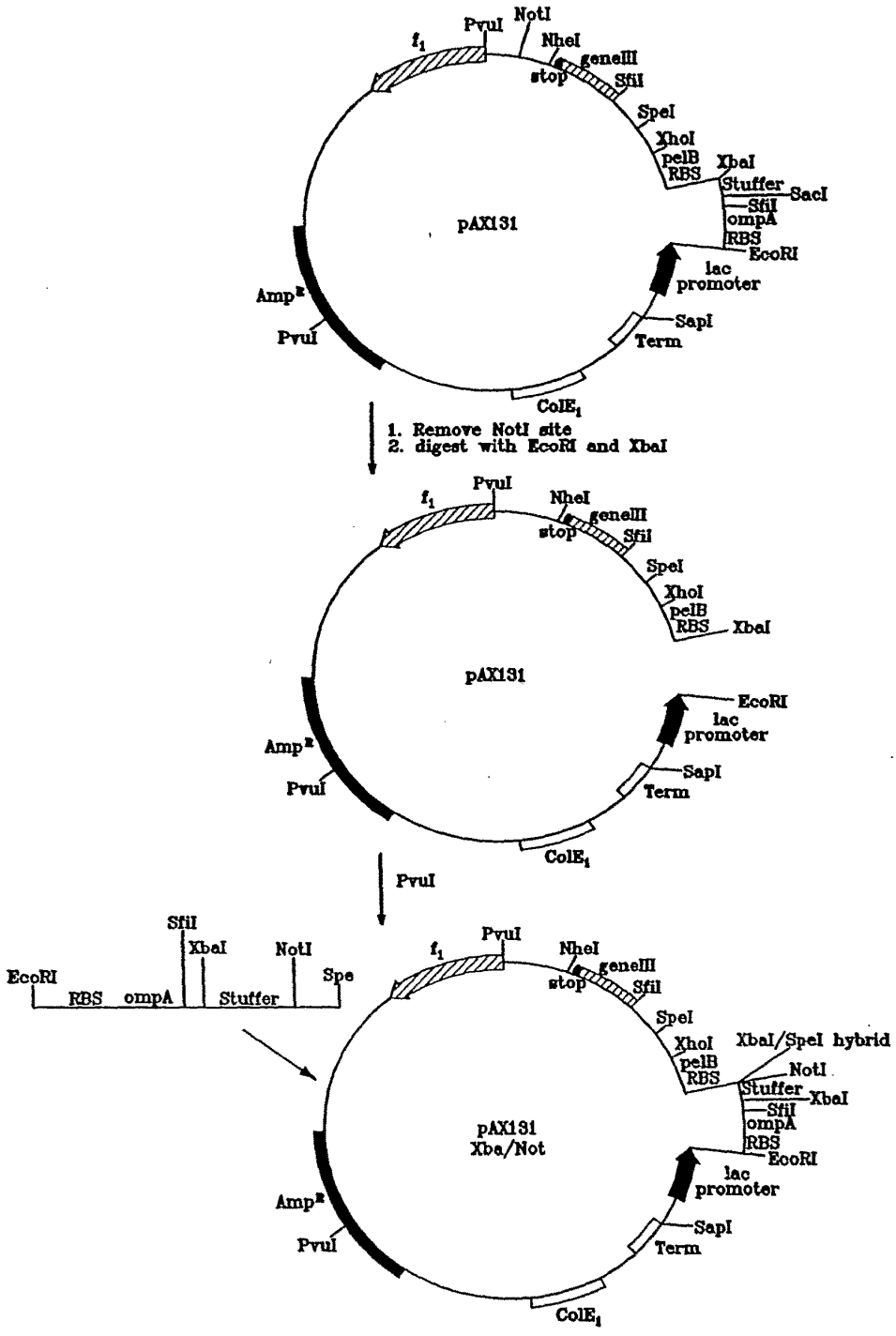


FIG. 17

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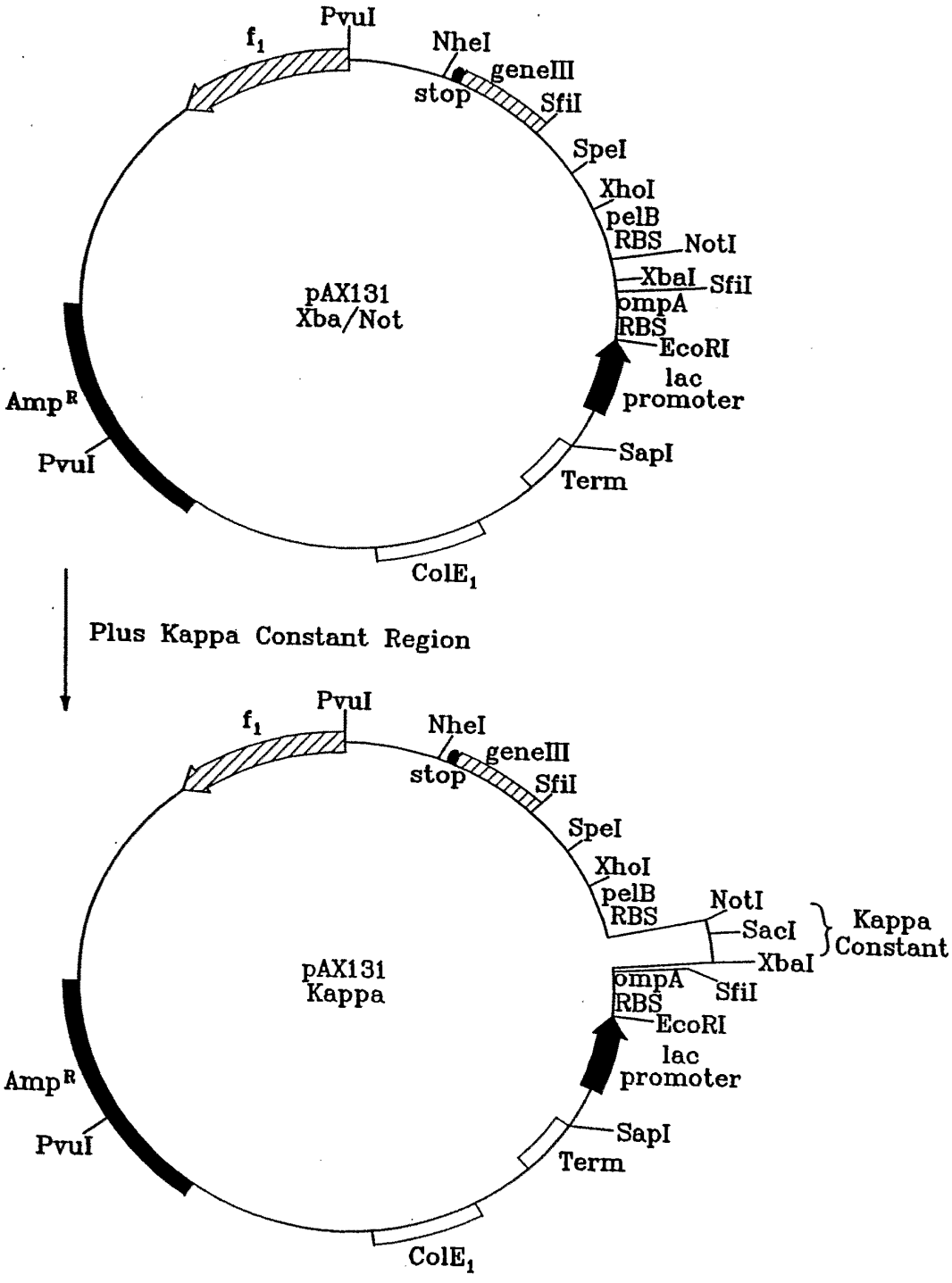


FIG. 18

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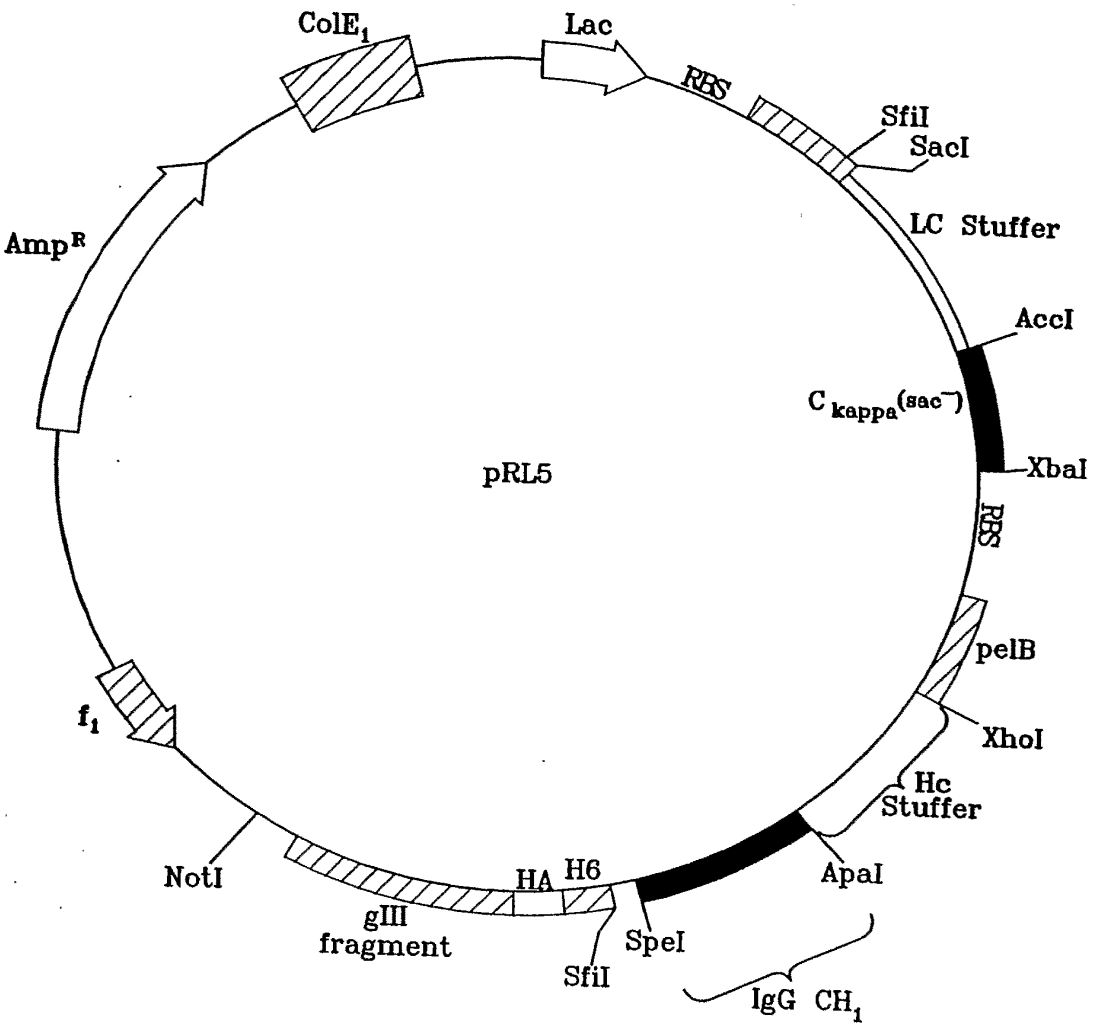


FIG. 19

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Page 7341

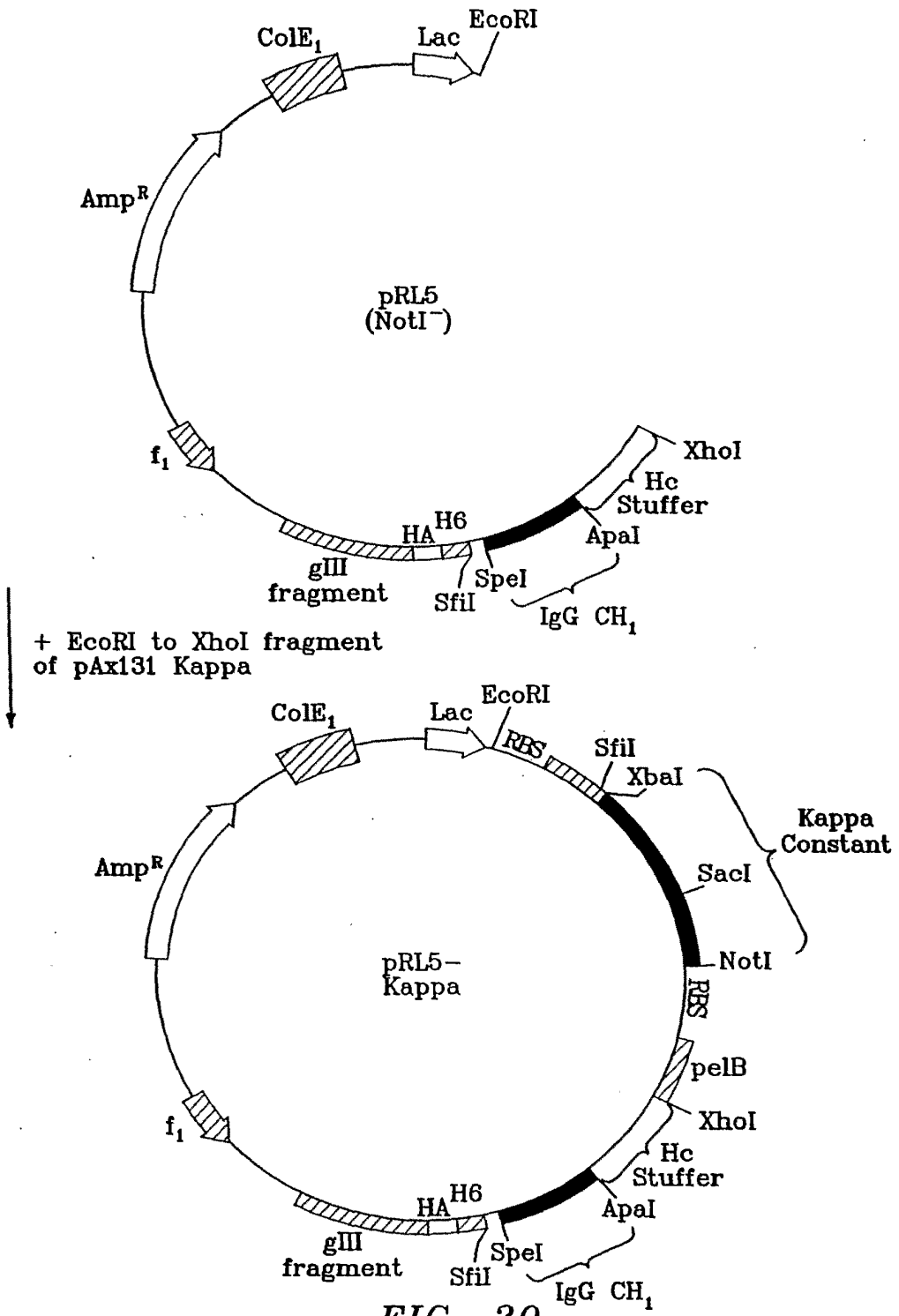


FIG. 20

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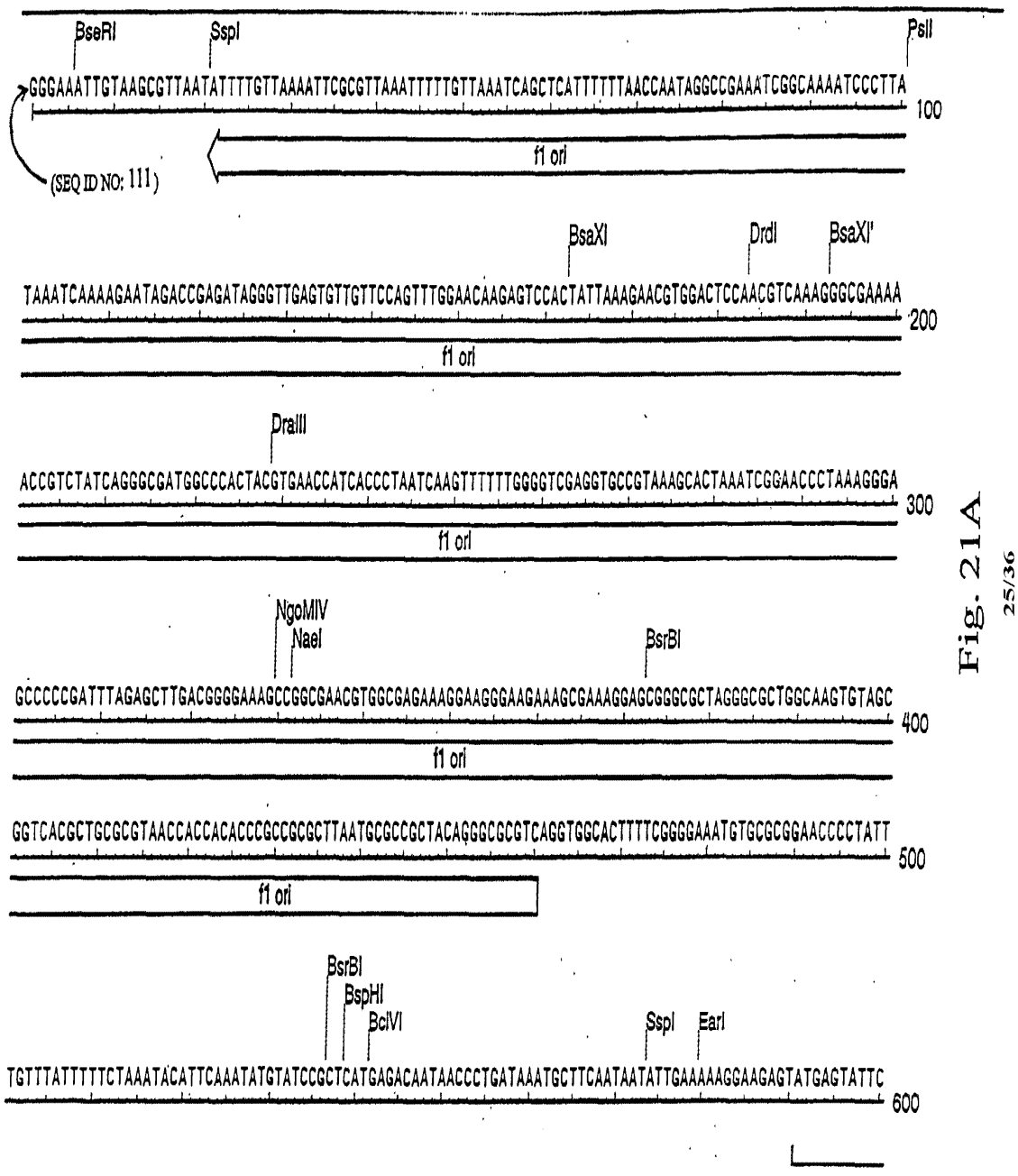


Fig. 21A

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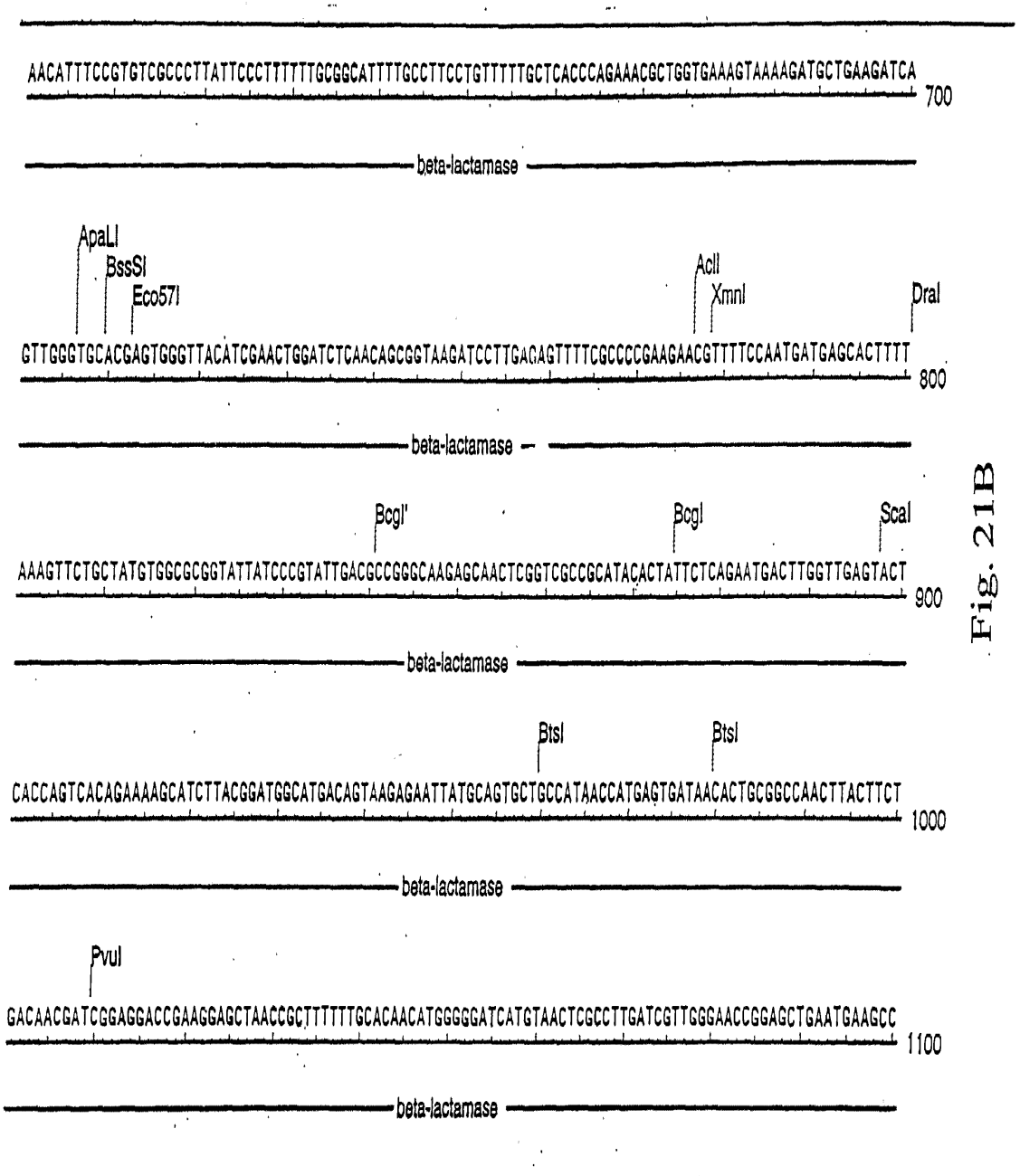


Fig. 21B

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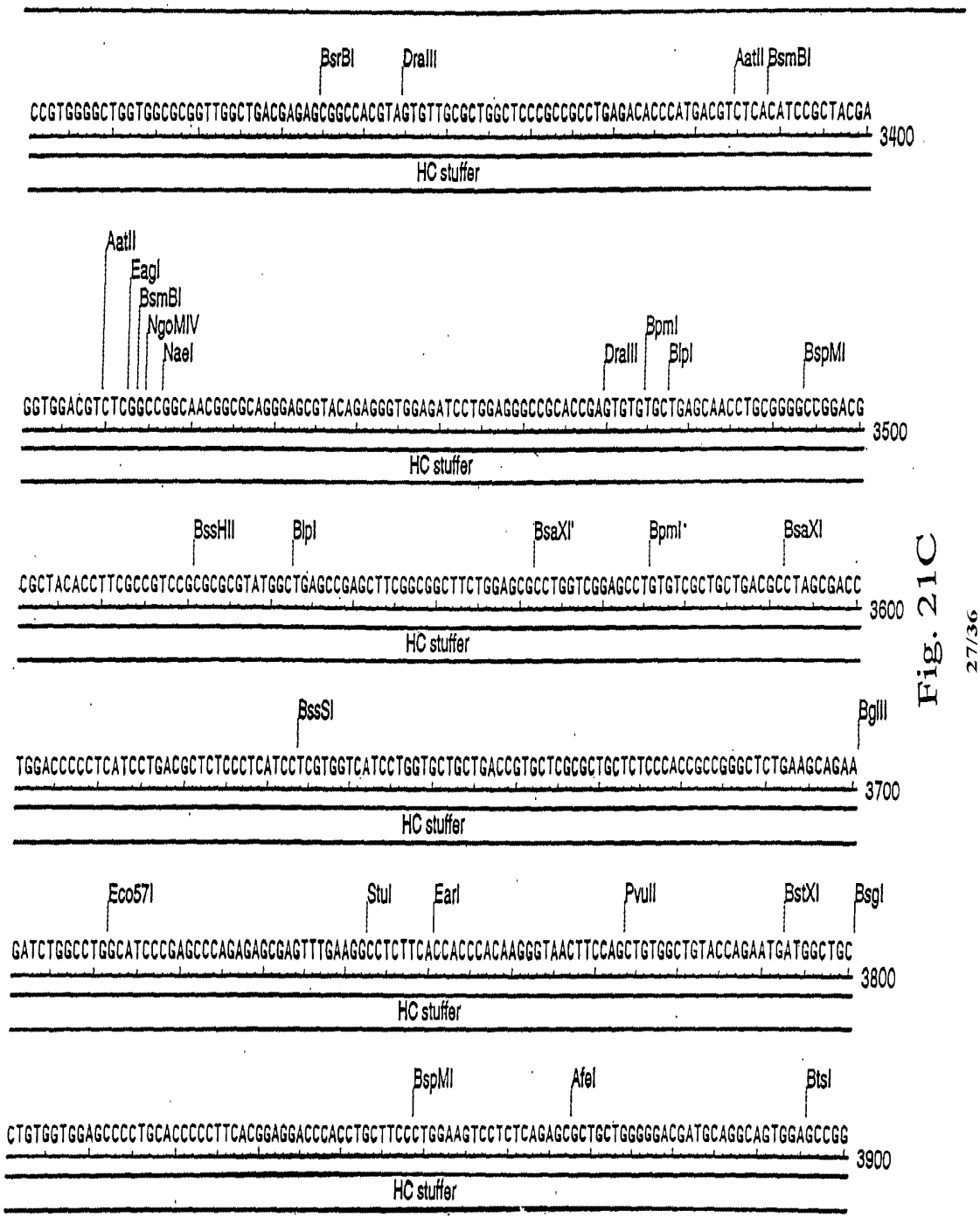


Fig. 21C

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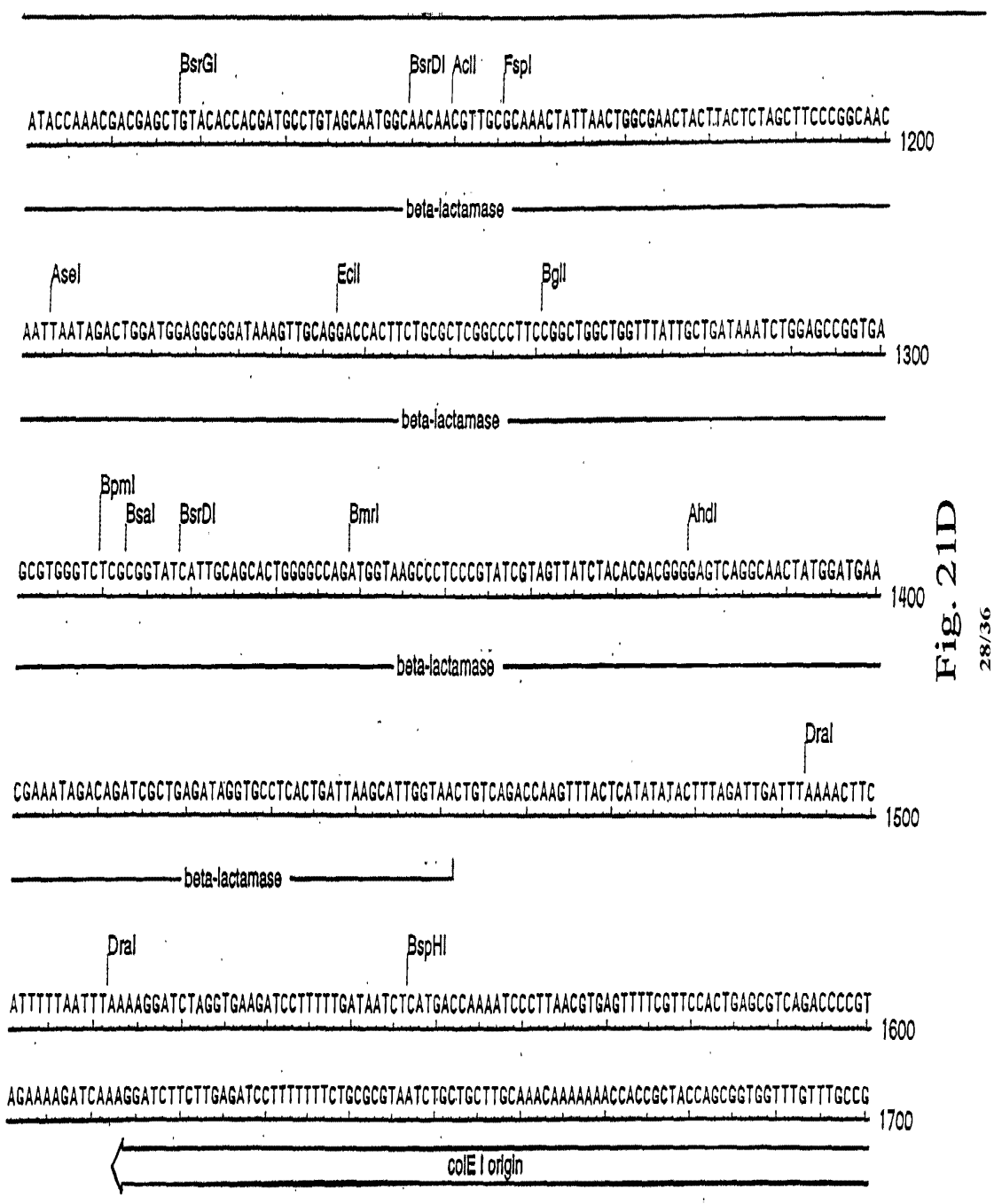


Fig. 21D

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WO 2004/050017

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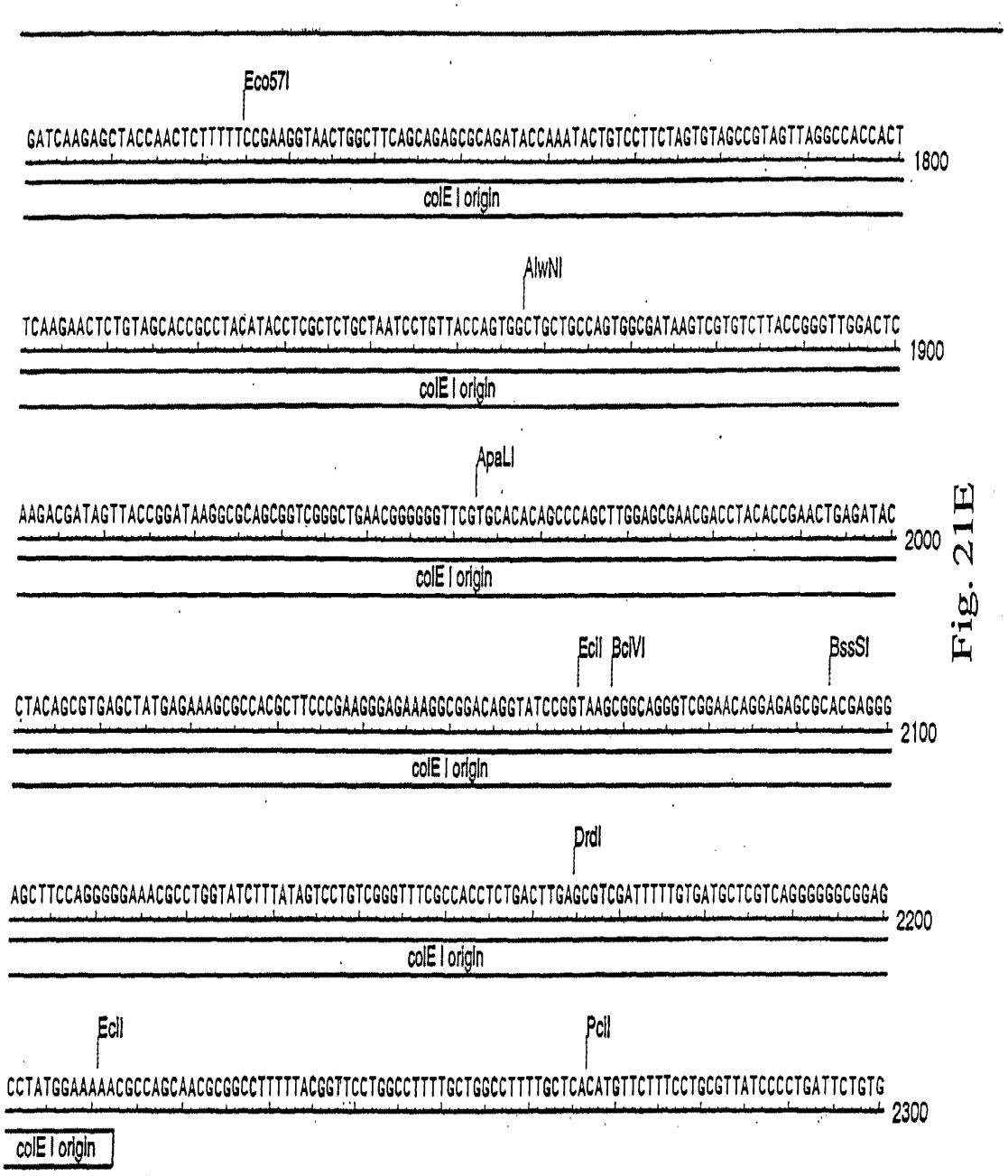


Fig. 21E

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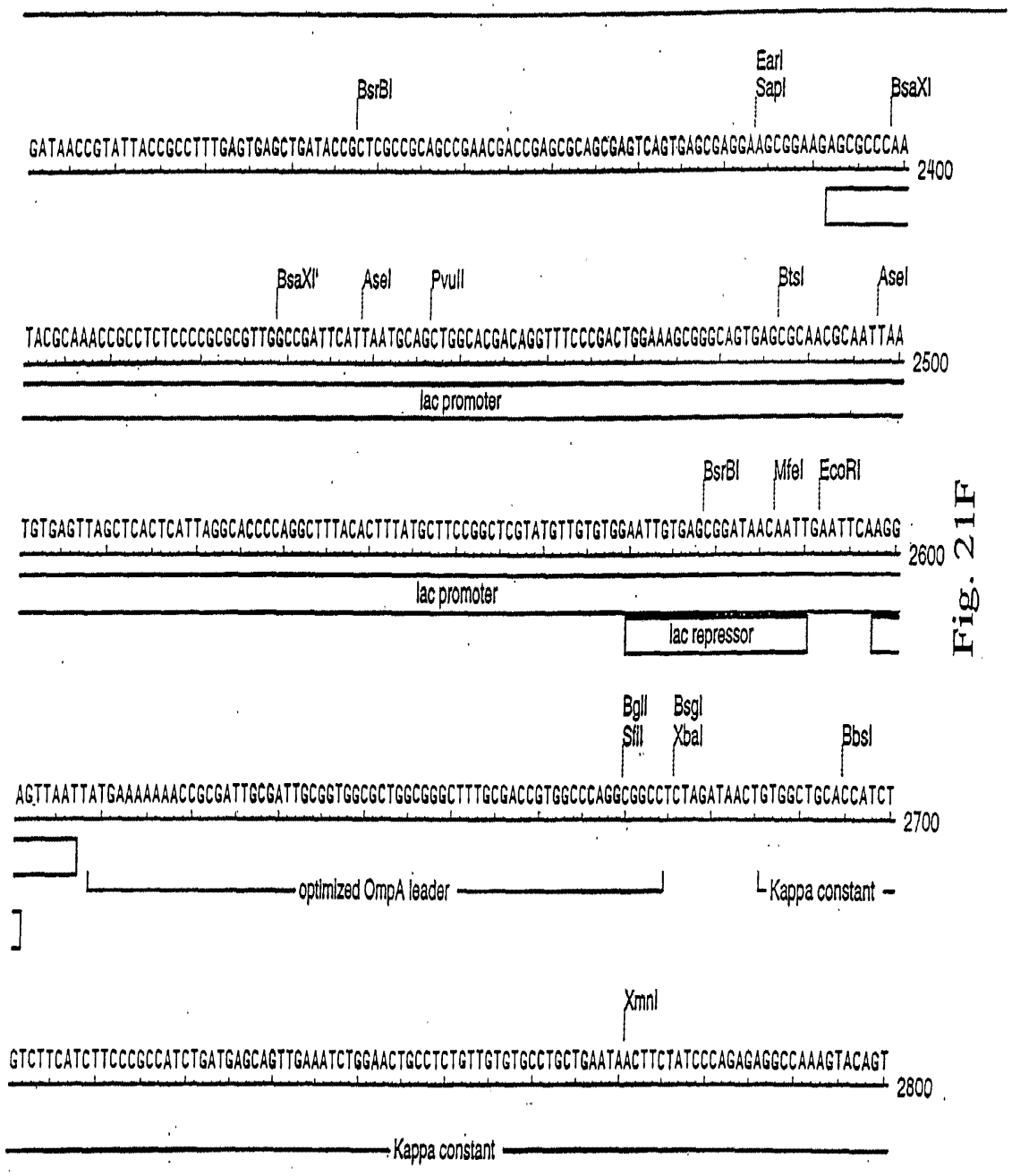


Fig. 21F

Samsung Bioepis Exhibit No. 1002
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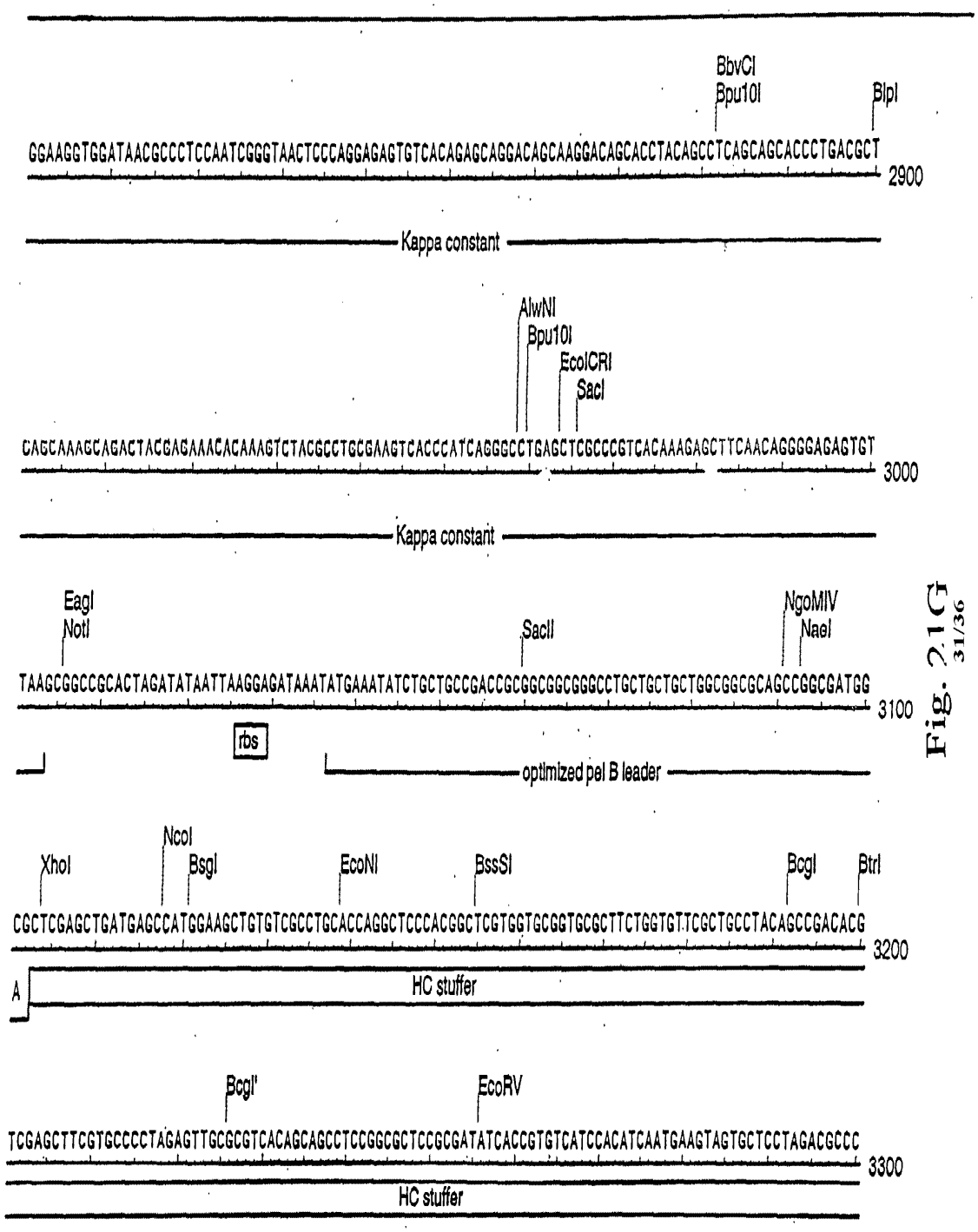


Fig. 21G 31/36

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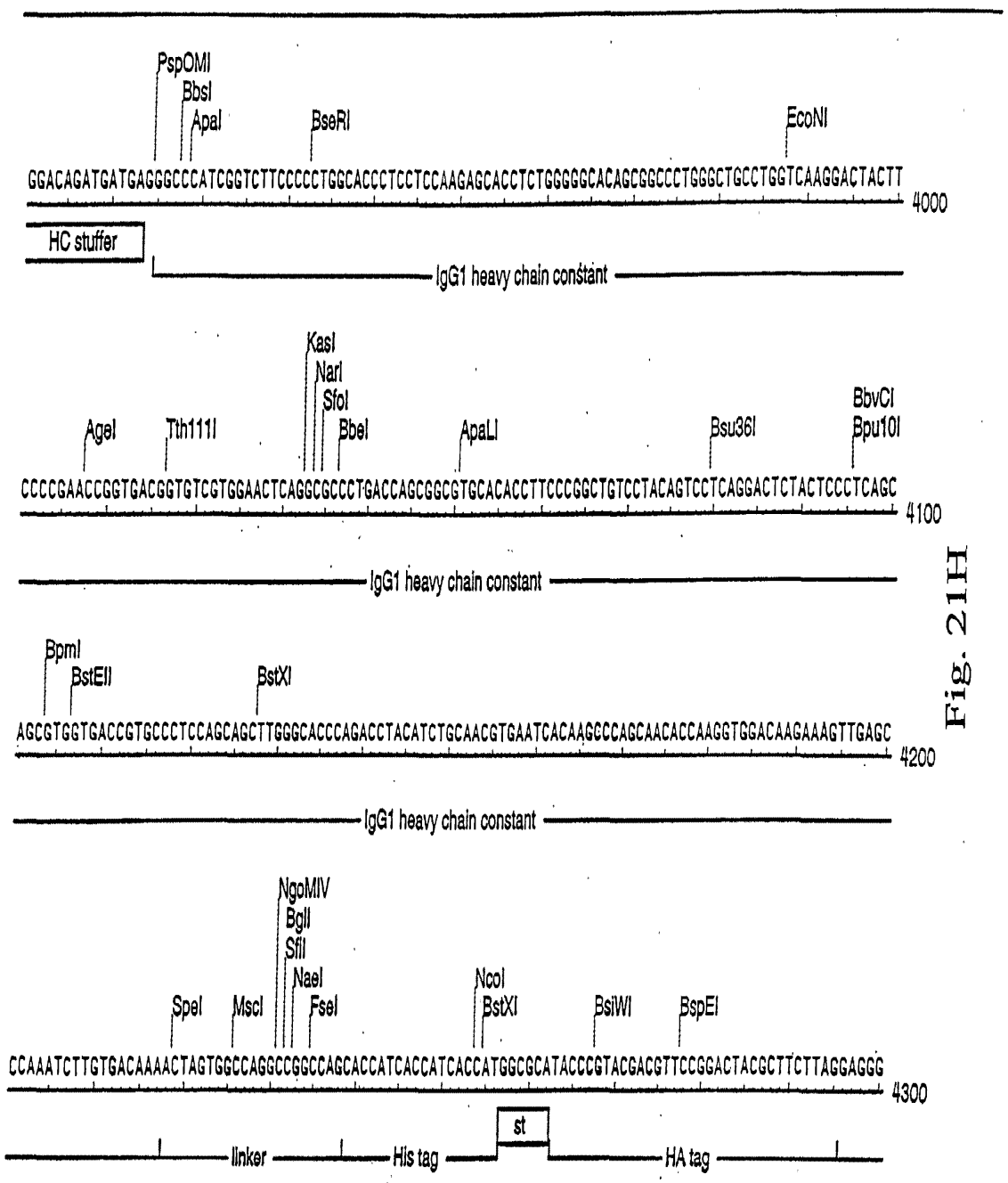


Fig. 21H

Samsung Bioepis Exhibit No. 1002
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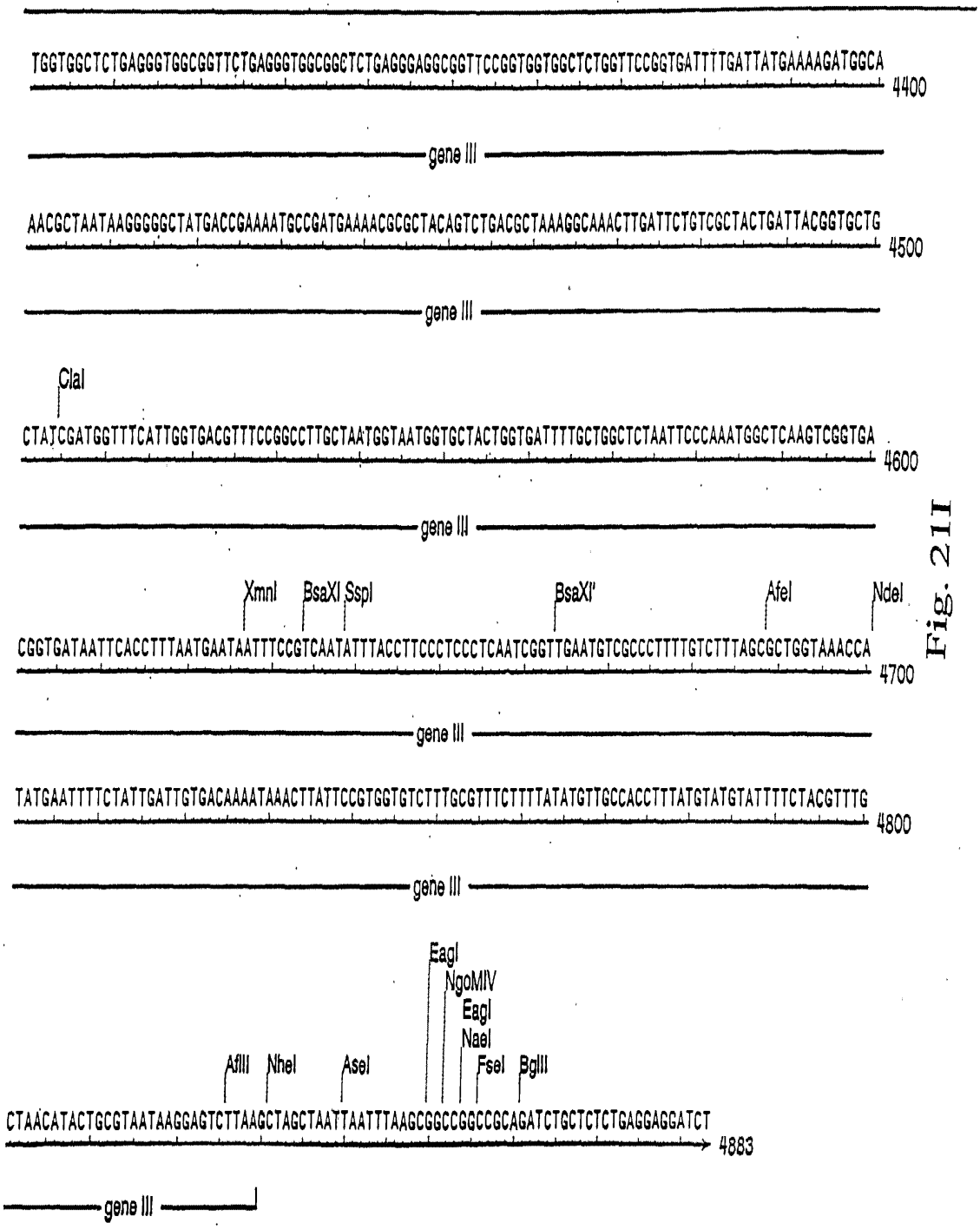


Fig. 21I

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116 Light Chain (SEQ. ID NO. 122):

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSLPVTKSFNRGEC.

Variable Region of 116 Light Chain (SEQ. ID NO. 123):

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK

116 Heavy Chain (SEQ. ID NO. 124):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVTITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK **{ VEPKSCDKTHTCPPCP }** *APELLGGP*

end CH1 constant domain — hinge region — tail region

Variable Region of 116 Heavy Chain (SEQ. ID NO. 125):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVTITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTTVTV
SS

Clone 116. The light chain sequence is as given. The heavy chain may have several forms depending on the final antibody form. For example: the minimum sequence ending with the CH1 constant domain may result in Fab assembly but the heavy chain/light chain interaction will not be stable. Commonly, a portion of the hinge region containing a cysteine (the underlined bold portion of hinge region) may be included for a covalent interaction between the heavy and light chains. Fab'_2 association would need further cysteines such as an entire IgG_1 hinge region (bold). In this example, clone 116 was cloned in a Xoma pING3302 modified vector which includes a transition tail region (italicized).

Figure 22

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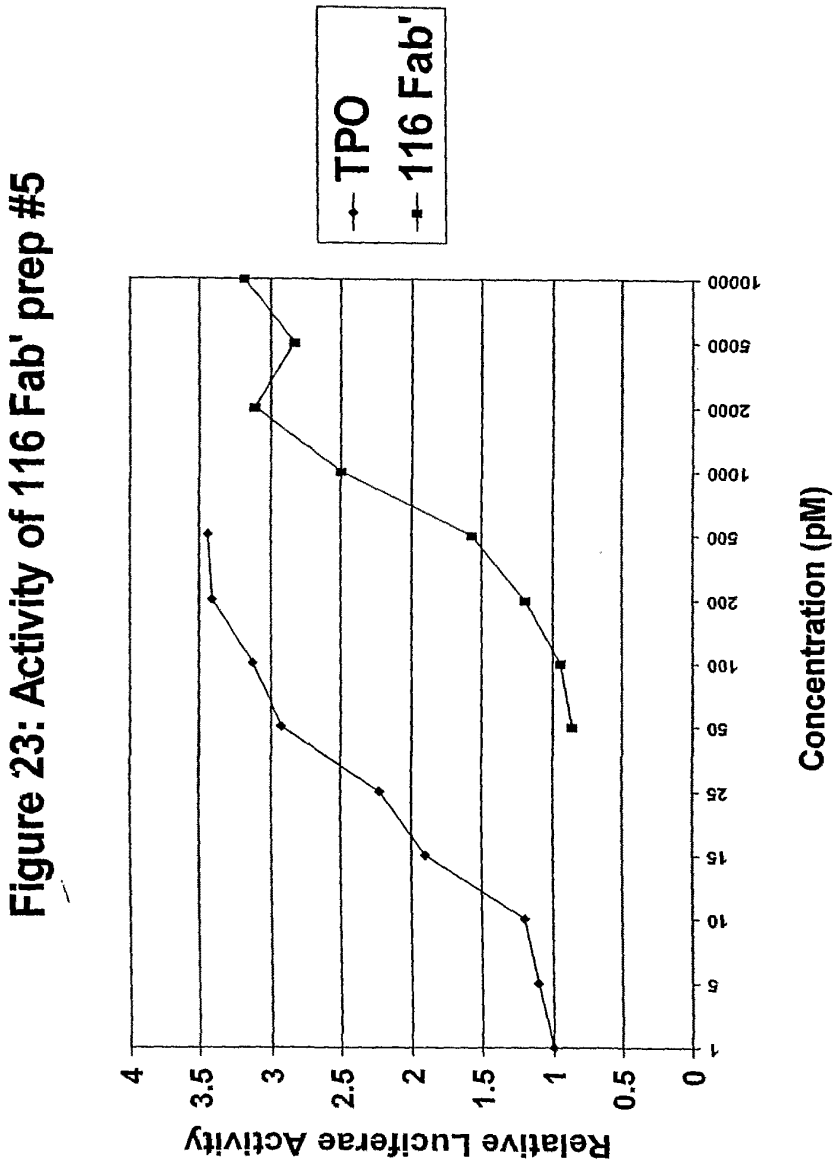


Figure 23: Activity of 116 Fab' prep #5

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Figure 24

Sample Sequences of Heavy Chain CDR2 clones

Gly-Ile-Phe-**xxx-xxx**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**xxx- xxx**-Gly (SEQ. ID NO. 126)
TT backbone randomized TPO peptide randomized TT backbone

Clone	Amino Acid Sequence
HR2-14	Gly-Ile-Phe-**Ser-Pro**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Ala-Gly**-Gly (SEQ. ID NO. 127)
HR2-20	Gly-Ile-Phe-**Pro-Gln**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-His**-Gly (SEQ. ID NO. 128)
HR2-23	Gly-Ile-Phe-**Pro-Asn**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Thr-Gly**-Gly (SEQ. ID NO. 129)
HR2-28	Gly-Ile-Phe-**Lys-Gly**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Pro-Gly**-Gly (SEQ. ID NO. 130)
HR2-43	Gly-Ile-Phe-**Pro-Pro**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Ala-Val**-Gly (SEQ. ID NO. 131)
HR2-44	Gly-Ile-Phe-**Pro-Arg**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-Leu**-Gly (SEQ. ID NO. 132)
HR2-48	Gly-Ile-Phe-**Pro-Arg**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-Leu**-Gly (SEQ. ID NO. 133)
HR2-50	Gly-Ile-Phe-**Pro-Tyr**-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-**Lys-Arg**-Gly (SEQ. ID NO. 134)

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ARTHRITIS & RHEUMATISM
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D43

The Efficacy and Safety of Rituximab in Patients With Active Rheumatoid Arthritis Despite Methotrexate Treatment

Results of a Phase IIb Randomized, Double-Blind, Placebo-Controlled, Dose-Ranging Trial

Paul Emery,[1] Roy Fleischmann,[2] Anna Filipowicz-Sosnowska,[3] Joy Schechtman,[4] Leszek Szczepanski,[5] Arthur Kavanaugh,[6] Artur J. Racewicz,[7] Ronald F. van Vollenhoven,[8] Nicole F. Li,[9] Sunil Agarwal,[9] Eva W. Hessey,[10] and Timothy M. Shaw,[10] for the DANCER Study Group

Objective. **To examine the efficacy and safety of different rituximab doses plus methotrexate (MTX), with or without glucocorticoids, in patients with active rheumatoid arthritis (RA) resistant to disease-modifying antirheumatic drugs (DMARDs), including biologic agents.**

Methods. **A total of 465 patients were randomized into 9 treatment groups: 3 rituximab groups (placebo [n = 149], 500 mg [n = 124], or 1,000 mg [n = 192] on days 1 and 15) each also taking either placebo glucocorticoids, intravenous methylprednisolone premedication, or intravenous methylprednisolone premedication plus oral prednisone for 2 weeks. All patients received MTX (10–25 mg/week); no other DMARDs were permitted.**

Results. **Significantly more patients who received 2 500-mg or 2 1,000-mg infusions of rituximab met the American College of Rheumatology 20% improvement criteria (achieved an ACR20 response) at week 24 (55% and 54%, respectively) compared with placebo (28%; $P < 0.0001$). ACR50 responses were achieved by 33%, 34%, and 13% of patients, respectively ($P < 0.001$), and ACR70 responses were achieved by 13%, 20%, and 5% of patients ($P < 0.05$). Changes in the Disease Activity Score in 28 joints (−1.79, −2.05, −0.67; $P < 0.0001$) and moderate to good responses on the European League Against Rheumatism criteria ($P < 0.0001$) reflected the ACR criteria responses. Glucocorticoids did not contribute significantly to the primary efficacy end point, ACR20 response at 24 weeks. Intravenous glucocorticoid premedication reduced the frequency and intensity of first infusion–associated events; oral glucocorticoids conferred no additional safety benefit. Rituximab was well tolerated; the type and severity of infections was similar to those for placebo.**

Conclusion. **Both rituximab doses were effective and well tolerated when added to MTX therapy in patients with active RA. The primary end point (ACR20 response) was independent of glucocorticoids, although intravenous glucocorticoid premedication improved tolerability during the first rituximab infusion.**

Although cytokine-specific biologic agents have improved the treatment of rheumatoid arthritis (RA), some patients have an inadequate response to such therapies and, in clinical practice, most have only a

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Drs. Emery, Fleischmann, and van Vollenhoven have received consulting fees or honoraria (less than $10,000 per year) from Hoffmann-La Roche or Genentech. Dr. Kavanaugh has conducted clinical trials for Genentech. Drs. Agarwal and Li and Mr. Shaw own stock in Genentech and Hoffmann-La Roche.

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partial response (1). The frequency of remission has remained <50% for patients with established RA, many of whom display "moderate" disease activity, according to their Disease Activity Score (DAS) (2). In order to achieve additional significant gains in RA therapy, new targets need to be identified. One such target is the B cell (3).

Rituximab is a monoclonal antibody that selectively depletes CD20+ B cells via 3 putative mechanisms: antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and promotion of CD20+ B cell apoptosis (4–6). CD20 is not expressed on stem cells or plasma cells (7,8); this is consistent with the observation that B cell recovery and Ig production were not compromised after a single course of rituximab (9).

Rituximab has shown efficacy in the treatment of CD20+ B cell non-Hodgkin's lymphoma (NHL) (10). More recently, a role for rituximab and B cell modulation has been investigated in rheumatoid factor (RF)–positive patients with active RA. In a phase IIa study, 2 1,000-mg infusions of rituximab (combined with methotrexate [MTX] and a 2-week treatment with glucocorticoids) significantly improved RA symptoms for up to 1 year compared with MTX alone (9). Moderate to good responses according to the European League Against Rheumatism (EULAR) criteria were also evident at 2 years in 33% of the rituximab plus MTX group (11).

To date, no studies have investigated the influence of the rituximab dosage or the role of glucocorticoids in the clinical efficacy and/or tolerability of rituximab regimens in RA. The objective of this study was therefore to examine these variables in patients with active RA who had an inadequate response to disease-modifying antirheumatic drugs (DMARDs) other than MTX or biologic agents, and who currently had an inadequate clinical response to MTX.

PATIENTS AND METHODS

Role of the funding source. This study was funded by Roche, and Roche scientists contributed to the study design, collection of data, and data analysis. The manuscript was written by Dr. Emery. The agreement to submit the manuscript and the approval of the content were made jointly by the authors.

Study design. This phase IIb, randomized, double-blind, double-dummy, placebo-controlled, international multifactorial trial of 9 different treatment regimens examined 2 dosage levels (and rituximab placebo) of both rituximab and glucocorticoids in a 3 × 3 configuration (Figure 1) with concomitant MTX in patients with active RA. Patients were evaluated at 4-week intervals between baseline and week 24.

Patients. Outpatients were eligible for study if they were between 18 and 80 years of age and had presented at least 6 months prior to randomization with moderate or severe RA

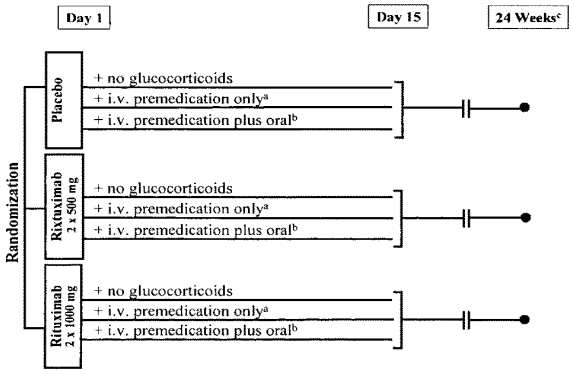


Figure 1. Study design. All groups received weekly methotrexate 10–25 mg parenterally or orally. a = 100 mg methylprednisolone administered intravenously (IV) prior to rituximab infusions on days 1 and 15. b = 100 mg methylprednisolone administered IV prior to rituximab infusions on days 1 and 15, and prednisone administered orally at 60 mg on days 2–7 and at 30 mg on days 8–14. c = primary end point: percentage of patients who met the American College of Rheumatology 20% improvement criteria at week 24.

(diagnosed according to the American College of Rheumatology [ACR; formerly, the American Rheumatism Association] revised criteria [22]) despite ongoing treatment with MTX at a dosage of 10–25 mg/week (orally or parenterally) for at least 12 weeks before randomization, with a stable dosage during the last 4 weeks. Active disease was defined as a swollen and tender joint count ≥8 and either an erythrocyte sedimentation rate ≥28 mm/hour or a C-reactive protein (CRP) serum level ≥1.5 mg/dl.

Patients must have failed prior treatment, manifesting as a lack or loss of response to treatment with at least 1 but not more than 5 DMARDs (other than MTX) and/or biologic response modifiers. Patients discontinued DMARDs (except MTX) and biologic therapy at least 4 weeks before randomization and discontinued infliximab, adalimumab, or leflunomide at least 8 weeks before randomization. Patients who were already taking oral glucocorticoids (≤10 mg/day of prednisone or equivalent) were included if the dosage had been stable for at least 4 weeks before trial entry, and patients who had received intraarticular or parenteral glucocorticoids were included, provided that the most recent administration was >4 weeks before screening. Concomitant treatment with nonsteroidal antiinflammatory drugs was permitted if the dosage had been stable for at least 2 weeks prior to entry. Concomitant treatment with any DMARD (other than MTX), anti–tumor necrosis factor α, or other biologic therapy was prohibited.

Patients were excluded if they had significant systemic involvement secondary to RA, evidence of significant other illnesses or laboratory abnormalities, a history of severe allergic or anaphylactic reactions to humanized or murine monoclonal antibodies, or previous treatment with rituximab or any lymphocyte-depleting therapies. In addition, patients were excluded if they had a history of recurrent significant infection.

The study was performed in accordance with the Declaration of Helsinki. All participating sites received ap-

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Table 1. Randomization of trial medication and disposition of patients at 24 weeks*

	Treatment†		
	Rituximab placebo, RF+/RF−	Rituximab 2 500-mg doses, RF+/RF−	Rituximab 2 1,000-mg doses, RF+/RF−
Glucocorticoid placebo	42/21	41/0	43/22
IV glucocorticoid premedication‡	42/0	41/0	42/20
IV glucocorticoid premedication + oral glucocorticoid§	44/0	42/0	43/22
Total no. of patients randomized¶	149	124	192
Patients withdrawn, no. (%)	52 (35)	11 (9)	27 (14)
Insufficient response	46 (31)	8 (6)	16 (8)
Adverse events	0	3 (2)	6 (3)
All other reasons#	6 (4)	0	5 (3)
Completed 24 weeks, no. (%)	97 (65)	113 (91)	165 (86)

* RF = rheumatoid factor; IV = intravenous.
† Rituximab or rituximab placebo was administered as 2 infusions: 1 on day 1 and 1 on day 15. All patients received 10–25 mg of methotrexate orally or parenterally on a weekly basis.
‡ Methylprednisolone 100 mg IV on days 1 and 15.
§ Methylprednisolone 100 mg IV on days 1 and 15 plus oral prednisone 60 mg on days 2–7 and 30 mg on days 8–14 (total oral prednisone dose 570 mg).
¶ Patients received at least 1 dose of rituximab.
In the rituximab placebo and rituximab groups receiving 2 1,000-mg infusions, 4 patients (3%) and 4 patients (2%), respectively, refused treatment.

proval from their governing institutional review board (or equivalent). See Appendix A for additional members of the Dose-Ranging Assessment: International Clinical Evaluation of Rituximab in Rheumatoid Arthritis (DANCER) Study Group and their locations. All patients provided written informed consent. The primary study population for efficacy analysis was composed of RF-positive patients. A limited number of RF-negative patients were also included for an initial exploratory analysis of the safety of rituximab in this group.

Study treatment. Rituximab was administered by intravenous (IV) infusion in RF-positive patients: placebo, 500 mg or 1,000 mg on days 1 and 15 (total dose 0 mg, 1,000 mg, and 2,000 mg). Glucocorticoids were administered as placebo methylprednisolone, given IV 30–60 minutes before the infusion of rituximab (or rituximab placebo) on days 1 and 15, premedication methylprednisolone 100 mg, given IV on days 1 and 15 (250 mg prednisone equivalent), or premedication methylprednisolone 100 mg, given IV on days 1 and 15 plus 60 mg of oral prednisone on days 2–7 and 30 mg on days 8–14 (total glucocorticoid dose 820 mg prednisone equivalent) (Table 1). RF-negative patients received either placebo or rituximab (2 1,000-mg infusions), with or without glucocorticoids. All patients received a weekly regimen of MTX (10–25 mg orally or parenterally) with folate (≥5 mg/week).

Trial end points. The primary end point of the trial was the proportion of RF-positive patients who met the ACR 20% improvement criteria (achieved an ACR20 response) (defined as a 20% improvement in the tender and swollen joint counts and a 20% improvement in 3 of the 5 other ACR improvement criteria) (13) at week 24. Secondary and exploratory analyses examined group differences in ACR50 and ACR70 responses, DAS 28-joint assessment for swelling and tenderness (DAS28) (14), EULAR responses (15), fatigue, as measured by the Functional Assessment of Chronic Illness Therapy–Fatigue (FACIT-F) subscale (16), and the effect on individual parameters of the ACR improvement criteria. The level of physical functioning and disability was assessed using the disability index (DI) of the Health Assessment Questionnaire (HAQ) (17).

Safety outcomes were expressed as the incidence of adverse events, graded according to National Cancer Institute (NCI) Common Toxicity Criteria (CTC), version 2.0 (18). CD19+ counts, measured by fluorescence-activated cell sorting, were used as a marker of the onset and duration of peripheral B cell depletion (7). Effects on Ig levels, protective antibody titers, and human antichimeric antibody (HACA) levels were also evaluated.

Statistical analysis. The trial was planned to recruit 440 patients: 40 RF-positive patients per group (a total of 360 patients), who constituted the primary efficacy population, and 20 RF-negative patients in 4 of the 9 groups (a total of 80 patients), for exploratory analysis. Using a 2-sided 5% significance level, it was calculated that a sample size of 360 RF-positive patients would provide at least 80% power to detect differences between the proportions of patients who achieved an ACR20 response in the rituximab 2 1,000-mg and 2 500-mg infusion groups, as well as dose-response trends across the 3 pooled rituximab groups. Randomization was stratified by region (US versus non-US) and by RF status. Steroids were included in the main effects model of ACR20 response at 24 weeks to determine whether a significant effect of the steroid regimen had been achieved. The study was powered to test for an association between ACR20 response at week 24 and rituximab treatment while controlling for corticosteroid usage and region (US or non-US) for RF-positive patients, using a logistic regression model (main effects model). It was not powered to determine any individual effects of these controlling factors.

Primary analyses were based on intent-to-treat (ITT), with nonresponders imputed for all categorical end points (e.g., ACR and EULAR responses), and the last observation was carried forward for continuous variables (e.g., ACR improvement criteria and DAS28). All patients who dropped out of the study were considered nonresponders for purposes of categorical end points. A main-effect logistic regression model

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Table 2. Patient demographics and baseline disease characteristics*

	Rituximab placebo	Rituximab 2 500-mg infusions	Rituximab 2 1,000-mg infusions
Safety population, no.	149	124	192
Demographics			
Female, %	80	83	80
Age, mean years	51.1	51.4	51.1
Duration of RA, mean years	9.3	11.1	10.8
Previous DMARDs (other than MTX), mean no.	2.2	2.5	2.5
Prior anti-TNFα treatment, %	26	33	28
MTX dose, mean mg/week	15.6	16.0	14.9
US patients, %	33	33	34
Primary ITT population, no.	122	123	122
Disease characteristics			
Swollen joint count, mean no.	21	22	22
Tender joint count, mean no.	35	33	32
Baseline HAQ DI score, mean	1.7	1.8	1.7
RF, mean IU/liter	437	421	437
ESR, mean mm/hour	40	45	41
Serum CRP, mean mg/dl	3.3	3.2	3.0
DAS28, mean	6.8	6.8	6.7

* RA = rheumatoid arthritis; DMARDs = disease-modifying antirheumatic drugs; MTX = methotrexate; TNFα = tumor necrosis factor α; ITT = intent to treat; HAQ = Health Assessment Questionnaire; DI = Disability Index; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; DAS28 = Disease Activity Score in 28 joints.

was used to evaluate ACR20 response at week 24 in the primary efficacy population across all study groups, with terms for rituximab, glucocorticoid usage, and region (US or non-US). This model also studied any effect of adding RF-negative patients to the analysis population. Contrast tests were conducted for rituximab 2 1,000-mg infusions versus placebo, and rituximab 2 500-mg infusions versus rituximab 2 1,000-mg infusions, in addition to a dose-response relationship in the ACR20 response across the upper- and lower-dose rituximab and placebo groups. No comparisons between the rituximab 2 500-mg infusions and placebo were originally planned.

RESULTS

Patient demographics and characteristics. A total of 465 patients were treated, 380 RF-positive patients and 85 RF-negative patients; this constituted the safety population (Table 1). Patients in each of the rituximab groups were generally well matched in terms of demographic parameters and region, baseline disease characteristics, and previous DMARD/biologic treatments (Table 2). Antimalarial agents had been taken by 38–68% of patients across treatment groups, sulfasalazine by 38–68% and immunosuppressive agents (cyclosporine, azathioprine, and/or leflunomide) by 24–44%. Overall, 29% of patients had previously taken biologic agents (infliximab, adalimumab, etanercept, or anakinra), with no significant differences between the randomized groups.

Of the 465 patients, 65% in the placebo group, 91% in the rituximab 2 500-mg infusions group, and 86% in the rituximab 2 1,000-mg infusions group completed the 24-week primary analysis period (Table 1). Fewer patients discontinued rituximab than placebo as a result of insufficient therapeutic response, but rituximab was associated with more withdrawals because of adverse events. Nine patients (all in rituximab groups) withdrew for safety reasons (mainly as a result of infusion-related events). One patient in the 2 500-mg rituximab infusions plus IV glucocorticoid group died due to a cerebrovascular accident.

The primary ITT efficacy population was composed of 367 RF-positive patients; the remaining RF-positive patients were excluded from this analysis for reasons related to accidental unblinding, drug dispensing errors, and unverifiable data.

Efficacy outcomes. The proportion of patients in the primary ITT efficacy population who achieved an ACR20 response at 24 weeks, the primary end point of the trial, was significantly greater in each of the rituximab groups than in the placebo group ($P < 0.0001$) (Figure 2A). At week 24, 55% of the patients in the rituximab 2 500-mg infusions group and 54% of those in the 2 1,000-mg infusions group had achieved an ACR20 response, compared with 28% in the placebo group. There was no statistically significant difference in the odds of achieving an ACR20 response between the groups taking 2 1,000-mg infusions of rituximab versus 2 500-mg infusions of rituximab (odds ratio 0.93, $P = 0.768$). Analyses of the proportions of patients who achieved an ACR50 or ACR70 response showed a similar pattern. Compared with placebo, a significantly greater proportion of patients treated with either dosage

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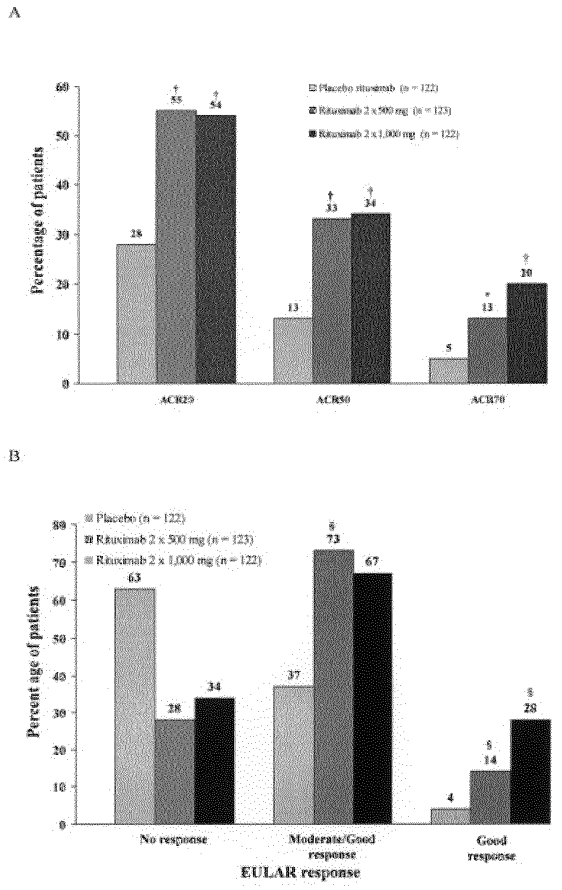


Figure 2. Patients who experienced responses according to **A,** the American College of Rheumatology 20% (ACR20), 50% (ACR50), and 70% (ACR70) improvement criteria and **B,** the European League Against Rheumatism (EULAR) criteria at 24 weeks. Results are expressed using pooled data for glucocorticoid dose. Patients who withdrew or who had insufficient data from which to calculate an ACR score were classified as nonresponders. * = $P = 0.029$; † = $P \leq 0.001$; § = $P < 0.0001$, versus placebo.

of rituximab achieved an ACR50 response ($P \leq 0.001$ versus placebo in each rituximab dosage group) and an ACR70 response ($P = 0.029$ for rituximab 2 500-mg infusions group and $P \leq 0.001$ for rituximab 2 1,000-mg infusions group). Although ACR20 and ACR50 responses were similar for both rituximab dosages, ACR70 responses were numerically more frequent in the rituximab 2 1,000-mg infusions group than in the 2 500-mg infusions group (Figure 2A).

Primary analysis within the main-effect model showed that glucocorticoid treatment had no significant effect on ACR20 response ($P = 0.166$). Dose-contrast tests between individual glucocorticoid treatments showed that the probability of achieving an ACR20 response in any of the main treatment groups was not significantly different for glucocorticoid placebo versus IV glucocorticoids ($P = 0.62$) or IV and oral glucocorticoids ($P = 0.18$). Patients who received glucocorticoids were, however, more likely to achieve an ACR20 response by week 4 than those who received no glucocorticoids. Consequently, since glucocorticoid treatment was not a prerequisite for the efficacy of rituximab, efficacy results are expressed using pooled glucocorticoid dose cohorts within each rituximab group.

The time course for achieving an ACR response showed early separation of rituximab from placebo. The proportion of patients who achieved an ACR20 response in the placebo group leveled off at ~30% at week 4, while in the rituximab groups, it continued to increase, leveling off at ~55% at 12 weeks. The same was essentially true for ACR50 and ACR70 responses, except in the rituximab 2 1,000-mg infusions group, in which ACR70 was seen in 10% of patients at week 12 and increased to 20% at week 24, suggesting a time-related effect on achievement of higher-level responses at this dose.

Changes in the DAS28 at week 24 reflected ACR response findings. Analysis of variance showed the adjusted mean change in the DAS28 from baseline to be significantly greater in patients treated with rituximab 2 500-mg and 2 1,000-mg infusions (−1.79 and −2.05, respectively) than in patients treated with placebo (−0.67; $P < 0.0001$). Similarly, EULAR responses in patients treated with rituximab at either dosage were significantly greater than those seen in patients who received placebo (Figure 2B). Compared with placebo, moderate or good EULAR responses were achieved in a significantly greater proportion of patients treated with rituximab 2 500-mg infusions ($P < 0.0001$) and rituximab 2 1,000-mg infusions ($P < 0.0001$), with a trend toward a higher proportion of patients achieving a good EULAR response with 2 1,000-mg infusions of rituximab.

CRP levels fell by a mean of 1.7 mg/dl between baseline and week 24 in patients treated with the active rituximab regimens. The corresponding mean change in CRP in the rituximab placebo group was 0.1 mg/dl. These differences in favor of the active rituximab groups were observed after week 4 and continued to week 24. In patients who were RF-positive at baseline, the proportion with RF titers below the level of detection by week 24 was 1.6% in the placebo group and 12% and 9% in the rituximab 2 500-mg infusions and 2 1,000-mg infu-

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Table 3. Most frequently reported adverse events in the safety population (n = 465)*

Adverse events	Rituximab placebo (n = 149)	Rituximab 2 500-mg infusions (n = 124)	Rituximab 2 1,000-mg infusions (n = 192)
All events	105 (70)	100 (81)	164 (85)
Exacerbation of RA	44 (30)	21 (17)	27 (14)
Headache	19 (13)	14 (11)	21 (11)
Nausea	13 (9)	8 (6)	19 (10)
URI	9 (6)	10 (8)	12 (6)
Nasopharyngitis	8 (5)	7 (6)	10 (5)
Arthralgia	5 (3)	5 (4)	11 (6)
Diarrhea	8 (5)	7 (6)	6 (3)
Fatigue	8 (5)	5 (4)	8 (4)
Hypertension	4 (3)	5 (4)	12 (6)
Rigors	3 (2)	5 (4)	13 (7)
Dizziness	6 (4)	4 (3)	10 (5)
Serious noninfection adverse events	2 (1)†	9 (7)‡	9 (5)§
Serious infections	2 (1)	0	4 (2)

* All adverse events affecting ≥5% of patients in any one group are shown. Values are the number (%). RA = rheumatoid arthritis; URI = upper respiratory tract infection.
† Food allergy and exacerbation of RA in 1 patient each.
‡ Cerebral infarction, fatal cerebrovascular accident unrelated to study treatment, convulsion, myocardial infarction, supraventricular tachycardia, drug hypersensitivity (occurring within 24 hours of rituximab infusion), failure of implant, lower limb fracture, and interstitial lung disease in 1 patient each.
§ Epilepsy, serotonin syndrome, chest pain, generalized edema (occurring within 24 hours of rituximab infusion), exacerbation of RA, status asthmaticus, intestinal obstruction, metrorrhagia, and thromboangiitis obliterans in 1 patient each.

sions groups, respectively. This reflected a mean increase in total RF titers in the placebo groups (ranging from 7.1% to 37.4% among placebo groups) compared with a mean decrease in the active rituximab groups (ranging from 11.5% to 47.9% among rituximab groups).

Patient-reported outcomes mirrored the improvements in clinical and laboratory findings in the primary ITT population. Changes in the mean DI scores of the HAQ between baseline and week 24 were −0.43 and −0.49 in the rituximab 2 500-mg and 2 1,000-mg infusions groups, respectively, compared with −0.16 in the rituximab placebo group. The proportions of patients with an improvement of −0.22 from baseline (minimum clinically important difference [MCID] [19]) were 63%, 67%, and 34%, respectively in these 3 groups. Furthermore, changes in fatigue, measured as the percentage improvement in the FACIT-F score between baseline and week 24, were 20% and 28% in the rituximab 2 500-mg and 2 1,000-mg infusions groups, respectively, compared with 4% in the placebo group. In both rituximab groups, the mean change from baseline in the FACIT-F score exceeded 4 points, which is the MCID (16). This was not the case in the placebo group.

The inclusion of data from RF-negative patients in the analyses did not alter the primary outcome. In the combined RF-positive and RF-negative population, 52% of the rituximab 2 1,000-mg doses group achieved an ACR20 response at week 24 compared with 32% of the placebo group. An exploratory analysis of RF-negative patients alone proved inconclusive, since it was confounded by an unusually high placebo response (52% versus 48% in the rituximab group) and a relatively small sample size (21 and 63 patients, respectively).

There was a significant difference at week 24 between the absolute proportions of US and non-US patients who achieved an ACR20 response across treatment groups, including placebo ($P = 0.002$), with an ACR20 response being more frequent in the non-US region. Between regions, the efficacy of rituximab relative to placebo (i.e., the difference between placebo and active drug responses) was similar, and consequently, there was no significant interaction between rituximab and region ($P = 0.797$). An exploratory analysis of the 2 regions showed that the only major baseline difference between the US and non-US populations was the proportion of patients previously exposed to biologic therapy (55% of US versus 23% of non-US patients). While there was no identified interaction between prior use of biologic therapies and rituximab treatment, ACR20 response rates were generally lower in patients with prior exposure to biologic therapies.

Safety outcomes. Overall, 70% of patients treated with placebo reported at least 1 adverse event at any time during the study period, compared with 81% and 85% of patients in the rituximab 2 500-mg and 2

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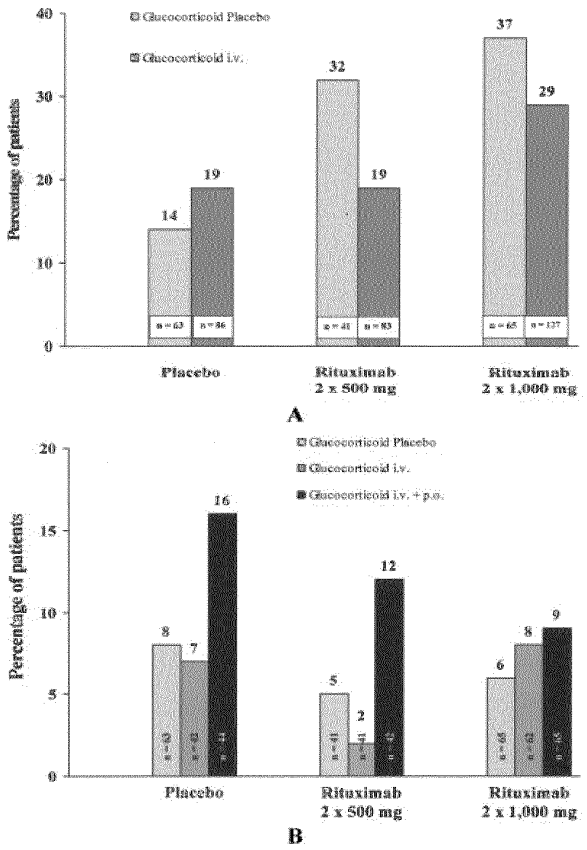


Figure 3. Effect of glucocorticoids on the frequency of acute infusion reactions after **A,** the first infusion and **B,** the second infusion. IV = intravenous; PO = by mouth.

1,000-mg infusions groups, respectively. The majority (82%) of events in each group were mild to moderate in severity (NCI CTC grades 1–2). A similar proportion of patients reported severe (NCI CTC grade 3) events in each group (18% of patients receiving placebo and 17% and 18% of patients in the rituximab 2 500-mg and 2 1,000-mg infusions groups, respectively). The most frequently reported adverse events in each treatment group are shown in Table 3 and were mainly associated with the first infusion. Exacerbation of RA, reported as an adverse event, distinguished rituximab placebo from both doses of active rituximab, reflecting the efficacy of rituximab relative to placebo. There were no unexpected adverse events in the RF-negative subgroup.

Infusion-associated events. Infusion-associated events (defined as any adverse event that occurred during or within 24 hours of rituximab infusion) occurred with the first infusion in 18% of patients who received placebo and in 31% and 38% of patients who received rituximab 2 500-mg and 2 1,000-mg infusions, respectively.

Acute infusion reactions. Symptoms or signs suggesting an acute infusion reaction (pruritus, fever, urticaria/rash, chills, pyrexia, rigors, sneezing, angioneurotic edema, throat irritation, cough and bronchospasm, hypotension, or hypertension) were more commonly associated with the first infusion of rituximab compared with placebo. Overall, acute infusion reactions were reported at the first infusion in 17% of placebo-treated patients compared with 23% and 32% of patients who received 2 500-mg or 2 1,000-mg rituximab, respectively. However, premedication with IV glucocorticoid significantly reduced both the incidence and severity of acute infusion reactions. Without glucocorticoid premedication, these events were reported by 14% of the placebo group, 32% of the rituximab 2 500-mg infusions group, and 37% of the rituximab 2 1,000-mg infusions group, as compared with 19%, 19%, and 29% of these groups, respectively, following IV glucocorticoid premedication (Figure 3A).

The number of patients who experienced these symptoms following the second infusion of the treatment course was substantially lower in all groups, irrespective of glucocorticoid premedication (Figure 3B). The addition of 2 weeks of oral prednisone treatment did not appear to provide any further reduction of acute infusion reactions with the second infusion.

Two adverse events occurred within the first 24 hours of the first infusion: 1 case of drug hypersensitivity reaction occurred in the rituximab 2 500-mg infusions group, and 1 case of generalized edema occurred in the rituximab 2 1,000-mg infusions group. Both events occurred in patients who had not received IV glucocorticoid premedication. Five patients (2.6%) in the 2 1,000-mg infusions rituximab group were withdrawn from treatment because of acute infusion-associated events following the first infusion. Four of these patients had not received glucocorticoid premedication.

Infection-related adverse events. Adverse events classed as infections and infestations were reported in 28% of the placebo group and in 35% of each of the active rituximab groups. The type and severity of infections were similar across the placebo and active rituximab groups (the most common being respiratory tract infections, urinary tract infections, and nasopharyngitis),

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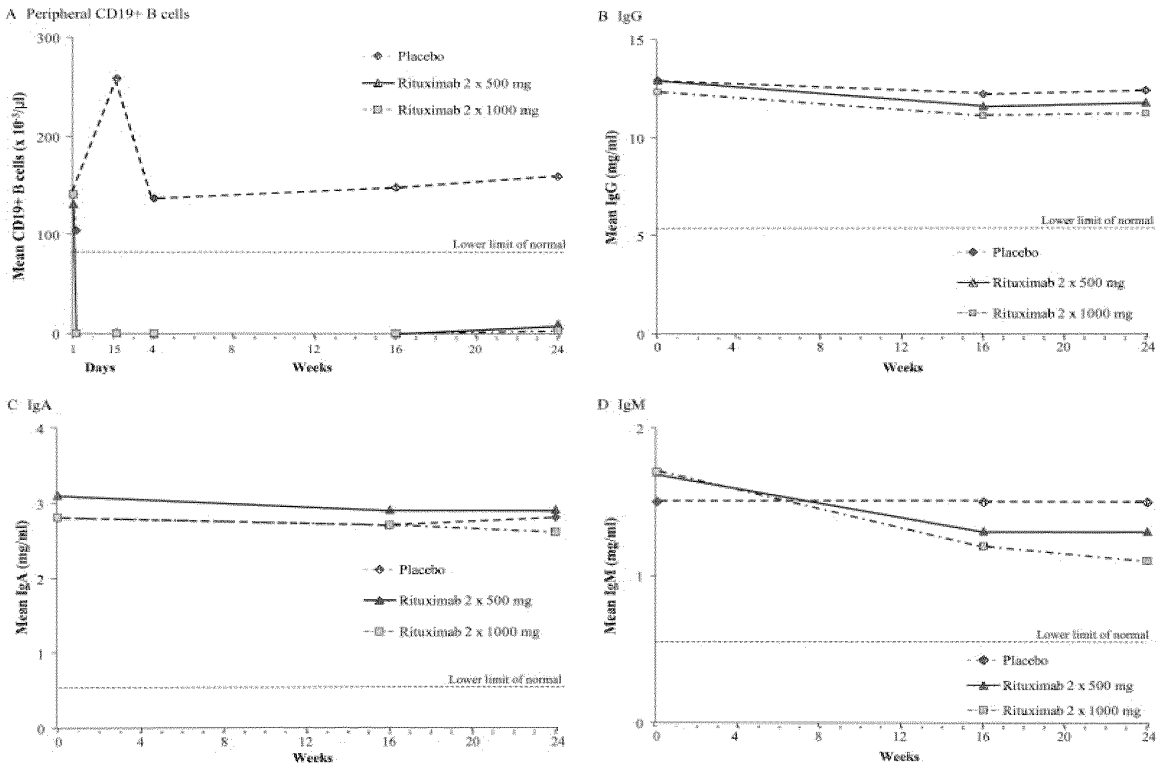


Figure 4. Levels of **A,** peripheral blood CD19+ B cells, **B,** IgG, **C,** IgA, and **D,** IgM during the 24-week treatment period.

so the pattern of infections did not distinguish between placebo- and rituximab-treated patients.

Six serious infections were reported. Two (1%)occurred in the placebo group (pneumonia and respiratory tract infection) and 4 (2%) in the rituximab 2 1,000-mg infusions group (2 cases of pyelonephritis, and 1 each of bronchitis and epiglottitis). These events resolved without sequelae. No serious infections occurred in the rituximab 2 500-mg infusions group. The rate of serious infections per 100 patient-years was 3.19 for the rituximab placebo group, 0 for the 2 500-mg infusions of rituximab group, and 4.74 for the 2 1,000-mg infusions of rituximab group.

Serious adverse events. A total of 26 serious adverse events were reported, 4 (3%) in the placebo group (3%), 9 in the rituximab 2 500-mg infusions group (7%), and 13 in the rituximab 2 1,000-mg infusions group (7%), with no consistent pattern or signal. Of these, 20 were noninfection events in 2, 9, and 9 patients in the placebo, rituximab 2 500-mg, and rituximab 2 1,000-mg infusions groups (1%, 7%, and 5%, respectively) (Table 3). Five events were nervous system disorders, while others were distributed evenly across body systems in no discernible pattern. There was 1 fatal event, which occurred in the rituximab 2 500-mg infusions group. A 73-year-old woman died of a cerebral infarction 167 days after the first infusion of rituximab. This patient had a history of atrial fibrillation, hypertension, diabetes mellitus, and hyperlipidemia; at the time of enrollment into the trial, she was receiving concomitant celecoxib, MTX, folic acid, lisinopril, atorvastatin, and sertraline.

Pharmacodynamics. In the placebo group, peripheral B cell levels remained stable around baseline levels throughout the study period, after an initial transient increase during the 2-week glucocorticoid dosing period between rituximab placebo infusions. Treatment with rituximab was associated with nearly complete

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depletion of peripheral blood B cells, which persisted throughout the 24-week study period. Peripheral blood B cell recovery began after week 16 (Figure 4A).

Although peripheral blood B cell levels were depleted in rituximab-treated patients, mean and median Ig levels (IgG, IgA, and IgM) remained above the laboratory lower limit of normal at all times; the greatest change from baseline occurred in IgM (Figures 4B–D). Anti-tetanus titers remained stable.

Antibodies to the test agent, i.e., HACA (analyzed up to and including week 24), were detectable in 0.7%, 4.2%, and 2.7% of the placebo, rituximab 2 500-mg, and rituximab 2 1,000-mg infusions groups, respectively. There were no serious adverse events in the group of patients with HACAs. Moreover, 6 of the 10 rituximab-treated patients with positive HACAs at week 24 achieved ACR20 responses at that time.

DISCUSSION

Results of the DANCER trial have confirmed that a single course of rituximab, given as 2 infusions 2 weeks apart, is highly effective over 24 weeks in the treatment of active RA in patients who have shown an incomplete response to MTX. Followup of these patients beyond the primary end point is ongoing, and radiologic outcomes (not measured in this study) will be addressed in future studies. Rituximab has a novel mode of action that results in the depletion of B cells, and it is therefore distinct from other biologic therapies for RA that target T cells and their related cytokines. The present study corroborates earlier studies that support a role for B cells in the inflammatory processes that underlie RA (9,10,20–24).

Both rituximab dosages were effective, with 54–55% of patients achieving an ACR20 response by week 24, and a high proportion achieving ACR50 or ACR70 responses. Importantly, clinical improvements in rituximab-treated patients were reflected in patient- and physician-reported outcome measures, as well as in changes in laboratory parameters. Moreover, the beneficial effects of rituximab were often evident early in the course of treatment.

In general, the efficacy results from the DANCER study broadly mirror those from a phase IIa study of rituximab in 161 RF-positive RA patients (9), although in the latter, a higher ACR20 response rate was observed. A possible explanation for this discrepancy is the prior use of biologic therapies at baseline (an indication of refractory disease). In the phase IIa study, only ~10% of patients had been exposed to a biologic agent, whereas in the DANCER trial, the proportion was 30–40% in the active rituximab groups. A subanalysis of DANCER trial patients who received active rituximab with no prior exposure to biologic therapies determined an ACR20 response rate of 62% for this group, which is closer to previously reported rates (9). Previous use of biologic agents may also be a contributory factor to the lower absolute ACR response observed in US patients compared with non-US patients in this study. A greater proportion of US patients had received such treatment and may therefore have constituted a more refractory population. Nevertheless, rituximab was similarly effective relative to placebo both in patients who had received prior biologics and those naive to these therapies.

No dose-response relationship was established, based on the ACR20 criteria, for the 2 rituximab doses studied. There were, however, trends to indicate that the dose may influence the achievement of high-level response (i.e., ACR70 response and good EULAR response), and further evaluation of both rituximab doses is warranted.

The use of concomitant glucocorticoids with rituximab was based on the successful application of similar regimens in patients with NHL (10) and the initial trials in patients with RA (9,20,21). This trial has, however, established that glucocorticoids play no significant role in determining the primary end point of an ACR20 response at 24 weeks in RA patients treated with rituximab, although they may enhance the early response (up to 4 weeks) by virtue of their antiinflammatory properties. Consequently, the use of glucocorticoids does not appear to be a prerequisite for a clinical response to rituximab.

Both dosages of rituximab were generally well tolerated, with a low incidence of serious adverse events, including serious infections. The majority of adverse events were infusion associated, mild to moderate in intensity, and easily managed. Premedication with IV glucocorticoids did, however, reduce the incidence and severity of infusion-associated events following the first infusion of rituximab. The incidence of reactions following the second infusion was low in all groups, with 2 weeks of oral glucocorticoid treatment providing no additional safety benefit over IV glucocorticoid premedication alone. Overall, the incidence of infusion-related adverse events in RA patients appears to be lower than that observed in NHL patients (who may be prone to such events because of elevated levels of white blood cells or tumor lysis) (10,25).

All patients had depleted B cell levels following rituximab treatment, with peripheral blood B cell levels falling to below the lower limit of normal. Mean and

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median Ig levels stayed within the normal range, but there was a downward trend specifically with IgM; however, there was no significant change in acquired immunity. Overall, the incidence of immunogenicity (HACAs) was low and had no apparent clinical relevance over the primary 24-week course of this trial, but patients continue to be monitored for this, together with all other pharmacodynamic parameters, including CD19 and Ig levels.

The DANCER study is a novel design for the study of RA: 9 treatment groups with a logistic regression main-effects model as its primary means of analysis. The complexity of the study was driven by a need to understand the effects of different components of a potential therapeutic regimen (i.e., rituximab dose and effects of glucocorticoids). Both objectives were met, but several factors must be taken into account when interpreting the results. First, extrapolation of these data to the larger RA population must be done with care, given the relatively small number of patients involved. In particular, specific comparison between any of the individual 9 groups is inappropriate; however, valuable guidance concerning both the use of glucocorticoids and the dosage of rituximab has been obtained. Second, it should be acknowledged that the present data represent just 24 weeks of followup; longer-term followup will be required to better characterize safety and, possibly, to identify potential differences between rituximab dosages. Last, with regard to the subset of RF-negative patients, small numbers of patients and a large placebo effect prevent the drawing of firm conclusions about the safety and efficacy of rituximab in this population.

In conclusion, both dosages of rituximab were highly effective and generally well tolerated when given with continuing MTX to patients with active RA. The study has also shown that concomitant glucocorticoids are not a prerequisite for the efficacy of rituximab in RA. However, premedication with IV glucocorticoids does appear to ameliorate acute infusion reactions. Both dosages of rituximab explored in this study warrant further differential exploration and longer-term followup.

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REFERENCES

1. Van Vollenhoven RF, Klareskog L. Clinical responses to tumor necrosis factor α antagonists do not show a bimodal distribution: data from the Stockholm tumor necrosis factor α followup registry. Arthritis Rheum 2003;48:1500–3.
2. Feltelius N, Fored CM, Blomqvist P, Bertilsson L, Geborek P, Jacobsson LT, et al. Results from a nationwide postmarketing cohort study of patients in Sweden treated with etanercept. Ann Rheum Dis 2005;64:246–52.
3. Edwards JC, Cambridge G. Prospects for B cell-targeted therapy in autoimmune disease [review]. Rheumatology (Oxford) 2005;44: 151–6.
4. Anderson DR, Grillo-Lopez A, Varns C, Chambers KS, Hanna N. Targeted anti-cancer therapy using rituximab, a chimaeric anti-CD20 antibody (IDEC-C2B8) in the treatment of non-Hodgkin's B-cell lymphoma [review]. Biochem Soc Trans 1997;25:705–8.
5. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 2000;6:443–6.
6. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994;83:435–45.
7. Roitt I, Bostoff J, Male D. Immunology. 2nd ed. London: Gower Medical Publishing; 1989. p. 14.1–10.
8. Sell S, Max EE. Immunology, immunopathology and immunity. 6th ed. Washington, DC: ASM Press; 2001. p. 101–49.
9. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell–targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004;350:2572–81.
10. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998;16: 2825–33.
11. Emery P, Sheeran T, Lehane PB, Saiedabadi N, Shaw TM. Efficacy and safety of rituximab at 2 years following a single treatment in patients with active rheumatoid arthritis [abstract]. Arthritis Rheum 2004;50 Suppl 1:S659.
12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
13. Felson DJ, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727–35.
14. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight–joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.
15. Van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis: comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism criteria. Arthritis Rheum 1996;39:34–40.
16. Cella D, Yount S, Sorensen M, Chartash E, Sengupta N, Grober J. Validation of the Functional Assessment of Chronic Illness Therapy Fatigue Scale relative to other instrumentation in patients with rheumatoid arthritis. J Rheumatol 2005;32:811–9.
17. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980;23:137–45.
18. National Cancer Institute. Common Toxicity Criteria, version 2.0. URL: http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf.
19. Strand V, Cohen S, Crawford B, Smolen JS, Scott DL. Patient-

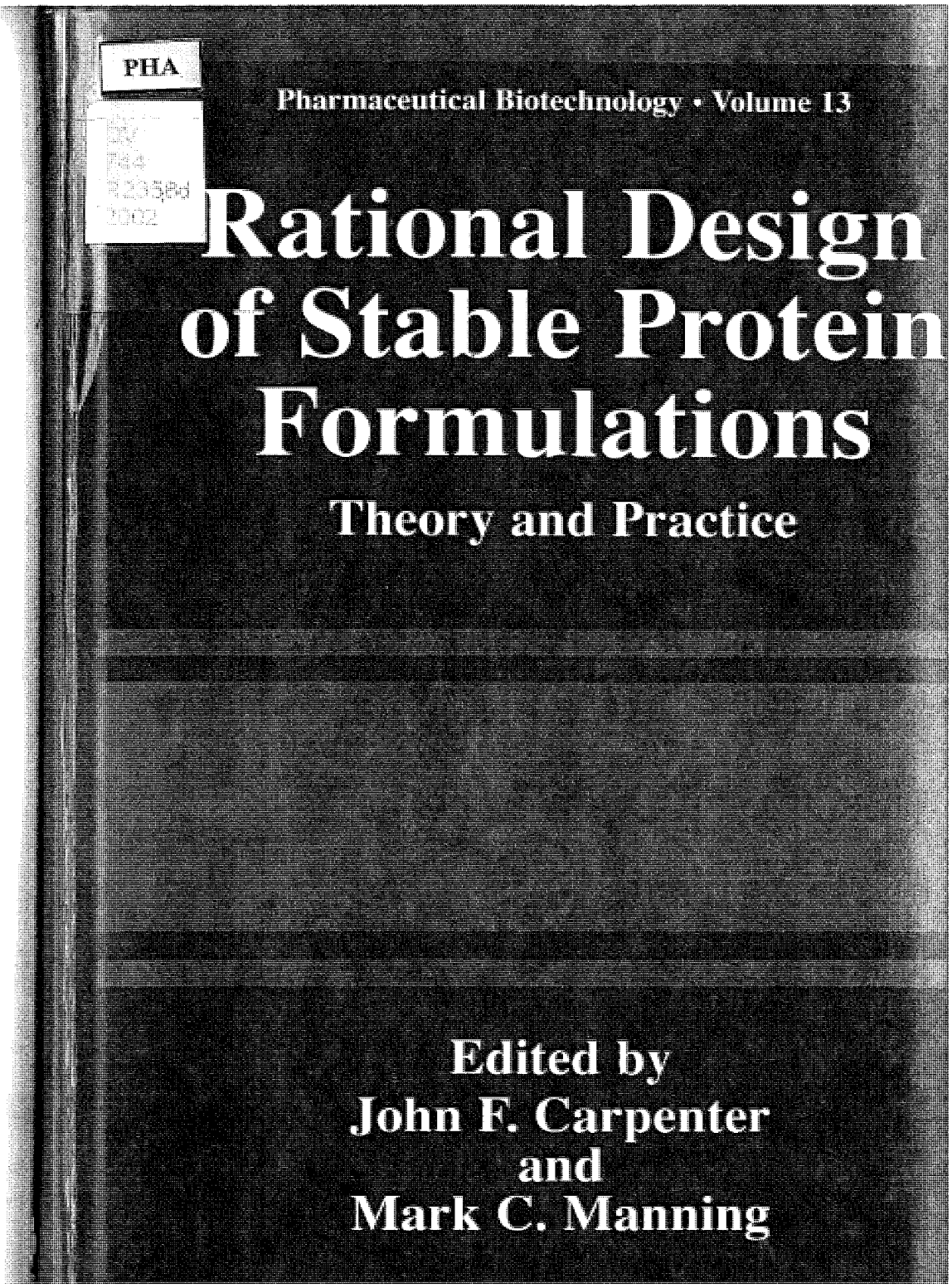
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reported outcomes better discriminate active treatment from placebo in randomized controlled trials in rheumatoid arthritis. Rheumatology (Oxford) 2004;43:640–7.
20. Edwards JC, Cambridge G. Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. Rheumatology (Oxford) 2001;40:205–11.
21. Leandro MJ, Edwards JC, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. Ann Rheum Dis 2002;61:883–8.
22. De Vita S, Zaja F, Sacco S, de Candia A, Fanin R, Ferraccioli G. Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenic role of B cells. Arthritis Rheum 2002;46:2029–33.
23. Kneitz C, Wilhelm M, Tony HP. Improvement of refractory rheumatoid arthritis after depletion of B cells. Scand J Rheumatol 2004;33:82–6.
24. Kramm H, Hansen KE, Gowing HE, Bridges A. Successful therapy of rheumatoid arthritis with rituximab: renewed interest in the role of B cells in the pathogenesis of rheumatoid arthritis. J Clin Rheumatol 2004;10:28–32.
25. Kimby E. Tolerability and safety of rituximab (MabThera) [review]. Cancer Treat Rev 2005;31:456–73.

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Rational Design of Stable Protein Formulations

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Theory and Practice

Edited by

John F. Carpenter

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1

Practical Approaches to Protein Formulation Development

Byeong S. Chang and Susan Hershenson

INTRODUCTION

As is the case with other pharmaceuticals, formulation development is one of the critical steps in developing a protein as a therapeutic product. Development of stable protein formulations may require even more resources and effort than conventional small molecule pharmaceuticals. Proteins typically have more stability issues as a result of their complexity and delicate structural stability. Fortunately, a great deal of research regarding protein stability has been conducted and this information is readily available in the literature (reviewed by Manning et al., 1989; Chen, 1992; Ahern and Manning, 1992a, 1992b; Arakawa et al., 1993; Cleland et al., 1993; Wang and Pearlman, 1993; Pearlman and Wang, 1996; Volkin and Middaugh, 1997). Ultimately, it would be ideal to be able to develop a pure pharmaceutical containing only the native protein. However, it is not practical to have only the native form of a protein in the formulation because the protein must be purified from a complex biological mixture containing a pool of other proteins which includes misfolded, denatured, and degraded forms of the same protein. Furthermore, a major challenge is to maintain the integrity of the purified protein during routine pharmaceutical processing, storage, handling, and delivery to the patient. One could envision achieving this goal by developing a formulation with

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perfect stability, i.e., no physical and chemical change in the protein. Because proteins are complex molecules composed of numerous reactive chemical groups and delicate three-dimensional structures, identifying a set of conditions to keep all components stable is virtually impossible. In general, commercial therapeutic protein formulations are developed under the assumption that some degree of physicochemical changes will occur during storage and handling.

Realizing that it is impossible to develop a perfectly stable formulation, especially while meeting an aggressive product development timeline, the main objective then becomes one of maintaining the appropriate safety and efficacy of the product. In order to achieve this objective, it is imperative to understand the broad spectrum of degradation pathways affecting proteins, and to have available equipment and expertise in an extensive repertoire of analytical methods. Formulation development focuses on determining the potential degradation pathways, assessing the significance of each and optimizing variables to minimize the degradation products that are clinically significant.

Regulatory guidelines also are critical elements for guiding formulation development. They provide information about how to conduct studies and obtain useful results for evaluating formulations. The results obtained allow formulation scientists to write an appropriate developmental pharmaceutics section in regulatory filings. The guidelines also help to evaluate the significance of some inevitable degradation products that are produced during manufacturing, shipping and storage. For example, if the degradation products have properties comparable to those of the desired product with respect to activity, efficacy, and safety, they can be classified as product-related substances. This classification is significantly different from considering the degradation product as an impurity when there is not sufficient supporting evidence to justify classification as a product-related substance (Appendix, Regulatory Document 1). Understanding of these practical issues of regulatory requirements is critical for formulation scientists during design, implementation, evaluation and reporting of their studies.

In addition to insight into the scientific and regulatory issues, developing commercial formulations requires a clear understanding of the potential market. For example, indication, patients, method of delivery, frequency of dosing, typical dose requirement, market distribution and other business-related information will provide directions for the design of a successful formulation. Also, it is important to consider the competitiveness of the formulation as compared to other products available in the market.

In this chapter, an overview of critical factors affecting the design of therapeutic protein formulations and a general guide to developing commercially viable dosage forms for protein pharmaceuticals will be discussed. Since the majority of practical issues are not covered very well in the scientific literature, this chapter also includes information from regulatory guidance documents (see Appendix), labels from marketed products and routine industrial experience.

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PREPARATION FOR FORMULATION DEVELOPMENT

Resource Requirements for Formulation Development

A list of resources that should be available before starting formulation development is summarized in Table 1. Some resources may not be as critical as others, depending on the nature of problems encountered, but it is important to have sufficient resources to discover major formulation issues as early as possible in the product development process. Without the appropriate equipment and personnel, the development of an acceptable formulation can be greatly hampered, potentially to such a degree that the product is never brought to market. Of course, not all of the resources need to be at the company developing the therapeutic protein, but if outside contractors are employed it is essential that timely access to resources is available. In addition, the quality of raw materials should be carefully evaluated because unexpected impurities may introduce unnecessary

Table 1.
Resource Requirements for Initial Protein Formulation Development

Resources	Requirement	Example
Purified protein	Representative of manufacturing process; sufficient quantity to cover dose bracket, formulation variables, and stress conditions; minimum complication by impurity (precipitation of impurity, degradation by impurity like proteolytic cleavage).	Purified bulk, sample from final purification process
Qualified excipients	Pharmaceutically acceptable quality, manufacturers with qualified production procedures and sufficient scale, specifications on critical impurities, quality that can be carried on to clinical studies and commercial distribution.	USP, Ph. Eur, JP
Access to fill finish facility	Capability to sterilize container/closure components; fill/finish under aseptic environment; head-space purge system; drying equipment	Sterile hood, filling machine, lyophilizer
Analytical instruments	Structural analyses; concentration determination, chromatographic analyses; electrophoresis; bioassays; other microcharacterizations.	CD, UV, fluorescence, HPLCs, mass-spectrometry, SDS-PAGE
Facility to accommodate stability studies	Controlled temperature, controlled light exposure, controlled relative humidity, devices to provide controlled agitation.	Freezer, refrigerator, incubator, light chamber, RH incubator, agitator

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Table 2.
Examples of Information Useful for Designing Formulation Studies

Information	Examples
Clinical indication	Site of treatment (self-administration, office visit, hospital), methods of delivery, concomitant medication, competition.
Patient population	Age, strength, tolerability, capability to manipulate devices, sensitivity to excipient
Typical routes of delivery	Injectables (IV, SC, IM, IP, ICV, IT, IO), topical, inhalation, nasal, oral, etc.
Dose requirement	PK profile, frequency of dosing, variable vs. fixed dose, single-dose/multidose
Drug interaction	Co-administration with other drug, dilution or reconstitution with other solution; presence of undesirable compounds like reducing sugars, preservatives
Typical dosage forms	Liquid, lyophilized, spray-dried, aerosol by liquid or powder, other novel carrier; stability, physical properties, reconstitution art
Container/closure	Vial/stoppers, prefilled syringes, prefilled cartridges, dual-chamber cartridges, blister packages, product contact material, leacheates, breakage, light sensitivity, moisture penetration
Delivery device	Syringes, prefilled-syringes, pen injectors, auto-injectors, needle-free injectors, inhalation devices, infusion pumps

complications in the stability profile that is determined during formulation development and testing.

Useful Information for Designing Formulations

The configuration of a protein formulation is affected by how the drug will be used as a product. If such information is available when designing formulation studies, it is recommended to consider the limitations and challenges associated with each application. Examples of such information are listed in Table 2.

PREFORMULATION DEVELOPMENT

It is important to understand the critical properties of a protein before starting large studies to design and test the final formulation. Preformulation studies are designed to learn about the protein's susceptibility to a variety of pharmaceutically relevant stresses. The main objectives of preformulation research

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Table 3.
Information Obtained from Pre-formulation Studies for Protein Pharmaceuticals

Characterization	Examples
Physical properties	Primary, secondary, tertiary and quaternary structures, solubility, viscosity, self-association, hydrophobicity, molecular weight, extinction coefficient, glycosylation, effects of ionic strength, etc.
Biological properties	Substrate or receptor affinity, in vitro activity model, *in vivo* preclinical model, etc.

include: general characterization of the product; investigation of potential stability issues; development of relevant analytical methods; establishment of a stability profile with stability-indicating assays; and identification of major formulation challenges. A summary of these issues will be presented in this section.

Characterization of Protein Pharmaceuticals

There is available in the literature extensive coverage of analytical methods and their principles for the characterization of proteins (e.g., Jones, 1993; Reubsaet et al., 1998; Herron et al., 1995). General points to consider when characterizing a protein as an active ingredient are listed in Table 3. As a protein's biological activity is dependent on its structure, significant emphasis has been given to structural properties and stability against various stresses.

Accelerated Stability Studies

In order to predict potential stability problems within a short period of time and to develop appropriate analytical methods, proteins are exposed to stronger-than-real stresses and various degradation products induced by the stresses are examined. The results obtained from these so-called "accelerated stability studies" might also be useful to predict the kinetics of the degradation processes under real handling conditions, when there are not sufficient real-time results available because of time and resource constraints. However, the accelerated stability study is not acceptable to determine expiry of the product, so it is best used to rank order the importance of different degradation pathways. Approaches and cautions in the extrapolation of data from accelerated stability testing to real-time stability and normal handling conditions are discussed in detail below.

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Table 4.
Various Conditions Used to Accelerate Protein Degradation

Stresses	Routine ranges	Practical applications	Problems to monitor
Temperature	0–50°C	Storage, shipping, handling, delivery	Structural changes (precipitation, aggregation, recovery loss), solubility, increased reaction rates for all degradations
Light	>1.2 million lux hrs illumination, >200 watt hrs/square meter UV energy	Light exposure, container, package	Oxidation, cleavage
Freezing	Multiple freeze-thaw, liquid nitrogen freeze	Frozen storage, accidental freezing, lyophilization	Precipitation, aggregation, pH change, crystallization of excipients
Oxidation	Oxygen purge, peroxide spike	Storage, excipient stability, impurity	Oxidations, inactivation
Humidity	0–100% RH	Storage, container integrity, powder	Moisture content, moisture related degradations
Mechanical stresses	Vortex, agitation, shear-stress ($3000\,s^{-1}$)	Manufacturing, filling, shipping, handling, delivery	Precipitation, aggregation, recovery loss
Other denaturants	—	Impurities, pH, denaturing excipients	Precipitation, aggregation, recovery loss, structural changes

Conditions to accelerate various degradation reactions in protein products and potential problems to monitor are listed in Table 4. Proteins contain numerous amino acid side chains and delicate three-dimensional structures, which can be susceptible to different stresses. Therefore, it is important to test the protein under a variety of physical and chemical stresses in order to provide a good simulation of the degradation products that can be generated.

Development of Analytical Methods

A brief summary of typical analytical methods is presented in Table 5. It is essential to have a wide range of analytical methods available to identify and characterize degradation products. The analytical methods should be further selected and customized to accommodate the specific needs for each protein product.

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Table 5.
Typical Methods Used to Characterize Proteins and Degradation Products

Methods	Examples	Applications
Column chromatography	HPLC, FPLC, low pressure LC; size-exclusion, reversed-phase, ion-exchange, hydrophobic, affinity columns; coupled with UV, fluorescence, RI, and other analytical instruments as detectors	Most physical and chemical degradations, excipient impurities, leacheates
Electrophoresis	SDS-PAGE, native PAGE, isoelectric focusing, capillary electrophoresis, etc.	Degradations with changes in size and/or charge
Spectroscopy	CD, fluorescence, FTIR, UV, Raman, NMR, etc.	Structural changes, chemical modifications of side groups
Thermal analysis	Differential scanning calorimetry, thermogravimetric analysis, thermomechanical analysis, etc.	Protein structure, lyophilized cake structure, powder characterizations
Light scattering/turbidity	Dynamic light scattering, other light scattering devices, turbidity, particle size determination, particle counter, etc.	Aggregation, precipitation, molecular weight determination
Other microcharacterization methods	Peptide mapping, peptide sequencing, amino acid analysis, mass spectrometry, other specific analyses for individual reactive groups	Identification of impurities and chemical degradation, analysis of complex proteins, e.g., antibody and glycoprotein

Evaluation of the Significance of Problems

As stated earlier, it would be ideal to have a pure protein in an absolutely stable formulation. In reality, however, scientists have to design formulation based on compromises that deal with several different potential problems. To make matters worse, the formulation needs to be recommended long before it is evaluated fully, because of typical aggressive timelines in the industry. In general, the formulation will have to be optimized based on assumptions and extrapolations of results obtained during a limited time. Therefore, it is important to utilize the given time efficiently, by collecting as much relevant information as possible for evaluating the significance of each problem.

Due to the marginal stability of proteins, it is possible to create rapidly a variety of degradation products during accelerated stability testing. However, not

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all the degradation products that are observed will be significant under normal handling, shipping and storage conditions. Furthermore, the rank order of different degradation processes under accelerated stability testing will NOT be the same under practical handling conditions, because each reaction has a different temperature dependency, i.e., reaction order and activation energy. Another important thing to keep in mind when evaluating different degradation processes is the contribution to the pharmaceutical quality of the product. Critical degradation products should be designated not on the quantity obtained during accelerated stability studies, but on a comprehensive understanding of their contributions to the quality of the product.

Quantitative Assessment. Usually, the rate of each degradation reaction during real storage conditions (e.g., at 4–8°C) should be very slow. In order to predict the rate under real storage conditions, reaction rates obtained under accelerated stability conditions can be extrapolated by using the Arrhenius equation.

Predicting the correct reaction rate requires a proper understanding of the reaction order, because the amount of the degradation product does not linearly increase over time unless the reaction follows zero-order kinetics. One way to determine the reaction order is to calculate the linearity between the concentration of residual native protein and time. The reaction order can be calculated from:

$$dC/dt = kC^n \tag{1}$$

where, C is the protein concentration, t is the time, k is the reaction rate constant, n is the reaction order. For a zero order reaction $n = 0$ and $C_0 - C = k \cdot t$. For a first order reaction $n = 1$ and $\log C_0 - \log C = k \cdot t$. For a second order reaction $n = 2$ and $1/C - 1/C_0 = k \cdot t$. For each reaction order a characteristic transformation of concentration will show a linear relationship with time, e.g., log C will show a linear relationship with time for a first order reaction, whereas $1/C_0$ will be linear versus time for second order reactions.

After obtaining the reaction rate constants for different temperatures, the activation energy can be found by using with the Arrhenius equation.

$$k = A \cdot e^{(-Ea/RT)} \tag{2}$$

The activation energy is obtained by plotting (log k) vs (1/T) and determining the slope of the plot. Using the activation energy, the reaction rate constant for real storage condition, e.g., 2–8°C for refrigerated storage, can be estimated by extrapolation.

Care must be taken when extrapolating the rate constants because there are numerous cases when the Arrhenius relationship does not apply. The Arrhenius reaction applies only to irreversible reactions where the product is accumulated as a single quantifiable species. If the degradation product is the result of serial

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reactions, the reaction order and rate constant will be determined only by the rate-limiting reaction at the testing condition. If the rate-limiting reaction changes or another significant variable is introduced at the extrapolated condition, then the extrapolated rate constant will be in error. Such complications routinely can be found in various degradation pathways that are affected by temperature-sensitive reactions such as structural changes, pH changes, by physical changes such as the glass transition of an amorphous phase in a lyophilized formulation and by changes in reactants like dissolved oxygen. For example, proteins have relatively high activation energies for structural changes. Thus, many reactions that are dependent on a major perturbation of the native protein structure tend to cause substantial damage under accelerated storage conditions, e.g., higher temperature. However, many of these reactions are not necessarily major problems when the product is stored at 2–8°C where the protein maintains its native conformation. In contrast, degradation reactions with lower activation energies, which might not be coupled to protein conformational changes (e.g., oxidation of surface methionine residues), tend to be much more problematic in the development of protein formulations.

Results from stresses (e.g., agitation) other than heat imposed in accelerated stability studies potentially could be extrapolated in a similar way to storage and actual handing conditions. The major challenge is the ability to assess quantitatively the magnitude of the stress so that the stress-stability relationship can be established. To date there are not published guidelines for such quantitation.

Qualitative Assessment. Not all of the degradation products are equivalent in terms of their contribution to the pharmaceutical quality of the protein product. Therefore, a qualitative assessment of each degradation product is important when weighing their significance. Regardless of their quantity, some degradation products are generally less acceptable than others. If the degradation product comprises the safety of the product, then it should be considered less acceptable. For example, even at levels of a few percent or less of the total protein population, non-native aggregates can cause adverse reactions in patients such as immune responses and even anaphylactic shock. Also, if the degradation reaction results in the inactivation of protein, it needs to be considered more important than other degradation reactions that do not affect the activity. For example, certain chemical changes (e.g., deamidation) may not alter the activity of a given product, whereas other reactions (e.g., oxidation) may render that product inactive. The relative impact of each degradation product on safety and efficacy cannot be predicted and must be determined for each protein therapeutic.

Information useful for the qualitative evaluation of degradation products includes the identity, clinical and preclinical experiences with the degradation product, biological activity, stability and potential side effects. Regulatory documents also provide clear guidance about what assessment is necessary for protein degradation products (Appendix, Regulatory Documents 2,3). The criteria for

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identification, reporting and qualification of degradation products are set up based on the total patient exposure and percentage of the degradation products (Appendix, Regulatory Document 3). When certain degradation products cannot be prevented from forming in the formulation, their inclusion in the product must be qualified, which may require that additional information on safety and efficacy be obtained as recommended in the regulatory guidelines (Appendix, Regulatory Documents 2,3). Obtaining this information requires a significant amount of additional work, which may include clinical studies.

FORMULATION DEVELOPMENT

As discussed above, the critical parameters affecting the pharmaceutical quality of a protein therapeutic are defined during the preformulation studies. In formulation development, the effects of formulation variables on the defined critical parameters are examined to optimize protein stability. Which variables are most important depends of the formulation type chosen. For example, resistance to agitation and/or accidental freezing is a critical property for an aqueous formulation, which would not be a concern for a lyophilized formulation. Similarly, choosing excipients that provide a glassy matrix to stabilize the protein is only a concern for dried formulations.

Formulation Options for Protein Pharmaceuticals

Different types of formulations need to be developed based on clinical needs, patient compliance, delivery method, stability of the drug, storage and distribution, and market competitiveness. Having a clear plan about what type of formulation is desired will allow one to design better formulation studies. Liquid formulations have been generally preferred due to the convenience of manufacturing and use. However, protein drugs may not be stable enough to be handled as a liquid formulation. Dried formulations (e.g., lyophilized) or suspension formulations (e.g., insulin zinc suspension) have been successfully used to overcome stability problems. In addition, specific applications and delivery may demand the appropriate type of formulation, e.g., a spray-dried powder for pulmonary delivery. As details of the stability issues are discussed in other chapters in this book, only practical issues regarding these different types of formulations will be summarized here.

Liquid Formulations. It is important to understand that developing conditions to keep proteins stable in a liquid form for a pharmaceutically relevant

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storage time (e.g., two years) is not a simple task. For most proteins, including relatively stable ones, at least some degradation should be expected even during refrigerated storage. Unless there is strong evidence supporting that the protein remains stable for two years and the evidence is supported by a broad spectrum of analytical methods, one needs to be very careful about the final decision to market a liquid formulation. Physical or chemical changes that have low activation energy should be monitored especially carefully under real storage conditions. If there is a degradation product that can significantly affect the pharmaceutical quality at its minimum concentrations (e.g., formation of particulates) great care must be taken to assure that such a product does reach unacceptable levels during real-time storage. If long-term storage studies are not implemented until late in the product development process, there is a risk that problems due to unacceptable levels of degradation products will not be discovered in time to test alternative formulations. In addition to stability during storage, temporary exposure to temperatures outside the recommended conditions should also be tested because it can affect the quality of the drug. Also, results obtained from multiple freeze-thawing cycles will be useful to determine if the formulation can accommodate unexpected freezing during distribution and storage. Likewise, sensitivity to agitation or surface denaturation needs to be understood to support formulation choices as well as shipping and handling guidelines. For formulations containing low protein concentration, special attention is required to avoid impurity-related stability issues (e.g., oxidation fostered by metal contaminants in excipients).

Solid Dosage Forms. Recent improvements in devices designed for easier use of lyophilized products, e.g., dual chamber syringes, dual chamber cartridges and convenient reconstitution devices, have helped pharmaceutical industries to develop lyophilized products without too many concerns surrounding patient compliance issues. Because lyophilized products have less stability-related issues and have a much greater potential tolerance for room-temperature storage, many biopharmaceutical industries consider the lyophilized formulation as a default option. In fact, lyophilization has been widely utilized to overcome various stability issues of labile proteins. However, it is important to note that lyophilization can present its own challenges, particularly in designing appropriate formulations and economic cycles. Detailed discussion about the rational design of stable lyophilized formulations is available in another chapter in this book.

In addition to the required stability, a successful lyophilized formulation should also have the desired physical properties of the dried powder, e.g., ruggedness of the cake and the maintenance of the physical states for each of the ingredients. For example, crystallization of an initially amorphous excipient during storage can lead to unacceptable degradation of the protein product and loss of cake structure. Furthermore, optimal water content for protein stability should be studied and controlled during storage. It is preferable to define an acceptable

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range of water contents to provide flexibility in the manufacturing process. To assure that the appropriate water content is maintain, the integrity of container/packaging should be monitored in a high relative humidity environment.

The identity and volume of the reconstitution medium should also be clearly defined, because the use of injectable fluids other than the recommended one may compromise the quality of the product (e.g., solution tonicity or protein stability). Reconstitution should be convenient and rapid, and the stability of the reconstituted solution during handling and delivery needs to be demonstrated.

Only recently have spray-dried formulations become commonly used with the advent of novel protein delivery technologies. For example, spray-dried powders have been useful for developing products that require uniform particle size (e.g., pulmonary inhalation for systemic delivery). Similar issues to the lyophilized formulation apply to the spray-dried formulation, and the details of development of spray-dried formulations are presented in another chapter in this book. In addition to protein stability, physical properties pertinent to powder processing and powder stability (e.g., flowablity, hygroscopicity, agglomeration, density and crystallization of excipients) need to be well characterized.

Single Dose and Multidose Forms. Most protein pharmaceuticals are marketed as single dose forms. However, multidose formulations are useful when the dose needs to be split (e.g., dose titration or dose combination). Multidose formulations are distinguished by the presence of preservatives in the formulation, which prevent microbial contamination and/or growth during multiple disruptions of container closure integrity. In general, the addition of preservative(s), regardless of the preservative used, significantly changes the stability profiles of proteins (Maa and Hsu, 1996; Fransson et al., 1997; Lam et al., 1997). In some extreme cases, visible precipitation and aggregation have been reported. Therefore, the effect of various preservatives on the stability of protein should be carefully examined. Other experiments required to qualify a multidose formulations include the preservative effectiveness test and the stopper self-sealing test. Detailed procedures and specification are available from the USP (U.S. Pharmacopeia), the Ph. Eur. (European Pharmacopeia) and the JP (Japan Pharmacopeia).

Desired properties of the ideal preservative include: effectiveness at low concentration against a wide variety of organisms; chemical stability; solubility; compatibility with the protein drug, excipients and auxiliary agents; free from objectionable odor, taste, color and stinging; and non-toxic and non-sensitizing both internally and externally at the required concentration. Also, it must not absorb, penetrate, or interact with containers or closures (Thompson, 1998). For further details on preservatives, readers are referred to Regulatory Document 4 in the Appendix and Thompson (1988). Examples of typical preservatives used for parenteral protein pharmaceuticals are benzyl alcohol, phenol, *m*-cresol and benzalkonium chloride.

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Typical Protein Stability Problems: Causes and Solutions

Table 6 summarizes typical stability problems observed during protein formulation development and potential methods to solve each problem. The list does not represent the complexity of multiple problems that can be experienced with a given protein. Formulation research should be designed to handle each protein based on its unique stability profile.

Optimization of Formulation Variables

Overview of the Process. The optimization of formulation variables for product stability is the most critical part of protein formulation development. Various formulation excipients and buffers (Table 7) can be utilized and must, therefore, be chosen to maximize the pharmaceutical quality of the product (i.e., stability and activity) without introducing significant side effects. Among the listed formulation variables, the most powerful one is pH. Problems associated with the physical properties of a protein, e.g., precipitation due to solubility and/or stability, are generally very difficult to manage by other formulation means. Optimization of pH is a simple but very useful solution for such problems (Kolvenbach et al., 1997). Most chemical reactions also are affected by pH, e.g.,

Table 6.
Typical Stability Problems Observed in Protein Pharmaceuticals

Problems	Potential causes	Possible solutions
Non-covalent aggregation	Solubility, structural changes, heat, shear, surface, denaturants, impurities	pH, ionic additives, amino acids, surfactants, protein concentration, raw material purity
Covalent aggregation	Disulfide scrambling, other unknown mechanisms	pH, inhibit non-covalent aggregation
Deamidation	pH < 5.0 or pH > 6.0	pH optimization
Cyclic imide	pH around 5	pH optimization
Cleavages	Protease impurity, other unknown mechanisms	pH, product purity, inhibitors
Oxidation	Active oxygen species, free radicals, metals, light, impurity	Excipient purity, free-radical scavenger, active oxygen scavengers
Surface denaturation, adsorption	Low protein concentration, specific affinity, protein hydrophobicity	Surfactants, protein concentration, pH

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Table 7.
Important Components of Protein Formulations

Formulation Variables	Desired attributes	Examples
pH	Provides good physical properties of protein, minimize degradations	
Stabilizer	Inhibit degradations, effective at low concentrations	Surfactants, sugars, salts, antioxidants
Solubilizer	Improve the solubility, effective at low concentrations	Salts, amino acids, surfactants
Buffer	Good buffering capacity, stable to temperature change, stable to freezing, good safety record	Phosphate, acetate, histidine, glutamate
Tonicity modifier; bulking agent	Inert, good safety record	Sodium chloride, sorbitol, mannitol, glycine

deamidation, cyclic imide formation, disulfide scrambling, peptide bond cleavage, and oxidation (see reviews listed above). Other functional excipients should be also carefully evaluated for the benefit of the product (e.g., use of sucrose to stabilize protein during lyophilization and storage in the dried solid).

In addition to their intended use, excipient candidates should be also qualified as appropriate pharmaceutical ingredients. Generally, it is preferred to select excipients that have been used in marketed products with a relevant route of delivery. Further, it is preferable if these excipients have been used with similar frequency of dosing, history of chronic use and similar patient populations. Otherwise, the approval and safety of the excipients need to be carefully examined. If the excipient is considered safe with a solid scientific basis or has a proven clinical safety record, it can be considered equivalent to approved excipients. The list of excipients used for parenteral pharmaceuticals is available in the literature (Powell et al., 1998; Nema et al., 1997; Appendix Regulatory Document 5). When it is necessary to introduce other excipients with minimum safety records, a significant risk associated with the excipients will be added to the product development and additional pre-clinical and clinical studies may be needed.

Another important requirement in qualifying an excipient is the purity of the raw material. Depending on the historical use as a pharmaceutical ingredient, several different pharmaceutical grades are available, e.g., USP (U.S. Pharmacopeia), Ph. Eur. (European Pharmacopeia) and JP (Japan Pharmacopeia). These pharmaceutical grade materials should be considered as a primary resource, but the quality provided may not be good enough for specific product development. For example, significant stability problems can be found with some impurities

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even at concentrations below their specification, e.g., metal ions, peroxides, proteases and reducing sugars. These problems are more prominent in low protein concentration products due to a high impurity-protein ratio, although problems like visible precipitation of the protein may be independent of protein concentration. If adjustment in the existing specification is necessary, it is critical to look into the availability of GMP quality raw materials with modified specifications as early as possible, once the potential problem is identified.

The use of excipients derived from animals (e.g., Tweens) or humans (e.g., human serum albumin) should be avoided if possible due to the risk associated with transmissible diseases like bovine spongiform encephalopathy, Creutzfeldt-Jakob Disease, hepatitis virus and HIV. Numerous regulatory guidelines have been issued to discourage the use of animal/human-derived excipients (Appendix Regulatory Documents 6,7,8). When animal-derived excipients have to be included in the product, the manufacturer will need to demonstrate that their selection is fully justified.

Design of the Study. A design for the formulation optimization study should be in place before commencing the work. A typical study protocol will include the following information:

- Study title
- Study objective
- Source and quality of drug substance and excipients
- Material preparation
- Formulation matrix
  - Formulation variables
  - Protein concentrations or bracket
- Analytical methods
- Storage conditions (temperature, light, humidity)
- Additional sample handling conditions (temporary exposure to stresses, container orientation, etc.)
- Sampling schedule and expected duration of the study
- Plan for data analysis and report

The study can be more efficiently designed by utilizing experimental design software packages, which will help to minimize the resources required for sample preparation, analyses, and data analysis.

Necessary Studies for Formulation Development

Storage Stability Study. Documenting that the formulation will keep the protein stable until the desired expiry can be the most time-consuming part of

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formulation development. The expiry requirements are determined by distribution not regulatory requirements. One year is probably too short for effective manufacturing and distribution through normal channels. In general, a shelf life of 18 month is considered acceptable for commercialization. Results obtained from accelerated stability studies are useful for predicting potential degradation products and appropriate analytical methods, but whatever the actual shelf life, it must be supported by sufficient real-time storage data to obtain regulatory approval. Thus, it is important to establish a final formulation and start the real-time storage studies as early as possible during product development.

Process Development. Formulations that can be prepared on a small scale without experiencing any problems may encounter significant problems during the scale-up of the process. For example, mixing solutions in a large stainless steel tank, pumping solutions through stainless steel tubing, filtration and filling through a high-speed filling machine can introduce unexpected stresses to the protein. An increase in the formation of particulates, along with a loss of proteins due to surface adsorption and aggregation, has routinely been observed. It is important to expose the formulation to equivalent stresses and make sure that no formulation adjustment is necessary to accommodate the manufacturing processes. Again, this testing should be done as early in the development process as possible.

Transportation, Handling and Delivery Study. Unexpected environmental changes can be encountered during the distribution and handling of products (e.g., accidental freezing, exposure to temperatures different from the recommended conditions, vigorous agitation, etc). It is critical to develop the formulation with these stresses in mind because they can compromise the quality of the product.

Also, during administration to the patient, proteins can be exposed to different types of stresses introduced by the device and the routes of delivery. Examples include incompatibility with the delivery device (e.g., protein aggregation induced by exposure to tubing surfaces) and/or concomitant medication (e.g., protein aggregation induced by co-administered antibiotics).

Preclinical and Clinical Studies. Results documenting the maintenance of the biophysical and biochemical properties of the protein are essential for a final formulation decision. Before finalizing the formulation, it is also important to confirm that it does not affect critical *in vivo* biological properties of the protein (e.g., activity, pharmacokinetic profile, and toxicity profile). Maintenance of a protein's biophysical properties can be examined using various structural analyses (e.g., circular dichroism, fluorescence and infrared spectroscopies, etc.). It is possible to determine the biochemical equivalence of the protein pharmaceutical by *in vitro* activity and/or preclinical *in vivo* bioassays. Results supporting the toxicity profile of the formulation can be generated by both preclinical and clinical studies.

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Strategies to Overcome Difficult Formulation Problems

Occasionally, problems are encountered that are difficult to overcome with conventional formulation approaches. It is possible to conduct additional studies to confirm that the problem is pharmaceutically acceptable. On the other hand, unique formulation approaches can be introduced to address specific problems. Examples of this approach are provided below.

Qualification of Degradation Products. If decreasing the amount of degradation product below the specified threshold is not feasible, then the degradation product can be defined as a drug substance by demonstrating that it does not affect the pharmaceutical quality of the drug product, i.e., safety and efficacy (Appendix, Regulatory Document 1). If there is not sufficient information available, additional qualification studies recommended in the regulatory guidance can be carried out (see the discussion above). The experience of having these degradation products in the materials used for clinical studies can sometimes be very useful because the results obtained can provide useful insight into the clinical implication of the degradation products.

Site-directed Mutagenesis to Improve Properties. Some problems related to the intrinsic properties of a protein cannot be overcome unless a change in the sequence is introduced. After careful research is carried out to identify the problematic region or residue, the protein can be engineered for better physical and/or chemical properties. For serious problems like precipitation due to insolubility or poor stability, changes in protein sequence have been proven effective for improving physical properties (Murby et al., 1995; Roig and Kennedy, 1995).

Chemical Modifications. The physical properties of proteins can be improved by modifying problematic amino acid side chains with small compounds or large polymers (e.g., attachment of polyethylene glycol) (Fagain, 1995; Guerra et al., 1998; Francis et al., 1998). In addition, there are other desirable improvements that can be achieved by the conjugation chemistry (e.g., enhanced pharmacokinetic profiles, reduced immunogenecity, enhanced adsorption, etc.).

Unconventional Dosage Forms. Some protein stability problems can be resolved by introducing novel approaches in the formulation. Examples include suspension formulations (Defelippis et al., 1998), microencapsulation with cyclodextrin (Brewster et al., 1991), suspension of dry-powder in non-aqueous vehicle (Knepp et al., 1998), and use of non-aqueous vehicles with hydrophobic ion-pairing (Manning et al., 1995).

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FORMULATION IN COMMERCIAL PRODUCT DEVELOPMENT

Critical Formulation Decisions During Pharmaceutical Development

A brief summary of the important phases of a commercial product development is shown in Figure 1. Ideally, a stable formulation would be available during the early discovery research period to ensure that the protein is in its active and stable form during critical feasibility studies. However, development of a stable formulation for each drug candidate is not practically possible because such an approach would take too much time and too many resources. Generally, formulation development starts after the decision is made to start clinical trials. A

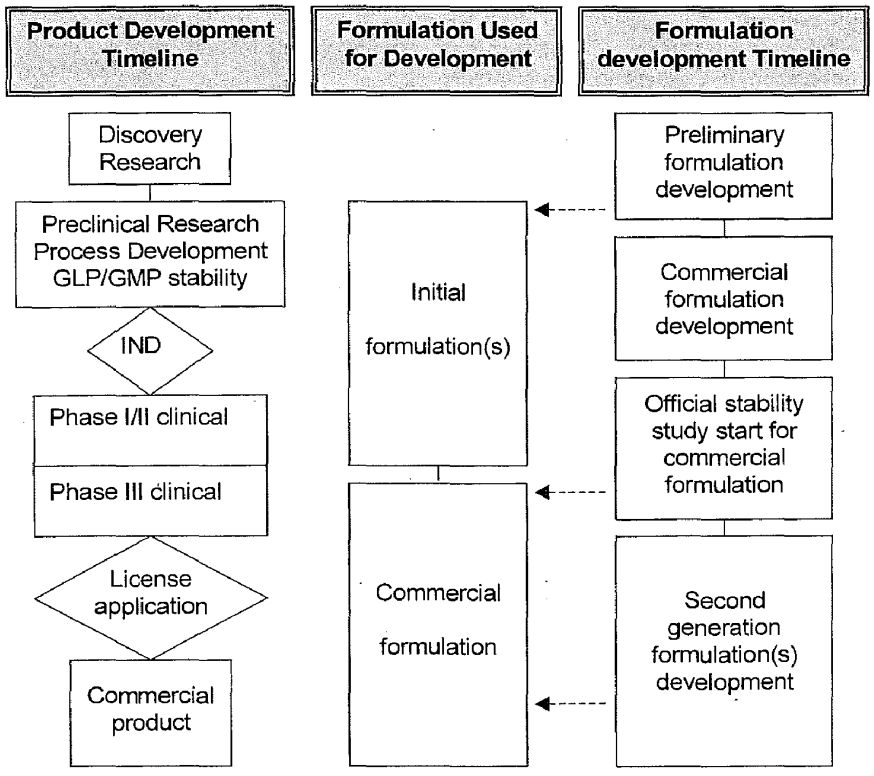


Figure 1. Diagram showing commercial formulation development process.

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reliable formulation is required to support various preclinical and clinical studies. The formulation can be further improved later to satisfy the clinical, marketing, and regulatory needs. Although formulation development does not necessarily appear rate-limiting during this improvement period, it is important to understand that changing a formulation may require much more supporting work, including additional clinical trials, which can take years.

Formulation for Early Preclinical and Clinical Studies

When a promising protein drug candidate is identified from preliminary feasibility studies, a decision will be made to introduce the drug to clinical studies. In order to obtain regulatory approval to initiate the clinical trials, it is required to demonstrate safety, manufacturing capability, stability of the drug and reproducibility in studying the drug. A sufficiently stable formulation is necessary at this stage because it is important to maintain the quantity and quality of the protein for all research studies from which results will be used for registration of the product with regulatory agencies. Therefore, formulation development becomes a rate-limiting process at this early stage. Formulations can be stored frozen or lyophilized for this purpose. At this stage in product development, the shelf-life requirement is determined by the logistics of supplying drug for clinical trials.

Commercial Formulation

Commercially viable and market competitive formulations have some common features. Most of all, the formulation should maintain the safety and efficacy profile of the protein drug during all the handling and uses specified on the label. Since commercial distribution channels are not equipped for frozen products, shipping and storage at refrigerated temperature or higher are required. Sufficient shelf life needs to be determined under conditions to which the product will be exposed in the commercial distribution system. Various systematic studies need to be carried out to comply with regulatory requirements for registration. It takes 1–2 years to collect all of these results, so the commercial formulation is developed while preclinical studies or early clinical trials (with the preliminary formulation) are in progress. If possible, the commercial formulation should be introduced before the pivotal clinical trial because clinical experience is the most effective way to confirm the safety and efficacy aspects of the formulation. In addition, formulation changes after this point may introduce formidable challenges to the clinical program and to obtaining regulatory approval.

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Regulatory Issues in Formulation Development

Incorporating regulatory guidelines into the formulation development is not only useful to develop high quality pharmaceutical formulations for maximum benefit to the patients, but also critical to prepare complete documents necessary for regulatory approval for commercialization of the drug. Readers who wish to obtain a comprehensive understanding of the guidelines are referred to the relevant documents published by regulatory agencies. In this section, a brief summary of the guidelines related to the formulation issues is provided.

Guidelines for Stability Studies. Comprehensive guidelines can be obtained from regulatory agencies (Appendix, Regulatory Documents 9–12) and review articles in the literature (Grimm, 1998; Kommanaboyina and Rhodes, 1999; Matthews, 1999). These guidelines generally cover the formal stability studies for bulk drug material, in-process samples and final formulated product. Although some issues may not be relevant for the earlier formulation development work, these guidelines provide key elements for designing stability studies. The guidelines provide information such as what types of experiments should be included in the study protocol, how to propose a stability-indicating profile, and what analytical results are needed to define the purity of the product and the molecular characteristics of degradation products. Another important piece of information provided in the guidelines is the definition of proper experimental conditions, e.g., definition of storage temperature, humidity, light strength, and accelerated and stressed conditions. The guidelines also provide the official protocols to evaluate the results obtained from stability studies.

Results Required to Apply for Regulatory License for a Drug Product. Detailed information regarding the formulation is presented at the Development Pharmaceutics section in the CMC section of regulatory applications (Appendix, Regulatory Docoments 13–16). All of the essential information required to apply for regulatory approval of protein formulations can be found in Note for Guidance on Development Pharmaceutics (Appendix Regulatory Document 17) and Development of Pharmaceutics for Biotechnological and Biological Products (Appendix, Regulatory Document 8). It is important to note that this is currently a European requirement although the international harmonization process may eventually introduce it to U.S. regulatory requirements. A brief summary of the important formulation information is shown in Table 8.

Results Required to File Formulation Amendments. A formulation change can introduce substantial potential adverse effects on the identity, strength, quality, purity or potency of the product as related to the safety or effectiveness of the product. For this reason, it is required that a supplement to the approved license application be submitted. This supplement should include a detailed description of the proposed change. It should also include methods and results for the studies performed to evaluate the effect of the change on the

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Table 8.
Summary of Information Included in Regulatory Applications

	General information	Comments specific to proteins
Active substance (protein)	Compatibility with excipients and other combined products, and physicochemical characteristics	Structural elements responsible for the biological activity; good coverage of degradations occurring during storage, manufacturing, and delivery.
Excipients	Function of each excipients, justification for their inclusion, and compatibility with other excipients, choice of quality	
Formulated products	Overage, physicochemical parameters, components with appropriate results supporting intended purpose; compatibility with diluent, device, and other drugs in contact before delivery; critical physical properties	Stability of structure in terms of biological activity; formulation optimization for both manufacturing process and stability
Packaging material	Integrity of container and closure during storage, reconstitution, admixture, dilution; sorption to container, leaching, and dose reproducibility	Adsorption, denaturation at the interface, and aggregation of the surface-denatured protein; reconstitution of dry-powder formulation
Manufacturing process	Manufacturing process for the preparation of formulation and its justification, appropriate method of sterilization and justification	Results to support the quality and stability of the protein during the manufacturing process; membrane filtration under aseptic conditions sufficient

product's identity, strength, quality, purity, and potency of the product, as related to the product's safety or effectiveness (Appendix, Regulatory Documents 18–20). When the degradation profile is changed qualitatively or quantitatively, it is recommended to follow the impurity-related guidelines discussed above (Appendix, Regulatory Documents 2,3). The manufacturer must obtain approval of the supplement by FDA prior to distribution of the product made using the change. In general, the following studies (Table 9) can be carried out to confirm

Table 9.
Studies Needed to Support Change in Formulation

Research requirements	Supporting results
Purity	Stability, compatibility, Structural analyses
Potency	*In vitro*/*in vivo* bioassays, preclinical and/or clinical pharmacokinetic comparability , clinical efficacy
Safety	Preclinical safety, clinical safety

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that the change in the formulation will not affect the safety and effectiveness c the product. It is recommended to consult the regulatory representative to fin out how much additional information may be needed for the approval of th changed formulation.

APPENDIX: LIST OF REGULATORY DOCUMENTS

1. International Conference on Harmonization: Guidance on specifications: Te procedures and acceptance criteria for Biotechnological/Biological Produc Federal Register: August 18, Volume 64, Number 159, pp. 44928–449 (1999).
2. International Conference on Harmonization: Impurities in New Drug Prc ucts. FDA Q3B (11/97).
3. Guidance for Industry. ANDAs: Impurities in Drug Products. Draft guidan FDA (12/98).
4. Note for Guidance on Inclusion of Antioxidants and Antimicrobial Pres vatives in Medicinal Products (CPMP/CVMP/QWP/115/95) EMEA (7/9
5. Inactive ingredient guide: inactive ingredients for currently marketed dı products. (1996) FOI Services, Inc. Rockville, MD.
6. Note for guidance on minimizing the risk of transmitting animal spongifc encephalopathy agents via medicinal products. EMEA (4/99).
7. Note for guidance on plasma-derived medicinal products. EMEA (7/98).
8. Development Pharmaceutics for Biotechnological and Biological Produ Annex to Note for Guidance on Development Pharmaceutics (CPM QWP/155/96). EMEA (10/1999).
9. International Conference on Harmonization: Stability testing of new d substances and products. Federal Register, Sept. 22, Volume 59, Number 1 pp. 48754–48759 (1994).
10. International Conference on Harmonization: Final guideline on stab testing of biotechnological/biological products. Federal Register, July Volume 61, Number133, pp. 36466–36469 (1996).
11. Guideline for Industry: Stability testing for drug substances and drug p ucts: draft guidances. FDA, (6/98).
12. International Conference on Harmonization: Guidelines for the photost ity testing of new drug substances and products. Federal Register, May Volume 62, Number. 95, pp. 27115–2712 (1997).
13. Guideline for Submitting Documentation for the Stability of Human D and Biologics FDA. (2/87).
14. Guidance for Industry. For the Submission of Chemistry, Manufacturing Controls and Establishment Description Information for Human Pla

Samsung Bioepis Exhibit No. 1002
Page 7400

Derived Biological Products, Animal Plasma or Serum-Derived Products. FDA (2/99).
15. Guidance for Industry. INDs for Phase 2 and 3 Studies of Drugs, Including Specified Therapeutic Biotechnology-Derived Products Chemistry, Manufacturing, and Controls Content and Format (Draft guidance) FDA (2/99).
16. Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products, FDA (11/95).
17. Note for Guidance on Development of Pharmaceutics (CPMP/BWP/328/99). EMEA (01/1998).
18. Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products, April, 1996.
19. Guidance for Industry: Changes to an Approved Application. For Specified Biotechnology and Specified Synthetic Biological Products, 21 CFR 601.12, 314.70; July 24, 1997, Vol 62. No. 142.
20. Guidance for Industry: Changes to an Approved Application: Specified Biotechnology and Specified Synthetic Biological Products; July 1997.

REFERENCES

Ahern, T.J. and Manning, M.C., 1992a. Stability of protein pharmaceuticals, Part A: Chemical and physical pathways of protein degradation. *Pharm. Biotech. Ser.* Volume 2. Plenum Press, N.Y.

Ahern, T.J. and Manning, M.C., 1992b. Stability of protein pharmaceuticals, Part B: In vivo pathways of degradation and strategies for protein stabilization. *Pharm. Biotech. Ser.* Volume 3. Plenum Press, N.Y.

Arakawa, T., Prestrelski, S., Kinney, W., and Carpenter, J.F., 1993. Factors affecting short-term and long-term stabilities of proteins. *Adv. Drug Delivery Rev.* 10:1.

Brewster, M.E., Hora, M.S., Simpkins, J.W., and Bodor, N., 1991. Use of 2-hydroxypropyl-beta-cyclodextrin as a solubilizing and stabilizing excipient for protein drugs. *Pharm. Res.* 8:792.

Cleland, J.L., Powell, M.F., and Shire, S.J., 1993. The development of stable protein formulations—A close look at protein aggregation, deamidation and oxidation. *Crit. Rev. Ther. Drug* 11:60.

Chen, T., 1992. Formulation concerns of protein drugs. *Drug Dev. Ind. Pharmacy*, 18:1311.

Defelippis, M.R., Bakaysa, D.L., Bell, M.A., Heady, M.A., Li, S., Pye, S., Youngman, K.M., Radzuik, J., and Frank, B.H., 1998. Preparation and characterization of a cocrystalline suspension of [Lys(B28),Pro(B29)] human insulin analogue. *J. Pharm. Sci.* 87:170.

Fagain, C.O., 1995. Understanding and increasing protein stability. *Biochimica et Biophysica Acta.* 1252:1.

Samsung Bioepis Exhibit No. 1002
Page 7401

Francis, G.E., Fisher, D., Delgado, C., Malik, F., Gardiner, A., and Neale, D., 1998. PEGylation of cytokines and other therapeutic proteins and peptides: the importance of biological optimisation of coupling techniques *Int. J. Hematology.* 68:1.

Fransson, J., Hallen, D., and Florin-Robertsson, E., 1997. Solvent effects on the solubility and physical stability of human Insulin-like Growth Factor I. *Pharm. Res.* 14:606.

Grimm, W., 1998. Extension of the international conference on harmonization tripatic guieline for stability testing of new drug substances and products to countries of climatic zones III and IV. *Drug Dev. Indust. Pharm.* 24:313.

Guerra, P.I., Acklin, C., Kosky, A.A., Davis, J.M., Treuheit, M.J., and Brems, D.N., 1998. PEGylation prevents the N-terminal degradation of megakaryocyte growth and development factor *Pharm. Res.* 15:1822.

Herron, J.N., Jiskoot, W., and Crommelin, D.J.A., 1995. Physical methods to characterize pharmaceutical proteins. *Pharm. Biotech. Ser.* Volume 7. Plenum Press, N.Y.

Jones, A.J.S., 1993. Analysis of polypeptides and proteins *Adv. Drug Del. Rev.* 10:29.

Knepp, V.M., Muchnik, A., Oldmark, S., and Kalashnikova, L., 1998. Stability of nonaqueous suspension formulations of plasma derived factor IX and recombinant human alpha interferon at elevated temperatures. *Pharm. Res.* 15:1090.

Kolvenbach, C.G., Narhi, L.O., Philo, J.S., Li, T., Zhang, M., and Arakawa, T., 1997. Granulocyte-colony stimulating factor maintains a thermally stable, compact, partially folded structure at pH 2 *J. Pept. Res.* 50:310.

Kommanaboyina, B. and Rhodes, C.T., 1999. Trends in stability testing with emphasis on stability during distribution and storage. *Drug Dev. Indust. Pharm.* 25:857.

Lam, X.M., Patapoff, T.W., and Nguyen, T.H., 1997. The effect of benzyl alcohol on recombinant human interferon-gamma *Pharm. Res.* 14:725.

Maa,Y.F. and Hsu, C.C., 1996. Aggregation of recombinant human growth hormone induced by phenolic compounds *Int. J. Pharm.* 140:155.

Manning, M.C., Matsuura, J.E., Kendrick, B.S., Meyer, J.D., Dormish, J.J., Vrkljan, M., Ruth, J.R., Carpenter, J.F., and Shefter, E., 1995. Approaches for increasing the solution stability of proteins *Biotech. Bioeng.* 48:506.

Manning, M.C., Patel, K., and Borchardt, R.T., 1989. Stability of protein pharmaceuticals. *Pharm. Res.* 6:903.

Matthews, B.R., 1999. Regulatory aspects of stability testing in Europe. *Drug Dev. Indust. Pharm.* 25:831.

Murby, M., Samuelsson, E., Nguyen, T.N., Mignard, L., Power, U., Binz, H., Uhlen, M., and Stahl, S., 1995. Hydrophobility engineering to increase solubility and stability of a recombinant protein from respiratory syncytial virus. *Eur. J. Biochem.* 230:38.

Nema, S., Washkuhn, R.J., and Brendel, R.J., 1997. Excipients and their use in injectable products *PDA J. Pharm. Sci. Technol.* 51:166.

Note for Guidance on Inclusion of Antioxidants and Antimicrobial Preservatives in Medicinal Products, 1997. (CPMP/CVMP/QWP/115/95) EMEA (7/97).

Pearlman, R. and Wang, Y.J., 1996. Formulation, characterization, and stability of protein drugs: case histories. *Pharm. Biotech. Ser.* Volume 9. Plenum Press, N.Y.

Powell, M.F., Nguyen, T., and Baloian, L., 1998. Compendium of excipients for parenteral formulations. *PDA J. Pharm. Sci. Technol.* 52:238.

Samsung Bioepis Exhibit No. 1002
Page 7402

Reubsaet, J.L.E., Beijnen, J.H., Bult, A., Van-Maanen, R.J., Marchal, J.A.D., and Underberg, W.J.M., 1998. Analytical techniques used to study the degradation of proteins and peptides: chemical instability *J. Pharm. Biomed. Anal.* 17:955.

Roig, M.G. and Kennedy, J.F., 1995. Perspectives for biophysicochemical modifications of enzymes. *J. Biomaterials Sci. Polymer Ed.* 7:1.

Thompson, J.E., 1998. Practical Guide to Contemporary Pharmacy Practice. Lippincott Williams & Wilkins, Hagerstown, MD.

Volkin, D.B., Mach, H., and Middaugh, C.R., 1997. Degradative covalent reactions important to protein Stability. *Molec. Biotech.* 8:5.

Wang, Y.J. and Hanson, M.A., 1988. Parenteral formulations of proteins and peptides: stability and stabilizers. *J. Parent. Sci. Technol.* 42:SS4.

Wang, Y.J. and Pearlman, R., 1993. Stability and characterization of protein and peptide drugs: case histories. *Pharm. Biotech. Ser.* Volume 5. Plenum Press, N.Y.

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5

Rational Design of Stable Lyophilized Protein Formulations: Theory and Practice

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INTRODUCTION

For ease of preparation and cost containment by the manufacturer, and ease of handling by the end user, an aqueous therapeutic protein formulation usually is preferred. However, with many proteins it is not possible—especially considering the time constraints for product development—to develop sufficiently stable aqueous formulations. Unacceptable denaturation and aggregation can be induced readily by the numerous stresses to which a protein in aqueous solution is sensitive; e.g., heating, agitation, freezing, pH changes, and exposure to interfaces or denaturants (Arakawa et al., 1993; Cleland et al., 1993; Brange, 2000; Bummer

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and Koppenol, 2000). Furthermore, even under conditions that thermodynamically greatly favor the native state of proteins, aggregation can arise during months of storage in aqueous solution (e.g., Gu et al., 1991; Arakawa et al., 1993; Chen et al., 1994; Volkin and Middaugh, 1996; Chang et al., 1996a). In addition, several chemical degradation pathways (e.g., hydrolysis and deamidation) are mediated by water. In aqueous formulations, the rates of these and other (e.g., oxidation) chemical degradation reactions can be unacceptably rapid on the time scale of storage (e.g., 18–24 months) for pharmaceutical products (Manning et al., 1989; Cleland et al., 1993; Goolcharran et al., 2000; Bummer and Koppenol 2000).

In contrast, a properly lyophilized formulation can maintain adequate physical and chemical stability of the protein during shipping and long-term storage even at ambient temperatures. As will be outlined in this chapter, developing stable lyophilized protein formulations should be a rational, straightforward process, which for most proteins should be rapid. With liquid formulation development, it may only be possible to obtain adequate protein stability after lengthy studies. Furthermore, sometimes there are conflicting conditions (e.g., pH) needed to slow sufficiently multiple degradation pathways in aqueous solution. Considering these issues plus the fact that formulation scientists now have to deal with numerous proteins and/or variants of a given protein, lyophilization should be considered as a primary mode for product development. Only if a parallel effort to develop an aqueous formulation is successful, will a final lyophilized product not be needed.

Rapid formulation development has important financial ramifications. A drug product has a finite patent life, during which time the company has an exclusive market. Considering that even a moderately successful drug product has annual sales of hundreds of millions of dollars, potentially millions of dollars in sales are lost for each day of delay in bringing a product to market. Unfortunately there are often delays because the formulations designed during early stage development and clinical trials (e.g., frozen) were not adequate for the final product. With a rational approach to formulation development, pharmaceutical scientists and process engineers can minimize the risk of this problem and the time needed to obtain a successful, final formulation. The success of such efforts depends on frank, open communication between the groups involved. For example, it is critical that the formulation scientists learn from the process engineers the issues for large-scale lyophilization runs, which are usually conducted in units that do not have the capacity to match processing parameters obtained in a small-scale research lyophilizers.

Despite the best efforts of the scientists and engineers, all too often delays in formulation development arise because sufficient resources are not invested in product development. For example, sometimes, purchase of essential equipmen

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(e.g., a differential scanning calorimeter), which costs a minute fraction of a day's sale of product, is not allowed. To avoid unnecessary delays in product launch, which can have disastrous consequences for the company and for patients, it is essential that companies appreciate that product development is ultimately a key limiting factor in getting a therapeutic to market. Hence, development efforts need to be as well funded as the usually much more visible drug discovery research programs. If a product is not stable, it will not be marketed, no matter how dramatic an impact it can have on human health and the financial status of the company.

MINIMAL CRITERIA FOR A SUCCESSFUL LYOPHILIZED FORMULATION

Research over the past several years has demonstrated that five criteria define the minimal conditions necessary for a successful lyophilized protein formulation (Table 1).

The first four criteria can be met with use of the appropriate excipients and lyophilization cycle design. For information on the proper design of lyophilization cycles, the reader is directed to the numerous previous reviews in this area (Franks, 1990; Pikal, 1990; Nail and Gatlin, 1993; Gatlin and Nail, 1994; Carpenter and Chang, 1996; Rey and May, 1999; Cappola, 2000). For the current chapter we will only consider cycle design in terms of the interplay between formulation physical properties (e.g., collapse temperature) and process parameters (see below). The last criterion listed in Table 1 requires insight into the unique physicochemical properties of each therapeutic protein, which will be explained in more detail below. We will discuss in turn why each of these criteria is important. Then we will present an explanation of how to design rationally a formulation to meet these criteria.

Table 1.
Minimal Criteria for a Successful Lyophilized Protein Formulation

1. Protein unfolding during freezing and drying is inhibited.
2. The glass transition temperature of the product exceeds the planned storage temperature (e.g., $T_g > 30°C$).
3. The water content is relatively low (e.g., 1% by mass).
4. A strong, elegant cake structure is obtained (i.e., collapse and meltback are avoided).
5. Steps are taken to minimize specific routes of protein chemical degradation (e.g., product vials are sealed under nitrogen to reduce the rate of methionine oxidation).

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Inhibition of Lyophilization-Induced Protein Unfolding

The stresses of freezing and drying cause protein unfolding, and the formulation must be designed to inhibit unfolding at each step (Prestrelski et al., 1993a,b; Carpenter et al., 1993; Prestrelski et al., 1995; Constantino et al., 1995, 1998; Griebenow and Klibanov, 1995; Allison et al., 1996, 1998, 1999, 2000; Chang et al., 1996b; Krielgaard et al., 1998a, 1999; Chen et al., 1999; Bell, 1999; Carrasquillo et al., 2000). Even if the formulation excipients and/or intrinsic thermodynamic stability of the protein prevent denaturation during freezing, unfolding can arise during subsequent drying (Carpenter et al., 1993; Prestrelski et al., 1993b; Allison et al., 1998; Carrasquillo et al., 2000). Conversely, once a protein unfolds during freezing, it will not regain native structure during dehydration.

For many proteins, unfolding during lyophilization leads to clinically unacceptable, non-native aggregates, even when samples are rehydrated immediately after lyophilization (Prestrelski et al., 1993a, 1995; Allison et al., 1996; Krielgaard et al., 1998a, 1999; Costantino et al., 1998). Aggregates are not necessarily formed during freezing and drying. Rather, during rehydration refolding of structurally perturbed protein molecules competes with formation of non-native protein aggregates (Prestrelski et al., 1993a). Aggregation can be minimized by including stabilizing excipients (e.g., sucrose or trehalose) in the formulation to inhibit lyophilization-induced unfolding (Prestrelski et al., 1993a, 1995; Allison et al., 1996; Krielgaard et al., 1998a, 1999; Costantino et al., 1998). Furthermore, fostering refolding during rehydration (e.g., with surfactants) can reduce aggregation (Chang et al., 1996c; Zhang et al., 1995, 1996).

In addition to minimizing protein aggregation during lyophilization/rehydration, maximizing retention of native protein structure in the dried solid is essential for optimizing long-term storage stability (Prestrelski et al., 1995; Chang et al., 1996b; Krielgaard et al., 1998a, 1999; Allison et al., 2000; Cleland et al., 2001). Both chemical and physical degradation in the dried solid can be accelerated if protein unfolding is not inhibited during lyophilization. With chemical degradation, a non-native structure may provide an environment conducive to covalent modification of one or more residues. For example, exposure of a methionine, which is normally buried deep in the interior of the native protein, on the surface of an unfolded dried protein may foster oxidation. Increased levels of aggregates noted after storage and rehydration of unfolded proteins could be due to formation of non-native intermolecular contacts within the dried solid, perturbation of refolding during rehydration because of chemical degradation, and/or other undefined processes.

Infrared spectroscopy has been used routinely to compare the secondary structures of a protein in lyophilized formulations to that of the native protein in aqueous solution (Prestrelski et al., 1993a,b; Prestrelski et al., 1995; Dong et al.,

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1995; Constantino et al., 1995, 1998; Griebenow and Klibanov, 1995; Allison et al., 1996, 1998, 1999, 2000; Chang et al., 1996b; Krielgaard et al., 1998a, 1999; Carpenter et al., 1998; Chen et al., 1999; Carrasquillo et al., 2000). This method should be considered essential in the development of stable lyophilized formulations, because it allows one to assess rapidly the effectiveness of formulations at inhibiting protein unfolding. Technical details about how to employ infrared spectroscopy to design stable lyophilized protein formulations can be found in the papers cited above.

Storage at Temperatures Below Formulation Glass Transition Temperature

In the dried powder, the protein is a component of an amorphous phase that includes amorphous excipients and water. If this glassy matrix is held below its characteristic glass transition temperature (T_g), the rate of diffusion-controlled reactions, including protein unfolding/aggregation and chemical degradation, are greatly reduced, relative to rates noted at temperatures $>T_g$ (Roy et al., 1991; Franks, 1990; Franks et al., 1991; Pikal, 1994, 1999). T_g can be determined with differential scanning calorimetry (DSC) or other thermal scanning methods (Nail and Gatlin, 1993; Chang and Randall, 1992; Craig and Royall, 1998; Verdonck et al., 1999).

Obtaining a formulation T_g in excess of the planned storage temperature (e.g., room temperature) is absolutely essential for optimal protein stability (e.g., Franks et al., 1991; Pikal, 1994, 1999; Carpenter and Chang, 1996; Duddu and Dal Monte, 1997). The T_g of a given amorphous phase is dependent on the T_g and mass percent of each component, including water (Angell, 1995; Franks et al., 1991; Levine and Slade, 1988, 1992; Pikal, 1994, 1999). Compared to excipients, dried proteins have relatively high T_g's (e.g., >150°C; Angell, 1995). Thus, with all other factors being held constant, the formulation T_g varies directly with the mass fraction of protein. However, care must be taken that the mass fraction of protein is not so high that there are not adequate levels of stabilizing excipients to prevent protein unfolding during lyophilization (Cleland et al., 2001; and see below).

Fortunately, sucrose and trehalose, which are the preferred excipients for inhibiting lyophilization-induced protein unfolding (see below), also provide a glassy matrix with acceptably high T_g values. For example, with water contents of 1% the T_g for pure sucrose and trehalose are about 100 and 65°C, respectively (Crowe et al., 1998).

It has now been documented with several proteins, that simply storing the formulation at temperature below T_g alone does not assure optimal stability. A

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native protein structure is also required. For example, proteins lyophilized in dextran alone are usually unfolded, but in a glassy matrix with a relatively high T_g (e.g., > 75° C). Yet they still degrade at relatively rapid rates compared to those for native protein molecules lyophilized with either sucrose or trehalose (Kriel-gaard et al., 1998a, 1999; Lueckel et al., 1998; Allison et al., 2000; Yoshioka et al., 2000). On pharmaceutical time scales of several months of storage many degradative reactions are not coupled to the glass transition of a formulation. This is because on these times scales there is still significant molecular mobility, even at temperatures well below (e.g., more than 30° C) the T_g (Hancock et al., 1995; Duddu et al., 1997; Pikal, 1999; Yoshioka et al., 1999).

The Water Content is Relatively Low

Because of its very low T_g (–135°C), water is a potent plasticizer for glasses; increasing water content in the dried formulation will greatly reduce T_g. For example, increasing the water content of pure sucrose from 1 to about 3–4% (g H_20/100 g dried powder) is sufficient to reduce the T_g to below room temperature (Crowe et al., 1998). It is critical to achieve a sufficiently low water level for a given formulation such that T_g exceeds the planned storage temperature. The lyophilization cycle dictates the initial water content (see reviews listed above). The most important parameter is the temperature for secondary drying, when the unfrozen water is desorbed (Pikal et al., 1990).

Water can also be transferred to the product from the vial stoppers during storage (Pikal and Shah, 1992; DeGrazio and Flynn, 1992; Hora and Wolfe, 1999). This effect can be dramatic. For example, let's consider a formulation containing 10 mg of dried protein, 40 mg of sucrose and initial water content of 1% by weight. The total amorphous fraction containing protein and sucrose has 0.50 mg of water. If 1.0 mg of water was transferred from the stopper to the product, the water content of this fraction would increase from 1% to 3.0%. This increase would be sufficient to lower the formulation T_g to below room temperature (Crowe et al., 1998). The risk of transfer of moisture from stoppers can be minimized by drying the stoppers before use, and, if acceptable for a given product, using stoppers coated with a material such as Teflon (see Hora and Wolfe, 1999).

A Strong, Elegant Cake Structure is Obtained

Often the most desired cake has strong, porous structure, without macroscopic collapse or meltback. This structure has a high surface area to volume

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ratio, which aids in the rapid dissolution of product upon addition of water. A detailed account of how to obtain such a cake structure is beyond the scope of the current chapter, but is available in several previous reviews (see above). For the current purposes it is sufficient to focus on the impact of formulation composition on avoiding collapse or meltback. When a product is frozen, the protein and amorphous excipients (e.g., sucrose) are dispersed between ice crystals and any excipient used as a crystalline bulking agent (e.g., glycine). To obtain an appropriate cake structure during lyophilization, the product temperature during primary drying, when the water in ice is sublimed, must be below the characteristic collapse and eutectic melting temperatures of amorphous and crystalline solutes, respectively. Above the eutectic temperature, the melting of crystalline solutes leads to massive loss of porous structure and macroscopic dissolution of the frozen matrix into a "puddle". Above the collapse temperature, which closely coincides with the glass transition temperature (T_g') of the maximally freeze-concentrated amorphous phase, the amorphous phase cannot support its own weight. The result is also a loss of pore structure and a macroscopic shrinkage or collapse of the cake.

It should be noted that the T_g' thermal event, which can be measured with differential scanning calorimetry, is also referred to as a softening event (T_s), rather than the actual glass transition temperature of the freeze-concentrated amorphous phase (Shalaev and Franks, 1995). Whatever the exact nature of the thermal event, it can be detected with differential scanning calorimetry as a second order increase in the baseline of the thermogram, which usually occurs just prior to the onset of the endotherm for the melting of ice (e.g., Her and Nail, 1994). The transition can also be measured with electrothermal methods (Her et al., 1994).

Another powerful method, which is essential for rational development of lyophilized formulations, for determining collapse and eutectic melting temperatures is freeze-drying microscopy (e.g., Nail et al., 1994). With this approach, the formulation of interest is directly examined visually for its performance during a simulated freezing, annealing and drying cycle. All of the critical phase changes, including ice formation, solute crystallization, eutectic melting and collapse, can be detected easily, and the temperature of their occurrence can be measured accurately.

Formulation composition dictates collapse temperature. Each pure amorphous excipient has a characteristic T_g' and collapse temperature; the collapse temperature for the formulation is the mass averaged temperatures of all of the components in the amorphous phase. It is important to design a formulation with maximum collapse temperature, because the rate of drying is directly proportional to the sample temperature during lyophilization. To allow for a reasonable drying time the T_g' should not be lower than –40° C. The T_g' values for pure sucrose is –32° C, while that of pure trehalose is –30° C (Skrabanja et al., 1994; Chang and

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Randall, 1992). In contrast, glucose, which should also be avoided because it is a reducing sugar, has a T_g' of −43° C. The collapse temperature of pure protein is about −10° C, which means increasing the protein:sugar mass ratio will increase collapse temperature. Finally, collapse temperature will be decreased if salts and excipients are not maximally crystallized. For example, glycine has a T_g' of −37° C, and its contribution to the amorphous phase can reduce collapse temperature to impractically low values (Carpenter and Chang, 1996). This problem can be avoided by using the appropriate annealing (see Carpenter and Chang, 1996) of the frozen product to maximize crystallization.

Meltback can be avoided by using crystalline solutes with relatively high eutectic melting temperatures. For example, eutectic melting temperatures for mannitol and glycine are −1 and −4° C, respectively. In contrast, additives such as calcium chloride have very low eutectic melting temperatures (e.g., −51° C for calcium chloride). If mannitol or glycine is used as a crystalline bulking agent, the T_g' of the amorphous excipient phase, which is lower that the eutectic melting temperatures of these excipients, will be the value that dictates the temperature of primary drying.

Collapse can also occur during secondary drying, when unfrozen water is desorbed, if the temperature is increased too rapidly. As water is removed from the amorphous phase the T_g of this phase increases. Thus, product temperature can be raised gradually and collapse can be avoided if product temperature at a given time point does not exceed the T_g. Directions for optimizing secondary drying can be found in previous reviews mentioned above.

Steps Taken to Minimize Specific Routes of Protein Chemical Degradation

It is essential that the major routes of chemical, as well as physical, degradation be characterized carefully for each protein, because all of the criteria listed above can be met and a protein might still be damaged during long term storage in the dried solid. For example, methionine oxidation is a common degradation pathway for therapeutic proteins (Manning et al., 1989; Ahern and Manning, 1992; Cleland et al., 1993; Goolcharran et al., 2000). Even in a formulation that prevents protein unfolding and has a T_g exceeding the storage temperature methionine oxidation can proceed at an unacceptably rapid rate. If a methionine residue is on the surface of the protein, maintaining native structure would not prevent this residue's exposure to reactive oxygen species in the dried solid. But why would the glassy matrix not sufficiently retard the mobility of the reactive species to prevent the reaction from occurring? On pharmaceutical time scales (i.e., of many months) relevant motion in a glass is not arrested unless the storage

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temperature is about 50° C below the T_g (e.g., Hancock et al., 1995; Pikal, 1994; Pikal, 1999). Therefore, the mobility of a relatively small reactive oxygen species and the oxidation of methionine residues are probably not coupled to the glass transition of the formulation. An intriguing alternative, but not mutually exclusive, explanation has been suggested by Steve Prestrelski (personal communication). He proposes that reactive oxygen species accumulating during long-term storage might not be causing damage just in the dried solid. Rather upon rehydration oxygen radicals rapidly react with the protein. This is an important area for future research.

Fortunately, despite the rather complicated and poorly understood theoretical aspects of unacceptable methionine oxidation rates in dried protein formulations, from a practical viewpoint dealing with the problem is relatively straightforward. Methionine oxidation can be minimized by sealing vials under nitrogen and/or using formulation additives (e.g., free methionine) to compete with protein residues for reactive oxygen species. These approaches should be effective whether the oxidation of residues occurs in the dried solid, during rehydration or at both times.

RATIONAL DESIGN OF STABLE LYOPHILIZED FORMULATIONS

Two critical practical issues need to be considered when choosing excipients for a therapeutic protein formulation. 1) From a financial standpoint, scientists should focus on using excipients and processing approaches that do not need to be licensed from a patent holder. Acceptable protein stability can be achieved readily using excipients and processing methods that are well known to someone, to use the legal term, "skilled in the art of protein formulation." Clearly, however, the specific applications of such compounds and processes to a given protein drug product and/or a class of proteins often can be patented. Such patents may be critical to the company developing that product.

2) From a regulatory standpoint (and financial perspective, also), the formulation scientist should choose from among excipients that are already used in approved parenteral products. For protein stabilizers, the best choices are the disaccharides, sucrose and trehalose. For bulking agents, the best choices are glycine, mannitol and hydroxyethyl starch. For surfactants, usually the Tweens (20, 40 or 80) are preferred.

Based on these practical concerns and the criteria for a successful lyophilized protein product (Table 1), prototypic rational formulations are presented in Table 2. If the formulations suggested are employed, and the criteria in Table 1 are met, then most likely the product will have acceptable stability.

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Fortunately, despite the rather complicated and poorly understood theoretical aspects of unacceptable methionine oxidation rates in dried protein formulations, from a practical viewpoint dealing with the problem is relatively straightforward. Methionine oxidation can be minimized by sealing vials under nitrogen and/or using formulation additives (e.g., free methionine) to compete with protein residues for reactive oxygen species. These approaches should be effective whether the oxidation of residues occurs in the dried solid, during rehydration or at both times.

RATIONAL DESIGN OF STABLE LYOPHILIZED FORMULATIONS

Two critical practical issues need to be considered when choosing excipients for a therapeutic protein formulation. 1) From a financial standpoint, scientists should focus on using excipients and processing approaches that do not need to be licensed from a patent holder. Acceptable protein stability can be achieved readily using excipients and processing methods that are well known to someone, to use the legal term, "skilled in the art of protein formulation." Clearly, however, the specific applications of such compounds and processes to a given protein drug product and/or a class of proteins often can be patented. Such patents may be critical to the company developing that product.

2) From a regulatory standpoint (and financial perspective, also), the formulation scientist should choose from among excipients that are already used in approved parenteral products. For protein stabilizers, the best choices are the disaccharides, sucrose and trehalose. For bulking agents, the best choices are glycine, mannitol and hydroxyethyl starch. For surfactants, usually the Tweens (20, 40 or 80) are preferred.

Based on these practical concerns and the criteria for a successful lyophilized protein product (Table 1), prototypic rational formulations are presented in Table 2. If the formulations suggested are employed, and the criteria in Table 1 are met, then most likely the product will have acceptable stability.

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Table 2.
Example of Rational Lyophilized Protein Formulation

1. Buffer that does not acidify during freezing (e.g., Tris, histidine, citrate)
2. Specific ligands/pH that optimizes thermodynamic stability of protein
3. Trehalose or sucrose to inhibit protein unfolding and provide glassy matrix
4. Bulking agent (e.g., mannitol, glycine or hydroxyethyl starch)
5. Nonionic surfactant to reduce protein aggregation

It is important to emphasize that not all components listed in Table 2 may be needed for a given protein product. For example, it may not be necessary to include a nonionic surfactant to aid in reducing protein aggregation. If there is not clear evidence that a given component is beneficial for a formulation, then that component should not be included in the formulation. Also, some components that are used because of "tradition" and/or for purposes such as final formulation tonicity can cause great difficulties in lyophilization. The most common example is the use of NaCl as a tonicity modifier. During lyophilization, NaCl can greatly reduce the collapse temperature of a formulation, if a fraction of the salt does not crystallize (Her et al., 1994). Crystallization of NaCl during freezing and annealing can be inhibited by other excipients (e.g., bulking agents and stabilizing sugars). Because of the low collapse temperature, a low temperature cycle must be used, which increases production time and costs. Also, even if formulations with NaCl can be lyophilized successfully in small research lyophilizers, there is great risk that a large fraction of vials will collapse during large scale manufacturing runs. Thus, if at all possible, NaCl should not be used in lyophilized formulations. Alternative tonicity modifiers include mannitol and glycine, which can also serve as crystalline bulking agents.

Every protein and product has unique characteristics, some of which may cause difficulty in designing stable formulations. Thus, the suggestions in Table 1 should be considered only as a good starting point in formulation development. For the remainder of this chapter we will discuss the rationale for the choice of each of the formulation components, their mechanisms of action and other practical approaches that can be used to increase protein stability.

Choice of Buffer

In terms of protein stability, the main concern with choice of buffer is the potential of certain buffer salts to precipitate during freezing and cause large changes in pH. For example, crystallization of the dibasic form of sodium

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phosphate results can result in a pH < 4 (van den Burg, 1959; van den Burg and Rose, 1959; Anchordoquy and Carpenter, 1996). Thus, whenever possible, sodium phosphate buffer should be avoided. Although somewhat obvious, it is important to realize that a sodium phosphate system will be present if one starts with potassium phosphate buffer salts and NaCl, as is the case with phosphate buffered saline. Alternative buffers that do not appear to have major pH changes during freezing include Tris and histidine (Anchordoquy and Carpenter, 1996).

A clear example of the detrimental effects of buffer acidification on the stability of a lyophilized protein formulation has been reported by Lam et al. (1996). These researchers documented that a succinate buffer with an initial pH of 5 exhibited a pH drop of 1–2 units during lyophilization. The result was a reduced stability of interferon-γ during storage of the lyophilized formulation, relative to that noted for formulations prepared with glycolate buffer, which does not undergo freezing-induced acidification.

Specific Ligands/pH that Optimizes Thermodynamic Stability of Protein

Often experience gained during purification, intermediate storage protocols and preformulation studies can provide great insight into specific solution conditions (e.g., pH, ionic strength, buffer type) that confer the greatest stability to a given protein. Before choosing the appropriate "general" stabilizers, which are effective at protecting most proteins, it is absolutely essential that the formulation be optimized for the specific factors that increase the physical and chemical stability of a given protein. For example, simply avoiding extremes in pH can drastically reduce the rate of deamidation (Manning et al., 1989; Goolcharran et al., 2000). Also, specific ligands that increase protein physical stability (e.g., by increasing the free energy of unfolding) should be investigated. The stabilizing effects of heparin and other polyanions on growth factors (e.g., Chen et al., 1994; Volkin and Middaugh, 1996) and calcium on DNase (Chen et al., 1999) provide good examples. As will be described below, increasing the free energy of unfolding directly correlates with increased resistance of proteins to denaturation during freezing. In addition, at least in the case of DNase, there was also dramatic increases in storage stability of the lyophilized protein in formulation containing calcium, which were not due to increased structural stabilization during lyophilization (Chen et al., 1999). The mechanism for this effect is unknown.

Moreover, sometimes protein unfolding during freeze-drying can be minimized by optimizing initial solution pH (e.g., Prestrelski et al., 1995). However, in other cases it has been found that initial solution pH does not impact the degree of unfolding arising during lyophilization (Costantino et al., 1995; Carrasquillo et al., 2000). For each protein it is necessary to investigate the effect of initial pH

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(and potential pH changes during freezing) on structural retention during lyophilization. Infrared spectroscopy can be used to monitor secondary structure in the dried solid, and the resulting data can be used to choose the initial pH that results in the most native-like secondary structure in the dried solid.

Trehalose or Sucrose to Inhibit Protein Unfolding and Provide Glassy Matrix

In this section we will discuss the rationale for choice of stabilizing excipients. Then we will describe the mechanisms for protection of proteins by additives during freezing and drying. Mechanistic insight is important for a clear understanding of protein stabilization during lyophilization and for guiding critical practical choices, such as determining the level of disaccharide needed for optimal protein protection. Finally, we will address this and other practical issues in the use of stabilizing excipients to inhibit protein unfolding during freezing and drying.

Rationale for Choice of Stabilizing Sugar. Among the numerous compounds tested, it appears that the most effective stabilizers of proteins during lyophilization are disaccharides (reviewed in Carpenter et al., 1999). Trehalose and sucrose are the best choices for a stabilizing disaccharide for therapeutic proteins. Both sugars: 1) protect proteins during both freezing and dehydration; 2) are nonreducing; 3) tend to remain amorphous during lyophilization; and 4) have been used in approved parenteral therapeutic products. There are, however, some important differences in the physicochemical properties of these sugars. 1) Trehalose has a higher T_g at a given moisture content than sucrose and, thus, for formulations containing trehalose it may be easier to obtain an appropriate cake structure with an economical lyophilization cycle (Crowe et al., 1998). However, a skilled process engineer should be able to design economical, effective cycles for formulations containing either sugar. In addition, the condition of having a T_g greater than the product storage temperature will hold at higher residual water contents for trehalose. In products with a relatively high protein concentration, the protein could contribute to an increased T_g, which serves to minimize the advantages of trehalose. 2) Trehalose is more resistant than sucrose to acid hydrolysis. Hydrolysis of these disaccharides produces reducing sugars, which must be avoided. Usually this is not a problem, unless very low pH's (ca. < 4) are employed. It should be noted that acid catalyzed hydrolysis of sucrose can occur even in lyophilized solids (Shalaev et al., 2000), suggesting that buffer acidification during freezing (see below) could ultimately result in formation of reducing sugars and resulting damage to proteins in a lyophilized formulation. 3)

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Sucrose appears to be somewhat more effective at inhibiting unfolding during lyophilization (Allison et al., 1999). This difference has been most obvious when there is a relatively high protein concentration and a need to employ a relatively high initial concentration of sugar. Evidence to date indicates that less effective stabilization by trehalose is due to the greater propensity of this sugar to phase separate from polymers (Isutzu et al., 1996) and proteins (S.D. Allison, T.W. Randolph, B.S. Chang and J.F. Carpenter, unpublished observation) during freezing and drying. Whether or not this is a problem with a given formulation cannot be predicted. Hence, the relative capacities of sucrose versus trehalose to inhibit lyophilization-induced unfolding of must be examined for each protein. The final choice of sugar for a given protein product should be based on a direct comparison of sucrose and trehalose as stabilizers during the lyophilization cycle and storage in the dried solid.

Reducing sugars, such as maltose or lactose, should not be used. These compounds may effectively inhibit protein unfolding during the lyophilization cycle, but during storage in the dried solid they can degrade proteins via the Maillard reaction between carbonyls of the sugar and free amino groups on the protein (Hageman, 1992; Li et al., 1996).

Mechanism for Freezing Protection. Many different compounds, such as sugars, polyols, certain amino acids, methylamines and salting-out salts, nonspecifically stabilize proteins during freezing or freeze-thawing ("cryoprotection"). The same compounds have also been shown in nonfrozen aqueous systems to increase protein thermodynamic stability (e.g., increase resistance to temperature- or chaotrope-induced unfolding). Protection of proteins by these compounds during freezing and freeze-thawing is due to the same universal thermodynamic mechanism that Timasheff and colleagues have defined for solute-induced protein stabilization in nonfrozen aqueous solution (reviewed in Carpenter and Crowe, 1988; Carpenter and Chang, 1996; Carpenter et al., 1999; Timasheff, 1998).

Usually relatively high concentrations (ca. > 0.3 M) of solute are needed to stabilize proteins because the interactions of the solute with the protein are relatively weak. Stabilizing solutes are excluded preferentially from the surface of the protein. This statement does not mean that no solute molecules bind to the surface of the protein, but rather that there is a lower concentration of solute in the immediate vicinity of the protein than in the bulk solution. The chemical potential of the protein is increased in the presence of a preferentially excluded solute, which is the basis for the effect of solute of protein thermodynamic stability. Considering a two-state model for protein folding (N $\leftarrow \rightarrow$ D), the native state is favored thermodynamically (e.g., under physiological conditions) because it has a lower free energy than the denatured state. The key to increasing the thermodynamic stability of the native state is to increase the free energy barrier

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between the native and denatured states. So how can increasing the chemical potential of the native state result in stabilization? The degree of preferential exclusion and concomitant increase in protein chemical potential correlates directly with the protein surface area. The denatured state has a much greater surface area than the native state. Thus, in the presence of a preferentially excluded solute, the magnitude for the increase in protein chemical potential will be much greater for the denatured than that for the native state; the native state will be stabilized.

This same mechanism applies to the inhibition of smaller scale structural expansions of the native state, which may be sufficient to promote irreversible protein aggregation, by compounds such as sucrose (e.g., Chang et al., 1996a; Kendrick et al., 1997, 1998; Kim et al., 2000). Many proteins are known to form non-native aggregates from species with conformations that are not greatly different from the most compact native state. One example is the molten globule that has perturbed tertiary structure, but native secondary structure. However, even expanded species within that native state ensemble can participate in intermolecular interactions leading to non-native protein aggregates (e.g., Chang et al., 1996a; Kendrick et al., 1997, 1998; Kim et al., 2000). Preferentially excluded solutes shift the equilibrium between protein species towards that with the lowest surface area, i.e., the most compact species in the native state ensemble. As a result aggregation is inhibited.

Timasheff's preferential interaction mechanism also explains the influence of solutes on the degree of assembly of multimeric proteins. Preferentially excluded solutes tend to induce polymerization and stabilize native oligomers since the formation of contact sites between constituent monomers serves to reduce the surface area of the protein exposed to the solvent. Polymerization reduces the thermodynamically unfavorable effect of preferential solute exclusion. In this case, the assembled protein is much more stable than the constituent monomer because of the direct solute effects, but also because of the increase in oligomer stability gained by intersubunit contacts (e.g., Neet and Timm, 1994). In one example of this effect during lyophilization, maintenance of the native tetramer during freezing has been shown to increase the resistance of lactate dehydrogenase to dissociation and inactivation during subsequent drying (Anchordoquy and Carpenter, 1996; Anchordoquy et al., 2001).

How do we know that the preferential exclusion mechanism is actually operative in the frozen state? It is not possible to measure directly the protein-solute interaction in the frozen state. However, the effects of solutes on protein chemical potential and the resulting protein stabilization can be inferred from a freezing study with hemoglobin, in which the protein was partitioned into separate polyethylene glycol-rich and dextran-rich solution phases (Heller et al., 1996). In a phase separated system, thermodynamic equilibrium requires that chemical potential of each component be equal in both phases. Hence, there should be

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equivalent effects of the different solute conditions in the two phases on the increase in free energy of protein unfolding. In other words, the impact of preferentially excluded solutes on protein stability is equal in the two phases. As predicted from the preferential exclusion mechanism, the degree of structural protection of hemoglobin during freezing (as observed directly in the frozen state with infrared spectroscopy) was equivalent in both phases (Heller et al., 1996).

Mechanism for Inhibition of Dehydration-Induced Unfolding. The interaction of a protein's residues with water are intimately involved with the formation of the native, globular protein structure, and if the protein is dehydrated it will unfold. It has been documented by numerous studies that sucrose and trehalose prevent dehydration-induced unfolding by hydrogen bonding to the dried protein in place of the lost water (e.g., Carpenter and Crowe, 1989; Prestrelski et al., 1993a; Allison et al., 1999; Costantino et al., 1998; Wolkers et al., 1998; Tzannis and Prestrelski, 1999). This so called "water replacement mechanism" is supported by several different observations.

For example, with infrared spectroscopy, it has been found that the band at 1583 cm^{-1} in the spectrum for lysozyme, which is due to hydrogen bonding of water to carboxylate groups, is not present in the spectrum for the dried protein (Carpenter and Crowe, 1989; Remmele et al., 1997; Allison et al., 1999). When lysozyme is dried in the presence of trehalose or sucrose, the carboxylate band is retained in the dried sample, indicating that the sugar is hydrogen bonding in the place of water. Similar results have been obtained with α-lactalbumin and sucrose (Prestrelski et al., 1993a). The magnitude of the retention of the carboxylate band correlates directly with the level of trehalose or sucrose in the lyophilized formulation, as well as with the degree of inhibition of unfolding (Allison et al., 1999). These effects of sugars on proteins in the dried solid are not due to the presence of increased amounts of water in the formulations dried with sugars (Allison et al., 1999; Tzannis and Prestrelski, 1999). The level of water in formulations dried with the sugars is as low as that for the protein lyophilized from just buffer or water, and is so low that the hydration shell of the protein is essentially completely removed (Prestrelski et al., 1993a; Krielgaard et al., 1998a, 1999; Allison et al., 1999).

Furthermore, Farhat et al., (1998) used infrared spectroscopy to study dried gelatin-sugar mixtures and the hydration behaviors of the mixtures, and concluded that the sugars hydrogen bond to the dried protein. Costantino et al. (1997) studied water sorption of proteins lyophilized with sucrose or trehalose and found that the solid-state interactions between protein and sugar reduced the availability of water binding sites. Tzannis and Prestrelski (1999) found that water sorption behavior of spray-dried protein formulations containing stabilizing levels of sucrose indicated hydrogen bonding between dried protein and the sugar. However, if an excessively high sucrose:protein ratio was employed, due to

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formation of protein- and sugar-rich phases, hydrogen bonding of sucrose to protein and resulting protein stabilization were reduced. Also, Suzuki et al. (1998; 1999) found that there was a high degree of stabilization of lactate dehydrogenase when sucrose remained amorphous and hydrogen bonded to the dried protein. Crystallization of sucrose abolished hydrogen bonding between the sugar and dried protein, as well as protein stabilization.

Further support for the water replacement mechanisms comes from two studies that compared the relative effectiveness of saccharides of increasing molecular weight for inhibiting lyophilization-induced unfolding. Tanaka and colleagues (1991) found that the capacity to protect catalase during freeze-drying was inversely related to saccharide size. They suggested that as the size of the saccharide increases, steric hindrance interferes with hydrogen bonding between the saccharide and the dried protein. In support of this contention, the carboxylate band is only minimally detectable in the infrared spectrum of lysozyme freeze-dried in the presence of glassy dextran and the protein is unfolded (Allison et al., 1999). With infrared spectroscopy, Prestrelski et al. (1995) found that as the molecular weight of a carbohydrate additive was increased the capacity to inhibit unfolding of interleukin-2 during lyophilization decreased, and the level of protein aggregation after rehydration increased. Also, it was clear that protection of the protein did not correlate directly with the formation of a glass (all samples were found to be amorphous) or with the glass transition temperature of the sample (the T_g increased as carbohydrate molecular weight increased). Rather, there was a negative correlation between stabilization and molecular weight, which is to be expected if protection during drying is due to the water replacement mechanism.

An alternative mechanism for stabilization of proteins during dehydration states that proteins are simply mechanically immobilized in a glassy, solid matrix during dehydration (e.g., Franks, 1991). The restriction of translational and relaxation processes is thought to inhibit protein unfolding, and spatial separation between protein molecules (i.e., "dilution" of protein molecules within the glassy matrix) is proposed to prevent aggregation. It is clear that protective additives must partition with the protein into the amorphous phase of the dried sample. If the compound crystallizes during lyophilization it does not inhibit protein unfolding (Carpenter et al., 1993; Izutsu et al., 1993; Krielgaard et al., 1999). Also, spatially separating protein molecules can help favor refolding over aggregation during rehydration (Allison et al., 1998, 2000). However, as evidenced by the failure of glassy dextran matrices to prevent unfolding (Prestrelski et al., 1995; Krielgaard et al., 1999; Allison et al., 1998, 1999, 2000), simply forming a glassy solid is not sufficient for inhibiting dehydration-induced unfolding.

Practical Considerations. The protein mass in a unit dose (or multidose) vial of therapeutic protein is dictated by the amount to be given to the patient.

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With a lyophilized product, which can be reconstituted to a volume different from the prelyophilization volume, the initial protein concentration can be varied without changing the total protein mass. Changes in initial protein concentration can affect relative loss of protein due to interaction with vial surfaces (e.g., Page et al., 2000), protein stability during processing, the collapse temperature of the formulation, the T_g of the final dried formulation and the lyophilization process itself. Thus, protein concentration can be an important variable to investigate during formulation development.

Increasing initial protein concentration leads to apparent increased resistance to denaturation during freezing (Strambini and Gabellieri, 1996; Chang et al., 1996c; Krielgaard et al., 1998b). This phenomenon can be demonstrated by determining the percentage protein aggregated after freeze-thawing, which varies inversely with protein concentration. Increasing protein concentration can directly reduce freezing-induced protein unfolding because one component of protein damage during freezing appears to involve protein denaturation during formation of the ice-water interface (Strambini and Gabellieri, 1996; Chang et al., 1996c; Krielgaard et al., 1998b). Assuming that only a finite number of protein molecules can be unfolded per unit area at this interface (Krielgaard et al., 1998b), increasing the initial protein concentration will lead to a smaller percentage of damaged molecules. Of course, other factors such as direct cold denaturation and freeze concentration of salts may predominate over surface denaturation during freezing. For practical purposes, it is not necessary to sort out the relative contribution of the various stresses to freezing-induced damage. Rather, is important to include realative high protein concentrations during early formulation development in order to increase the "intrinsic" resistance of the protein to denaturation. The greater the intrinsic stability of the protein, the lower the concentration of excipient that will be needed.

Another advantage of employing a relatively high initial protein concentration is that increasing protein : disaccharide mass ratio increases both formulation collapse temperature and T_g' of the final dried product. In addition, the formulation volume will be reduced, which will reduce the duration and costs of lyophilization.

Of course, if the ratio of excipient to protein is not sufficiently high, there will not be adequate stabilization of the protein during freezing and dehydration. Freezing protection depends on the initial bulk concentration of the sugar, and if the given protein is freeze-labile sometimes concentrations exceeding 5% (wt/vol) are needed to maximize stabilization. However, often the stability of the protein is sufficient during freezing that the level of sugar needed for optimal protection during lyophilization is dictated by that required for inhibition of unfolding during dehydration. Protection during drying depends on the final mass ratio between the sugar and the protein (Pikal, 1994; Carpenter and Chang, 1996;

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Cleland et al., 2001). Recently it has been demonstrated that the sugar:protein mass ratio predicts not only degree of native structural retention during lyophilization, but also the long-term storage stability of a lyophilized protein (Cleland et al., 2001). Generally, a weight ratio of sugar to protein of at least 1:1 is required for inhibiting lyophilization-induced unfolding, with optimal stability being reached at around 3–5:1. In practice, with the protein concentration held constant, a range of sugar concentrations can be tested during formulation screening to discern the optimal concentration needed for retention of native protein in the dried solid. In general, the optimal sugar concentration for stabilizing the protein during lyophilization will also provide storage stability, if the final dried powder has a T_g well above the storage temperature. Finally, it should be stressed that the minimal effective level of sugar should be employed, because of the effect of changing protein:excipient ratio on glass transition temperatures. Also, if excess levels of sugar are employed there is an increased risk of sugar crystallization if a product should happen to be held at temperatures near or above the formulation T_g (e.g., Krielgaard et al., 1999).

Bulking Agent (e.g., Mannitol, Glycine or Hydroxyethyl Starch)

If the product has a relatively low mass of protein per vial, often it will necessary to have a bulking agent in the formulation to prevent the protein from being lost from the vial during drying and to form the product cake. Mannitol and glycine are examples of bulking agents, which can also serve as tonicity modifiers, that usually crystallize to a substantial degree during lyophilization (Pikal, 1994; Carpenter and Chang, 1996). A disaccharide protein stabilizer will need to be used in combination with these bulking agents. One drawback of mannitol and glycine is that often an annealing step is required to assure maximum crystallization. If a fraction of the bulking agent remains amorphous during lyophilization, there may be problems with obtaining a suitable cake structure, as well as a risk of excipient crystallization during subsequent storage in the dried solid (e.g., Carpenter and Chang, 1996; Carpenter et al., 1997). An alternative, amophous bulking agent is the polymer hydroxyethyl starch, which is used clinically as a plasma volume expander. Its main advantages in lyophilization are that it has a high collapse temperature (ca. −10°C), it forms strong cakes and it has a relatively high T_g for a given moisture content (e.g., >200°C at 2% residual moisture). However, as is the case with dextran, the large polymer hydroxyethyl starch does not inhibit protein unfolding during dehydration, and may actually foster additional protein unfolding due to phase separation from the protein during freezing and drying. Again, stabilizing disaccharides must be used with hydroxyethyl starch to inhibit lyophilization-induced protein unfolding.

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Nonionic Surfactant to Inhibit Aggregation

Surfactants are often included in protein formulations to minimize interfacial denaturation and aggregation, e.g., at vial surfaces, due to bubble entrainment during filling or because of agitation (Chang et al., 1996c; Krielgaard et al., 1998b; Page et al., 2000). For a lyophilized product, a surfactant can be beneficial to minimize protein aggregation during vial filling, freezing and rehydration. Generally, a surfactant will not inhibit protein unfolding during dehydration (Krielgaard et al., 1998a). The mechanisms by which surfactants inhibit protein aggregation will be described in a separate chapter in this volume. For the current discussion it is sufficient to stress that a surfactant should not be included in a lyophilized product, unless there is direct evidence that increases recovery of native protein in the rehydrated sample. Surfactants can decrease the free energy of unfolding of some proteins, which may cause the compound to actually foster aggregation. Also, they have been shown to inhibit the assembly of small soluble aggregates into higher order soluble oligomers and insoluble aggregates (e.g., Krielgaard et al., 1998b). This effect can manifest itself during lyophilization and rehydration. As a result, the presence of a surfactant can cause an undesirable increase in the level of soluble aggregates.

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REFERENCES

Ahern, T.J., and Manning, M.C., 1992. *Stability of Protein Pharmaceuticals: Part. A. Chemical and Physical Pathways of Protein Degradation*, Plenum Press, New York.

Allison, S.D., Dong, A., and Carpenter, J.F., 1996. Counteracting effects of thiocyanate and sucrose on chymotrypsinogen secondary structure and aggregation during freezing, drying and rehydration. *Biophys. J.* 71:2022.

Allison, S.D., Randolph, T.W., Davis, A., Middleton, K., and Carpenter, J.F., 1998. Effects of drying methods and additives on structure and function of actin: Mechanisms

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Page 7423

of dehydration-induced damage and its inhibition. *Arch. Biochem. Biophys.* 35 171.

Allison, S.D., Randolph, T.W., Chang, B.S., and Carpenter, J.F., 1999. Hydrogen bondi between sugar and protein is responsible for inhibiting dehydration-induced prote unfolding. *Arch. Biochem. Biophys.* 365:289.

Allison, S.D., Manning, M.C., Randolph, T.W., Middleton, K., Davis, A., and Carpent J.F., 2000. Optimization of storage stability of lyophilized actin using combinatio of disaccharides and dextran. *J. Pharm. Sci.* 89:199.

Anchordoguy, T.J., and Carpenter, J.F., 1996. Polymers protect lactate dehydrogena during freeze-drying by inhibiting dissociation in the frozen state. *Arch. Bioche. Biophys.* 332:231.

Anchordoquy, T.J., Izutsu, K.I., Randolph, T.W., and Carpenter, 2001. Maintenance of qu ternary structure in the frozen state stabilizes lactate dehydrogenase during freez drying. *Arch. Biochem. Biophys.* (In Press).

Angell, C.A., 1995. Formation of glasses from liquids and biopolymers. *Science* 267:19

Arakawa, T., Prestrelski, S., Kinney, W., and Carpenter, J.F., 1993. Factors affecting sho term and long-term stabilities of proteins. *Adv. Drug Delivery Rev.* 10:1.

Bell, J.A., 1999. X-ray crystal structures of a severely desiccated protein. *Prot. Sci.* 8:20

Bummer, P.M., and Koppenol, S., 2000. Chemical and physical considerations in prote and peptide stability, in: *Protein Formulation and Delivery*, E.J. McNally, ed., Mar Dekker, New York.

Brange, J., 2000. Physical stability of proteins, in: *Pharmaceutical Formulation Dev opment of Peptides and Proteins*, S. Frokjaer and L. Hovgaard, eds., Taylor a Francis, London.

Cappola, M.L., 2000. Freeze-drying concepts: The basics, in: *Protein Formulation a Delivery*, E.J. McNally, ed., Marcel Dekker, New York.

Carpenter, J.F., and Chang, B.S., 1996. Lyophilization of protein pharmaceuticals, *Biotechnology and Biopharmaceutical Manufacturing, Processing, and Preservati* K.E. Avis and V.L. Wu, eds., Interpharm Press, Buffalo Grove, IL.

Carpenter, J.F., Izutsu, K., and Randolph, T.W., 1999. Freezing- and drying-induced str tural changes in proteins and their inhibition by stabilizing additives. *Pharmace cal Freeze-Drying*, L. Rey and J.C. May, eds., Marcel Dekker, New York.

Carpenter, J.F., Pikal, M.J., Chang, B.S., and Randolph, T.W., 1997. Rational design stable lyophilized protein formulations: Some practical advice. *Pharm. R* 14:969–975

Carpenter, J.F., Prestrelski, S., and Arakawa, T., 1993. Separation of freezing- and dryi induced denaturation of lyophilized proteins by stress-specific stabilization: Enzyme activity and calorimetric studies. *Arch. Biochem. Biophys.* 303:456.

Carpenter, J.F., Prestrelski, S.J., and Dong, A., 1998. Application of infrared spectrosc to development of stable lyophilized protein formulations. *Eur. J. Pharm. Biopha* 45:231.

Carpenter, J.F., and Crowe, J.H., 1988. The mechanism of cryoprotection of proteins solutes. *Cryobiology* 25:244.

Carpenter, J.F., and Crowe, 1989. Infrared spectroscopic studies on the interaction of c bohydrates with dried proteins. *Biochemistry* 28:3916.

Samsung Bioepis Exhibit No. 1002
Page 7424

Chang, B.S., and Randall, C.S., 1992. Use of subambient thermal analysis to optimize protein lyophilization. *Cryobiology* 29:632.

Chang, B.S., and Fisher, N.L., 1995. Development of an efficient single-step freeze-drying cycle for protein formulations. *Pharm. Res.* 12:831.

Chang, B.S., Beauvais, R.M., Arakawa, T., Narhi, L.O., Dong, A., Aparisio, D.I., and Carpenter, J.F., 1996a. Formation of an active dimer during storage of interleukin-1 receptor antagonist in aqueous solution. *Biophys. J.* 71:3399.

Chang, B.S., Beauvais, R.M., and Carpenter, J.F., 1996b. Physical factors affecting the storage stability of freeze-dried interleukin-1 receptor antagonist: Glass transition and protein conformation. *Arch. Biochem. Biophys.* 331:249.

Chang, B.S., Kendrick, B.S., and Carpenter, J.F., 1996c. Surface-induced denaturation of proteins during freezing and its inhibition by surfactants. *J. Pharm. Sci.* 85:1325.

Chang, B.S., Kendrick, B.S., Carpenter, J.F. 1996c. Surface-induced denaturation of proteins during freezing and its inhibition by surfactants. *J. Pharm. Sci.* 85:1325.

Chen, B-L., Arakawa, T., Hsu, L., Narhi, L., Tressel, T.J., and Chen, S.L., 1994. Strategies to suppress aggregation of recombinant keratinocyte growth factor during liquid formulation development. *J. Pharm. Sci.* 83:1657.

Chen, B., Costantino, H.R., Liu, J., Hsu, C.C., and Shire, S.J., 1999. Influence of calcium ions on the structure and stability of recombinant human deoxyribonuclease I in the aqueous and lyophilized states. *J. Pharm. Sci.* 88:477.

Cleland, J.L., Powell, M.F., and Shire, S.J., 1993. The development of stable protein formulations—A close look at protein aggregation, deamidation and oxidation. *Crit. Rev. Ther. Drug* 11:60.

Cleland, J.L., Lam, X., Kendrick, B.S., Yang, J., Yang, T-Z., Overcashier, D., Brooks, D., Hsu, C., and Carpenter, J.F., 2001. A specific molar ratio of stabilizer to protein is required for storage stability of a lyophilized monoclonal antibody. *J. Pharm. Sci.* 90:310.

Carrasquillo, K.G., Sanchez, C., Griebenow, K., 2000. Relationship between conformational stability and lyophilization-induced structural changes in chymotrypsin. *Biotechnol. Appl. Biochem.* 31:41.

Costantino, H.R., Curley, J.G., Wu, S., and Hsu, C.C., 1997. Water sorption behavior of lyophilized protein-sugar systems and implications for solid-state interactions. *Int. J. Pharm.* 166:211.

Costantino, H.R., Griebenow, K., Mishra, P., Langer, R., and Klibanov, A.M., 1995. Fourier-transform infrared spectroscopic investigation of protein stability in the lyophilized form. *Biochim. Biophys. Acta* 1253:69.

Costantino, H.R., Carrasquillo, K.G., Cordero, R.A., Mumenthaler, M., Hsu, C.C., and Griebenow, K., 1998. Effect of excipients on the stability and structure of lyophilized recombinant human growth hormone. *J. Pharm. Sci.* 87:1412.

Craig, D.Q., Royall, P.G., 1998. The use of modulated temperature DSC for the study of pharmaceutical systems: potential uses and limitations. *Pharm. Res.* 15:1152.

Crowe, J.H., Carpenter, J.F., and Crowe, L.M., 1998. The role of vitrification in anhydrobiosis. *Annu. Rev. Physiol.* 60:73.

DeGrazio, F., Flynn, K., 1992. Lyophilization closures for protein based drugs. *J. Parenter. Sci. Technol.* 46:54.

Samsung Bioepis Exhibit No. 1002
Page 7425

Dong, A., Prestrelski, S.J., Allison, S.D., and Carpenter, J.F., 1995. Infrared spectroscopic studies of lyophilization- and temperature-induced protein aggregation. *J. Pharm. Sci.* 84:415.

Duddu, S.P., and Dal Monte, P.R., 1997. Effect of glass transition temperature on the stability of lyophilized formulations containing a chimeric therapeutic monoclonal antibody. *Pharm. Res.* 14:591.

Duddu, S.P., Zhang, G., Dal Monte, P.R., 1997. The relationship between protein aggregation and molecular mobility below the glass transition temperature of lyophilized formulations containing a monoclonal antibody. *Pharm. Res.* 14:596.

Farhat, I.A., Orset, S., Moreau, P., Blanchard, J.M.V., 1998. FTIR study of hydration phenomena in protein-sugar systems. *J. Coll. Interface Sci.* 207:200.

Franks, F., 1990. Freeze-drying: From empiricism to predictability. *Cryo-Letters* 11:93.

Franks, F., Hatley, R.H.M., and Mathias, S.F., 1991. Material science and the production of shelf stable biologicals. *BioPharm* 4(9):38.

Gatlin, L.A., and Nail, S.L., 1994. Protein purification process engineering. Freeze-drying: A practical overview. *Bioprocess Technol.* 18:317.

Goolcharran, C., Khossravi, M., and Borchardt, R.T., 2000. Chemical pathways of protein and peptide degradation, in: *Pharmaceutical Formulation Development of Peptides and Proteins*, S. Frokjaer and L. Hovgaard, eds., Taylor and Francis, London.

Griebenow, K., and Klibanov, A.M., 1995. Lyophilization-induced reversible changes in the secondary structure of proteins. *Proc. Natl. Acad. Sci. USA* 92:10969.

Gu, L.D., Erds, E.A., Chiang, H.-S., Calderwood, T., Tsai, K., Visor, G.C., Duffy, J., Hsu, W.C., and Foster, L.C., 1991. Stability of interleukin-1 beta (IL-1 beta) in aqueous solution: analytical methods, kinetics, products, and solution formulation implications. *Pharm. Res.* 8:485.

Hageman, M., 1992. Water sorption and solid state stability of proteins. in: *Stability of Protein Pharmaceuticals. Part A. Chemical and Physical Pathways of Protein Degradation*, T. Ahern and M.C. Manning, eds., Plenum Press, New York.

Hancock, B.C., Shamblin, S.L., and Zografi, G., 1995. Molecular mobility of amorphous pharmaceutical solids below their glass transition temperature. *Pharm. Res.* 12:799.

Heller, M.C., Carpenter, J.F., and Randolph, T.W., 1996. Effects of phase separating systems on lyophilized hemoglobin. *J. Pharm. Sci.* 85:1358.

Her, L.M., Jefferis, R.P., Gatlin, L.A., Braxton, B., and Nail, S.L., 1994. Measurement of glass transition temperatures in freeze concentrated solutions of non-electrolytes by electrical thermal analysis. *Pharm. Res.* 11:1023.

Her, L.M., and Nail, S.L., 1994. Measurement of glass transition temperatures of freeze-concentrated solutes by differential scanning calorimetry. *Pharm. Res.* 11:54.

Her, L.M., Deras, M., and Nail, S.L., 1995. Electrolyte-induced changes in glass transition temperatures of freeze-concentrated solutes. *Pharm. Res.* 12:768.

Hora, M.S., and Wolfe, S.N., 1999. Critical steps in the preparation of elastomeric closures for biopharmaceutical freeze-dried products, in: *Freeze-Drying/Lyophilization of Pharmaceuticals and Biological Products*, L. Rey and J.C. May, eds., Marcell Dekker, New York.

Izutsu, K., Yoshioka, S., Kojima, S., Randolph, T.W., and Carpenter, J.F., 1996. Effects of sugars and polymers on crystallization of poly (ethylene glycol) in frozen solutions: Phase separation between incompatible polymers. *Pharm. Res.* 13:1393.

Samsung Bioepis Exhibit No. 1002
Page 7426

Izutsu, K., Yoshioka, S., and Teroa, T., 1993. Decreased protein-stabilizing effects of cryoprotectants due to crystallization. *Pharm. Res.* 10:1232.

Kendrick, B.S., Chang, B.S., Arakawa, T., Peterson, B., Randolph, T.W., Manning, M.C., and Carpenter, J.F., 1997. Preferential exclusion of sucrose from recombinant interleukin-1 receptor antagonist: Role in restricted conformational mobility and compaction of native state. *Proc. Nat. Acad. Sci.* 94:11917.

Kendrick, B.S., Carpenter, J.F., Cleland, J.L., and Randolph, T.W., 1998. A transient expansion of the native state precedes aggregation of recombinant human interferon-gamma. *Proc. Nat. Acad. Sci.* 95:14142.

Kim, Y., Wall, J.S., Meyer, J., Murphy, C., Randolph, T.W., Manning, M.C., Solomon, A., and Carpenter, J.F., 2000. Thermodynamic modulation of light chain amyloid fibril formation. *J. Biol. Chem.* 275:1570.

Kreilgaard, L., Frokjaer, S., Flink, J.M., Randolph, T.W., and Carpenter, J.F., 1998a. Effects of additives on the stability of recombinant human Factor XIII during freeze-drying and storage in the dried solid. *Arch. Biochem. Biophys.* 360:121.

Kreilgaard, L., Jones, L., Randolph, T.W., Frokjaer, S., Flink, J., Manning, M.C., and Carpenter, J.F., 1998b. Effects of Tween 20 on agitation- and freeze-thawing-induced aggregation of recombinant Factor XIII. *J. Pharm. Sci.* 87:1597.

Kreilgaard, L., Frokjaer, S., Flink, J.M., Randolph, T.W., and Carpenter, J.F., 1999. Effects of additives on the stability of *Humicola lanuginosa* lipase during freeze-drying and storage in the dried solid. *J. Pharm. Sci.* 88:281.

Lam, X.M., Costantino, H.R., Overcashier, D.E., Nguyen, T.H., and Hsu, C.C., 1996. Replacing succinate with glycolate buffer improves the stability of lyophilized interferon-gamma. *Int. J. Pharm.* 142:85.

Levine, H., and Slade, L., 1988. Thermomechanical properties of small-carbohydrate-water glasses and "rubbers": Kinetically metastable systems at subzero temperatures. *J. Chem. Soc. Faraday Trans.* 1(84):2619.

Levine, H., and Slade, L., 1992. Glass transitions in foods, in: *Physical Chemistry of Foods*, H.S. Shartxberg and R.W. Hartel, eds., Marcel Dekker, NewYork.

Li, S., Patapoff, T.W., Overcashier, D., Hsu, C., Nguyen, T.H., and Borchardt, R.T., 1996. Effects of reducing sugars on the chemical stability of human relaxin in the lyophilized state. *J. Pharm. Sci.* 85:873.

Lueckel, B., Helk, B., Bodmer, D., and Leuenberger, H., 1998. Effects of formulation and process variables on the aggregation of freeze-dried interleukin-6 (IL-6) after lyophilization and on storage. *Pharm. Dev. Technol.* 3:337.

Manning, M.C., Patel, K., and Borchardt, R.T., 1989. Stability of protein pharmaceuticals. *Pharm. Res.* 6:903.

Nail, S.L., and Gatlin, L.A., 1993. Freeze-drying: Principles and practice, in: *Pharmaceutical Dosage Forms: Parenteral Medications*, K.E. Avis, H.A. Lieberman and L. Lachman, eds., Marcel Dekker, New York.

Nail, S.L., Her, L.M., Proffitt, C.P., and Nail L.L., 1994. An improved microscope stage for direct observation of freezing and freeze drying. *Pharm. Res.* 11:1098.

Neet, K.E., and Timm, D.E., 1994. Conformational stability of dimeric proteins: quantitative studies by equilibrium denaturation. *Prot. Sci.* 3:2167.

Page C., Dawson, P., Woollacott, D., Thorpe, R., Mire-Sluis, 2000. Development of a lyophilization formulation that preserves the biological activity of the platelet-

Samsung Bioepis Exhibit No. 1002
Page 7427

inducing cytokine interleukin-11 at low concentrations. *J. Pharm. Pharmacol.* 52:19.

Pikal, M.J., 1990. Freeze-drying of proteins. Part I: Process design. *BioPharm* 3(8):18.

Pikal, M.J., 1994. Freeze-drying of proteins, in: *Formulation and Delivery of Proteins and Peptides*, J.L. Cleland and R. Langer, eds., Symposium Series 567, American Chemical Society, Washington, DC.

Pikal, M.J., 1999. Mechanisms of protein stabilization during freeze-drying and storage: The relative importance of thermodynamic stabilization and glassy state relaxation dynamics, in: *Freeze-Drying/Lyophilization of Pharmaceuticals and Biological Products*, L. Rey and J.C. May, eds., Marcell Dekker, New York.

Pikal, M.J., and Shah, S., 1992. Moisture transfer from stopper to product and resulting stability implications. *Dev. Biol. Standard.* 74:165.

Pikal, M.J., Shah, S., Roy, M.L., and Putman, R., 1990. The secondary drying stage of freeze-drying: Drying kinetics as a function of temperature and chamber pressure. *Int. J. Pharm.* 60:203.

Prestrelski, S.J., Tedeschi, N., Arakawa, T., and Carpenter, J.F., 1993a. Dehydration-induced conformational changes in proteins and their inhibition by stabilizers. *Biophys. J.* 65:661.

Prestrelski, S.J., Arakawa, T., and Carpenter, J.F., 1993b. Separation of freezing- and drying-induced denaturation of lyophilized proteins by stress-specific stabilization: II. Structural studies using infrared spectroscopy. *Arch. Biochem. Biophys.* 303:465.

Prestrelski, S.J., Pikal, K.A., and Arakawa, T., 1995. Optimization of lyophilization conditions for recombinant interleukin-2 by dried state conformational analysis using Fourier transform infrared spectroscopy. *Pharm. Res.* 12:1250.

Remmele, R.L. Jr., Stushnoff, C., and Carpenter, J.F., 1997. Real-time *in situ* monitoring of lysozyme during lyophilization using infrared spectroscopy: dehydration stress in the presence of sucrose. *Pharm. Res.* 14:1548.

Rey, L., and May, J.C., 1999. *Freeze-drying/Lyophilization of Pharmaceuticals and Biological Products*, Marcel Dekker, New York.

Roy, M.L., Pikal, M.J., Rickard, E.C., and Maloney, A.M., 1991. The effects of formulation and moisture on the stability of a freeze-dried monoclonal antibody-vinca conjugate: A test of the WLF glass transition theory. *Dev. Biol. Standard.* 74:323.

Shalaev, E.Y., and Franks, F., 1995. Structural glass transitions and thermophysical processes in amorphous carbohydrates and their supersaturated solutions. *J. Chem. Soc. Faraday Trans.* 91:1511.

Shalaev, E.Y., Lu, Q., Shalaeva, M., and Zografi, G., 2000. Acid-catalyzed inversion of sucrose in the amorphous state at very low levels of residual water. *Pharm. Res.* 17:366.

Skrabanja, A.T.P., de Meere, A.L.J., de Ruiter Rien, and van der Oetelaar, P.J.M., 1994. Lyophilization of iotechnology products. *PDA J. Pharm. Sci. Technol.* 48:311.

Strambini, G.B., and Gabellieri, E., 1996. Proteins in frozen solutions: Evidence of ice-induced partial unfolding. *Biophys. J.* 70:971.

Suzuki, T., Imamura, K., Fujimoto, H., and Okazaki, M., 1998. Relationship between hermal stabilizing effect of sucrose on LDH and sucrose-LDH hydrogen bond. *J. Chem. Eng. Japan* 31:565.

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Page 7428

Suzuki, T., Imamura, K., Fujimoto, H., and Okazaki, M., 1999. Role of sucrose-LDH hydrogen bond for thermal stabilizing effect of sucrose on freeze-dried LDH. *Drying Technol.* 17:1429.

Tanaka, T., Takeda, T., and Miyajama, R., 1991. Cryoprotective effect of saccharides on denaturation of catalase during freeze-drying. *Chem. Pharm. Bull.* 39:1091.

Timasheff, S.N., 1998. Control of protein stability and reactions by weakly interacting cosolvents: The simplicity of the complicated. *Adv. Prot. Chem.* 51:355.

Tzannis, S.T., and Prestrelski, S.J., 1999. Moisture effects on protein-excipient interactions in spray-dried powders. Nature of destabilizing effects of sucrose. *J. Pharm. Sci.* 88:360.

van den Berg, L., 1959. The effect of addition of sodium and potassium chloride to the reciprocal system: KH_2-PO_4-Na_2HPO_4-H_2O on pH and composition during freezing, *Arch. Biochem. Biophys.* 84:305–315.

van den Berg, L., and Rose, D., 1959. The effect of freezing on the pH and composition of sodium and potassium solutions: The reciprocal system KH_2-PO_4-Na_2HPO_4-H_2O. *Arch. Biochem. Biophys.* 81:319.

Verdonck, E., Schaap, K., and Thomas, L.C., 1999. A discussion of the principles and applications of modulated temperature DSC (MTDSC). *Int. J. Pharm.* 192:3.

Volkin, D.B., and Middaugh, C.R., 1996. The characterization, stabilization, and formulation of acidic fibroblast growth factor. *Pharm. Biotechnol.* 9:181.

Wolkers, W.F., van Kilsdonk, M.G., and Hoekstra, F.A., 1998. Dehydration-induced conformational changes of poly-L-lysine as influenced by drying rate and carbohydrates. *Biochim. Biophys. Acta* 1425:127.

Yoshioka, S., Aso, Y., Kojima, S., and Tanimoto, T., 2000. Effect of polymer excipients on the enzyme activity of lyophilized bilirubin oxidase and beta-galactosidase formulations. *Chem. Pharm. Bull.* 48:283.

Yoshioka, S., Aso, Y., and Kojima, S., 1999. The effect of excipients on the molecular mobility of lyophilized formulations, as measured by glass transition temperature and NMR relaxation-based critical mobility temperature. *Pharm. Res.* 16:135.

Zhang, M.Z., Wen, J., Arakawa, T., and Prestrelski S.J., 1995. A new strategy for enhancing the stability of lyophilized protein: The effect of reconstitution medium on keratinocyte growth factor. *Pharm. Res.* 12:1447.

Zhang, M.Z., Pikal, K., Nguyen, T., Arakawa, T., and Prestrelski, S.J., 1996. The effect of the reconstitution medium on aggregation of lyophilized recombinant interleukin-2 and ribonuclease A. *Pharm. Res.* 13:643.

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8

High Throughput Formulation: Strategies for Rapid Development of Stable Protein Products

Rajiv Nayar and Mark C. Manning

INTRODUCTION

With the growing demand for new and innovative medicines, drug companies are spending record amounts of money on research and development. In the U.S. alone, research and development (R&D) investments are expected to exceed $24 billion in 1999, with investments in biotechnology totaling about $7 billion (Pharmaceutical Research and Manufacturers of America, 1999). As a result, new technologies are increasing the efficiency of the drug discovery process, and the drug pipelines have more products than ever in development. Nearly 350 biotechnology-related products are currently in clinical trials and over 50 are on the market (Pharmaceutical Research and Manufacturers of America, 1999). Pharmaceutical companies can now typically assess potential activities of up to 100,000 compounds a day using high throughput screening systems. In the biotechnology arena, recent advances in genomics, functional genomics, proteomics, bioinformatics and pharmacogenomics are facilitating the development of protein drug candidates at a much faster rate than was possible during the early years of the biotechnology industry. Couple these changes with the impending

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publication of the complete sequence of the human genome by 2001 and there is a potential for an additional 15,000 protein drugs from the predicted 150,000 potential genes in the human genome.

This new wave of protein drugs will include compounds that can function as growth factors, act as specific stimulators or suppressors of certain functions or exhibit activities that have not been observed before. Many experts anticipate a golden age of protein-based therapeutics in the twenty-first century. As various research enterprises gear up to search for these new candidates using high throughput cellular and molecular screening assays, there undoubtedly will be a need for a corresponding ability to formulate these drugs much more quickly than in the past. In addition, there will be increased use of novel delivery systems to administer these protein therapeutics efficiently, making stabilization even more challenging. In short, the pharmaceutical biotechnology industry must find new ways to speed up the drug development process, in order to keep pace with the drug discovery process. We think that the limiting factor for introduction of new biotechnology-derived products will not be finding new drug candidates, but efficient and rapid formulation of these compounds into acceptable dosage forms for delivery and testing in humans.

The potential of many new drug candidates flowing through the pipeline will require implementation of an entirely new approach towards product development. The particular challenge addressed here is the demand that will be placed upon formulation groups, where there will be increased expectations to develop stable formulations of:

more proteins,
in a shorter period of time,
with less material, and
with little, if any, increase in personnel.

Therefore, one must consider new strategies for meeting such challenges.

This chapter is intended to serve as a forum for discussing and evaluating what factors must be considered in trying to implement new approaches to formulating protein pharmaceuticals in a rapid, rational fashion. As a comprehensive strategy for developing stable formulations of protein pharmaceuticals is not currently available, we have assembled what we consider to be the key elements necessary to accomplish this critical task. This chapter should not be viewed as a finished product, but as a work-in-progress, encouraging pharmaceutical scientists to comment on the concept and to modify the approach to their particular situations.

Again, the overarching goal is to possess a unified strategy for accommodating many more proteins at one time, while meeting shortening timelines. It should be expected that there would be a limited supply of well-characterized material and a fixed personnel head count. We envision a scheme that we term "high throughput formulation" or HTF. It is based upon our current

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understanding regarding stabilization of proteins, but it also identifies areas where technical improvements must be made, especially in the area of analytical methodology and software. If the HTF strategy is successful, it should be possible to match the output of stable formulations by development groups with potential leads by discovery groups.

OVERALL STRUCTURE OF THE HTF APPROACH

The HTF concept includes four key elements, which are briefly outlined in Table 1. The heart of the HTF strategy is a decision plan, where the formulation scientist must decide on (a) the specific excipients to be used as well as (b) the final dosage form. Central to the HTF scheme is the use of a limited set of additives as well as dosage forms. Especially for lyophilized or freeze-dried formulations, our understanding of the behavior is such that some standard formulations can be envisioned (see Chapter 5). Also, the choice of basic solution conditions will be based upon preformulation studies that examine the behavior of the protein as a function of pH, ionic strength, and buffer type (see Chapters 1 and 3).

The second key aspect is an increased role for software and information databases. The preformulation studies could require a significant number of independent experiments to be conducted. Therefore, experimental design software should be used to reduce the number of experimental trials and optimize the results through statistical evaluation. In addition, there are a number of databases and associated programs for assessing physical and chemical properties based upon primary sequence of the protein through a number of websites (e.g. SWISS-PROT). These can provide insight into protein structure and other critical characteristics (e.g., pI, hydrophobicity and hydroflexibility plots and overall charge), and even identify residues that might be sensitive to chemical decomposition. We believe such capabilities will expand over time, and will play an increasingly important role in setting a rational plan for preformulation studies. Finally, we anticipate that specific protein stability databases will emerge, allowing scientists to gather data on specific development issues, without searching all of the open literature. Because information on protein stability comes from a multitude of disciplines (e.g., biochemistry, biophysics, food science, engineering, pharmaceutics, surface science and polymer science), open searches often miss crucial publications and documents. The challenge is not unlike Internet search engines trying to find a very specific subset of information on an ever-increasing world wide web. As these resources become more available and refined, they will emerge as invaluable tools for development of pharmaceutically acceptable formulations.

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Table 1.
Key Elements of the HTF Approach

1. Decision Plan to Finalize Choice of Excipients and Dosage Form
   - A. Choice of Dosage Form
     1. Frozen Solution
     2. Liquid Formulation
     3. Lyophilized Formulation
     4. Protein Suspension
     5. Specialized Drug Delivery System
   - B. Choice of Excipients
     1. Proper pH/Buffer Species
     2. Salt
     3. Stabilizer
     4. Bulking Agent (for lyophilized formulations)
     5. Surfactant
     6. Chelators
2. Use of Software and Databases to Assist in Formulation Development
   - A. Experimental Design Software
   - B. Software and Databases for Analysis of Structure/Function/Properties/Stability
   - C. Protein Stability Databases
3. Availability of Essential Analytical Methods
4. Design of Appropriate Stability Testing Protocols

The third aspect of HTF is assembling the correct analytical methods to assay the stability, both chemical and physical, of a wide range of proteins. The mechanisms by which proteins degrade have been widely reviewed (Manning et al., 1989; Pearlman and Nguyen, 1992; Cleland et al., 1993), so they will not be discussed here. However, given this backdrop, we will describe certain key methods that must be available in order to conduct proper HTF studies. All of the required instruments do not necessarily need to be available in-house, as many can be accessed through contract organizations and through academic collaborations. In addition, it should be noted that there exists a tremendous opportunity to modify existing analytical techniques for use with smaller sample sizes and for rapid, parallel sampling. One of the expected limitations will be lack of well-characterized material. Whereas access to a minimum of 200–500 mg of protein for formulation studies is desirable, the genomic approach will tax the ability of process scientists to provide adequate amounts of purified proteins for all compounds under development. Therefore, in some cases, the total amount of protein available may be an order of magnitude less than the optimum. This situation should serve as an incentive to identify new approaches for gathering stability data. We will discuss below possible new methodologies that would be useful in the HTF scheme, but have not yet been applied to formulation development.

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Finally, one must have a clear plan for stability testing, along with minimum criteria for a "stable" formulation. Certainly, that definition may change as a product matures. Minimum stability for the initial clinical studies may be quite different than the expectations for the final formulation tested in Phase III. Presently, there are debates within companies on how robust a preliminary formulation development or purification process must be in order to demonstrate efficacy in humans. Some companies will accept any minimal formulation in order to initiate clinical trials. However, even these formulations would likely have to have at least six to twelve months of shelf life. Our goal in the HTF approach is to develop a final formulation as early as possible in the drug development and testing process. If this goal can be accomplished, then fewer resources will need to be diverted to redesign the formulation later.

ROLE OF AN ESTABLISHED DECISION TREE FOR FORMULATION DESIGN

The central feature in the HTF scheme is a rational decision plan for choosing both the formulation components and the final dosage form. This decision tree must be flexible enough to accommodate a wide variety of proteins, it must allow for both liquid and solid dosage forms, and it must provide a rationale at each decision point. This framework allows the pharmaceutical scientist to decide what types of formulations are worth considering, both in terms of stability and the demands and desires of the marketing and clinical groups.

Often the design of a formulation of a protein pharmaceutical is not well developed. Companies attempt to expedite formulation selection by neglecting critical issues in formulation and stability. It is not uncommon to find sub-optimal formulations such as phosphate-buffered saline, possibly with the addition of human serum albumin as a ubiquitous protein stabilizer. For a few proteins, this approach has been sufficient, even resulting in commercially successful products, such as erythropoietin and granulocyte colony stimulating factor. However, there is a distinct danger in assuming that such successes with minimal formulations are generally applicable. Moreover, the current regulatory environment, and concerns in the general public surrounding the use of human-derived products in pharmaceuticals, makes the use of human serum albumin increasingly more difficult or even not acceptable. Therefore, there is pressure for any company selling a product that contains albumin to develop an albumin-free formulation. The same pressures are forcing companies to move from animal-derived surfactants to those obtained from vegetable sources. In addition, there is a realization that albumin, being a protein, is not indefinitely stable, and should have an expiration date above and beyond that of the active ingredient. All of these issues affect the

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list of possible excipients that can be employed in a protein pharmaceutical product.

Constraints on a Pharmaceutically Acceptable Protein Formulation

Before we discuss the choice of dosage forms and excipients in the context of maximizing protein stability, we need to recognize the myriad of constraints placed upon the formulation by the rest of the company and by regulatory bodies. The first constraint, given the global nature of the pharmaceutical industry, is to have excipients that are acceptable in the major markets. This includes avoiding animal-derived additives, as discussed above. It also means that the list of possible additives is effectively limited to those already found in approved products in North America, Europe, and Japan. While this is not an immutable rule, few companies are willing to bear the added cost of getting a new excipient on the market while seeking approval for a new drug product. A major impact of this constraint is to move away from formulations containing albumin, which is still found in a number of protein products. One can anticipate that these older formulations will disappear in the coming years and be reformulated into second-generation products. A recent example has been the development of an albumin-free formulations for recombinant Factor VIII.

The second consideration is the tonicity of the final formulation. If a protein drug is to be administered by intravenous bolus injection or subcutaneously, rather than by continuous infusion, there are strict isotonicity and pH considerations that have to be met for a pain-free injection. Similarly, there are constraints on the choice of excipients, as some (e.g., citrate) have been shown to be irritating upon injection. This is one example of restrictions being set by the clinical groups, which should be clearly defined prior to the start of the formulation process.

Likewise, the eventual route of administration must be taken into account. A convenient administration system is highly desirable for ease of use in the clinical setting, so it is both a marketing and clinical issue. For example, in the case of a subcutaneous injection, there is a maximal volume (~1 ml.) that can be given to a patient without discomfort. Given the expected dose range, this will define the protein concentration that must be used. For other routes of administration, the ability to manipulate the protein concentration as a variable may be possible. Otherwise, it will be fixed, and the decision point regarding protein concentration is removed from the process described below.

The fourth issue is to work with a well-characterized bulk drug substance. This criterion will require cooperation and collaboration with the process science groups involved in fermentation and purification. Variation in the quality of the

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starting material will compromise the validity of any preformulation or stability work done to date, as well as potentially invalidate data from toxicological tests.

The fifth constraint to be determined is the minimal stability required to complete proof-of-principle investigations, meaning animal studies and initial clinical trials. These investigations can extend for up to one year. Hence, at least a one-year shelf life for even the initial formulation may be required.

Finally, the manufacturing of the protein product must be possible at reasonable costs and allow adaptation to standard manufacturing processes. It is imperative that manufacturing economic analysis be conducted as soon as possible in the drug development process, including an assessment of whether sufficient manufacturing capability exists if the product is successful. Novel formulations that require special manufacturing setups and processes may not offer the advantages of speed which is necessary when one is confronted with development of numerous protein drug candidates. Most likely, specialized dosage forms and/or drug delivery systems will only be investigated with partners who possess the technology and capacity to solve these critical manufacturing issues. In addition, as the in-house pilot plants are challenged with manufacturing multiple products, with limited facilities and resources, there undoubtedly will be pressures to outsource the first clinical manufacturing because of both cost-effectiveness and timescale issues.

Proper Choice of Dosage Form

Considering all the therapeutic protein products on the market, it appears that three dosage forms are viable. The most preferred would be a solution formulation that is typically stored in the refrigerator and preferably in a pre-filled syringe. Second, a frozen solution loaded in a syringe or vial and thawed at the site of administration. Third, a lyophilized formulation, which can be stored at room temperature and reconstituted when needed. Each one of these dosage forms offers various advantages and disadvantages, in the speed of development, manufacturing, packaging and shipment logistics, and in administration of the product to the patient.

Generally, a frozen protein formulation offers the advantages of rapid development provided the protein is stable after multiple freeze/thaw cycles. It is also important to note that freezing can lead to selective precipitation of certain buffer species, producing possibly large pH shifts (van den Berg, 1959; van den Berg and Rose, 1959). This variation in pH, and the potential for damage at the water-ice interface (Chang et al., 1996; Hsu et al., 1995; Krielgaard et al., 1998), might limit the use of such a dosage form. Furthermore, there are disadvantages associated

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with a frozen formulation concerning the packaging complexities involved in labeling, storage and maintaining the cold chain during transportation of a frozen product. These factors can add significantly to the development costs.

The second option for dosage form is a stable liquid formulation that can be stored in the refrigerator or at room temperature. This dosage form offers the advantages of potentially rapid development and ease of administration. However, for most proteins maintaining physical and chemical stabilities in aqueous solution for an extended period of time is extremely difficult. In addition, because the protein is free in solution there is potential for adsorption to the vial, whether it is made of glass or some type of plastic. A liquid product will have to be shown to be impervious to damage at the air-water interface during transportation, because agitation-induced denaturation and aggregation is a common problem with therapeutic proteins. There is also the possibility of leaching of metals and organics from the container and stopper, which is why some of the vials will need to be inverted during stability testing. These problems have been reported for drug products of all types (Airaudo et al., 1990; Nedich, 1983). Leached materials are particularly problematic with plastic vials and non-Teflon-coated stoppers. With glass vials the leachables could include ionic species that alter the pH and ionic strength of the solution during storage. Finally, the integrity of the container closure must be demonstrated, in order to ensure sterility of the product. For multi-use vials, preservatives can be added, but some are known to foster degradation of proteins (Lam et al., 1997; Rodrigues-Silva et al., 1999). Therefore, development of a stable liquid protein formulation has to be considered very carefully. Determination of the primary pathways responsible for decomposition of the active therapeutic protein is critical for developing this particular dosage form. All of these issues can add significant costs in terms of resources and analytical testing.

Most protein pharmaceuticals currently on the market are sold as lyophilized formulations. Placing proteins in the dried state significantly increases the shelf life, if the proper formulation and lyophilization cycle are used. The combined stresses of drying and freezing are inherently damaging to proteins, and only proper formulation will ensure that a stable product will be obtained. Fortunately, enough is understood now about freezing and drying of proteins that rational design of lyophilized formulations can be undertaken. Moreover, there are some formulations that seem to work well for a variety of proteins. We suggest that part of the strategy for rapidly identifying viable formulations is to make liberal use of some standard protein formulations. The rationale for these compositions has been described in detail in a number of review articles and book chapters, and is discussed in Chapter 5 (MacKenzie, 1976; Franks, 1990; Pikal, 1985, 1994; Carpenter and Chang, 1996; Carpenter et al., 1997). An approach towards developing a lyophilized formulation is described below.

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As other formulation possibilities become more developed, one can modify the decision tree to accommodate them. For example, one can imagine alternatives to lyophilization for obtaining solid dosage forms. Both spray-drying and spray-freeze drying are being studied. However, it is expected that the formulation strategy for these processes will be quite similar to that used for lyophilization.

The most likely future additions to the dosage form list will be suspensions and specialized drug delivery systems. Protein suspensions can be produced by a number of different methods. The protein could be dried and suspended in a non-aqueous medium. Alternatively, the protein could be salted-out, using preferentially excluded solutes, such as ammonium sulfate or sucrose, leading to an aqueous vehicle containing most of the protein in its native state but in a solid form, because the solubility limit has been exceeded. The same effect might be accomplished by adjusting the pH to be near the isoelectric point of the protein. For the current version of HTF, we have chosen not to include these dosage forms until their use becomes more widespread and manufacturing facilities are developed to accommodate production of clinical batches.

The same can be said of specialized drug delivery systems, such as polymeric microspheres. Poly lactide/poly glycolide microspheres for sustained delivery of peptides have been in use for years. Recently, reports of their use for proteins, such as human growth hormone, have appeared. These systems bring their own specific stability issues (Cleland, 1998) in addition to making analysis of the stability of the active ingredient more challenging (Yang et al., 1999).

As discussed above, input from various departments, such as discovery research, pharmacokinetics, toxicology, purification, manufacturing, clinical and marketing, must be considered when deciding on the most appropriate dosage form for a given product. In the past, the drug development process has been more or less sequential, going from research to development to clinical, to manufacturing, and then to marketing. A change in strategy is required where there is more interactive, simultaneous communication between the various drug development departments. With the proper information from the other units, one should rapidly be able to decide on dosage form and develop candidate formulations.

Preformulation Studies

The exquisite sensitivity of protein structure, function, and stability to the primary sequence does not readily lend itself to a generic approach for protein formulation. Therefore, determination of the relative importance of various degradation pathways and elucidation of instability mechanisms for a given protein are essential. Even for closely related proteins, the relative stability and major

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pathways for degradation might be quite different. Thus, the preformulation studies of new protein entities become very important and crucial tasks in the overall HTF strategy.

Preformulation refers to experimental studies designed at understanding the chemical and physical behavior of the protein or drug candidate. The major focus is on the solubility and stability as a function of a number of extrinsic factors, such as pH, protein concentration, ionic strength, buffer composition and temperature. This information will guide the appropriate choice of excipients, buffers and final protein concentration. Although a number of studies could be envisioned, HTF requires that these studies be very specific and focused. Any information that may narrow the choices would be essential for speedy formulation development. Relevant data can be gathered from the process-engineering group responsible for purification or literature studies on similar proteins, even if it is anecdotal. The final dosage form must also include constraints from marketing, clinical and regulatory groups addressing issues related to eventual approval of a pharmaceutically acceptable formulation for market. Ultimately, all of these data may be required to ascertain the most beneficial composition of excipients and the most appropriate dosage form for the protein product in hand.

Typical time scales for preformulation can range from one to three months depending on what assay systems have been established by the research groups and whether they can be utilized as stability-indicating. Therefore, early involvement of all of the formulation experts is critical for rapid development of lead formulations during the developmental phase. An additional driving force is also the fixed time required to generate the stability data to validate the formulation choice.

Proper Choice of Excipients

Liquid Formulations. If a solution dosage form is indicated, then there will be a finite set of possible excipients, restricting choices to those that are found in approved products and have been shown to be effective in protein formulations. For solution formulations, a list of possible excipients is given in Table 2.

Given that most protein formulations will exist at pH values between 4 and 9, there is a limited set of buffers that will exhibit sufficient buffering capacity. A number have been used in protein formulations and they are listed in Table 2. The main criteria for selection are good buffering capacity at the desired pH and lack of ability to accelerate specific chemical and physical reactions. Some proteins can preferentially bind certain buffer species, altering the conformational stability of the protein. In addition, some buffers (e.g., phosphate, Tris) have been

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Table 2.
Possible Excipients for Use in Liquid Formulations

Excipient Class	Choices
Buffers	Histidine, Succinate, Acetate, Citrate Phosphate, Tris, Carbonate
Salts	Sodium Chloride, Calcium Chloride Magnesium Chloride
Non-Specific Stabilizers	Sucrose, Trehalose, other sugars, Amino acids (e.g., lysine, glycine)
Specific Stabilizers	depends upon the protein
Surfactants	Tween 20, Tween 80, Pluronic F-68 Sodium Dodecyl Sulfate
Chelators	EDTA

reported to catalyze certain hydrolytic reactions, such as deamidation (Patel et al., 1990).

Because proteins are polyelectrolytes, the ionic strength of the medium can often have an effect on structural stability. The most common salt used in protein formulations is sodium chloride. For an isotonic product, often it is advantageous to keep the concentration of salt as low as possible. Otherwise, the ability to employ stabilizing excipients will be limited.

Structural stabilization of proteins by specific ligands or cofactors in solution formulations can be important for formulation optimization. The mechanism by which these compounds increase thermodynamic stability is described by Timasheff (1992, 1995, 1998) and in other Chapters in this book. Several pharmaceutically important proteins have been stabilized by addition of specific ligands (including metals ions such as calcium), including fibroblast growth factor (Volkin et al., 1993) and DNAse (Chen et al., 1999).

To stabilize proteins (both in aqueous solution and during freezing) with non-specific compounds (e.g., sugars), relatively high concentrations (ca. > 0.2 M) of ligand (solute) are needed to affect protein stability. The mechanism of such stabilization is described in more detail in Chapters 2 and 3. The main point for the current discussion is that in the presence of nonspecific stabilizing excipients fewer protein molecules are unfolded at any point in time, either partially or completely, which reduces the amount of aggregation-competent species and the rate of aggregation (Kendrick et al., 1997, 1998). Addition of small amounts of a specific ligand should theoretically accomplish the same outcome. The most effective non-specific stabilizers tend to be disaccharides, such as sucrose and trehalose. However, certain salts, amino acids, and polymers are preferentially excluded as well. Still, unless there is evidence for advantage in use of a

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particular compound from this group, sucrose and trehalose should remain the first-line choices.

Finally, one must decide on whether to include a surfactant in the formulation. Surfactants have been shown to inhibit protein aggregation during agitation (e.g., Bam et al., 1995; Krielgaard et al., 1998). The mechanisms by which surfactants stabilize proteins are discussed in a separate Chapter. The surfactant often will be most effective at or above the critical micelle concentration. In Table 2, only the surfactants that have been approved for use in parenteral products in the U.S. have been listed. Note that one potential drawback of including surfactants in the formulation are contaminants (e.g., peroxides) that could cause chemical degradation of proteins (e.g., oxidation).

Lyophilized Formulations. It can be assumed that most proteins will not exhibit sufficient stability in aqueous solution to allow a liquid formulation to be developed. Our understanding of the basic requirements for obtaining a stable lyophilized protein formulation is relatively well developed. The question then is what combination of excipients will allow such a formulation to be prepared. The minimal composition includes a buffer species, an additive capable of forming an amorphous glassy state and inhibiting lyophilization-induced unfolding in which the protein remains entrapped, a bulking agent to provide cake stability, and possibly a surfactant to retard surface-induced damage and/or promote refolding. The rationales for the minimal criteria for a stable lyophilized formation and the choice of appropriate excipients are described in detail in another Chapter.

While a comprehensive formulation development algorithm is yet to be published, following the HTF process outlined above, one can imagine obtaining a rational approach towards selecting final candidate formulation that can be used routinely, even with new classes of therapeutic proteins. Clearly, the details of each case will be guided by the outcomes of the preformulation studies. However, even without a complete data set from the preformulation experiments, implementation of a few standard formulations should be possible. Ultimately, we believe there will be a relatively small number (six of less) of generic, standard lyophilized formulations that will work for nearly all proteins. Each of these will have a corresponding lyophilization cycle that can be used to achieve optimal results. When the HTF process has evolved to this optimal state, the personnel demands be greatly reduced (in terms of full-time equivalents, or FTEs) and the time lines should shrink significantly from the current average of nearly one year.

Estimates of Resources Needed for Formulation Development

The current estimates of the resources required and the possible timelines for formulation development are given in Table 3. Specialized formulations

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Table 3.
Estimated Resources Needed for Development of Final Stable Formulations of Protein Pharmaceuticals

Resource	Current Status	Initial HTF Targets	Optimal HTF Targets
• Personnel			
Standard Formulations	2 FTE	2 FTE	2 FTE
Complex Formulations	3–5 FTE 3–4 FTE	3 FTE	
• Time Scale			
Standard Formulations	12 months	6–9 months	3–6 months
Complex Formulations	12–24 months	12 months	6–9 months
• Minimal Amount of Material			
Standard Formulations	200–500 mg	100–200 mg	50–100 mg
Complex Formulations	400–2000 mg	200–500 mg	100–200 mg

(suspensions and controlled release dosage forms) will require more resources and less aggressive time lines (see Table 3). However, even this situation should improve as more of these types of products enter the marketplace. We anticipate that a significant number of these types of products will be approved over the next ten years. By then, most large pharmaceutical companies will have acquired the necessary expertise to develop them at rates comparable to the standard formulations of today. Once the HTF scheme has evolved to this stage, a company should be able to develop final formulations quickly and easily, even if personnel levels are relatively constant.

Interestingly, while a streamlined approach towards final formulation selection is the heart of the HTF strategy, it is the one aspect that can be introduced into any current product development scheme almost immediately, as it can be accomplished solely based on the current state of understanding of protein stabilization. Other aspects of the HTF package will demand more time to implement, as they will require significant advances to be made, particularly in the areas of analytical methodology and information technology. These two features of the HTF scheme are described below.

USE OF SOFTWARE AND DATABASES TO ASSIST IN THE HTF PROCESS

Unless formulation scientists have access to all pertinent information, critical choices for formulation components could be delayed or, even worse, incorrect. Therefore, we propose that a key aspect of the HTF process is to develop an integrated information system that can assist the scientist in making critical

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decisions. Each and every step in the formulation development process is predicated upon having the most recent and comprehensive information on the physicochemical properties of the protein under development, as well as on the effects of additives on protein structure and stability. Clearly, the latter part of this goal is ever evolving as our knowledge of protein chemistry increases. Therefore, the final HTF scheme will involve a central database of essential information. While a large amount of this type of information is currently available, it appears in a multitude of sources, generated by researchers in disparate disciplines, ranging from materials science to biochemistry to food science to pharmaceutics. This makes access to the data difficult. Unless efforts are made to assemble this data in a cohesive package soon, the challenge will become even greater. Once the task is accomplished, sufficient information should be available to predict the performance of certain types of formulations.

In the absence of such resources, one can still make use of existing software and databases. These are summarized below. Briefly, they cover three areas: predictive protein algorithms based upon primary sequence, sequence homology analysis software, and programs aimed at predicting relative stability of proteins. Summaries of the locations and availability of these programs and databases has recently been published (Cook, 1999; Apweiler, 1999).

Predictive algorithms allow one to analyze a primary sequence and predict a variety of chemical and physical properties. For example, there has been enormous effort to develop schemes whereby one can predict the three-dimensional folding of a protein based solely on its sequence. Although there have been some successes, it has been demonstrated in double-blind studies that analysis of new sequences, when they have no clearly related partner in the existing database, fail to provide an accurate prediction of the folded conformation (Orengo et al., 1999; Vencloyas et al., 1999). When the analysis can be guided by sequence homology, the accuracy rises dramatically. Likewise, one can imagine developing structure-stability relationships, based solely upon primary sequence. Some studies of this nature have been reported. For example, Pandit and co-workers relate the frequency of certain dipeptide sequences to instability in proteins (Reddy, 1996; Guruprasad et al., 1990). The result is an algorithm that predicts the relative stability of a protein. Although the degradation criteria are based upon intracellular stability, this approach does provide researchers with an expectation of the overall fragility of the protein in question.

In another example of this type of sequence analysis, Roger et al. (1986) identified regions in proteins that were high in proline, acidic side chains (Asp and Glu), serine, and threonine. The presence of these regions was associated with more rapid degradation *in vivo*. Similar correlations should exist for *in vitro* stability. Even programs to predict likely deamidation sites (where Asn is converted into Asp-like residues) would be helpful, especially as our ability to remove those sites by recombinant DNA technology improves. There is now a

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solid basis on which to predict both primary and secondary structure effects on deamidation rates (Wright, 1991; Xie and Schowen, 1999), which is considered in more detail in Chapter 4.

Given that numerous protein sequences, whether determined directly or extrapolated from nucleotide data, are available in databases that can be accessed via the world wide web, focused studies generating data on specific instabilities (e.g., deamidation, oxidation, aggregation) could lead to similar structure-stability relationships. The number of available sequences has been doubling every 18–24 months over the last ten years (Cook, 1999). Similarly, there has been an explosion in algorithms and software to analyze these data. Many focus on sequence homology and identification of functional and genetic relationships. Given these advances, it is not unreasonable to expect that stability-indicating profiles could be developed as well.

In addition to stability predictions, many programs are available to calculate important chemical and physical properties such as the isoelectric point, the hydrophobicity profile, the positioning of secondary structure elements, and the conformational flexibility. Knowledge of these properties would help the formulation scientist anticipate the behavior of a new molecule provided for development into a potential drug product. Furthermore, algorithms to predict correlations of these physical properties with physical and chemical stabilities of therapeutic proteins should be straightforward to design and implement with current programs.

ESSENTIAL ANALYTICAL METHODS

One must have access to certain equipment in order to characterize properly protein degradation pathways and effects of formulation variables on protein stability. In addition, we will discuss some areas where technological advancements must be made in order to allow formulation scientists to work with smaller amounts of protein. While a number of reliable and accurate stability-indicating assays exist, there needs to be efforts to miniaturize some of them. This will allow development of proteins where the amount of material is limited, e.g., less than 100 milligrams total.

The first analytical methods that are needed are those that can quantify the protein concentration. As determination of accurate extinction coefficients is straightforward, ultraviolet absorption spectroscopy is inexpensive and preferred over chemical methods, such as the Lowry method. One also needs to be able to monitor the global structure of the protein. Circular dichroism (CD) spectroscopy allows one to follow changes in both secondary and tertiary structure of proteins in solution (Manning, 1994). Infrared spectroscopy can also be used to detect

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secondary structural changes (see below), and fluorescence and derivative U\ spectroscopies can be used to study tertiary structure.

The second category of required analytical methods are those that monitc retention of native primary structure, namely, chromatographic methods, suc as reversed phase HPLC and ion exchange HPLC, or alternative methods lik capillary electrophoresis. Among the most useful applications of these HPL(methods is the generation of a "tryptic map", which is accomplished by digest ing the protein with a sequence-specific enzyme (e.g., trypsin) and separating th resulting peptide fragments by reversed phase HPLC. The pattern then become a sensitive fingerprint of the protein. Any chemical modification of the protei will cause one or more of the peaks to shift, indicating damage to the protein Mass spectrometry can be used to determine the specific chemical modificatio leading to the peak shift.

Among the most important degradation pathways for proteins is aggrega tion. A number of methods are available to characterize and quantify aggregatio levels in proteins, including polyacrylamide gel electrophoresis (PAGE), siz exclusion chromatography (SEC), light scattering techniques and analytical ultra centrifugation. Although each of these methods has its advantages and disac vantages, all four should be considered essential, as they can be complementary They allow both qualitative and quantitative characterization of the soluble an insoluble aggregates in a bulk substance or final formulation.

For lyophilized formulations, a certain set of equipment is considered to b the minimum required for conducting formulation development properly. Thes include an infrared (IR) spectrometer, a Karl Fischer titrator, a differential scar ning calorimeter (DSC), and x-ray diffractometer. The IR instrument will allo\ one to determine the extent of structural damage (at a secondary structure level in the solid state. In addition, it can be used to monitor the secondary structur composition in the original solution (prior to lyophilization), in the frozen soli and in the reconstituted product (Carpenter et al., 1998). X-ray diffraction c solids allows one to determine the crystallinity of a solid sample. In formulation where the bulking agent is crystallizing during the lyophilization process, on needs to know whether the crystallization is complete. Incomplete crystallizatio can lead to degradation during storage (cf. Carpenter et al., 1997). The Ka Fischer apparatus allows one to measure the amount of water in the final cak Typically, moisture contents need to be at 1–2 % in order for the product to hav the optimal glass transition temperature (T_g) and maximal stability. Finally, DS(is required to measure the T_g of the final formulation. The storage temperatur must below the T_g. As the minimal acceptable storage temperature for lyophilize formulations is room temperature, a minimum T_g would be about 40° C. In add tion to these instruments, access to a microscope equipped with a lyophilizatio stage is valuable.

The issue of how to develop formulations with limited amounts of materi is a critical concern. One can imagine adapting a number of existin

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technologies to assist in assessing the viability of a given formulation while using very small amounts of material. These would include:

1. microtiter plate assays,
2. robotics to assist in sample preparation and analysis,
3. ELISA or other antibody-based recognition assays for intact drug,
4. multi-sample adaptations of existing analytical techniques (e.g., DSC or analytical ultracentrifuge)
5. nanoseparation methodology (the so-called "lab-on-a-chip" approach),
6. surface-immobilized analytical methods, such as the BIAcore.

Each of these methods could provide significant increases in the capability to monitor the stability of a protein in a microformulation (less than 10 μg of active drug). For example, new chip technologies (either DNA- or protein-based) are becoming powerful tools that are increasingly used in all areas of drug discovery. They offer significant advantage over older technologies in terms of speed, sensitivity and cost. It is important to consider the use or implementation of these tools in formulation studies. One can envision protein chips doing for the study of proteins what DNA chips have done to the study of genes. That is, they can be used to identify critical parameters required for rapidly developing a stable formulation. Similarly, any of the other methods could be modified to assist the formulation scientist.

Finally, there has been a steady improvement in protein analysis tools such as mass spectrometry and more powerful and versatile biochemical techniques, which are not only more sensitive but offer the advantages of speed and low cost. So with the emerging technological advances in analytics and implementation of these enabling technologies in preformulation and formulation studies, one can envision a more time and resource efficient process for developing stable protein pharmaceuticals. Most of these technologies are being developed to meet the needs of efficient drug discovery research. The challenge here will be the speed at which the formulation scientists adapt these technologies into their programs. Unfortunately, these tools have not yet reached their maturity, so the implementation of micro-analytical methods for preformulation and formulation is crucial for developing HTF strategies for protein pharmaceuticals.

STABILITY PROTOCOLS

In addition to formal storage stability protocols that would be acceptable to regulatory agencies for real-time data, one must have standard approaches for assessing the possible long-term fate of the candidate formulations. Given the aggressive time line of the HTF strategy, one must rely, to some degree, upon accelerated storage studies to guide decision about suitable excipients. These

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could be done as isothermal studies, storing the protein at room temperatu and 40° C, for example. In certain cases, exposure to higher temperature such as 50° C or even 60° C, may be warranted. However, it is important to no that at higher temperatures, there is the distinct possibility that the rat determining pathway may change compared to that at the projected stora conditions.

An alternative to isothermal testing that needs to be considered is the use non-iosothermal stress testing. In this procedure, the sample is exposed to a line increase in temperature over time (or approximately linear with respect to time Samples are taken at set intervals and assayed for protein damage. Using an alg rithm developed by Lee and Stavchansky (1998), it is possible to determine t Arrhenius parameters in a single experiment, which would otherwise require least three separate isothermal studies. This approach has been demonstrated f a pentapeptide (Lee and Stavchansky, 1998), and should be extended to larg proteins in the future.

UNIFIED STRATEGY FOR HTF

The requirements for HTF can be summarized in four points. First, integ tion of speed, sensitivity and cost can be achieved by having in place go analytical methodologies for monitoring both chemical and physical instabil mechanisms. These methods can serve as tools for elucidating the mechanism instability of protein drugs and also as stability indicating assays during analy of the final formulation. Second, identification of the instability mechanism proteins can lend itself to developing a fast-track stable liquid protein formu tion, although the disadvantages of addressing biocompatibility and transpor tion issues have to be addressed with such dosage forms. A third requirement HTF is to have obviously a safe and a convenient dosage form for administ tion. Coupled with this criterion should be the requirement for manufacturi the formulation using standard processes, so that if needed the production can out-sourced to a contract facility.

Finally, technological advances will be a major driver for HTF and in gene biotechnology development. The ability to work at low microgram to milligr scale of protein is essential for the ability to start the process before the d candidate achieves development status. In many cases, companies may initi neglect formulation and stability issues that will haunt them in later stages development. The development of successful formulations is dependent u the ability to study both the *in vitro* and *in vivo* characteristics of the prot drug, as well as its intended application in the clinic. Thus, it is essential the formulation group to work closely with the discovery research,

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pharmacokinetics and the toxicology departments prior to the decision to move the drug into full-scale development. Resolving the difficulties in the early research and development stages will not only help in targeting the appropriate drug candidate for development, but also speed up the selection of a pharmaceutically acceptable formulation for clinical trials. What we propose is the involvement of the formulation group in a multi-disciplinary environment where interactions start early in the research environment. The appropriate lead candidates would be selected not only based on efficacy studies, but also on pre-formulation studies. We envision these studies to take one to three months to establish appropriate profiles for formulation development. As a result, the lead formulation can have the advantages of demonstrating efficacy and potential stability of the protein drug.

During the development process of a biotechnology drug we envision formulation to take typically three to six months. This period would encompass preformulation studies and evaluation of the formulation options determined by the planned clinical studies. Hence, protein formulation and purification can be developed concurrently using limited quantities of the product, and identification of the lead formulation could occur at the time of establishment of the purification process. What we propose is not a sequential development pathway for protein drugs, but a concurrent scenario where formulation design is initiated very early in the drug development pipeline. We are quite confident that the HTF can be coupled successfully with high throughput screening strategies of protein drugs and facilitate drug development within the inherent time and resource constraints of the pharmaceutical industry.

REFERENCES

Airaudo, C.B., Gayte-Sorbier, A., Momburg, R., and Laurent, P., 1990. Leaching of antioxidants and vulcanization accelerators from rubber closures into drug preparations, *J. Biomater. Sci., Polymer Ed.* 1:231.

Aldous, B.J., Auffret, A.D., and Franks, F., 1995. The crystallization of hydrates from amorphous carbohydrates, *Cryo-Letters* 16:181.

Anchordoquy, T.J. and Carpenter, J.F., 1996. Polymers protect lactate dehydrogenase during freeze-drying by inhibiting dissociation in the frozen state, *Arch. Biochem. Biophys.* 332:231.

Apweiler, R., 1999. Introduction to molecular biology databases, in: *EBI Online Manual on Molecular Biology Databases*, R. Apweiler, R. Lopez, and B. Marx, eds., URL: www.ebi.ac.uk/swissprot/Publications/mbd1.html.

Bam, N., Cleland, J., and Randolph, T.W., 1995. Stability of protein formulations: investigation of surfactant effects by a novel EPR spectroscopic technique, *Pharm. Res.* 12:1.

Samsung Bioepis Exhibit No. 1002
Page 7448

Bam, N., Cleland, J.L., Yang, J., Carpenter, J.F., Manning, M.C., and Randolph, T.W., 1998. Tween protects recombinant human growth hormone against agitation-induced damage via hydrophobic interactions, *J. Pharm. Sci.* 87:1554.

Bell, L.N., Touma, D.E., White, K.L., and Chen, Y.H., 1998. Glycine loss and Maillard browning as related to the glass transition in a model food system, *J. Food Sci.* 63:625.

Carpenter, J.F. and Chang, B.S., 1996. Lyophilization of protein pharmaceuticals, in *Biotechnology and Biopharmaceutical Manufacturing, Processing, and Preservation*, K.E. Avis and V.L. Wu, eds., Interpharm Press, Buffalo Grove, IL.

Carpenter, J.F., Pikal, M.J., Chang, B.S., and Randolph, T.W., 1997. Rational design of stable lyophilized protein formulations: some practical advice, *Pharm. Res.* 14:969.

Carpenter, J.F., Prestrelski, S.J., and Dong, A.,1998. Application of infrared spectroscopy to development of stable lyophilized protein formulations, *Eur. J. Pharm. Biopharm.* 45:231.

Chang, B.S. and Randall, C.S., 1992. Use of subambient thermal analysis to optimize protein lyophilization, *Cryobiology* 29:632.

Chang, B.S., Kendrick, B.S., and Carpenter, J.F., 1996. Surface-induced denaturation of proteins during freezing and its inhibition by surfactants, *J. Pharm. Sci.* 85:1325.

Chen, B., Costantino, H.R., Hsu, C.-C., and Shire, S.J., 1999. Influence of calcium ions on the structure and stability of recombinant human deoxyribonuclease I in the aqueous and lyophilized States, *J. Pharm. Sci.* 88:477.

Cleland, J.L., 1998. Solvent evaporation processes for the production of controlled release biodegradable microsphere formulations for therapeutics and vaccines, *Biotechnology Progress* 14:102.

Cleland, J.L., Powell, M.F., and Shire, S.J., 1993. The development of stable protein formulations: a close look at protein aggregation, deamidation, and oxidation, *Crit. Rev. Ther. Drug Carrier Systems* 10: 307.

Cook, J.L., 1999. Internet biomolecular resources, *Anal. Biochem.* 268:165.

Crowe, J.H., Carpenter, J.F., and Crowe, L.M., 1998. The role of vitrification in anhydrobiosis. *Ann. Rev. Physiol.* 60:73.

Franks, F., 1990. Freeze drying: from empiricism to predictability, *Cryo-Letters* 11:93.

Guruprasad, K., Reddy, B.V.B., and Pandit, M.W., 1990. Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting *in vivo* stability of a protein from its primary sequence, *Protein Eng.* 4:155.

Heller, M.C., Carpenter, J.F., and Randolph, T.W., 1999. Application of a thermodynamic model to the prediction of phase separations in freeze-concentrated formulations for protein lyophilization, *Arch. Biochem. Biophys.* 363:191.

Hsu, C.C., Nguyen, H.M., Yeung, D.A., Brooks, D.A., Koe, G.S., Bewley, T.A., and Pearlman, R., 1995. Surface denaturation at solid-void interface- a possible pathway by which opalescent particulotes form during the storage of lyophilized tissue-type plasminogen activator at high temperatures, *Pharm. Res.* 12:69.

Kendrick, B.S., Chang, B.S., Arakawa, T., Peterson, B., Randolph, T.W., Manning, M.C., and Carpenter, J.F., 1997. Preferential exclusion of sucrose from recombinant interleukin-1 receptor antagonist: role in restricted conformational mobility and compaction of native state, *Proc. Natl. Acad. Sci. USA* 94:11917.

Samsung Bioepis Exhibit No. 1002
Page 7449

Kendrick, B.S., Carpenter, J.F., Cleland, J.L., and Randolph TW., 1998. A transient expansion of the native state precedes aggregation of recombinant human interferon-gamma, *Proc. Natl. Acad. Sci. USA* 95:14142.

Koseki, T., Kitabatake, N., and Doi, E., 1990. Freezing denaturation of ovalbumin at acid pH, *J. Biochem.* 107:389.

Krielgaard, L., Jones, L. S., Randolph, T.W., Frokjaer, S., Flink, J.M., Manning, M.C., and Carpenter, J.F., 1998. Effect of Tween 20 on freeze-thawing and agitation-induced aggregation of recombinant human factor XIII, *J. Pharm. Sci.* 87:1597.

Lam, X.M., Patapoff, T.W., and Nguyen, T.H., 1997. The effect of benzyl alcohol on recombinant human interferon-gamma, *Pharm. Res.* 14:725.

Lee, J.C. and Timasheff, S.N., 1981. The stabilization of proteins by sucrose, *J. Biol. Chem.* 259:7193.

Lee, M.-L. and Stavchansky, S., 1998. Isothermal and nonisothermal decompositon of thymopentin and its analogs in aqueous solution, *Pharm. Res.* 15:1702.

Li, S., Schoneich, C., and Borchardt, R.T., 1995. Chemical instability of protein pharmaceuticals: mechanisms of oxidation and strategies for stabilization. *Biotechnol. Bioeng.* 48:490.

MacKenzie, A.P., 1976. The physico-chemical basis of the freeze-drying process, *Dev. Biol. Stand.* 36:51.

Manning, M.C., Patel, K., Borchardt, R.T., 1989. Stability of protein pharmaceuticals, *Pharm. Res.* 6:903.

Nedich, R.L., 1983. Selection of containers and closure systems for injectable products, *Am. J. Hosp. Pharm.* 40:1924.

Orengo, C.A., Bray, J.E., Hubbard, T., LoConte, L., and Sillitoe, I., 1999. Analysis and assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction, *Proteins: Struct., Func., Gen. Suppl.* 3:149.

Patel, K. and Borchardt, R.T., 1990. Chemical pathways of peptide degradation. II. kinetics of deamidation of an asparaginyl residue in a model hexapeptide, *Pharm. Res.* 7:703.

Pearlman, R. and Nguyen, T.H., 1992. Pharmaceutics of protein drugs, *J. Pharm. Pharmacol.* 44(Suppl 1):178.

Pharmaceutical Research and Manufacturers of America, 1999. PhRMA Annual Survey. URL: www.phrma.org.

Pikal, M.J., 1985. Use of laboratory data in freeze drying process design: Heat and mass transfer coefficients and the computer simulation of freeze-drying, *J. Parent. Drug Assoc.* 39:115.

Pikal, M.J., 1994. Freeze-drying of proteins, in: *Formulation and Delivery of Proteins and Peptides* (J.L. Cleland and R. Langer, Eds.) ACS Symposium Series. 567: 120.

Reddy, B.V., 1996. Structural distribution of dipeptides that are identified to be determinants of intracellular protein stability, *J. Biol. Struct. Dyn.* 14:201.

Rodrigues-Silva, R., Antunes, G.F., Velarde, D.T., and Santoro, M.M., 1999. Thermal stability studies of hyperimmune horse antivenoms, *Toxicon* 37:33.

Tanaka, T. Takeda, T., and Miyajama, R., 1991. Cryoprotective effect of saccharides on denaturation of catalase during freeze-drying, *Chem. Pharm. Bull.* 39:1091.

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Timasheff, S.N., 1992. Stabilization of protein structure by solvent additives, in: *Stability of Protein Pharmaceuticals. Part B. In Vivo Pathways of Degradation and Strategies for Protein Stabilization*, T.J. Ahern and M.C. Manning, eds., Plenum Press, New York.

Timasheff, S.N., 1995. Preferential interactions of water and cosolvents with proteins, in: *Protein-Solvent Interactions*, R.B. Gregory, ed., Marcel Dekker, New York.

van den Berg, L., 1959. The effect of addition of sodium and potassium chloride to the reciprocal system: KH_2-PO_4-Na_2HPO_4-H_2O on pH and composition during freezing, *Arch. Biochem. Biophys.* 84: 305.

van den Berg, L. and Rose, D., 1959. The effect of freezing on the pH and composition of sodium and potassium solutions: The reciprocal system KH_2-PO_4-Na_2HPO_4-H_2O, *Arch. Biochem. Biophys.* 81:319.

Vencloyas, C., Ginalski, K., and Fidelis, K., 1999. Addressing the issue of sequence-to-structure alignments in comparative modeling of CASP3 target proteins. *Proteins: Struct., Func., Gen.* Suppl. 3:231.

Volkin, D.B., Verticelli, A.M., Marfia, K.E., Burke, C.J., Mach, H., and C.R. Middaugh, C.R., 1993. Sucralfate and soluble sucrose octasulfate bind and stabilize acidic fibroblast growth factor, *Biochim. Biophys. Acta* 1203:18.

Wright, H.T., 1991 Sequence and structure determinants of the nonenzymatic deamidation of asparagine and glutamine residues in proteins, *Protein Eng.* 4:283.

Xie, M., and Schowen, R.L., 1999. Secondary structure and protein deamidation, *J. Pharm. Sci.* 88:8.

Yang, T.H., Dong, A., Meyer, J., Johnson, O.L., Cleland, J.L., and Carpenter, J.F., 1999. Use of infrared spectroscopy to assess secondary structure of human growth hormone within biodegradable microspheres, *J. Pharm. Sci.* 88:161.

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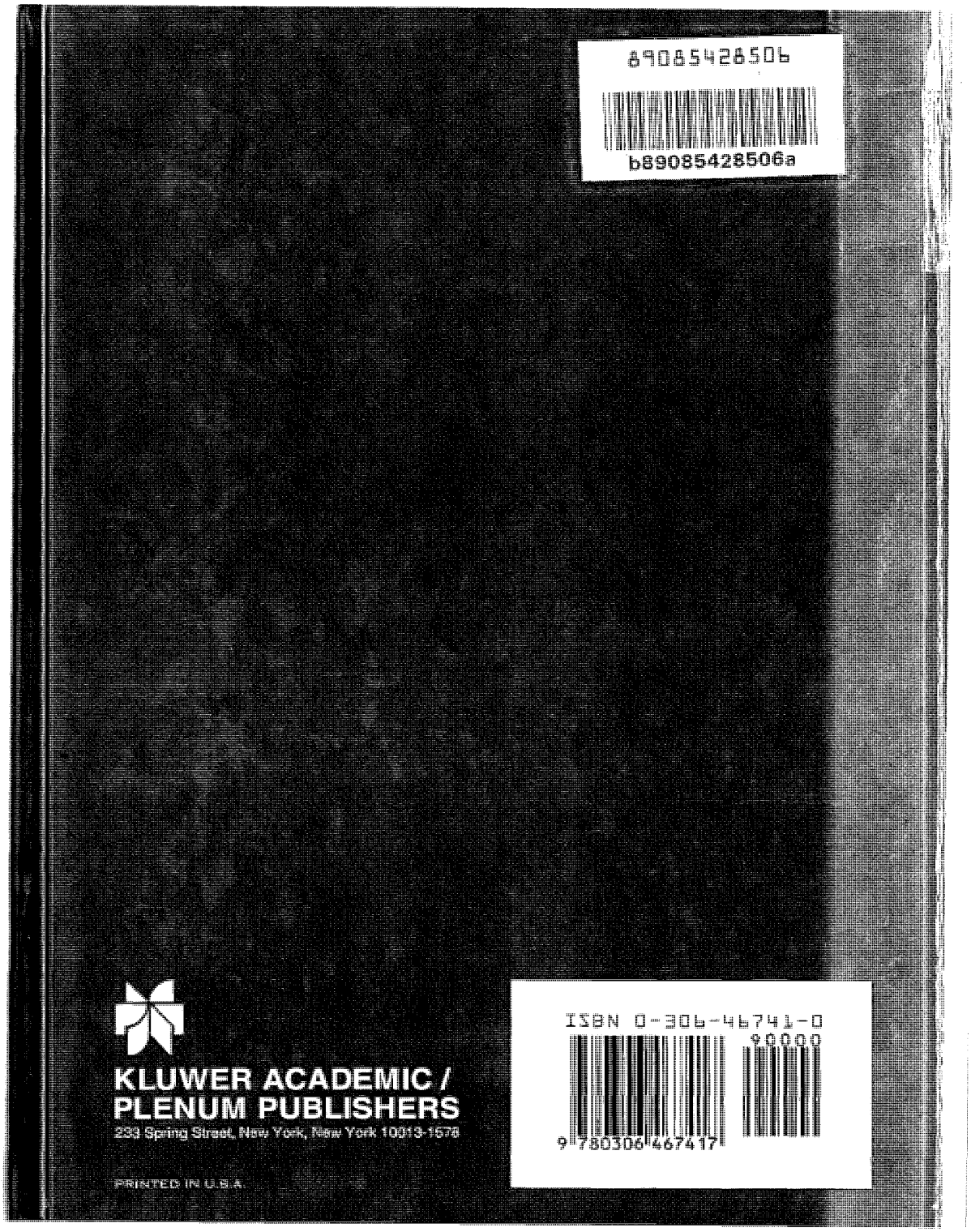
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NATURAL HISTORY OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Abstract *Background.* Paroxysmal nocturnal hemoglobinuria (PNH), which is characterized by intravascular hemolysis and venous thrombosis, is an acquired clonal disorder associated with a somatic mutation in a totipotent hematopoietic stem cell. An understanding of the natural history of PNH is essential to improve therapy.

Methods. We have followed a group of 80 consecutive patients with PNH who were referred to Hammersmith Hospital, London, between 1940 and 1970. They were treated with supportive measures, such as oral anticoagulant therapy after established thromboses, and transfusions.

Results. The median age of the patients at the time of diagnosis was 42 years (range, 16 to 75), and the median survival after diagnosis was 10 years, with 22 patients (28 percent) surviving for 25 years. Sixty patients have died; 28 of the 48 patients for whom the cause of death is known died from either venous thrombosis or hemorrhage. Thirty-one patients (39 percent) had one or more episodes of venous thrombosis during their illness. Of the 35 patients who survived for 10 years or more, 12 had a spontaneous clinical recovery. No PNH-affected cells were found among the erythrocytes or neutrophils of the patients in prolonged remission, but a few PNH-affected lymphocytes were detectable in three of the four patients tested. Leukemia did not develop in any of the patients.

Conclusions. PNH is a chronic disorder that curtails life. A spontaneous long-term remission can occur, which must be taken into account when considering potentially dangerous treatments, such as bone marrow transplantation. Platelet transfusions should be given, as appropriate, and long-term anticoagulation therapy should be considered for all patients. (N Engl J Med 1995;333:1253-8.)

PAROXYSMAL nocturnal hemoglobinuria (PNH) is an acquired disorder of hematopoiesis characterized by intravascular hemolysis and manifested by episodes of hemoglobinuria and life-threatening venous thromboses.[1] The cellular abnormality in this disorder is caused by a somatic mutation in a totipotent hematopoietic stem cell.[2,3] The cells derived from the abnormal clone are deficient in all surface proteins normally attached to the cell membrane by a glycosylphosphatidylinositol anchor.[4,5]

PNH was first described as a distinct clinical entity in 1882.[6] The cardinal diagnostic test, introduced in the late 1930s, is Ham's test,[7] which is based on the increased sensitivity of PNH-affected erythrocytes to lysis by complement. Deficiency of an antigen known as the membrane inhibitor of reactive lysis (CD59) is largely responsible for the hemolysis[8,9] and is implicated in the tendency for patients to have thromboses.[9] In the past two years the biochemical defect underlying PNH has been pinpointed at an early step in the biosynthesis of glycosylphosphatidylinositol molecules — namely, the transfer of *N*-acetylglucosamine to phosphatidylinositol.[10-12] The protein required for this step is encoded by a gene, *PIG-A*, that is somatically mutated in patients with PNH.[13-17]

Despite the remarkable progress in our understanding of this disorder, treatment has remained largely supportive. The only potentially curative therapy currently available is bone marrow transplantation,[18,19] which is associated with substantial morbidity and mortality.[20] To determine whether the management of PNH can be improved, it is important to know its natural history. We report the results of a long-term study (up to 48 years after the diagnosis) of a group of 80 patients with PNH seen at one institution between 1940 and 1970. This study has uncovered some surprising information on the natural history of the disorder and has identified relatively simple therapies that may reduce the associated morbidity and mortality.

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METHODS

Patients

Eighty patients with PNH were referred to Hammersmith Hospital in London between 1940 and 1970. In all patients the diagnosis was established or confirmed by a positive Ham's test.[21] This group of patients was last described in 1972.[22] Follow-up data have been obtained by contacting the patients' primary physicians and reviewing death certificates.

Complications and Causes of Death

The complications and causes of death reported were either unequivocally diagnosable on clinical grounds or were diagnosed after death. If a complication was not confirmed, it is not reported here. Thus, the reported incidence of complications is likely to be an underestimate, because many of the patients were seen at Hammersmith Hospital only infrequently. We compared the survival of the patients with the survival of a control group matched for sex and age.

Analysis of Blood Samples

In 1993 venous blood samples were obtained from five patients in either acid–citrate–dextrose or EDTA for the performance of Ham's tests and flow cytometric studies of the expression of glycosylphosphatidylinositol-linked proteins on erythrocytes and white cells, as previously described.[23,24] By definition, normal cells express normal levels of glycosylphosphatidylinositol-linked proteins, whereas PNH-affected cells are deficient in all these proteins.

RESULTS

Follow-up Studies

Sixty of the 80 patients were known to have died, and 6 patients were lost to follow-up before 1972. The remaining 14 patients were alive when last seen between

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1975 and 1994, and 9 of the 14 were known to be alive in 1994.

Clinical Presentation

The presenting features or initial diagnoses were reported previously.[22] The median age at the time of diagnosis was 42 years (range, 16 to 75). Forty-three (84 percent) of the 51 patients for whom reliable information was available had episodes of hemoglobinuria as a chief symptom at some time during their illness.

Course of Illness and Survival

The course of the disease in each of the 80 patients is shown in Figure 1. From the actuarial survival curve for the group, we calculated a median survival of approximately 10 years (Fig. 2). Twenty-five years after the diagnosis, 58 patients (72 percent) had died, and 22 (28 percent) were alive. The median age at the time of death was 56 years (range, 20 to 84), with a median interval of 10 years (range, 0 to 48) between diagnosis and death. Patient 10 received the diagnosis of PNH in 1939, had a spontaneous remission in 1949, and died of bronchial carcinoma in 1987, 48 years after the diagnosis.

Cause of Death

For 12 of the 60 patients who died (Table 1), the cause of death was unknown. In 28 of the other 48 patients (58 percent), the cause of death (either thrombosis or hemorrhage attributable to thrombocytopenia) was directly related to PNH. In the other 20 patients (42 percent), the cause of death was unrelated to PNH.

Venous Thrombosis

Thirty-one patients (39 percent) were known to have had one or more episodes of venous thrombosis during the course of their illness (Table 2), and several patients had repeated thromboses. In many patients the thrombosis was either fatal or life-threatening, and it was often unheralded.

Spontaneous Remission

Twelve patients (15 percent) had spontaneous clinical remissions (Fig. 1), with negative Ham's tests in all nine who were tested. We later obtained blood samples from five of these nine patients; in all five the erythrocytes expressed normal levels of glycosylphosphatidylinositol-linked proteins, with no demonstrable PNH-affected erythrocytes (Fig. 3A). In addition, of the four patients tested, none had PNH-affected neutrophils (Fig. 3A), but three of the four had small numbers of PNH-affected lymphocytes (Fig. 3B). Further analysis by double fluorescent staining demonstrated a subpopulation of PNH-affected lymphocytes among CD4+

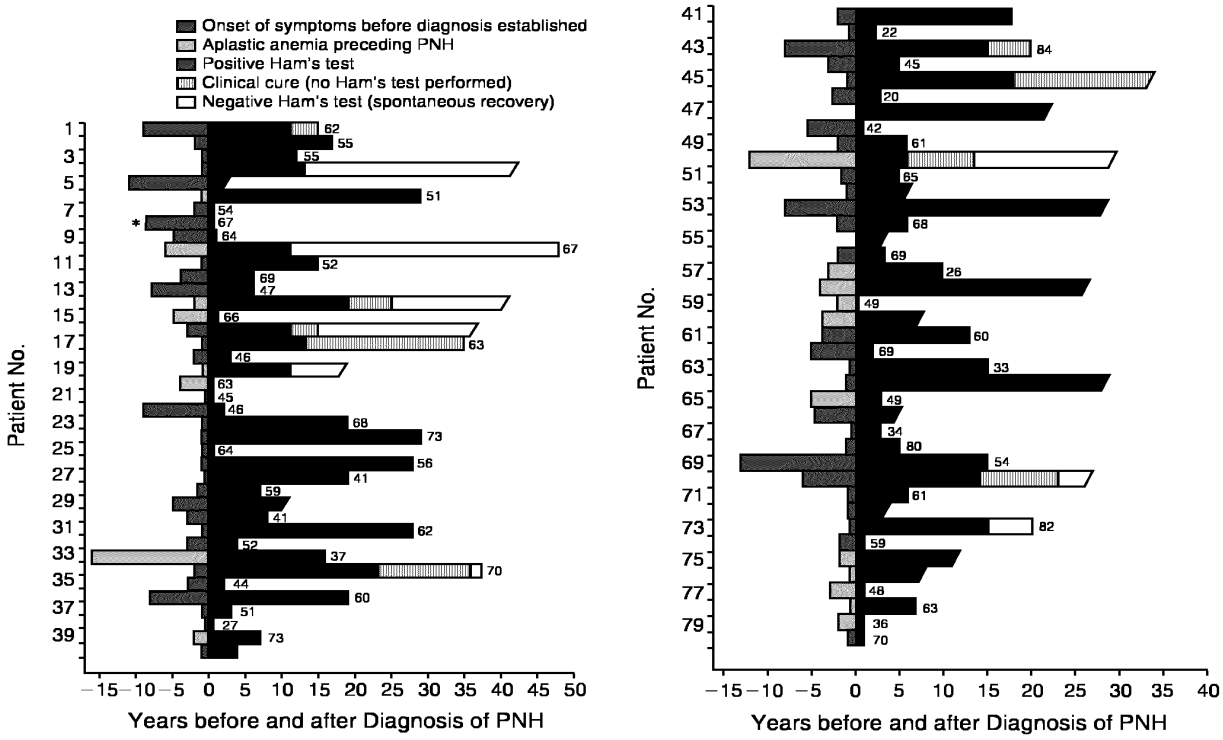


Figure 1. Course of Illness in 80 Consecutive Patients with PNH.

The x axis indicates the years before and after the diagnosis of PNH, which is denoted by the zero on the axis. Bars ending in a straight line indicate patients who died, and their ages at death are shown; bars ending in a diagonal line indicate surviving patients. Nine patients had complete clinical recovery, with negative Ham's tests. Three additional patients had protracted clinical remissions, but Ham's test was not repeated during remission. The asterisk indicates a prolonged but unspecified period of symptoms before the diagnosis was established.

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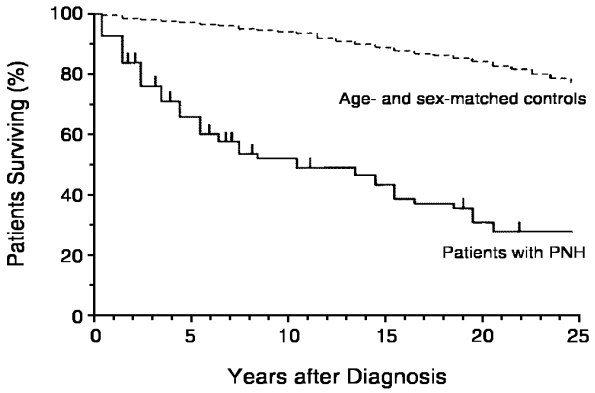


Figure 2. Actuarial Survival from the Time of Diagnosis in 80 Patients with PNH.

The median survival was 10 years. The expected survival of an age- and sex-matched control group is shown for comparison.

T cells, CD8+ T cells, and CD19+ B cells in all three patients.

Associated Disorders

Twenty-three patients (29 percent) received a diagnosis of aplastic anemia before the diagnosis of PNH. Nine of these patients subsequently had hemolytic PNH, with episodes of overt hemoglobinuria, and six did not; whether the remaining eight patients ever had hemoglobinuria could not be reliably determined.

At the time of the diagnosis, 64 of the 80 patients (80 percent) had thrombocytopenia (platelet count, <150,000 per cubic millimeter), and 38 patients (48 percent) had severe thrombocytopenia (platelet count, <50,000 per cubic millimeter). In addition, 44 patients (55 percent) had neutropenia (neutrophil count, <1500 per cubic millimeter).

In five patients the disorder progressed from hemolytic PNH to aplasia. Ham's test remained positive in four of these patients, but the fifth (Patient 34) subsequently had a negative Ham's test, with no PNH-affected erythrocytes or neutrophils. The patient died from bleeding due to severe thrombocytopenia 37 years after the diagnosis of PNH and at least 8 years after Ham's test had become negative.

Leukemia was not known to have developed in any of the patients.

Discussion

Clinical Features

In this group of 80 patients who were followed for up to 48 years, the median actuarial survival was 10 years, with 28 percent of the patients surviving for 25 years. Death was directly attributable to PNH or to bone marrow hypoplasia in 58 percent of the patients who died. Therapy with platelet concentrates should reduce mortality from hemorrhage due to thrombocytopenia.

At least 39 percent of the patients had venous thrombosis at some time during their illness. Many patients had initial thromboses that were life-threatening, but two (Patients 17 and 70), who probably had postpartum hepatic-vein thrombosis, recovered and survived for 34 and 27 years, respectively, after the thrombosis. One other patient (Patient 34) survived for 24 years after a thrombosis of the inferior mesenteric vein.

Spontaneous Remission

In some patients with PNH, the severity of the illness diminishes with time,[25] and some patients have a complete clinical remission,[22,26] although laboratory abnormalities may persist for years.[27] In this series 15 percent of the patients had spontaneous clinical remissions, and Ham's test became negative in the patients who were tested. Neither the severity of symptoms or complications nor the proportion of red cells lysed in Ham's test was correlated with an eventual remission. Among the patients with remissions, one (Patient 14) had severe, transfusion-dependent PNH and thromboses, and at

Table 1. Causes of Death in 60 Patients with PNH.

Cause of Death	No. of Patients
Probably related to PNH	
Venous thrombosis	
Hepatic vein	7
Inferior vena cava	1
Cerebral vein	1
Mesenteric vein	2
Pulmonary embolism	3
Hemorrhage	
Gastrointestinal	6
Subarachnoid	3
Intracerebral	2
Miscellaneous	
Liver failure	2
Intraabdominal event	1
Probably unrelated to PNH	
Arterial thrombosis	
Myocardial infarction	6
Cerebrovascular accident	2
Bronchopneumonia	3
Chronic obstructive airways disease plus cor pulmonale	2
Cardiac tamponade	1
Constrictive pericarditis	1
Renal failure	1
Amyloidosis	1
Lymphoma	1
Bronchial carcinoma	1
Epilepsy	1
Unknown	12

Table 2. Sites and Types of Thrombosis.

Site and Type of Thrombosis	No. of Patients
Intraabdominal	
Hepatic vein	8
Inferior vena cava	3
Mesenteric vein	4
Splenic vein	1
Renal vein	1
Unspecified	1
Other venous sites	
Cerebral vein	4
Pulmonary embolism	9
Deep vein	7
Superficial	3
Arterial	
Myocardial infarction	6
Cerebrovascular accident	2

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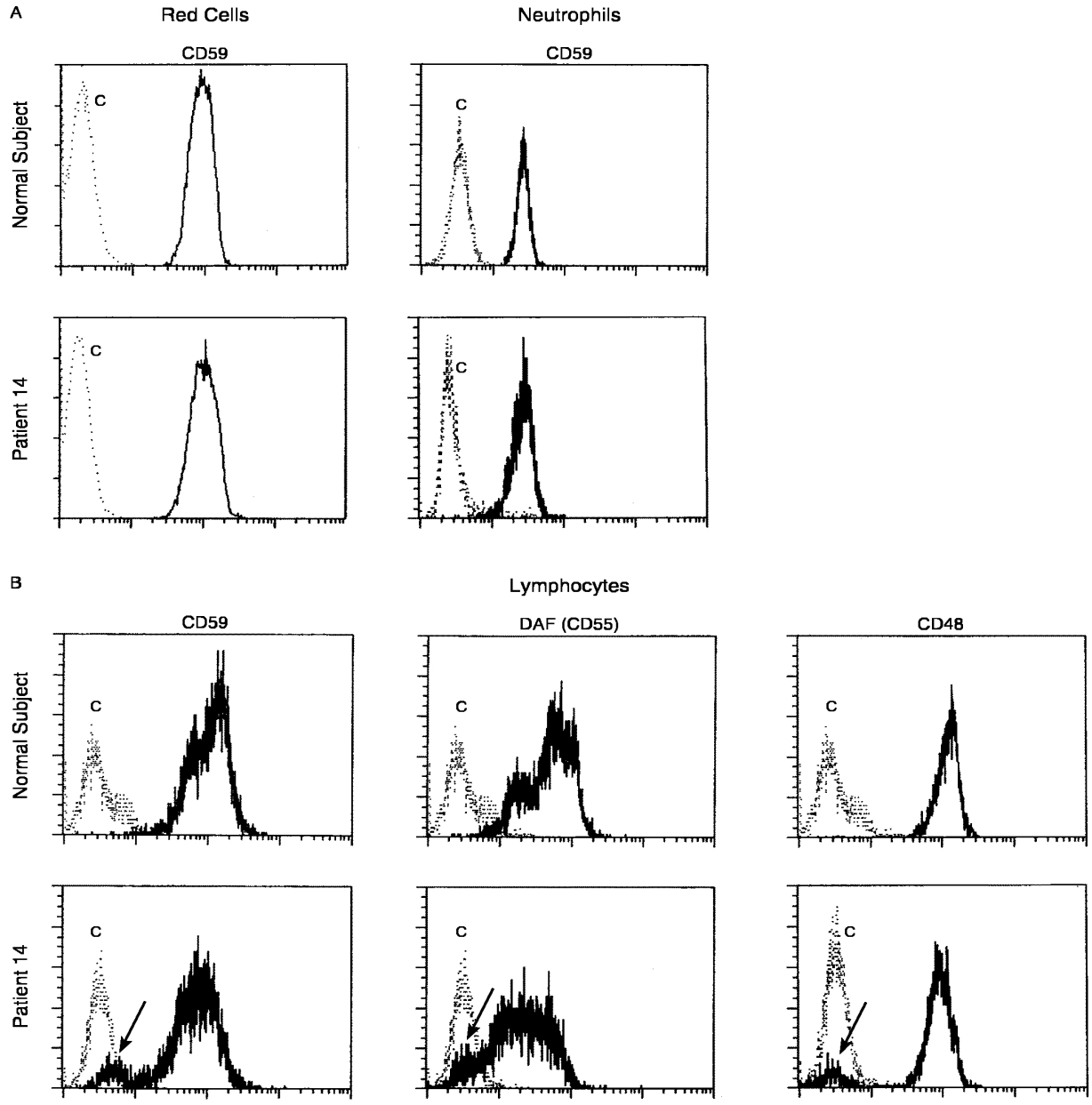


Figure 3. Flow-Cytometric Analysis of Blood Cells from a Normal Subject and Patient 14 after Spontaneous Recovery from PNH.

The analysis was carried out 20 years after the patient's clinical remission began and 17 years after her Ham's test had become negative. Panel A shows a unimodal distribution of cells with normal reactivity after reaction with anti-CD59 antibody (a molecule absent from the surface of PNH-affected cells). The distributions obtained with the patient's red cells and neutrophils are indistinguishable from those of the cells from a normal subject, demonstrating that no PNH-affected cells remain. C indicates distributions obtained in control experiments with an antibody directed against a molecule absent from normal blood cells.

Panel B shows the results of an analysis, similar to that in Panel A, carried out on peripheral-blood lymphocytes. The distributions of cells from a normal subject show that, as compared with the results of analyses with negative control antibody (C), all the lymphocytes reacted with all three antibodies used. The distribution is unimodal for CD48 and bimodal for CD59 and CD55. In contrast, the cells from Patient 14 have a small population of lymphocytes that are unreactive with all three antibodies (arrows), which indicates that these cells lack the corresponding antigen protein molecules on their surfaces. The fact that there is a peak of antigen-negative cells for three different proteins that are normally attached to the cell membrane by a glycosylphosphatidylinositol anchor means that the anchor is absent, which is characteristic of the PNH phenotype. The finding that the PNH clone was still present when there were no PNH-affected erythroid or myeloid cells in Patient 14 during a clinical and hematologic remission is consistent with the notion that this clone does not have a selective advantage once normal hematopoiesis has resumed. DAF denotes decay-accelerating factor.

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least two had over 50 percent lysis in earlier Ham's tests, suggesting that they had few residual normal stem cells at the height of their illness. The spontaneous remissions occurred between 10 and 20 years after the diagnosis of PNH. Of the 35 patients who survived 10 years or more, 12 (34 percent) eventually had remissions. A possible explanation for spontaneous remission is that the clones of PNH-affected cells have a finite life span, like normal somatic cells. Recovery may thus depend on the presence of normal stem cells capable of repopulating the bone marrow.[28]

There were no erythrocytes or neutrophils affected by PNH after recovery, but three of four patients tested had some residual abnormal lymphocytes. These results are consistent with the fact that lymphocytes have a much longer life span than myeloid and erythroid cells and that committed lymphocyte progenitor cells undergo division less frequently than the other cells. If the aging process postulated as being responsible for the gradual disappearance of clones of PNH-affected cells is related to the number of cell divisions, this may explain why abnormal lymphocytes persist for many years after the disappearance of abnormal neutrophils and erythrocytes.

PNH and Bone Marrow Failure

The data on this series of patients provide further evidence of the close relation between PNH and aplastic anemia. The coexistence of the two conditions in one patient was first reported in 1944,[29] with subsequent reports in 1952[30] and 1961.[31] Cultures of peripheral blood and bone marrow from patients with PNH consistently show reduced numbers of erythroid progenitor cells (erythroid burst-forming units) and myeloid progenitor cells (granulocyte–macrophage colony-forming units), even in the absence of pancytopenia,[32,33] as in patients with aplastic anemia.[34] In addition, PNH eventually develops in 10 to 31 percent of patients with aplastic anemia treated with immunosuppressive agents.[35] It is important to emphasize that PNH is not a complication of immunosuppressive treatment. Instead, the majority of patients with this disorder probably have an underlying aplastic process. Clones of PNH-affected cells may thus have a relative growth or survival advantage over the residual normal bone marrow in patients who have aplastic anemia.[1,28,36] It is conceivable that the mutation leading to the development of PNH occurs quite frequently but that, in the absence of marrow hypoplasia, the clone has difficulty establishing itself.[28]

PNH and Leukemia

Because of the reports of myelodysplasia and leukemia[1] — invariably, acute myeloid leukemia — in patients with PNH, it has been regarded as a preleukemic condition. Leukemia did not develop in any of our 80 patients, however, indicating that it is relatively rare in unselected patients with PNH. Acute myeloid leukemia eventually develops in approximately 5 percent of patients with aplastic anemia who survive the marrow aplasia.[35] Thus, aplastic anemia may predispose patients to both PNH and acute myeloid leukemia, but the development of PNH may not add to the risk of leukemia associated with uncomplicated aplastic anemia.

In most reported cases of leukemia in patients with PNH in whom the leukemic cells were studied,[37,38] the leukemic clone was derived from the PNH clone. According to a recent report of a patient with PNH who subsequently had a myelodysplastic syndrome, the blasts expressed normal levels of glycosylphosphatidylinositol-linked proteins,[39] indicating that the leukemic event may occur in the residual normal stem cells.

Management

The natural history of PNH is an important factor in decisions about therapy for individual patients. Thrombolytic treatment of hepatic-vein thrombosis with tissue plasminogen activator has been reported to be effective.[40] Oral anticoagulation therapy after venous thrombosis has been used since the 1950s.[25] In view of the high incidence of potentially fatal thrombosis, a strong case can be made for prophylactic anticoagulation treatment in all patients with PNH in whom there is no contraindication (such as severe thrombocytopenia). This approach may improve survival and reduce morbidity.

The only curative therapy at present is bone marrow transplantation, but it is available to only a small proportion of patients and is associated with substantial morbidity and mortality. The recent advances in the understanding of the pathogenesis of PNH and, in particular, of the molecular lesion open opportunities to explore new treatments, such as gene therapy. The possibility of spontaneous remission and of long-term survival must be taken into account when considering either bone marrow transplantation or other new treatments for this disorder.

We are indebted to the late Miss Eleanor Lloyd for her assistance in this project, to the patients, and to the physicians who originally referred the patients and provided follow-up data.

References

1. Rotoli B, Luzzatto L. Paroxysmal nocturnal haemoglobinuria. Baillieres Clin Haematol 1989;2:113-38.
2. Dacie JV. Paroxysmal nocturnal haemoglobinuria. Proc R Soc Med 1963; 56:587-96.
3. Oni SB, Osunkoya BO, Luzzatto L. Paroxysmal nocturnal hemoglobinuria: evidence for monoclonal origin of abnormal red cells. Blood 1970;36:145-52.
4. Ferguson MAJ. Glycosyl-phosphatidylinositol membrane anchors: the tale of a tail. Biochem Soc Trans 1992;20:243-56.
5. Rosse WF. Phosphatidylinositol-linked proteins and paroxysmal nocturnal hemoglobinuria. Blood 1990;75:1595-601.
6. Crosby WH. Paroxysmal nocturnal hemoglobinuria: a classic description by Paul Strübing in 1882, and a bibliography of the disease. Blood 1951;6:270-84.
7. Rosse WF. Dr. Ham's test revisited. Blood 1991;78:547-50.
8. Holguin MH, Fredrick LR, Bernshaw NJ, Wilcox LA, Parker CJ. Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. J Clin Invest 1989;84:7-17.
9. Yamashina M, Ueda E, Kinoshita T, et al. Inherited complete deficiency of 20-kilodalton homologous restriction factor (CD59) as a cause of paroxysmal nocturnal hemoglobinuria. N Engl J Med 1990;323:1184-9.
10. Armstrong C, Schubert J, Ueda E, et al. Affected paroxysmal nocturnal hemoglobinuria T lymphocytes harbor a common defect in assembly of *N*-acetyl-D-glucosamine inositol phospholipid corresponding to that in class A Thy-1⁻ murine lymphoma mutants. J Biol Chem 1992;267:25347-51.
11. Hillmen P, Bessler M, Mason PJ, Watkins WM, Luzzatto L. Specific defect in *N*-acetylglucosamine incorporation in the biosynthesis of the glycosylphosphatidylinositol anchor in cloned cell lines from patients with paroxysmal nocturnal hemoglobinuria. Proc Natl Acad Sci U S A 1993;90: 5272-6.

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12. Takahashi M, Takeda J, Hirose S, et al. Deficient biosynthesis of *N*-acetylglucosaminyl-phosphatidylinositol, the first intermediate of glycosyl phosphatidylinositol anchor biosynthesis, in cell lines established from patients with paroxysmal nocturnal hemoglobinuria. J Exp Med 1993;177:517-21.
13. Miyata T, Takeda J, Iida Y, et al. The cloning of PIG-A, a component in the early step of GPI-anchor biosynthesis. Science 1993;259:1318-20.
14. Takeda J, Miyata T, Kawagoe K, et al. Deficiency of the GPI anchor caused by a somatic mutation of the *PIG-A* gene in paroxysmal nocturnal hemoglobinuria. Cell 1993;73:703-11.
15. Bessler M, Mason PJ, Hillmen P, et al. Paroxysmal nocturnal haemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. EMBO J 1994;13:110-7.
16. Miyata T, Yamada N, Iida Y, et al. Abnormalities of *PIG-A* transcripts in granulocytes from patients with paroxysmal nocturnal hemoglobinuria. N Engl J Med 1994;330:249-55.
17. Bessler M, Mason PJ, Hillmen P, Luzzatto L. Somatic mutations and cellular selection in paroxysmal nocturnal haemoglobinuria. Lancet 1994;343:951-3.
18. Storb R, Evans RS, Thomas ED, et al. Paroxysmal nocturnal haemoglobinuria and refractory marrow failure treated by marrow transplantation. Br J Haematol 1973;24:743-50.
19. Kawahara K, Witherspoon RP, Storb R. Marrow transplantation for paroxysmal nocturnal hemoglobinuria. Am J Hematol 1992;39:283-8.
20. Armitage JO. Bone marrow transplantation. N Engl J Med 1994;330:827-38.
21. Luzzatto L, Hillmen P. Paroxysmal nocturnal haemoglobinuria. In: Dacie JV, Lewis SM, eds. Practical haematology. 8th ed. Edinburgh, Scotland: Churchill Livingstone, 1995:287-96.
22. Dacie JV, Lewis SM. Paroxysmal nocturnal haemoglobinuria: clinical manifestations, haematology, and nature of the disease. Ser Haematol 1972;5:3-23.
23. Hillmen P, Hows JM, Luzzatto L. Two distinct patterns of glycosylphosphatidylinositol (GPI) linked protein deficiency in the red cells of patients with paroxysmal nocturnal haemoglobinuria. Br J Haematol 1992;80:399-405.
24. Hillmen P, Bessler M, Crawford DH, Luzzatto L. Production and characterization of lymphoblastoid cell lines with the paroxysmal nocturnal hemoglobinuria phenotype. Blood 1993;81:193-9.
25. Crosby WH. Paroxysmal nocturnal hemoglobinuria: relation of the clinical manifestations to underlying pathogenic mechanisms. Blood 1953;8:769-812.
26. Paroxysmal nocturnal haemoglobinuria. In: Dacie JV. The haemolytic anaemias. London: J&A Churchill, 1954:412-50.
27. Charache S. Prolonged survival in paroxysmal nocturnal hemoglobinuria. Blood 1969;33:877-83.
28. Dacie J. Paroxysmal nocturnal haemoglobinuria. Sangre (Barc) 1980;25:890-5.
29. Dacie JV, Gilpin A. Refractory anaemia (Fanconi type). Arch Dis Child 1944;19:155-62.
30. Letman H. Possible paroxysmal nocturnal hemoglobinuria with pronounced pancytopenia, reticulocytopenia, and without hemoglobinuria simulating aplastic anemia. Blood 1952;7:842-9.
31. Dacie JV, Lewis SM. Paroxysmal nocturnal haemoglobinuria: variation in clinical severity and association with bone-marrow hypoplasia. Br J Haematol 1961;7:442-57.
32. Rotoli B, Robledo R, Luzzatto L. Decreased number of circulating BFU-Es in paroxysmal nocturnal hemoglobinuria. Blood 1982;60:157-9.
33. Moore JG, Humphries RK, Frank MM, Young N. Characterization of the hematopoietic defect in paroxysmal nocturnal hemoglobinuria. Exp Hematol 1986;14:222-9.
34. Kurnick JE, Robinson WA, Dickey CA. In vitro granulocytic colony-forming potential of bone marrow from patients with granulocytopenia and aplastic anaemia. Proc Soc Exp Med 1971;137:917-20.
35. Tichelli A, Gratwohl A, Wursch A, Nissen C, Speck B. Late haematological complications in severe aplastic anaemia. Br J Haematol 1988;69:413-8.
36. Rotoli B, Luzzatto L. Paroxysmal nocturnal haemoglobinuria. Semin Hematol 1989;26:201-7.
37. Devine DV, Gluck WL, Rosse WF, Weinberg JB. Acute myeloblastic leukemia in paroxysmal nocturnal hemoglobinuria: evidence of evolution from the abnormal paroxysmal nocturnal hemoglobinuria clone. J Clin Invest 1987;79:314-7.
38. Longo L, Bessler M, Beris P, Swirsky D, Luzzatto L. Myelodysplasia in a patient with pre-existing paroxysmal nocturnal haemoglobinuria: a clonal disease originating from within a clonal disease. Br J Haematol 1994;87:401-3.
39. van Kamp H, Smit JW, van den Berg E, Halie MR, Vellenga E. Myelodysplasia following paroxysmal nocturnal haemoglobinuria: evidence for the emergence of a separate clone. Br J Haematol 1994;87:399-400.
40. McMullin MF, Hillmen P, Jackson J, Ganly P, Luzzatto L. Tissue plasminogen activator for hepatic vein thrombosis in paroxysmal nocturnal haemoglobinuria. J Intern Med 1994;235:85-9.

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IMMUNOBIOLOGY

Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti–DC-SIGN antibody

Paul J. Tacken, I. Jolanda M. de Vries, Karlijn Gijzen, Ben Joosten, Dayang Wu, Russell P. Rother, Susan J. Faas, Cornelis J. A. Punt, Ruurd Torensma, Gosse J. Adema, and Carl G. Figdor

Current dendritic cell (DC)–based vaccines are based on ex vivo–generated autologous DCs loaded with antigen prior to readministration into patients. A more direct and less laborious strategy is to target antigens to DCs in vivo via specific surface receptors. Therefore, we developed a humanized antibody, hD1V1G2/G4 (hD1), directed against the C-type lectin DC-specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) to explore its capacity to serve as a target receptor for vaccination purposes. hD1 was cross-linked to a model antigen, keyhole limpet hemocyanin (KLH). We observed that the chimeric antibody-protein complex (hD1-KLH) bound specifically to DC-SIGN and was rapidly internalized and translocated to the lysosomal compartment. To determine the targeting efficiency of hD1-KLH, monocyte-derived DCs and peripheral blood lymphocytes (PBLs) were obtained from patients who had previously been vaccinated with KLH-pulsed DCs. Autologous DCs pulsed with hD1-KLH induced proliferation of patient PBLs at a 100-fold lower concentration than KLH-pulsed DCs. In addition, hD1-KLH–targeted DCs induced proliferation of naive T cells recognizing KLH epitopes in the context of major histocompatibility complex (MHC) classes I and II. We conclude that antibody-mediated targeting of antigen to DCs via DC-SIGN effectively induces antigen-specific naive as well as recall T-cell responses. This identifies DC-SIGN as a promising target molecule for DC-based vaccination strategies. (Blood. 2005;106:1278-1285)

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Introduction

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that play a key role in regulating antigen-specific immunity. DCs capture antigens, process them into peptides, and present these to T cells.[1] The interaction between DC and T-cell controls the type and magnitude of the resulting immune response. Recently, preclinical and clinical studies have exploited DCs in an attempt to improve vaccine efficacy.[2] Most of these studies involve ex vivo antigen loading of autologous monocyte-derived DCs that are readministrated to the patient, a laborious and costly procedure. A more direct strategy involves targeting of antigens specifically to antigen uptake receptors on the DC in vivo. Potential candidate receptors highly expressed by DCs include Fc receptors[3-5] and members of the C-type lectin family.[6,7] Whereas Fc receptors are expressed by many different cell types, the expression of some members of the C-type lectin family are more DC restricted.[8]

C-type lectins bind sugar residues in a calcium-dependent manner via a highly conserved carbohydrate recognition domain. C-type lectin receptors expressed by DCs are implicated in immunoregulatory processes, such as antigen capture, DC trafficking, and DC–T-cell interactions.[8] Based on the location of the amino (N) terminus, 2 types of membrane-bound C-type lectins can be distinguished on DCs. Type I C-type lectins have their N terminus located outside, while type II C-type lectins have their N terminus located inside the cell. Several studies have been conducted on antigen targeting to C-type lectin receptors for vaccination purposes, mainly focusing on the type I C-type lectins mannose receptor (MR)[9] and DEC-205.[6,10,11] Vaccines based on natural MR ligands have been shown to effectively induce humoral and cellular responses.[9] However, these ligands lack specificity for the MR, and may target multiple lectins with overlapping binding specificities, including soluble lectins and lectin receptors expressed by cells that are not specialized in antigen presentation. More specific receptor targeting can be obtained by the use of antibodies directed against specific C-type lectins, a strategy that has been successfully applied in MR[7,12] and DEC-205[6,10,11] targeting studies. Antibody-mediated targeting of antigen to the MR on human DCs results in antigen presentation and activation of naive T cells in vitro.[7,12] Moreover, in vivo studies on antibody-mediated targeting of DEC-205 in mice demonstrate presentation of the antigen to naive $CD4^+$ and $CD8^+$ T cells.[6,10]

DC-specific intercellular adhesion molecule 3 (ICAM-3)–grabbing nonintegrin (DC-SIGN) represents a member of the

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I.J.M.d.V. and K.G. contributed equally to this paper.

S.J.F., R.P.R., and D.W. are employed by Alexion Pharmaceuticals, whose potential product was studied in the present work.

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type II C-type lectin family. We have previously demonstrated that DC-SIGN is an endocytic receptor mediating antigen presentation.[13] A major advantage of targeting DC-SIGN over other C-type lectin receptors is its expression pattern. In humans, DC-SIGN expression is restricted to professional APCs and expression levels are high. Human DC-SIGN is abundantly expressed by DCs residing in lymphoid tissues and at mucosal surfaces, dermal DCs, and by specialized macrophages in placenta and lung.[14,15]

Targeting constructs that are to be used in humans should consist of antibodies that do not elicit immune responses directed against the antibody itself. Recent developments in antibody engineering provide the tools for the production of either humanized or human antibodies.[16] Undesired interactions between the targeting antibody and Fc receptors can be avoided by use of single-chain Fv constructs or composite immunoglobulin G (IgG) molecules,[17] thus enhancing targeting specificity.

Here we evaluate the effectiveness of targeting antigen to human DCs via DC-SIGN. For targeting purposes, keyhole limpet hemocyanin (KLH), a large globular protein containing a large array of immunogenic epitopes, was chosen as a model antigen. KLH is widely used in clinical DC-based vaccination trials for immunomonitoring purposes, and is thought to stimulate cytotoxic T-cell responses by recruiting bystander T-cell help.[2] KLH was chemically cross-linked to a humanized anti–DC-SIGN IgG2/IgG4 composite antibody (hD1), resulting in the chimeric hD1-KLH protein. The results demonstrate that hD1-KLH was capable of inducing T-cell responses at a 100-fold lower concentration than KLH alone.

Patients, materials, and methods

Antibodies and reagents

The following antibodies were used: AZN-D1 (IgG1, mouse antihuman DC-SIGN),[14] AZN-L19 (IgG1, mouse antihuman CD18),[18] W6/32 (IgG2a, mouse anti–HLA-A, –HLA-B, –HLA-C; ATCC, Manassas, VA), IVA-12 (IgG1, mouse anti–HLA-DR, –HLA-DP, –HLA-DQ; ATCC), mouse IgG1 isotype (R&D Systems, Abingdon, United Kingdom), total mouse IgG (Jackson ImmunoResearch; Brunschwig Chemie B.V., Amsterdam, the Netherlands), Alexa Fluor 647–labeled goat anti–human IgG (Molecular Probes, Leiden, the Netherlands), and Alexa Fluor 647–labeled goat anti–mouse IgG1 (Molecular Probes). Endotoxin-free KLH was purchased from Calbiochem (La Jolla, CA).

Recombinant antibodies

The humanized antihuman DC-SIGN antibody hD1V1G2/G4 (hD1) was generated by complementarity determining region (CDR) grafting of AZN-D1 hypervariable domains into human framework regions. The humanized variable heavy and variable light regions were then genetically fused with a human hybrid IgG2/IgG4 constant domain[17] and a human kappa chain constant domain, respectively. This construct was cloned into a mammalian expression vector and the final construct transfected into NSO cells. Stable transfectants were obtained using glutamate synthetase (GS) selection (Lonza Biologics, Portsmouth, NH). Supernatants containing hD1 were purified over a protein A column. An isotype control antibody, h5G1.1-mAb (5G1.1, eculizamab; Alexion Pharmaceuticals) containing the same IgG2/IgG4 constant region, is specific for the human terminal complement protein C5.[19]

Generation of hD1-KLH

The chemical cross-linker sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sSMCC; Pierce, Rockford, IL) was conjugated to KLH according to the manufacturer's protocol. Protected sulfhydryl groups were introduced to the hD1 antibody with N-succinimidyl-S-acetylthiopropionate (SATP; Pierce) and were reduced with hydroxylamine hydrochloride (Pierce) using the manufacturer's protocol. Subsequently, hD1 was added to sSMCC-treated KLH in phosphate-buffered saline (PBS, pH 7.4) and allowed to react for 16 hours at 4°C. Unbound sites were alkylated by adding iodoacetamide (Sigma-Aldrich, St Louis, MO) to a final concentration of 25 mM, followed by 30-minute incubation at room temperature. The protein mixture was loaded onto a Superose 6 column (24-mL bed volume; Amersham Pharmacia Biotech, Uppsala, Sweden), and fractions were collected and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Fractions containing hD1-KLH were pooled and fractions containing free hD1 were discarded. The efficiency of the cross-linking reaction was estimated by comparing the amount of hD1 relative to KLH before the reaction to the hD1–to–hD1-KLH ratio after cross-linking. We calculated that, on average, each KLH molecule had reacted with 10 hD1 molecules (data not shown). Endotoxin levels of the pooled hD1-KLH fractions were below detection levels (< 0.04 pg/μg protein) in the QCL-1000 *Limulus* amebocyte lysate assay (BioWhittaker, Walkersville, MD).

Monocyte-derived DCs and PBLs

Peripheral blood mononuclear cells (PBMCs) were obtained from buffy coats of healthy individuals and were purified using Ficoll density centrifugation. Peripheral blood lymphocytes (PBLs) and immature DCs (iDCs) were obtained from PBMCs as reported elsewhere.[14] In brief, PBMCs were allowed to adhere for one hour at 37°C. Nonadherent cells (PBLs) were gently removed, washed, and cryopreserved. The adherent monocytes were cultured in the presence of interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF, 500 and 800 U/mL, respectively; Schering-Plough International, Kenilworth, NJ) for 6 days to obtain immature DCs. Mature DCs (mDCs) were obtained by culturing iDCs in the presence of 2 μg/mL lipopolysaccharide (LPS) for 24 hours. DCs were cryopreserved until use. Unless indicated otherwise, cells were cultured in X-VIVO 15 medium (Cambrex, Verviers, Belgium) supplemented with 2% human serum.

Tritiated thymidine incorporation assays

Tritiated thymidine (1 μCi [0.037 MBq]/well; MP Biomedicals, Amsterdam, the Netherlands) was added to the cell cultures. Tritiated thymidine incorporation was measured after 16 hours in a β-scintillation counter. Proliferation indices higher than 2 were considered positive.

Clinical vaccination protocol

PBLs and monocyte-derived DCs were isolated from melanoma patients participating in a clinical vaccination trial, as described by de Vries et al.[20] Patients were determined to have stage IV disease according to the American Joint Committee on Cancer (AJCC) criteria.[21] The study was approved by the institutional review board (Radboud University Nijmegen Medical Centre, Commissie Mensgebonden Onderzoek). Informed consent was provided according to the Declaration of Helsinki. The vaccination protocol consisted of 2 parts. In the first part, antigen-pulsed mDCs were administered intravenously and intradermally, 3 times at biweekly intervals. In the second part, patients received 3 monthly intradermal vaccinations with peptides alone (100 μg) and KLH (2 μg). Patients who remained free of disease progression after the first vaccination cycle were eligible for maintenance cycles at 6-month intervals, each consisting of 3 biweekly intranodal vaccinations in a clinically tumor-free lymph node region under ultrasound guidance with mDCs alternately pulsed with wild-type or modified glycoprotein 100 (gp100) peptides,[20] tyrosinase peptides, and KLH. Clinical grade DCs for vaccination purposes were generated from PBMCs as described previously.[22]

Humoral responses to KLH

Humoral responses to KLH were determined by enzyme-linked immunosorbent assays (ELISA) as described by Holtl et al.[23] Briefly, 96-well plates were coated overnight at 4°C with the protein KLH (25 μg/mL) in PBS (0.1

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mL/well). Subsequently, plates were incubated with serial dilutions of patient serum, obtained before and during the third maintenance cycle, for one hour at room temperature. After extensive washing, human IgG-specific antibody labeled with horseradish peroxidase was allowed to bind for one hour at room temperature. Peroxidase activity was revealed using 3,3′ 5,5′ tetramethyl-benzidine as substrate and measured in a microtiter plate reader at 450 nm. A signal detected at 1:400 or higher dilution of serum was considered positive.

Cellular responses to KLH

Cryopreserved PBMCs, isolated from peripheral blood samples taken from the patients, were thawed, washed, and plated at 1×10^5 PBMCs per well of a 96-well tissue culture microplate either in the presence or absence of 10 μg/mL KLH. After 4 days of culture, a tritiated thymidine incorporation assay was performed.

Binding and internalization assays

Binding of hD1 and hD1-KLH to iDCs was assessed by immunofluorescence and flow cytometry. iDCs were incubated with or without 10 μg/mL hD1 or hD1-KLH. In some experiments, DC-SIGN was blocked by pretreating iDCs with 100 μg/mL AZN-D1. After a one-hour incubation at 4°C, cells were washed and incubated with Alexa Fluor 647–labeled anti–human IgG antibody. Cells were analyzed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences, San Jose, CA).

Internalization of hD1 by iDCs was determined by flow cytometry as described previously.[24] Briefly, iDCs were incubated with 10 μg/mL hD1, AZN-D1, AZN-L19, or mouse IgG1 and 5G1.1 isotype control antibodies at 4°C for 30 minutes, washed, and incubated for 0, 15, 30, or 45 minutes at 37°C. Subsequently, some of the cells were fixed, while others were fixed and permeabilized in PBS/0.1% (vol/wt) saponin (Sigma-Aldrich) before addition of the Alexa Fluor 647–labeled anti–human IgG secondary antibody. The amount of internalized antibody was calculated by subtracting the mean fluorescence in fixed cells (surface bound) from that recorded with fixed and permeabilized cells (internalized and surface bound) at the various time points.

Internalization of hD1 and hD1-KLH was confirmed by confocal laser scanning microscopy (CLSM). iDCs were incubated with 10 μg/mL hD1, hD1-KLH, AZN-D1, or isotype control 5G1.1 and mouse IgG1 antibodies for one hour at 37°C. Cells were fixed on poly-L-lysine–coated glass slides, followed by intracellular staining with Alexa Fluor 647–labeled secondary antibodies. Cells were imaged with a Bio-Rad MRC 1024 confocal system operating on a Nikon Optiphot microscope and a Nikon × 60 planApo 1.4 oil immersion lens (Bio-Rad, Hercules, CA). Pictures were analyzed with Bio-Rad Lasersharp 2000 and Adobe Photoshop 7.0 (Adobe Systems, Mountain View, CA) software.

KLH binding and internalization by iDCs was assessed by direct labeling of KLH using the Alexa Fluor 488 labeling kit (Molecular Probes). iDCs were incubated with 10 μg/mL labeled KLH for one hour at either 4°C or 37°C. Subsequently, cells were washed and analyzed by flow cytometry.

Live imaging of hD1-KLH and KLH uptake by iDCs

iDCs were labeled with LysoTracker Red (Molecular Probes) in PBS for 10 minutes at room temperature. Subsequently, cells were transferred to RPMI 1640 without phenol red (Gibco, Life Technologies, Breda, the Netherlands) supplemented with 1% human serum. Labeled cells were analyzed at 37°C with a Zeiss LSM 510 microscope equipped with a type S heated stage CO_2 controller and PlanApochromatic 63 × 1.4 oil immersion DIC lens (Carl Zeiss, Jena, Germany). KLH and hD1-KLH, directly labeled with the Alexa Fluor 488 labeling kit (Molecular Probes), were added to the medium at 10 μg/mL. Cells were imaged using Zeiss LSM Image Browser version 3.2 (Carl Zeiss) and processed with Image J version 1.32j software (National Institutes of Health, http://rsb.info.nih.gov/ij).

Targeting experiments

iDCs were incubated with hD1, hD1-KLH, or KLH for one hour at 4°C or 37°C. Where indicated, iDCs were matured with LPS. Subsequently, DCs were washed and cocultured with KLH-responsive PBLs (ratio 1:10) at 37°C. In some experiments, iDCs were matured with LPS before addition of PBLs. After 16 hours of coculture, IL-2, IL-4, IL-5, IL-10, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) were measured by cytometric bead array (CBA) (T helper 1 [Th1]/Th2 Cytokine CBA 1; BD Pharmingen, San Diego, CA) according to the manufacturer's instructions. After 4 days of coculture, a tritiated thymidine incorporation assay was performed.

Presentation of KLH epitopes over time

iDCs were incubated with 5 μg/mL KLH or 5 μg/mL hD1-KLH for one hour at 37°C. Following washing, KLH-responsive PBLs were added to the iDCs either on the same day, or 2 or 4 days later. Four days after addition of the PBLs, proliferative responses were determined in a tritiated thymidine incorporation assay.

Activation and expansion of naive T cells

Experiments were performed essentially as described previously.[7] Briefly, PBLs from a healthy donor were used as a source of T cells. Autologous DCs were incubated with 10 μg/mL hD1-KLH for one hour at 37°C, washed, and matured. Targeted mDCs were incubated with PBLs (ratio, 1:10) in the presence of IL-7 (10 ng/mL, day 0), followed by addition of IL-10 (10 ng/mL) on day 1 and IL-2 (20 U/mL) on day 2. IL-2 was added to the culture every 3 to 4 days. PBLs were restimulated each week. Restimulations were performed with iDCs treated with 5 μg/mL hD1-KLH after 1 week, and with iDCs treated with 2.5 μg/mL hD1-KLH after 2, 3, and 4 weeks. Finally, PBLs were harvested and cocultured with autologous mDCs that had been pulsed with KLH, as described in "Targeting experiments." Proliferative responses were determined by tritiated thymidine incorporation assays.

Results

Humanized anti–DC-SIGN antibody hD1 is internalized by iDCs

The CDRs of the mouse antihuman DC-SIGN antibody AZN-D1 were grafted onto a human IgG2/IgG4 composite antibody to generate a humanized antibody for DC-SIGN targeting, hD1. We have previously shown that the human hybrid IgG2/IgG4 constant domain prevents antibodies from binding to Fc receptors.[17] The binding affinity of hD1 for DC-SIGN was similar to that of AZN-D1 as determined by surface plasmon resonance (3.7 ± 0.7 nM and 3.8 ± 1.1 nM, respectively, data not shown).

Flow cytometric analysis revealed specific binding of hD1 to DC-SIGN on iDCs, as preincubation of iDCs with AZN-D1 efficiently reduced binding (Figure 1A). Furthermore, hD1 bound to DC-SIGN–expressing K562 cells after transfection with DC-SIGN cDNA, whereas it did not bind to untransfected K562 cells (data not shown). A time-course internalization experiment revealed that both the hD1 and the AZN-D1 antibodies were rapidly internalized by iDCs, although the AZN-D1 antibody was internalized slightly more efficiently than hD1. As expected, the control AZN-L19 antibody, directed against CD18, was not internalized (Figure 1B). Analysis by confocal microscopy confirmed internalization of the hD1 and AZN-D1 antibodies by iDCs, whereas the control antibodies 5G1.1, directed against human terminal complement protein C5, and mouse IgG1 isotype were not internalized (Figure 1C).

hD1-KLH is rapidly internalized and translocated to the lysosmal compartment

To further explore the potential of DC-SIGN as a targeting receptor for DC vaccination, we studied the uptake of KLH complexed to

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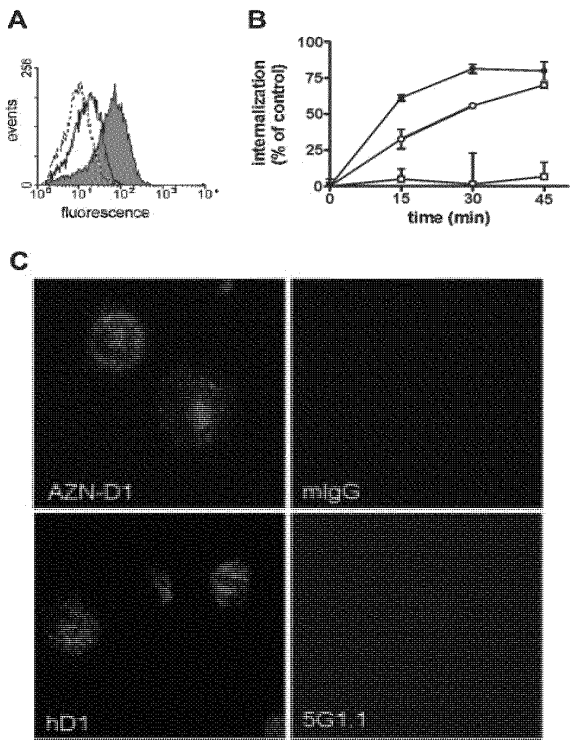


Figure 1. Binding of hD1 to DC-SIGN and internalization by DCs. (A) iDCs were treated with 10 μg/mL hD1 (gray shaded curve), 100 μg/mL AZN-D1 (open dotted curve), or pretreated with 100 μg/mL AZN-D1 followed by 10 μg/mL hD1 incubation (open solid curve), followed by incubation with an Alexa Fluor 647–labeled goat anti–human IgG antibody. Binding of hD1 was analyzed by flow cytometry. (B) iDCs were incubated with AZN-D1 (●), hD1 (○), or AZN-L19 (□) at 4°C for one hour, and transferred to 37°C. Cells were fixed at various time points, and stained with Alexa Fluor–labeled secondary antibodies with or without prior permeabilization. The mean fluorescence was determined by flow cytometric analysis, and the amount of internalized antibody was plotted as a percentage of the amount of total cell-associated antibody. Data represent experiments performed in triplicate ± SD. (C) Internalization of hD1 was confirmed by CSLM. iDCs were incubated with hD1, AZN-D1, or their isotype controls 5G1.1 and mouse IgG1 (mIgG) for one hour at 37°C. Cells were stained with Alexa Fluor 647–labeled secondary antibodies (blue), followed by microscopic analysis. The image represents the middle focal plane of the DCs, with iris set at 2 nm. Original magnification, ×600.

the humanized anti–DC-SIGN antibody hD1. As was observed with the hD1 antibody, hD1-KLH bound to iDCs (Figure 2A). hD1-KLH binding to DCs is mediated by DC-SIGN, as binding could be blocked by pretreating iDCs with AZN-D1 (Figure 2A). Analysis by CLSM revealed that hD1-KLH was rapidly internalized by iDCs (Figure 2B). After one hour of incubation at 37°C, hD1-KLH could be detected in 74% of the iDCs (data not shown). In contrast to hD1-KLH, KLH itself did not bind to iDCs, but was internalized by iDCs following one hour of incubation at 37°C (Figure 2C). These findings demonstrate that internalization of KLH by DCs is not receptor mediated, and likely depends on macropinocytosis.

Foreign antigens are taken up by DCs and shuttled, via endosomes, to the lysosomal compartment where they can be processed. In order to establish whether hD1-KLH reaches the lysosomal compartment, and to study uptake-kinetics and routing of KLH and D1-KLH, these proteins were directly labeled with Alexa Fluor 488 dye. Live imaging by CLSM revealed rapid uptake of hD1-KLH and KLH by iDCs. Both hD1-KLH and KLH were detected in the lysosomal compartment within one hour after their addition to the culture medium (Figure 2D; Supplemental Videos S1 and S2; see the Supplemental Videos link at the top of the online article, at the *Blood* website).

Induction of KLH responses by DC vaccination

In order to compare the antigen-presentation capacity of DCs pulsed with KLH to that of DCs targeted by hD1-KLH, we required KLH-specific T cells. To this end, PBLs were isolated from melanoma patients participating in a vaccination study. This study involved vaccination of patients with autologous DCs pulsed ex vivo with KLH and melanoma-associated antigens. Monocyte-derived DCs and PBLs from patients showing humoral as well as cellular responses against KLH were used for the targeting studies.

All 31 vaccinated patients participating in the clinical vaccination trial showed responses against KLH (data not shown). Humoral responses were readily detected in the patients' serum. KLH-specific IgGs were present after the first vaccination cycle, and their serum levels increased upon the second vaccination (Figure 3A). Cellular responses were analyzed using PBMCs obtained from patients one week before the start of the third vaccination cycle. Typically, PBMCs pulsed with KLH showed an increased proliferative response compared with unpulsed PBMCs, revealing the presence of KLH-reactive T cells in peripheral blood

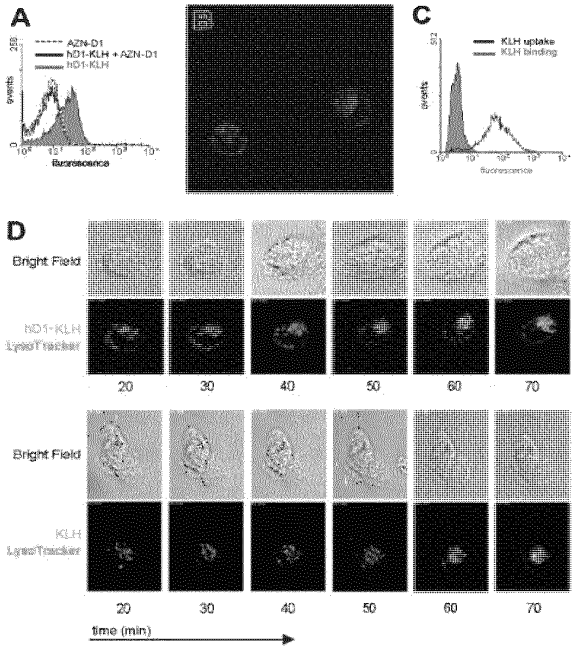


Figure 2. Binding and uptake of hD1-KLH by DCs. (A) iDCs were treated with 10 μg/mL hD1-KLH, 100 μg/mL AZN-D1, or pretreated with 100 μg/mL AZN-D1 followed by 10 μg/mL hD1 incubation, followed by incubation with an Alexa Fluor 647–labeled goat anti–human IgG antibody. Binding of hD1-KLH was analyzed by flow cytometry. (B) Internalization of hD1-KLH was confirmed by CSLM. iDCs were incubated with Alexa Fluor 647–labeled hD1-KLH (blue) for one hour at 37°C, followed by microscopic analysis. The image represents the middle focal plane of the DCs, with iris set at 2 nm. Original magnification, ×600. (C) iDCs were incubated with Alexa Fluor 488–labeled KLH for one hour at 4°C or 37°C. Subsequently, cells were fixed and analyzed by flow cytometric analysis. (D) iDCs were labeled with LysoTracker Red, followed by addition of Alexa Fluor 488–labeled (green) hD1-KLH or KLH. Cells were imaged by CSLM. Data represent bright field and corresponding fluorescent images of cells at various time points after addition of hD1-KLH or KLH to the culture medium. Original magnification, ×630.

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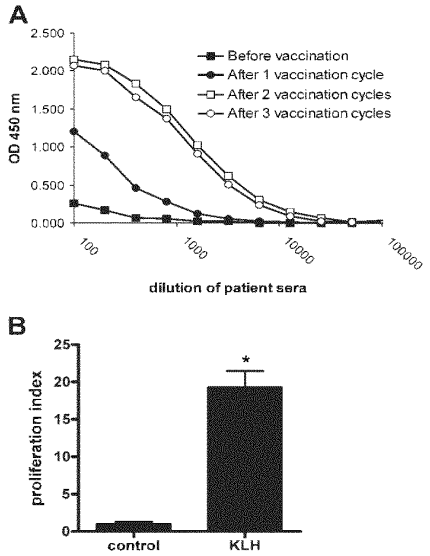


Figure 3. Humoral and cellular responses against KLH in patients vaccinated with KLH-pulsed DCs. (A) Patient serum was obtained both before the first, and after the first, second, and third vaccination cycles. Total IgG antibodies specific for KLH were detected by ELISA. Data represent optical density (OD) 450 values of serially diluted serum samples for a representative patient. (B) Patient PBMCs were isolated one week prior to the third vaccination cycle and cultured in the absence (control) or presence of 10 μg/mL KLH (KLH). Cellular responses were assessed in a tritiated thymidine incorporation assay. Data represent mean ± SD of experiments performed in 6-fold for a representative patient. Significant difference from control according to Student *t* test: $^*P < .001$.

(Figure 3B). These findings show that KLH-pulsed mDCs effectively induced both humoral and cellular responses in vivo.

Targeting of KLH to DC-SIGN results in presentation of KLH antigen epitopes

To demonstrate that antibody-mediated targeting of antigen to DC-SIGN leads to antigen presentation, autologous iDCs were incubated with 5 μg/mL KLH (± 0.63 nM) or hD1-KLH (± 0.53 nM) at 4°C for one hour, washed to remove unbound protein, and added to PBLs. Presentation of KLH epitopes to specific T cells was determined by measuring proliferative responses (Figure 4A-B) and the levels of cytokines secreted in the medium (Figure 4C). As endocytotic processes are inhibited at 4°C, and KLH does not bind to iDCs (Figure 2C), hD1-KLH can be internalized only after binding to DC-SIGN. iDCs incubated with hD1-KLH induced cellular responses by the PBLs, whereas iDCs incubated with KLH did not (Figure 4A-C), showing that targeting of KLH to DC-SIGN results in antigen presentation. We have previously demonstrated that the clinical vaccination protocol used in our studies results in a Th1-type immune response.[20] Indeed, stimulation of patient PBLs with hD1-KLH–treated iDCs resulted in enhanced secretion of IL-2, IFN-γ, and TNF-α (Figure 4C), while IL-4, IL-5, and IL-10 levels were not detectable (data not shown). As expected, iDCs incubated with KLH at 37°C induced proliferative responses, as did iDCs incubated with hD1-KLH (Figure 4A).

Presentation of KLH epitopes by iDCs incubated with hD1-KLH at 4°C was abolished by preincubation of iDCs with the hD1 antibody, but was unaffected by preincubation with the isotype control 5G1.1 (Figure 4B-C). This confirms that hD1-KLH targeted specifically to DC-SIGN, resulting in presentation of KLH epitopes. Cross-linking of hD1 to KLH was required for induction of DC-SIGN–mediated presentation of KLH epitopes, since iDCs incubated with both KLH and hD1 antibody did not induce cellular responses (Figure 4B-C).

Targeting KLH to DC-SIGN enhances its immunogenicity

To determine whether DC-SIGN targeting increases the efficiency of antigen presentation, immature monocyte-derived DCs from previously immunized patients were incubated at 37°C with various concentrations of KLH or hD1-KLH. Subsequently, iDCs were washed, and a portion of the iDCs was matured with LPS. hD1-KLH by itself did not induce maturation of iDCs (data not shown). Both hD1-KLH–treated iDCs (Figure 5A) and mDCs (Figure 5B) induced cellular responses at 100-fold lower concentrations than KLH-pulsed DCs. Thus, antigen targeting via DC-SIGN significantly enhances immunogenicity.

KLH epitopes are presented up to 4 days after targeting DC-SIGN

After antigen uptake in the periphery, DCs migrate to the draining lymph nodes to present the processed antigen to T cells. It is

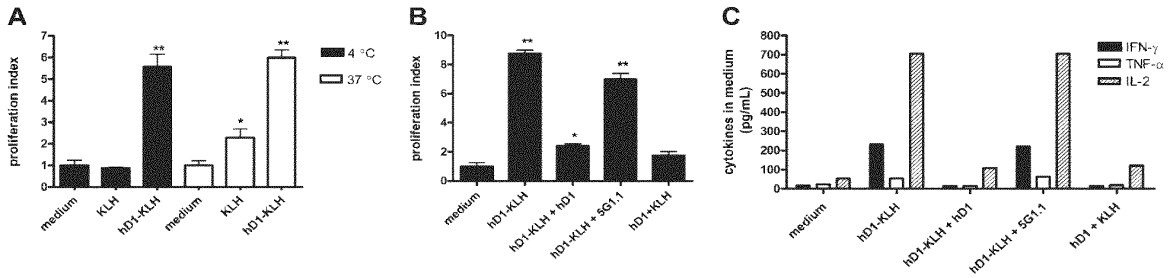


Figure 4. Antibody-mediated targeting of antigen to DC-SIGN results in antigen presentation. (A) iDCs were incubated with medium (control), 5 μg/mL KLH, or 5 μg/mL hD1-KLH for one hour at 4°C or 37°C. Subsequently, iDCs were washed and cocultured with autologous KLH-responsive PBLs, derived from the patients enrolled in the vaccination trial. After 4 days, cellular responses were assessed in a proliferation assay. Data are mean proliferation indices relative to medium control for experiments performed in triplicate ± SD. Significant difference from medium control according to analysis of variance (ANOVA) and Bonferroni test: $^*P < .05$; $^{**}P < .001$. (B) iDCs were incubated with medium (control), 5 μg/mL hD1-KLH, 5 μg/mL hD1-KLH and 100 μg/mL hD1 (hD1-KLH + hD1), 5 μg/mL hD1-KLH and 100 μg/mL 5G1.1 (hD1-KLH + 5G1.1), or 5 μg/mL hD1 and 5 μg/mL KLH (hD1 + KLH) for one hour at 4°C. Subsequently, iDCs were washed and cocultured with autologous KLH-responsive PBLs, derived from patients enrolled in the vaccination trial. After 4 days, cellular responses were assessed in a proliferation assay. Data are mean proliferation indices relative to medium control for experiments performed in triplicate ± SD. Significant difference from medium control according to ANOVA and Dunnett test: $^*P < .05$; $^{**}P < .01$. (C) Production of IFN-γ, TNF-α, and IL-2 in the coculture experiment described in panel B. After 16 hours of coculturing iDCs and PBLs, supernatants were taken and cytokine levels were determined. Data represent cytokine levels in pooled samples of experiments performed in triplicate.

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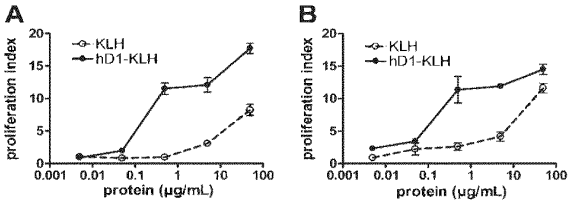


Figure 5. Targeting of antigen to DC-SIGN enhances its immunogenicity. (A) iDCs were incubated with medium, 5 μg/mL KLH, or 5 μg/mL hD1-KLH for one hour at 37°C. Subsequently, iDCs were washed and cocultured with autologous KLH-responsive PBLs, derived from patients enrolled in the vaccination trial. After 4 days, cellular responses were assessed in a proliferation assay. Data are mean proliferation indices relative to medium control for experiments performed in triplicate ± SD. (B) Experiment performed as described in panel A, except that antigen-treated iDCs were matured with LPS before addition of PBLs.

imperative that DCs present the antigens over a prolonged period of time to initiate a significant immune response. We evaluated the efficiency of antigen presentation over time following targeting by incubating patient PBLs with autologous iDCs that had been pulsed with KLH or hD1-KLH 0, 2, or 4 days earlier. The results demonstrate that DCs targeted with hD1-KLH presented KLH epitopes up to 4 days after targeting. Moreover, at all time points evaluated, DCs targeted with hD1-KLH induced stronger proliferative responses than DCs pulsed with KLH (Figure 6).

hD1-KLH targeted to DCs activates both major histocompatibility complex (MHC) class I– and II–restricted naive T cells recognizing KLH epitopes

To evaluate whether hD1-KLH, besides triggering recall responses, is able to activate naive T cells, hD1-KLH was tested in an autologous in vitro culture system. PBLs from a healthy donor were stimulated by repetitive coculturing with hD1-KLH–pulsed autologous DCs. IL-10 was added to the cultures to stimulate expansion of $CD8^+$ T cells.[25] After 5 rounds of stimulation, PBLs were cocultured with DCs pulsed with KLH or hD1 to evaluate KLH-specific proliferative responses. The results demonstrate that hD1-KLH–targeted DCs activated KLH-specific naive T cells (Figure 7). Presentation of KLH epitopes was mediated by MHC class I and class II, since both class I and class II blocking antibodies significantly reduced the proliferative response, while an antibody control did not. DCs pulsed with hD1 antibody did not induce proliferative responses by the PBLs (Figure 7), demonstrating that the T-cell response is specific for KLH epitopes rather than epitopes contained within the hD1 antibody.

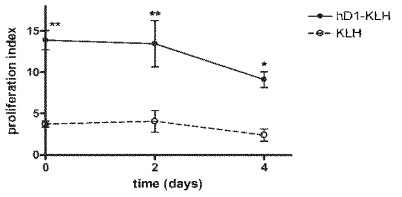


Figure 6. Antigen targeted to DC-SIGN is presented for at least 4 days. iDCs were incubated with medium, 5 μg/mL KLH, or 5 μg/mL hD1-KLH for one hour at 37°C. Subsequently, iDCs were washed and cultured for 0, 2, or 4 days before addition of autologous KLH-responsive PBLs, derived from patients enrolled in the vaccination trial. Four days after addition of PBLs, cellular responses were assessed in a proliferation assay. Data are mean proliferation indices relative to medium control for experiments performed in triplicate ± SD. Significant difference from KLH according to ANOVA and Bonferroni test: $^*P < .05$; $^{**}P < .01$.

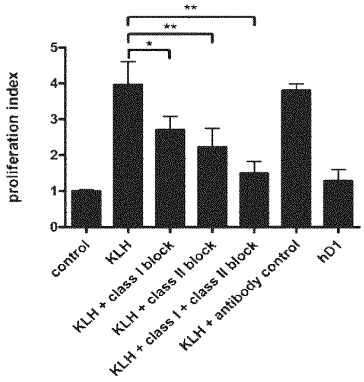


Figure 7. hD1-KLH–treated DCs activate naive T cells recognizing KLH epitopes. iDCs derived from a healthy donor were incubated with medium (control), 10 μg/mL KLH, or 10 μg/mL hD1 for one hour at 37°C. Subsequently, cells were washed and matured with LPS. mDCs were cocultured with autologous PBLs that had been repeatedly stimulated with DCs treated with hD1-KLH as described in "Patients, materials, and methods." Some cocultures of KLH-pulsed mDCs and PBLs were supplemented with w6/32 antibody (KLH + class I block), IVA-12 (KLH + class II block), both w6/32 and IVA-12 (KLH + class I + class II block), or total mouse IgG (KLH + antibody control). Data are mean proliferation indices relative to medium control for experiments performed in triplicate ± SD. Significant difference according to ANOVA, followed by the Student-Newman-Keuls test: $^*P < .01$; $^{**}P < .001$.

Discussion

In the present study, we explored the capacity of the type II C-type lectin DC-SIGN to function as a target receptor for vaccination purposes. The results demonstrate that antibody-mediated targeting of antigen to human DC-SIGN was 100-fold more efficient than pulsing DCs with antigen, and that antigen epitopes were presented for at least 4 days after targeting.

A critical step in designing a suitable targeting construct is the choice of targeting antibody. Various antibodies directed against the same receptor might bind distinct receptor epitopes, thus influencing biologic outcome. For example, not all antibodies directed against DC-SIGN are internalized following binding,[13] and the anti-MR antibody PAM-1 induces maturation of iDCs, in contrast to a different isotype-matched anti-MR antibody.[26] The mouse antihuman DC-SIGN antibody AZN-D1 has been previously described to be internalized by DCs,[13] a prerequisite for successful targeting. However, the use of murine antibodies in humans presents numerous problems, including a short half-life and high immunogenicity.[27] We were able to graft the CDR of AZN-D1 hypervariable domains onto a human composite IgG2/IgG4 antibody without loss of binding characteristics. The resulting hD1 antibody exhibited a similar binding affinity for DC-SIGN as AZN-D1, and did not induce maturation of iDCs (data not shown). hD1 binding induced rapid internalization of DC-SIGN, while internalization did not result in a lasting down-regulation of DC-SIGN expression (data not shown), thus minimizing the effect a therapeutic intervention might have on the biologic function of DC-SIGN. hD1 rapidly targeted antigen to the DC lysosomal compartment resulting in antigen presentation, and did not induce hD1-specific proliferative responses in the naive T-cell stimulation experiments. Thus, humanization of AZN-D1 resulted in an effective targeting antibody, and will facilitate introduction into clinical trials.

Vaccination strategies aimed at inducing cytotoxic T-cell help, such as antitumor therapies, require antigen presentation in the

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context of MHC class I. In addition, the induction of CTL responses requires bystander $CD4^+$ T-cell help.[28] Targeting DCs with hD1-KLH resulted in the activation of naive T cells in the context of both MHC class I and II. Although the major route for presentation of exogenous antigens is via class II, presentation via class I can occur via the process of cross-presentation. This process provides internalized proteins access to cytosolic proteasomes and their derived peptides access to the endoplasmatic reticulum (ER)–based class I processing machinery.[29] Particulate antigens taken up by phagocytosis have access to this machinery since phagosomes fuse with the ER soon after or during their formation.[30] Particulate antigens are more efficiently cross-presented than soluble antigens.[31,32] However, a recent study by Ackerman et al reveals that internalized soluble proteins can escape proteolysis and also gain access to the lumen of the ER.[33] This might explain how antibody-mediated targeting of the type I C-type lectins MR[7] and DEC-205[10] results in class I responses. Although we cannot exclude the possibility that, besides receptor-mediated endocytosis, a portion of our chimeric protein was taken up via macropinocytosis, our current findings strongly suggest that antibody-mediated targeting of DC-SIGN results in cross-presentation.

The technique commonly used in current clinical trials to load DCs with antigens involves ex vivo incubation with MHC class I and II binding peptides. Other techniques involve loading with tumor lysates or apoptotic tumor cells, and the introduction of genetic material to drive expression of specific antigens by the DC itself.[2] Ideally, vaccines should deliver antigens to the DCs in vivo. Antibody-mediated targeting of antigens to DC surface receptors to stimulate antigen presentation in vivo has been shown to be far more potent than immunization with antigen in complete Freund adjuvant or splenic DCs pulsed with antigen ex vivo.[6] However, mere targeting of antigens to DCs is not sufficient for induction of immunity. Antibody-mediated targeting of antigen to DEC-205 in mice leads to tolerance, and coadministration of a DC maturation stimulus is required to induce immunity.[6,10,11] These findings are consistent with DC-based vaccination studies in humans, showing that DC maturation is a prerequisite for induction of immunity.[20] Vaccination strategies for transplantation, allergy, autoimmunity, and chronic inflammatory diseases could exploit the finding that targeted iDCs induce tolerance. However, strategies aimed at inducing immunity will require a combination of antigens and DC activation factors. Agents that have been shown to activate DCs in vivo include anti-CD40 antibody,[11] α-galactosylceramide,[34] and the Toll-like receptor ligands LPS[35] and cytosine-phosphate-guanosine (CpG) oligonucleotides.[36] A better understanding of differences in DC subsets, their activation pathways, and antigen uptake receptors will provide the information necessary for development of effective vaccines.

In the present study, we used a neoantigen to study targeting efficiency. A major challenge in cancer therapy is to break immunologic tolerance to tumor-associated self-antigens. Tolerance can be the result of clonal deletion, active suppression of antigen-specific T cells by regulatory T cells, or inadequate activation stimuli provided by tumor cells upon antigen presentation, resulting in T-cell anergy.[37] One way of breaking tolerance is by disruption of negative regulatory mechanisms directly at the T-cell level. Antibody-mediated blockade of the cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) breaks tolerance against moderately immunogenic tumors[38] and improves the efficiency of tumor cell–based vaccines against poorly immunogenic tumors.[39] Depletion of $CD25^+$ regulatory T cells prior to blocking of CTLA-4 improves vaccine efficiency even further.[40] A second way of breaking tolerance is to recruit DCs as potent APCs. DCs can overcome the heightened threshold of anergic T cells[41] and restore responsiveness of tolerogenic tumor-specific T cells[42] in vitro. Moreover, studies in mice demonstrate that DC-based vaccines can break tolerance against (tumor-associated) self-antigens,[43-47] resulting in regression of established tumors.[47] Thus, a combination of antibody-mediated strategies targeting tumor-associated antigens to DCs and strategies modulating regulatory mechanisms at the T-cell level might provide effective cancer therapies.

In conclusion, antibody-mediated targeting of antigens to DC surface receptors represents an exciting way to induce immune responses. Our results demonstrate efficient delivery of antigen to DCs via DC-SIGN, resulting in naive as well as recall responses by T cells. These data expose DC-SIGN as a promising target molecule for antibody-mediated antigen delivery to DCs.

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References

1. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. Cell. 2001;106:255-258.
2. Figdor CG, de Vries IJM, Lesterhuis WJ, Melief CJM. Dendritic cell immunotherapy: mapping the way. Nat Med. 2004;10:475-480.
3. Dhodapkar KM, Krasovsky J, Williamson B, Dhodapkar MV. Antitumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. J Exp Med. 2002;195:125-133.
4. Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcγ receptors on dendritic cells. J Exp Med. 2002;195:1653-1659.
5. Regnault A, Lankar D, Lacabanne V, et al. Fcγ receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. J Exp Med. 1999; 189:371-380.
6. Bonifaz LC, Bonnyay DP, Charalambous A, et al. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. J Exp Med. 2004;199:815-824.
7. Ramakrishna V, Treml JF, Vitale L, et al. Mannose receptor targeting of tumor antigen pmel17 to human dendritic cells directs anti-melanoma T cell responses via multiple HLA molecules. J Immunol. 2004;172:2845-2852.
8. Figdor CG, van Kooyk Y, Adema GJ. C-type lectin receptors on dendritic cells and Langerhans cells. Nat Rev Immunol. 2002;2:77-84.
9. Keler T, Ramakrishna V, Fanger MW. Mannose receptor-targeted vaccines. Expert Opin Biol Ther. 2004;4:1953-1962.
10. Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. J Exp Med. 2002;196:1627-1638.
11. Hawiger D, Inaba K, Dorsett Y, et al. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J Exp Med. 2001;194:769-779.
12. He LZ, Ramakrishna V, Connolly JE, et al. A novel human cancer vaccine elicits cellular responses to the tumor-associated antigen, human chorionic gonadotropin β. Clin Cancer Res. 2004; 10:1920-1927.
13. Engering A, Geijtenbeek TBH, van Vliet SJ, et al. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. J Immunol. 2002;168:2118-2126.
14. Geijtenbeek TBH, Torensma R, van Vliet SJ, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell. 2000;100:575-585.
15. Soilleux EJ, Morris LS, Leslie G, et al. Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro. J Leukoc Biol. 2002;71:445-457.

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16. Hudson PJ, Souriau C. Engineered antibodies. Nat Med. 2003;9:129-134.
17. Mueller JP, Giannoni MA, Hartman SL, et al. Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells. Mol Immunol. 1997;34:441-452.
18. Stuyt RJL, Netea MG, Geijtenbeek TBH, Kullberg BJ, Dinarello CA, van der Meer JWM. Selective regulation of intercellular adhesion molecule-1 expression by interleukin-18 and interleukin-12 on human monocytes. Immunology. 2003;110: 329-334.
19. Thomas TC, Rollins SA, Rother RP, et al. Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. Mol Immunol. 1996; 33:1389-1401.
20. de Vries IJM, Lesterhuis WJ, Scharenborg NM, et al. Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. Clin Cancer Res. 2003;9: 5091-5100.
21. Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol. 2001;19:3635-3648.
22. de Vries IJM, Eggert AAO, Scharenborg NM, et al. Phenotypical and functional characterization of clinical grade dendritic cells. J Immunother. 2002;25:429-438.
23. Holtl L, Rieser C, Papesh C, et al. Cellular and humoral immune responses in patients with metastatic renal cell carcinoma after vaccination with antigen pulsed dendritic cells. J Urol. 1999; 161:777-782.
24. Mahnke K, Guo M, Lee S, et al. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. J Cell Biol. 2000;151:673-684.
25. Santin AD, Hermonat PL, Ravaggi A, et al. Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8(+) cytotoxic T lymphocytes. J Virol. 2000;74:4729-4737.
26. Chieppa M, Bianchi G, Doni A, et al. Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosuppressive program. J Immunol. 2003; 171:4552-4560.
27. Khazaeli MB, Conry RM, LoBuglio AF. Human immune response to monoclonal antibodies. J Immunother. 1994;15:42-52.
28. Bennett SRM, Carbone FR, Karamalis F, Miller JFAP, Heath WR. Induction of a CD8+ cytotoxic T lymphocyte response by cross-priming requires cognate CD4+ T cell help. J Exp Med. 1997;186: 65-70.
29. Ackerman AL, Cresswell P. Cellular mechanisms governing cross-presentation of exogenous antigens. Nat Immunol. 2004;5:678-684.
30. Guermonprez P, Saveanu L, Kleijmeer M, Davoust J, van Endert P, Amigorena S. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. Nature. 2003;425:397-402.
31. Carbone FR, Bevan MJ. Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. J Exp Med. 1990;171:377-387.
32. Reis e Sousa C, Germain RN. Major histocompatibility complex class I presentation of peptides derived from soluble exogenous antigen by a subset of cells engaged in phagocytosis. J Exp Med. 1995;182:841-851.
33. Ackerman AL, Kyritsis C, Tampe R, Cresswell P. Access of soluble antigens to the endoplasmic reticulum can explain cross-presentation by dendritic cells. Nat Immunol. 2005;6:107-113.
34. Fujii S, Shimizu K, Smith C, Bonifaz L, Steinman RM. Activation of natural killer T cells by α-galactosylceramide rapidly induces the full maturation of dendritic cells in vivo and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. J Exp Med. 2003;198:267-279.
35. De Smedt T, Pajak B, Muraille E, et al. Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. J Exp Med. 1996;184: 1413-1424.
36. Jakob T, Walker PS, Krieg AM, Udey MC, Vogel JC. Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of Th1 responses by immunostimulatory DNA. J Immunol. 1998;161:3042-3049.
37. Mapara MY, Sykes M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. J Clin Oncol. 2004;22:1136-1151.
38. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271:1734-1736.
39. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med. 1999;190:355-366.
40. Sutmuller RPM, van Duivenvoorde LM, van Elsas A, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25+ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med. 2001;194:823-832.
41. Heeg K, Wagner H. Induction of responsiveness in superantigen-induced anergic T cells: role of ligand density and costimulatory signals. J Immunol. 1995;155:83-92.
42. Wang H, Cheng F, Cuenca A, et al. Imatinib mesylate (STI-571) enhances antigen-presenting cell function and overcomes tumor-induced CD4+ T-cell tolerance. Blood. 2005;105:1135-1143.
43. Koido S, Kashiwaba M, Chen D, Gendler S, Kufe D, Gong J. Induction of antitumor immunity by vaccination of dendritic cells transfected with MUC1 RNA. J Immunol. 2000;165:5713-5719.
44. Mullins DW, Bullock TNJ, Colella TA, Robila VV, Engelhard VH. Immune responses to the HLA-A*0201-restricted epitopes of tyrosinase and glycoprotein 100 enable control of melanoma outgrowth in HLA-A*0201-transgenic mice. J Immunol. 2001;167:4853-4860.
45. Schreurs MWJ, Eggert AAO, de Boer AJ, et al. Dendritic cells break tolerance and induce protective immunity against a melanocyte differentiation antigen in an autologous melanoma model. Cancer Res. 2000;60:6995-7001.
46. Saha A, Chatterjee SK, Foon KA, et al. Dendritic cells pulsed with an anti-idiotype antibody mimicking carcinoembryonic antigen (CEA) can reverse immunological tolerance to CEA and induce antitumor immunity in CEA transgenic mice. Cancer Res. 2004;64:4995-5003.
47. Okano F, Merad M, Furumoto K, Engleman EG. In vivo manipulation of dendritic cells overcomes tolerance to unmodified tumor-associated self antigens and induces potent antitumor immunity. J Immunol. 2005;174:2645-2652.

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Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti–DC-SIGN antibody

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Submission in opposition proceedings

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Patent No.	EP2359834
Application No.	EP11001632.6
Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Requests:

- Oral proceedings are hereby requested auxiliarily.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Any annexes (other than citation) to an opposition letter - D39	AMG17203OP_D39.pdf	OTHER-1.pdf
2	Any annexes (other than citation) to an opposition letter - D40	AMG17203OP_D40.pdf	OTHER-2.pdf
3	Any annexes (other than citation) to an opposition letter - D41	AMG17203OP_D41.pdf	OTHER-3.pdf
4	Any annexes (other than citation) to an opposition	AMG17203OP_D42.pdf	OTHER-4.pdf

	letter - D42		
5	Any annexes (other than citation) to an opposition letter - D43	AMG17203OP_D43.pdf	OTHER-5.pdf
6	Any annexes (other than citation) to an opposition letter - D44	AMG17203OP_D44.pdf	OTHER-6.pdf
7	Any annexes (other than citation) to an opposition letter - D45	AMG17203OP_D45.pdf	OTHER-7.pdf
8	Any annexes (other than citation) to an opposition letter - D46	AMG17203OP_D46.pdf	OTHER-8.pdf
9	Facts and arguments	AMG17203OP_Reply to proprietors submission.pdf	OPPO.pdf

Signatures

Place:	**Munich, Germany**
Date:	**21 November 2018**
Signed by:	**Hans Doerries 13689**
Representative name:	**H. Ulrich Dörries**
Capacity:	**(Representative)**

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Date of mention of the grant in the European Patent Bulletin (Art. 97(3), Art. 99(1) EPC)	09 November 2016
Title of the invention	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
Proprietor of the patent	Alexion Pharmaceuticals, Inc.

Documents attached:

	Description of document	Original file name	Assigned file name
1	Auxiliary request in opposition	Track Amended Auxiliary Request 1 C411599EP ALS PXT.pdf	AUXREQ-1.pdf
2	Auxiliary request in opposition	Auxiliary Request 1 C411599EP ALS PXT.PDF	AUXREQ-2.PDF
3	Auxiliary request in opposition	Track Amended Auxiliary Request 2 C411599EP ALS PXT.pdf	AUXREQ-3.pdf
4	Auxiliary request in opposition	Auxiliary Request 2 C411599EP ALS PXT.PDF	AUXREQ-4.PDF
5	Any annexes (other than citation) to an opposition letter - Consolidated list of documents cited	Consolidated List of Documents.PDF	OTHER-2.PDF
6	Any annexes (other than citation) to an opposition letter - Submission under R116	Submission under R116 C411599EP ALS PXT.pdf	OTHER-1.pdf

Evidence filed subsequently:

D39	Other evidence	Dr Klibanov Declaration original file name: D39 Klibanov Declaration C411599EP.pdf attached as: Other-evidence-2.pdf
D41	Other evidence	Alberts et al, Molecular Biology of the Cell, Third Edition, 1994, page 585. original file name: D41 Alberts et al (1994) page 585 - C411599EP.PDF attached as: Other-evidence-3.PDF

Signatures

Place:	
Date:	**21 November 2018**
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AUXILIARY REQUEST 2

1. A pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH) wherein the composition is a 300 mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution, wherein the composition is administered via IV infusion and diluted to a final concentration of 5 mg/ml prior to administration.

AUXILIARY REQUEST 1

1. A pharmaceutical composition for use in treating a patient afflicted with paroxysmal nocturnal hemoglobinuria (PNH) wherein the composition is a 300mg eculizumab single-use dosage form comprising 30 ml of a 10 mg eculizumab/ml sterile, preservative free solution, wherein administration is by intravenous infusion.

Declaration by Dr. Ronald Taylor

I, Ronald Taylor, Ph.D., declare that:

1. I was employed as a Professor of Biochemistry and Molecular Genetics at the University of Virginia from 1973 to 2016. I retired in 2016 and I am now Professor Emeritus and I still have an active work schedule based on submitting papers for publication, giving invited talks, serving on a study section, and reviewing papers. For almost 30 years, my laboratory has had a productive record in the study and characterization of monoclonal antibodies (mAbs), including humanized mAbs, and their use in clinical applications. A major focus of my research is anti-CD20 mAbs. In collaboration with investigators at the NIH and the Mayo Clinic, we have participated in numerous pilot clinical trials testing the feasibility of changing amount and frequency of dosing for CD20 mAb therapies for CLL. My work has resulted in 199 publications in peer-reviewed journals. A current copy of my CV is provided as **Appendix A.**

2. I have reviewed U.S. Patent Application Publication No. 2005/0191298 ("**D1**"), which is titled "Method of Treating Hemolytic Disease" and published on September 1, 2005. **D1** discloses that patients with paroxysmal nocturnal hemoglobinuria (PNH) were treated by administering eculizumab in four weekly 600 mg intravenous infusions, followed one week later by a dose of 900 mg, and then 900 mg on a bi-weekly basis (**D1**, [0082]). **D1** further discloses that anti-C5 mAbs such as eculizumab "can be administered in a variety of unit dosage forms" (**D1**, [0058]-[0060], [0062]).

3. The skilled artisan would have understood that pharmaceutical unit dosage forms containing a mAb should not be shared among patients or returned to storage after opening, in order to prevent contamination of the dosage form and any unnecessary health risks to the patient being treated with the dosage form. The skilled artisan also would have understood that

wasting mAb increases medical costs to the patient without any commensurate benefit. Thus, the skilled artisan would have had reason to prepare a single-use dosage form of eculizumab that could be used to administer both of the doses disclosed by **D1** (600 mg and 900 mg) without leaving any leftover drug as unusable waste. The skilled artisan would have understood that the most efficient way to meet that goal would be to prepare a 300 mg single-use dosage form because that dosage form could be used to administer both of the doses disclosed by **D1**—*i.e.*, two, 300 mg single-use dosage forms to administer the 600 mg dose and three, 300 mg single-use dosage forms to administer the 900 mg dose. Therefore, a 300 mg single-use dosage form would have been the most obvious and convenient single-use dosage form for preparing both the 600 mg and 900 mg doses of eculizumab for treating PNH.

4. The skilled artisan also would have recognized that preparing a single dosage form that could flexibly be used to administer either 600 mg or 900 mg offered advantages over developing a separate dosage form for each of the 600 mg and 900 mg doses. Developing any given dosage form requires testing, validating, manufacturing, storing, and transporting that dosage form. The skilled artisan would have known that it would be more efficient to avoid the redundancy and simply find a dosage form that could be used to administer both desired doses rather than creating multiple different dosage forms that would each require individual testing and validation, and may each require different storage or transport set-ups. Accordingly, efficiency would have provided the skilled artisan with further reason to use a 300 mg single-use dosage form.

5. One example of aligning antibody dosage form and administered doses known as of 2006 is the administration of the therapeutic antibody rituximab. Rituximab was administered in doses of 500 or 1000 mg to treat rheumatoid arthritis (e.g., Emery *et al.*, *Arthritis & Rheumatism* 2006, *54(5)*, 1390-1400 ("**D43**") at p. 1390). Consistent with maximizing efficiency and minimizing waste, the dosage forms of rituximab available as of 2006 were supplied as 100 mg and 500 mg sterile, preservative-free, single-use vials—both of which could be used to administer either a 500 or 1000 mg dose without waste and with maximal efficiency (e.g., Rituximab 1997 Product Data Sheet ("**D44**") at p. 2, right column, "How Supplied" section).

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6. In preparing the 300 mg eculizumab single-use dosage form, the skilled artisan also would have considered the appropriate concentration at which to formulate the mAb. Eculizumab is a humanized mAb with a hybrid IgG2/IgG4 constant domain. U.S. Patent No. 6,407,213 ("**D42**") is titled "Method for Making Humanized Antibodies" and issued on June 18, 2002. **D42** relates to methods for making and using humanized antibodies, including antibodies with hybrid constant domains (**D42**, col. 8, ll. 47-51). **D42** states that such humanized "antibodies will typically be formulated … at concentrations of about 1 mg/ml to 10 mg/ml" (**D42**, col. 45, li. 57, to col. 46, li. 2). Thus, selection of 10 mg/ml as a concentration for a humanized mAb with a hybrid IgG2/IgG4 constant domain, such as eculizumab, would have been a typical, arbitrary choice among known concentrations. Similarly, U.S. Patent Application Publication No. 2005/0271660 ("**D22**") discloses using eculizumab at a concentration between 1 and 30 mg/ml in nebulized formulations (**D22**, Fig. 10, [0170]-[0172]). From this, the skilled artisan would have understood that eculizumab is sufficiently stable and biologically active to be used as a drug at concentrations including 10 mg/ml. Thus, **D22** reinforces that selection of 10 mg/ml as a concentration for eculizumab would have been a typical, arbitrary choice among known concentrations, regardless of the route of administration. **D44** reinforces the typical nature of a 10 mg/ml concentration because it discloses that rituximab was supplied at that concentration (e.g., **D44** at p. 2, right column, "How Supplied" section).

7. Additionally, in choosing among these *typical* concentrations, the skilled artisan would have had a reason to prepare the 300 mg single-use dosage form using a 10 mg/ml concentration of eculizumab in order to minimize the volume of the 600 mg and 900 mg doses required for treating PNH. For example, preparing the 600 mg and 900 mg doses taught by **D1** would require 60 ml and 90 ml, respectively, of a 300 mg single-use dosage form having 10 mg/ml of the mAb. But preparing the same 600 mg and 900 mg doses would require 600 ml and 900 ml, respectively, of a 300 mg single-use dosage form having 1 mg/ml of the antibody. The skilled artisan would have understood that smaller volumes would be more convenient for storage, transportation, and administration of the mAb. Further, the skilled artisan would have been reluctant to use concentrations higher than the 10 mg/ml taught by **D42** because increased aggregation was known to result from increased concentration of antibodies (e.g., Wang *et al*., *J. Pharm. Sci*. 2007, *96(1)*, 1-26 ("**D20**") at p. 9, left column, 2nd full paragraph).

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8. Given that the skilled artisan would have had reason to use a 300 mg single-use dosage form with a concentration of 10 mg/mL, the skilled artisan necessarily would have arrived at a 30 mL volume. The volume of a 10 mg/mL solution needed to prepare 300 mg is, and can only be, 30 mL.

I certify that the above statements are correct to the best of my knowledge and belief.

Keswick, Virginia Nov 20, 2018
Place and Date

Dr. Ronald Taylor

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Appendix A

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ronald P. Taylor

eRA COMMONS USER NAME (credential, e.g., agency login): RPTNIH

POSITION TITLE: Emeritus Professor of Biochemistry and Molecular Genetics, University of Virginia Health Science Center

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
City College of New York, New York, NY	B.S.	1966	Chemistry
Princeton University, Princeton, NJ	Ph.D.	1970	Physical Chemistry
University of Minnesota, Mpls., MN	Post-doc	1971-1973	Physical Chemistry

A. Personal Statement

For almost 30 years my laboratory has had a long and productive record in the study and characterization of monoclonal antibodies and their use in several different clinical applications. We have a considerable level of experience in studying complement activation by, as well as effector cell-mediated killing of, tumor cells opsonized with monoclonal antibodies. Our laboratory discovered and has substantially characterized the shaving/trogocytosis reaction: that is, the loss of CD20 that occurs when CLL patients are treated with the usual (high) doses of rituximab or of ofatumumab. We have demonstrated, in several pilot clinical studies as well as in vitro investigations that much lower doses of CD20 mAbs, given more frequently, may provide far more effective therapies for CLL. We have also now described, in some detail at the cellular level, the complex series of events that occur when mAb-opsonized nucleated cells are killed by complement. Our laboratory also first described the exhaustion of complement which follows high dose rituximab therapy in CLL treatment.

I was appointed Professor Emeritus as of July 1, 2016. Since then I have maintained on-going collaborations with investigators in the US: Dr. D. Portilla (UVA), Dr. C. Zent (Rochester), Dr. A. Wiestner (NIH), Dr. J. Lambris (Penn), Dr. J. Thurman (U Colorado), Dr. S. Ram (U Mass); and in Europe: Dr. T. Mollnes (Norway) Dr. M. Huber-Lang (Germany), Dr. A. Verschoor (Germany) and the Genmab Biotech Company (The Netherlands).

B. Positions and Honors

Professional Positions:

1973-1978, Assistant Professor, Dept. of Biochemistry, University of Virginia.
1978-1983, Associate Professor, Dept. of Biochemistry, University of Virginia.
1983-present, Professor, Dept. of Biochemistry and Molecular Genetics, University of Virginia.
1991-present, Member, Beirne B. Carter Center for Immunology Research, University of Virginia.
1997-present, Member, NCI Sponsored Cancer Center, University of Virginia.

Awards and Other Professional Activities:

1965, Phi Beta Kappa; 1966, ECAC Scholar-Athlete Award; 1966-1969, NDEA Graduate Fellow; 1970, NSF Graduate Fellow; 1972-1973, NIH Fellow; 1975-1980, NIH Research Career Development Award; 1992, Ad

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Hoc Study Section, Immunological Sciences, NIH; 1991-1993, Shannon Award, NIH; 1990-1994, Editorial Board, Arthritis and Rheumatism; 1990, Fellowship Subcommittee, Arthritis Fdn; 2008, DARPA Significant Technical Achievement Award; 2010-2015, Editorial Board, Haematologica; 2010-2014, Associate Editor, Journal of Immunology; Ad hoc reviewer: J. Immunology, J. Immunological Methods, J. Clin Invest, Blood, Mol. Immunology, Immunology, Science Translational Medicine, and many Cancer Journals: Reviewer, NIH: 2003-2004, Exptl. Immunol; 2004,Tumor Immunol./Immunotherapy; 2007-2018 (ongoing), Bench to Bedside NIH Study Section.

C. Contributions to Science (total publications: 199)

1. Our laboratory has pioneered the detailed and comprehensive evaluation of blood samples taken from patients before, during, and after CD20 therapy for CLL. This work led to our discovery of the shaving/trogocytosis reaction, as well as to the finding that immune effector functions in the body, such as complement, can be saturated/exhausted when CLL patients receive the usual high CD20 mAb doses (300 mg to 2 grams). This work also directly led to our in vitro analyses and mechanistic descriptions of trogocytosis, in which we demonstrated that it is mediated by Fcγ receptors on effector cells including monocyte/macrophages, neutrophils, and NK cells. We have also described the process in a mouse model and demonstrated that the reaction can be generalized to include other FDA-approved immunotherapeutic mAbs including cetuximab and traustuzumab. Finally, in collaboration with investigators at the NIH and the Mayo Clinic, we have been able to participate in numerous pilot clinical trials that have indicated the potential feasibility of changing CD20 mAb therapies for CLL to low doses (~35 mg) three times a week, which ultimately can be based on subcutaneous infusions. If this approach can be clearly validated and generalized, it would provide a very important and revolutionary paradigm shift that could benefit both patients and health-care providers, based on efficacy, costs, and convenience.

Most recently, in collaboration with several investigators we have also been studying how complement plays a role in the pathogenesis of several diseases and syndromes associated with inflammation and tissue damage, including malaria and kidney injury due to tubulointerstitial fibrosis. This work has the potential to lead to new and innovative strategies for disease treatment, based on selective inhibition of key steps in the complement cascade(s).

1. Kennedy, AD, Beum, PV, Solga, MD, DiLillo, DJ, Lindorfer, MA, Hess, CE, Densmore, JJ, Williams, ME, **Taylor, R.P**. Rituximab infusion promotes rapid complement depletion and acute CD20 loss in chronic lymphocytic leukemia. *J. Immunology* 172(5):3280-8, 2004. PMID: 14978136
2. Beum, P.V., Kennedy, A.D., Williams, M.E., Lindorfer, M.A., **Taylor, R.P.** The shaving reaction: Rituximab/CD20 complexes are removed from mantle cell lymphoma and chronic lymphocytic leukemia cells by THP-1 monocytes. *J. Immunology* 176:2600-2609, 2006. PMID: 16456022
3. Williams, M.E., Densmore, J.J., Pawluczkowycz, A.W., Beum, P.V., Kennedy, A.D., Lindorfer, M.A., Hamil, S.H., Eggleton, J.C., **Taylor, R.P.** Thrice-weekly low-dose Rituximab decreases CD20 loss via shaving and promotes enhanced therapeutic targeting in chronic lymphocytic leukemia. *J. Immunology* 177:7435-7443, 2006. PMID: 17082663
4. Glennie, M.J. ,French, R., Cragg, M.S., **Taylor, R.P.** Mechanisms of killing by anti-CD20 monoclonal antibodies. *Molecular Immunology* 44: 3823-3837, 2007. PMID: 17768100
5. Li, Y., Williams, M.E., Cousar, J.B., Pawluczkowycz, A.W. Lindorfer, M.A., **Taylor, R.P.** Rituximab/CD20 Complexes Are Shaved from Z138 Mantle Cell Lymphoma Cells in Intravenous and Subcutaneous SCID Mouse Models. *J. Immunology* 179: 4263-4271, 2007. PMID: 17785867
6. Beum, PV, Lindorfer, MA, **Taylor, R.P.** Within Peripheral Blood Mononuclear Cells, Antibody-Dependent Cellular Cytotoxicity of Rituximab-opsonized Daudi cells is Promoted by Natural Killer Cells and Inhibited by Monocytes Due to Shaving. *J. Immunology* 181: 2916-2924, 2008. PMID: 18684983
7. Aue, G, Lindorfer, MA, Beum, PV, Pawluczkowycz, AW, Vire, B, Hughes, T, **Taylor, R.P.**, Wiestner, A Fractionated Subcutaneous Rituximab Preserves CD20 Expression on Tumor Cells in Patients with Chronic Lymphocytic Leukemia. *Haematolgica* 95:329-332, 2010. PMCID: PMC2817038
8. Daubeuf, S, Lindorfer, MA, **Taylor, R.P.**, Joly, E and Hudrisier, D. The direction of plasma membrane exchange between lymphocytes and accessory cells by trogocytosis is influenced by the nature of the accessory cell. *J. Immunology* 184: 1897-1908, 2010. PMID: 20089699

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9. Beum, PV, Peek, EM, Lindorfer, MA, Beurskens, FJ, Engelberts, PJ, Parren, PHI, van de Winkel, JGJ and **Taylor, R.P.** Loss of CD20 and Bound CD20 Antibody From Opsonized B cells Occurs More Rapidly due to Trogocytosis Mediated by Fc Receptor-Expressing Effector Cells than due to Direct Internalization by the B cells. *J. Immunology*, 187:3438-3447,2011. PMID: 21841127.
10. Beurskens, FJ, Lindorfer, MA, Farooqui, M, Beum, PV, Engelberts, P, Mackus, WJM, Parren, PWHI, Wiestner, A, and **Taylor, R.P**. Exhaustion of Cytotoxic effector Systems May Limit Monoclonal Antibody-Based Immunotherapy in Cancer Patients. *J. Immunology*, 188:3532-3541, 2012. PMCID: PMC3311731
11. Taylor, R.P., Lindorfer, MA, and Zent, CWS. Letter to the Editor, New England Journal of Medicine. Anti-CD20 Antibody Therapy for B-Cell Lymphomas. N. Eng. J. Medicine, 367: 876, 2012. The letter is focused on the mechanism of action of CD20 mAbs, in response to a review written by D Maloney for the journal.
12. Mo, CC, Njuguna, Beum, PV, Lindorfer, MA, Vire, B, Lee, E, Marti, G, Wilson, WH, **Taylor, RP,** and Wiestner, A. Rapid clearance of rituximab may contribute to the continued high incidence of autoimmune hematologic complications of chemoimmunotherapy for chronic lymphocytic leukemia Haematologica,98: 1259-1263, 2013.
13. Taylor, RP. 2013. Gnawing at Metchnikoff's paradigm. *Blood* 122:2922-2924.
14. Baig, N. A., **R. P. Taylor**, M. A. Lindorfer, A. K. Church, B. R. LaPlant, A. M. Pettinger, T. D. Shanafelt, G.S. Nowakowski, and C. S. Zent. 2014. Induced resistance to ofatumumab-mediated cell clearance mechanisms, including complement-dependent cytotoxicity, in chronic lymphocytic leukemia. J.Immunol. 192:1620-1629
15. Lindorfer, MA, Bakker, J, Parren PWHI, **Taylor, RP**. Ofatumumab: a next-generation human therapeutic CD20 antibody with potent complement-dependent cytotoxicity. Handbook of Therapeutic Monoclonal Antibodies Wiley VCH 2014.
16. Zent, C. S**., R. P. Taylor**, M. A. Lindorfer, P. V. Beum, B. LaPlant, W. Wu, T. G. Call, D. A. Bowen, M. J. Conte, L. A. Frederick, B. K. Link, S. E. Blackwell, S. Veeramani, N. A. Baig, D. S. Viswanatha, G. J. Weiner, and T. E. Witzig. 2014. Chemoimmunotherapy for relapsed/refractory and progressive 17p13 deleted chronic lymphocytic leukemia (CLL) combining pentostatin, alemtuzumab, and low dose rituximab is effective and tolerable and limits loss of CD20 expression by circulating CLL cells. Amer.J.Hematol. 89: 757-765, 2014
17. Taylor, R.P. and M.A. Lindorfer Analyses of CD20 Monoclonal Antibody-Mediated Tumor Cell Killing Mechanisms: Rational Design of Dosing Strategies. Molecular Pharmacology 86;485-491, 2014
18. Taylor, R.P. and M.A. Lindorfer Blood Spotlight. Fcγ-receptor-mediated trogocytosis impacts mAb-based therapies: historical precedence and recent developments. Blood 125: 762-766, 2015.
19. Lindorfer, M.A., Cook, E.M., Reis, E.S., Ricklin, D., Risitano, A.M., Lambris, J.D., **Taylor, R.P.** Compstatin Cp40 blocks hematin-mediated deposition of C3b fragments on erythrocytes: Implications for treatment of malarial anemia. Clin Immunol., 171: 32-35, 2016.
20. Taylor, R.P Neutrophils: Positive or Negative? Commentary in Blood, 129: 2596-2597, 2017
21. Xavier, S., Sahu, R.K. Landes, S.G., Yu, J., **Taylor, R.P**., Ayyadevara, S., Megyyesi, J., Stallcup, W.B., Duffield, J.S., Reis, E.S., Lambris, J.D., Portilla, D. Pericytes and immune cells contribute to complement activation in tubulointerstitial fibrosis. Renal Physiology: 312: F516-F532, 2017.
22. Denk, S., **Taylor R.P**., Wiegner, R., Cook, E.M., Pfeiffer, K., Paschke, S., Eiseler, T., Weiss, M., Barth, E., Lambris, J.D., Kalbitz, M., Martin, T., Barth, H., Messerer, D.A.C., Gebhard, F., Huber-lang, M.S. Complement C5a-induced changes in neutrophil morphology during inflammation. Scan J. Immunol., 86: 143-155, 2017.
23. Taylor, R.P., Stoute, J.A., Lindorfer, M.A. Mechanisms of Complement Activation in Malaria, pp 31-49. In Complement Activation in Malaria Immunity and Pathogenesis, edited by J. A. Stoute. Springer Nature, 2018.
24. **Taylor, R.P.** ,Lindorfer, M.A. Mechanisms of Complement-Mediated Damage in Hematological Disorders. Semin Hematol 55: 118-123, 2018.
25. Jalal, D., Renner, B., Laskowski, J., Stites, E., Cooper, J., Valente, K., You, Z., Perrenoud, L., Le Quintrec, M., Muhamed, I., Christians, U., Klawitter, J., Lindorfer, M.A., **Taylor, R.P**., Holers, V.M., Thurman, J.M. Endothelial Microparticles and systemic complement activation in patients with Chronic kidney disease. J Amer Heart Assoc 7: 2018.

2. A second focus in our laboratory has been based on studying the basic scientific details by which mAbs promote CDC of targeted cells. This led to our discovery of "streamers," or tunneling nanotubules, that are released when the complement membrane attack complex penetrates the cell and large amounts of calcium enter the cytoplasm. We have also been able to describe the sequence of cellular events that occurs during the CDC process, based on 4-color movies generated in confocal microscopy experiments. This work, in collaboration with the biotech company Genmab, also led to our description of hexamer-forming mAbs that are

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far more effective than wild-type mAbs in promoting CDC. It is very likely that these next generation mAbs (for CD20 as well as other targets) will find clinical applications in the not-too-distant future.

23. Beum, PV, Lindorfer, MA, Beurskens, F, Stukenberg, PT, Lokhorst, HM, Pawluczkowycz, AW, Parren, PWHI, van de Winkel, JGJ, **Taylor, R.P.** Complement Activation on B Lymphocytes Opsonized with Rituximab and Ofatumumab Produces Substantial Changes in Membrane Structure Preceding Cell Lysis. *J. Immunology* 181: 822-832, 2008. PMID: 18566448
24. Pawluczkowycz, AW, Beurskens, FJ, Beum, PV, Lindorfer, MA, van de Winkel, JGJ, Parren, PWHL, **Taylor, R.P.** Binding of Submaximal C1q Promotes Complement Dependent Cytotoxicity (CDC) of B cells Opsonized with anti-CD20 mAbs Ofatumumab (OFA) or Rituximab (RTX). Considerably Higher Levels of CDC are Induced by OFA Than by RTX. *J. Immunology* 183: 749-758, 2009. PMID: 19535640
25. Beum, PV, Lindorfer, MA, Peek, EM, Stukenberg, PT, de Weers, M, Beurskens, FJ, Parren, PWHI, van de Winkel, JGJ, **Taylor,R.P.** Penetration of antibody-opsonized cells by the membrane attack complex of complement promotes Ca2+ influx and induces streamers. Eur J Immunol 41: 2436-2446, 2011
26. Baig, NA, **Taylor, R.P.**, Lindorfer, MA, Church, AK, LaPlant, ES, Nowakrowski, GS, Zent, CS. Complement dependent cytotoxicity (CDC) in chronic lymphocytic leukemia (CLL): Ofattumumab enhances alemtuzumab CDC and reveals cells resistant to activated complement. *Leukemia and Lymphoma,* 53:2218-2227, 2013 PMID: 22475085
27. Lindorfer, MA, Koehl, J, and **Taylor,R.P.** Interactions between the Complement System and Fcγ Receptors. Invited chapter for book, "IgG Fc: Linking Adaptive and Innate Immunity", edited by F. Nimmerhahn and M Ackerman, Elsevier Press, 2013.
28. Pokrass, M. J., M. F. Liu, M. A. Lindorfer, and **R. P. Taylor**. 2013. Activation of complement by monoclonal antibodies that target cell-associated α2-microglobulin: implications for cancer immunotherapy. *Mol.Immunol.* 56:549-560.
29. Lindorfer, MA, Beum, PV, **Taylor, RP**. CD20 mAb-Mediated Complement Dependent Cytotoxicity of Tumor Cells is Enhanced by Blocking the Action of Factor I. Antibodies 2: 598-616, 2013
30. Taylor, R. P. and M. A. Lindorfer. 2014. **T**he role of complement in mAb-based therapies of cancer. Methods 65:18-27.
31. Diebolder CA. Beurskens FJ. de Jong RN. Koning RI. Strumane K. Lindorfer MA. Voorhorst M. Ugurlar D. Rosati S. Heck AJ. van de Winkel JG. Wilson IA. Koster AJ. **Taylor RP**. Saphire EO. Burton DR. Schuurman J. Gros P. Parren PW. Complement is activated by IgG hexamers assembled at the cell surface. Science 343: 1260-1263, 2014.
32. Da Roit,F., Engelberts, P.W., **Taylor, R.P.,** Breij, E., Gritti,G., Rambaldi, A., Introna M., Parren,P.W.H.I., Beurskens,F.J., and Golay, J. Ibrutinib interferes with the cell-mediated anti-tumor activities of therapeutic CD20 antibodies: implications for combination therapy. Haematologica 100:77-86, 2015
33. Ewald T.J. van den Bremer, E., Beurskens, F.J., Voorhorst, M., Engelberts, P.J., de Jong, R.N., Cook, E.M. Lindorfer, M.A., **Taylor, R.P**., van Berkel, P.H., and Parren, P.H.I. Human IgG is produced in a pro-form that requires clipping of C-terminal lysines for maximal complement activation. mAbs*, 7, 4, 672, 201,2015*
34. Lindorfer, M.A., Cook, E.M., Tupitza, J.C., Zent, C.S., Burack, R., de Jong, E.N., Beurskens, F.J., Schuurman, J., Parren, P.W.H.I., **Taylor, R.P**. Real-time analysis of the detailed sequence of cellular events in mAb-mediated complement-dependent cytoxicity of B-cell lines and of chronic lymphocytic leukemia cells. Molecular Immunology 70: 13-23, 2016.
35. Taylor, R.P., and Lindorfer, M.A. Cytotoxic Mechanisms of Immunotherapy: Harnessing Complement in The Action of Anti-tumor Monoclonal Antibodies. Seminars in Immunology 28: 309-316, 2016
36. Cook, E.M., Lindorfer, M.A., van der Horst, H., Oostindie, S. Beurskens, F.J., Schuurman, J., Zent, C.S., Burack, R., Parren, P.W.H.I, **Taylor, R.P.** Antibodies that efficiently form hexamers upon antigen binding can induce complement-dependent cytotoxicity under complement-limiting conditions J Immunol 197: 1762-1775, 2016
37. **Taylor, R.P**., Lindorfer MA, Cook, E.M., Beurskens, F.J., Schuurman, J., Parren, P.W.H.I., Zent, C.S., Van DerMeid, K., Burack, R., Mizuno, M., Morgan, B.P. Hexamerization-enhanced CD20 antibody mediates complement-dependent cytotoxicity in serum genetically deficient in C9. Clinical Immunology 181: 24-28, 2017.
38. Lee, C.H., (many authors, including **Taylor, R.P.)** Georgiou, G. IgGFc domains that bind C1q but not Fcgamma receptors delineate the importance of complement-mediated effector functions. Nature Immunology 18: 889-898, 2017.

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Julian Lewis • Martin Raff • Keith Roberts
James D. Watson



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Bruce Alberts received his Ph.D. from Harvard University and is currently President of the National Academy of Sciences and Professor of Biochemistry and Biophysics at the University of California, San Francisco. *Dennis Bray* received his Ph.D. from the Massachusetts Institute of Technology and is currently a Medical Research Council Fellow in the Department of Zoology, University of Cambridge. *Julian Lewis* received his D.Phil. from the University of Oxford and is currently a Senior Scientist in the Imperial Cancer Research Fund Developmental Biology Unit, University of Oxford. *Martin Raff* received his M.D. from McGill University and is currently a Professor in the MRC Laboratory for Molecular Cell Biology and the Biology Department, University College London. *Keith Roberts* received his Ph.D. from the University of Cambridge and is currently Head of the Department of Cell Biology, the John Innes Institute, Norwich. *James D. Watson* received his Ph.D. from Indiana University and is currently Director of the Cold Spring Harbor Laboratory. He is the author of *Molecular Biology of the Gene* and, with Francis Crick and Maurice Wilkins, won the Nobel Prize in Medicine and Physiology in 1962.

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Front cover: The photograph shows a rat nerve cell in culture. It is labeled (*yellow*) with a fluorescent antibody that stains its cell body and dendritic processes. Nerve terminals (*green*) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

Dedication page: Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

Back cover: The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)

tein translocator. There is now strong evidence for a protein translocator. Normally, the pore in the translocator is plugged with the growing polypeptide chain that is in transit across the membrane; when the nascent chains are experimentally released from the ribosomes with the drug puromycin, however, the pores can be detected by the ion currents that flow through them. Although the pore is large enough to allow the passage of an unfolded polypeptide chain, it closes when the ribosome is removed from the membrane (Figure 12–42). Thus the pore seems to be a dynamic structure, opening when a ribosome with a growing polypeptide chain attaches to the membrane and closing when the ribosome detaches after the synthesis of the protein is completed.

The ER Signal Peptide Is Removed from Most Soluble Proteins After Translocation [31]

We have seen that in chloroplasts and mitochondria the signal peptides are cleaved from the precursor proteins once they have crossed the membrane. Similarly, amino-terminal ER signal peptides are removed by a signal peptidase on the luminal side of the ER membrane. The peptide by itself, however, is not sufficient to signal cleavage by the peptidase; this requires an adjacent cleavage site that is specifically recognized by the peptidase. We shall see below that ER signal peptides that are contained within the polypeptide chain rather than at the amino terminus do not have these recognition sites and are never cleaved; instead, they can serve to retain transmembrane proteins in the lipid bilayer after the translocation process has been completed.

The amino-terminal ER signal peptide of a soluble protein itself has two signaling functions: in addition to directing the protein to the ER membrane, it is thought to serve as a **start-transfer signal,** which remains bound to the translocation apparatus while the rest of the protein is threaded continuously through the membrane as a large loop. Once the carboxyl terminus of the protein has passed through the membrane, the signal peptide is released from the translocator pore, cleaved off by the signal peptidase, and rapidly degraded to amino acids by other proteases in the ER while the protein is released into the ER lumen (Figure 12–43).

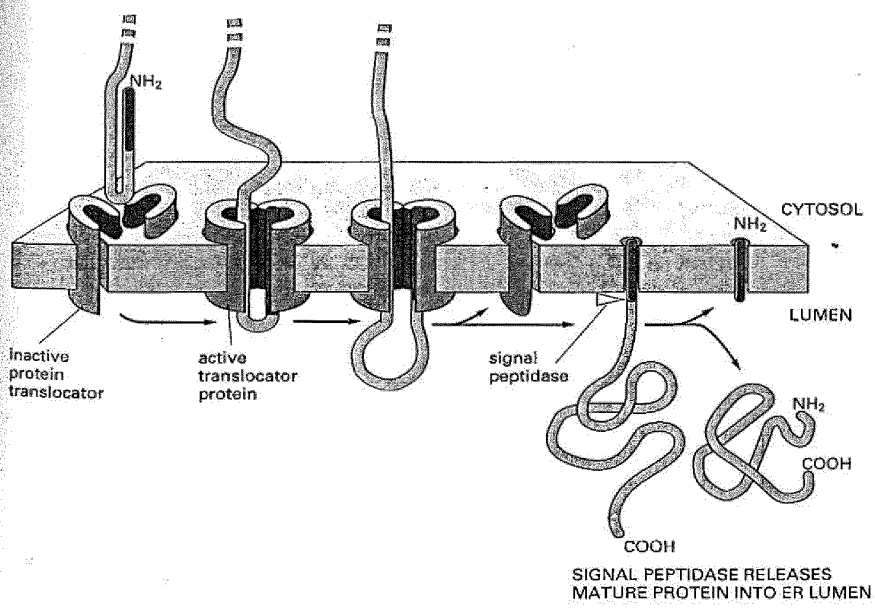


Figure 12–43 **The translocation of a soluble protein across the ER membrane.** In this hypothetical model the protein translocator in the membrane is postulated to exist in two alternative states—active or inactive. On binding an ER signal peptide (which acts as a start-transfer signal), the translocator adopts an active state and begins to transfer the polypeptide chain across the lipid bilayer as a loop. In this state it forms an aqueous pore across the membrane that can be detected electrophysiologically if the polypeptide chain is released (see Figure 12–42). After the protein has been completely translocated, the translocator reverts to an inactive conformation, which can no longer conduct ions across the membrane but is open to the lipid bilayer, allowing the hydrophobic signal peptide to diffuse out into the bilayer, where it is rapidly degraded. In this and the following two figures the ribosomes have been omitted for clarity.

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IN THE MATTER OF
European Patent No. EP 2 359 834

DECLARATION OF PROFESSOR ALEXANDER M. KLIBANOV

I, Dr. Alexander M. Klibanov, hereby declare as follows:

1. I am a Professor of Chemistry and Bioengineering at the Massachusetts Institute of Technology ("M.I.T."), where I have been teaching and conducting research for more than 39 years. I currently hold the Novartis Endowed Chair Professorship. I obtained an M.S. degree in Chemistry in 1971 and a Ph.D. in Chemical Enzymology in 1974, both from Moscow University in Russia.

2. Throughout my career as a practicing chemist, I have extensively researched, published, taught, and lectured in many areas of biological, medicinal, protein, and pharmaceutical formulation chemistry. For my professional accomplishments, I have received numerous honors. For example, I was elected to the U.S. National Academy of Sciences and the U.S. National Academy of Engineering; I have also received various awards and prizes from the American Chemical Society, as well as other national and international organizations. I serve on the Editorial Boards of over a dozen scientific journals, including the "Journal of Biological Chemistry and Molecular Pharmacology" and the "International Journal of Drug Design, Delivery and Safety." I have published some 315 scientific papers in various chemical disciplines, including protein stability and stabilization, as well as drug formulation and delivery. I have also founded six pharmaceutical companies and have been on the scientific advisory boards and/or boards of directors of those companies, as well as of many others. A number of these advisory and directorship activities have involved the formulation, stability, stabilization, administration, and pharmacological evaluation of protein-based therapeutic agents, including antibodies. A copy of my curriculum vitae is attached as Exhibit 1.

3. I have been asked by Alexion to provide information regarding the field of protein, including antibody, pharmaceutical formulations as of 2007 and what understanding and expectations a person skilled in that field would have had concerning their stability at that time.

4. One of the major challenges in developing a successful pharmaceutical formulation of a therapeutic antibody (and of any therapeutic protein) around 2007 was, and still is today, being able to create a composition that is stable enough for sufficiently long periods of time such that the antibody does not undergo deleterious physical or chemical degradation reactions or form aggregates prior to administration. If the antibody is not adequately stable in the composition, serious problems can arise, such as drastically diminished efficacy and adverse immune responses in the patient. Moreover, when developing an antibody for commercial therapeutic use, it is often critical to be able to formulate the antibody at a rather high concentration (thus allowing a low-volume dosage).

5. Based on my professional experience, in the 2007 time-frame (or even today) there was no assurance that a skilled person, when provided with a new therapeutic protein, including an antibody, would have been able to develop a suitably stable pharmaceutical composition to make it commercially viable. My experience in this regard is confirmed by relevant scientific publications around that time period, as discussed below.

6. For example, a 2002 book entitled "Rational Design of Stable Protein Formulations. Theory and Practice" (the "2002 book;" Ex. 2) describes the uncertainty that existed in the protein formulation field regarding the ability to develop a suitably stable liquid pharmaceutical composition for a new protein. The authors of the 2002 book state that "protein drugs may not be stable enough to be handled as a liquid formulation" and concede that "with many proteins, it is not possible – especially considering the time constraints with product development – to develop sufficiently stable aqueous formulations." (Ex. 2 at 10 and 109.) The authors suggested that there was no reasonable expectation at the time of being able to develop such a composition, especially a liquid one, *e.g.*, stating that "[i]t can be *assumed* that most proteins will not exhibit sufficient stability in aqueous solution to allow a liquid formulation to be developed." (Ex. 2 at 188.) Consequently, most pharmaceutical proteins, such as antibodies, commercially available even today are in a lyophilized form, which requires their reconstitution prior to administration, thereby making it far less convenient and more expensive than aqueous solutions.

7. As the 2002 book recognized and was understood both then and now, numerous factors influence the stability of an antibody (or any protein), thus making the development of a suitable pharmaceutical composition in each new specific case a unique challenge. The book explains that "the exquisite sensitivity of protein structure, function, and stability to the primary sequence does not readily lend itself to a generic approach for protein formulation" and that "[e]ven for closely related proteins, the relative stability and major pathways for degradation might be quite different." (Ex. 2 at 185-186.) In the 2007 time-frame, researchers like myself understood that therapeutic antibody (or protein in general) formulations, especially liquid ones, are ***not*** amenable to a "one size fits all" approach. Therefore, when given a new antibody and tasked with creating its suitable pharmaceutical composition, a person skilled in the art would have had little guidance in that endeavor. Indeed, the 2002 book recognized that developing such a stable composition "is not a simple task" and that "for most proteins, maintaining physical and chemical stabilities in aqueous solution for an extended period of time is extremely difficult" (Ex. 2 at 10-11 and 184).

8. A review article entitled "Formulation and delivery issues for monoclonal antibody therapeutics" published in 2006 in a peer-reviewed international journal had recognized the uniqueness of each therapeutic antibody's challenge when developing a suitably robust formulation: "A priori, one might assume that by finding a stable formulation for one of these antibody drugs, that such a formulation would be good for most[,] if not all, similar antibodies. If this were borne out by experience, there would be no need for a review such as this. Instead, each antibody seems to have a unique personality related to its requirements for

stability." (D21 at 690). For this reason, proteins typically have to be evaluated individually and stabilized on an empirical basis, which inevitably leads to unpredictability.

9. Another, 2007 review paper entitled "Antibody structure, instability, and formulation" points out that "antibodies, like other proteins, may generate a variety of degradants during production, processing, and storage both in liquid and solid states. Their tendency to generate such degradants depends very much on individual sequence, pI, hydrophobicity, and carbohydrate content." (D20 at 14). The review noted numerous variables affecting each of the many causes of antibody instability; because they were, in turn, influenced by a number of intertwining factors, as well as by the structure of the protein molecule itself, a person skilled in the art in 2007 would have recognized that predicting in advance whether or how a new antibody could be stabilized under relevant conditions at a particular concentration in a pharmaceutical composition was not possible: "It should be stressed that one formulation excipient stabilizing a specific antibody may not be suitable for another because of the difference in their sequence." (D20 at 14).

10. Given the complexity and the sheer number of the various modes of protein instability and the diverse mechanisms underlying each of them, there was no universal approach to successfully formulating an antibody in 2007 (nor is there one now). My both academic and industrial experience at the time was that the field of antibody formulation was difficult, case-specific, and uncertain. For these reasons, a person skilled in the art aiming to develop a liquid formulation of a new antibody with a sufficient storage stability at a high concentration would ***not*** have been able to predict which, if any, such formulation would meet the necessary requirements.

I do hereby declare that the facts stated above are true to the best of my knowledge.



Dr. Alexander M. Klibanov

November 16, 2018

Date

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Exhibit 1: Dr Klibanov Curriculum Vitae

Exhibit 2: Rational Design of Stable Protein Formulations, Pharmaceutical Biotechnology Volume 13, 2002 Kluwer Academic/Plenium Publishers; Chapters 1, 5 and 8 (D40).

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Curriculum vitae

ALEXANDER M. KLIBANOV

Date and Place of Birth: July 15, 1949, in Moscow (Russia)

Nationality: Naturalized U.S. Citizen (1983)

Education:

1974	Ph.D. in Chemical Enzymology, Moscow University
1971	M.S. in Chemistry, Moscow University

Honors:

2015	Tau Beta Pi's Leonardo Da Vinci Lecturer, MIT
2007-11 and 2014-	Novartis Chair Endowed Professorship, MIT
2012-13	Roger and Georges Firmenich Endowed Professorship, MIT
2011	MIT Biological Engineering Senior Class Faculty Award
2006	Distinguished GRUM Lecturer in Drug Discovery & Development, University of Montreal (Canada)
2004	UNAM Distinguished Lecturer, National University of Mexico (Mexico City)
2001	Walter Enz Lecturer in Pharmaceutical Chemistry, University of Kansas
2001	Elected a Corresponding Fellow of the Royal Society of Edinburgh (Scotland's National Academy of Science and Letters)
2000	Merck Distinguished Lecturer, Rutgers University
2000	Top 20 *Biotechnology & Bioengineering* Papers of the Last Forty Years
1998	Robert Lutz Lecturer, University of Virginia
1996	Perkin-Elmer Distinguished Lecturer, University of Pittsburgh
1995	Elected to the National Academy of Sciences of the U.S.A.
1995	Nathan O. Kaplan Memorial Lecturer in Biological Chemistry, University of California at San Diego
1994	R.W. Johnson PRI Lecturer, Pharmaceutical Research Institute
1994	Warren McCabe Lecturer, North Carolina State University
1993	Elected to the National Academy of Engineering of the U.S.A.
1993	Arthur C. Cope Scholar Award of the American Chemical Society
1993	Biotechnology Divisional Lectureship Award of the Institute of Food Technologists
1992	Charles Sabat Lecturer, Rutgers University
1992	Elected a Founding Fellow of the American Institute for Medical and Biological Engineering
1992	Louis C. Jordy Research Scholar Lecturer, Drew University
1991	International Enzyme Engineering Award
1991	Marvin J. Johnson Award of the American Chemical Society
1990	Monsanto Lecturer, Ohio State University
1990	NRC Distinguished Lecturer, Academia Sinica (Taiwan)
1989	Ipatieff Prize of the American Chemical Society
1989	Backer Lecturer, Groningen University (Holland)
1988	Dow Lecturer, University of Ottawa (Canada)
1987	Distinguished Scholar Lecturer, Hope College
1986	Leo Friend Award of the American Chemical Society
1984	Who's Who in Frontier Science and Technology

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1984	Sohio Lecturer, Case Western Reserve University
1982	American Men and Women of Science
1981-1983	Henry L. Doherty Career Development Professorship, MIT
1975	U.S.S.R. Ministry of Higher Education Prize

Professional Experience:

2007-11 and 2014-	Novartis Chair Endowed Professor of Chemistry and Bioengineering, MIT
2012-2013	Roger and Georges Firmenich Professor of Natural Products Chemistry Department of Chemistry, MIT
2000-present	Professor of Bioengineering Department of Biological Engineering, MIT
1988-present	Professor of Chemistry Department of Chemistry, MIT
1987-1988	Professor of Applied Biochemistry Department of Applied Biological Sciences, MIT
1983-1987	Associate Professor of Applied Biochemistry Department of Applied Biological Sciences (formerly Department of Nutrition and Food Science), MIT
1979-1983	Assistant Professor of Applied Biochemistry Department of Nutrition and Food Science, MIT
1977-1979	Postdoctoral Associate, Department of Chemistry University of California at San Diego
1974-1977	Research Chemist Department of Chemistry, Moscow University

Current Journal Editorial/Advisory Boards:

"Biocatalysis and Biotransformation", "Applied Biochemistry and Biotechnology", "Central European Journal of Chemistry", "Biotechnology Progress", "Biotechnology & Bioengineering", "Microbial Biotechnology", "Open Journal of Pharmacology", "Nanocarriers", "Journal of Antivirals and Antiretrovirals", "Open Access Academic Books in Chemistry", "Journal of Biological Chemistry and Molecular Pharmacology", "Recent Patents in Biotechnology", "Current Pharmaceutical Biotechnology"

Professional Societies:

U.S. National Academy of Sciences, U.S. National Academy of Engineering, American Chemical Society, Society for Applied Microbiology

Current Research Interests:

Enzyme chemistry and biotechnology
Medicinal and formulation chemistry
Protein drug delivery
Antimicrobial polymers
Enzymes as stereoselective catalysts in organic syntheses
Stabilization and formulation of macromolecular pharmaceuticals

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Publications:

1. Varfolomeyev, S.D., Klibanov, A.M., Berezin, I.V. 1971. Light-initiated enzymic activity caused by photostereoisomerization of *cis*-4-nitrocinnamoyl-α-chymotrypsin. FEBS Lett. 15: 118 -120.

2. Varfolomeyev, S.D., Klibanov, A.M., Martinek, K., Berezin, I.V. 1972. Light-sensitive catalysts. 4-Nitrocinnamoyl residue as a chromophoric reporter group in the α-chymotrypsin active center. Dokl. Acad. Nauk SSSR 203: 616-619.

3. Klibanov, A.M., Samokhin, G.P., Martinek, K., Berezin, I.V. 1974. Mechanochemistry of catalytic systems. Regulation by a mechanical action of the enzymatic properties of α-chymotrypsin covalently attached to a nylon fiber. Dokl. Acad. Nauk SSSR 218: 715-718.

4. Berezin, I.V., Varfolomeyev, S.D., Klibanov, A.M., Martinek, K. 1974. Light and ultrasonic regulation of α-chymotrypsin catalytic activity. Proflavin as a light- and sound- sensitive competitive inhibitor. FEBS Lett. 39: 329-331.

5. Berezin, I.V., Klibanov, A.M., Martinek, K. 1974. The mechanochemistry of immobilized enzymes. How to steer a chemical process at the molecular level by a mechanical device. Biochim. Biophys. Acta 364: 193-199.

6. Berezin, I.V., Klibanov, A.M., Goldmacher, V.S., Martinek, K. 1974. Mechanochemistry of catalytic systems. Regulation by a mechanical action of the enzymatic activity of trypsin entrapped in polyacrylamide gel. Dokl. Acad. Nauk SSSR 218: 367-370.

7. Klibanov, A.M., Martinek, K., Berezin, I.V. 1974. The effect of ultrasound on α-chymotrypsin. A novel approach to studying conformational transitions in active centers of enzymes. Biochemistry SSSR 39: 878-887.

8. Berezin, I.V., Klibanov, A.M., Klyosov, A.A., Martinek, K., Svedas, V.K. 1975. The effect of ultrasound as a new method of studying conformational transitions in enzyme active centers. pH- and temperature-induced conformational transitions in the active center of penicillin amidase. FEBS Lett. 49: 325-328.

9. Berezin, I.V., Klibanov, A.M., Martinek, K. 1975. Kinetic and thermodynamic aspects of catalysis by immobilized enzymes. Russ. Chem. Revs. 44: 17-47.

10. Martinek, K., Goldmacher, V.S., Klibanov, A.M., Berezin, I.V. 1975. Denaturing agents (urea, acrylamide) protect enzymes against irreversible thermoinactivation: a study with native and immobilized α-chymotrypsin and trypsin. FEBS Lett. 51: 152-155.

11. Martinek, K., Klibanov, A.M., Tchernysheva, A.V., Berezin, I.V. 1975. The stabilization of α-chymotrypsin by entrapment in polymethacrylate gels. Dokl. Acad. Nauk SSSR 223: 233-236.

12. Tchernysheva, A.V., Goldmacher, V.S., Klibanov, A.M., Martinek, K., Berezin, I.V. 1975. The catalytic activity and thermostability of α-chymotrypsin oligomers entrapped in cross-linked polymeric gels. Bull. Moscow Univ. 19: 428-431.

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13. Tchernysheva, A.V., Martinek, K., Klibanov, A.M., Mevkh, A.T., Berezin, I.V. 1975. The catalytic properties and thermostability of α-chymotrypsin in different polymethacrylate gels. Izvestia Acad. Nauk SSSR, Ser. Khim. No.8: 1764-1768.

14. Klibanov, A.M., Samokhin, G.P., Martinek, K., Berezin, I.V. 1976. Enzymatic mechano-chemistry: a new approach to studying the mechanism of enzyme action. Biochim. Biophys. Acta 438: 1-12.

15. Berezin, I.V., Klibanov, A.M., Samokhin, G.P., Martinek, K. 1976. Mechanochemistry of immobilized enzymes: a new approach to studies in fundamental enzymology. Regulation by a mechanical device of the catalytic properties of enzymes covalently attached to elastic polymeric supports. Meth. Enzymol. 44: 558-571.

16. Berezin, I.V., Klibanov, A.M., Goldmacher, V.S., Martinek, K. 1976. Mechanochemistry of immobilized enzymes: a new approach to studies in fundamental enzymology. Regulation by a mechanical device of the catalytic activity of enzymes trapped in polyacrylamide gel. Meth. Enzymol. 44: 571-576.

17. Klibanov, A.M., Kazanskaya, N.F., Larionova, N.I., Martinek, K., Berezin, I.V. 1976. A comparative study of the dynamic structures of the active centers of proteolytic enzymes by the ultrasonic method. The effect of ultrasound on α-trypsin, ß-trypsin, and trypsinogen. J. Bioorg. Chem. (Russ.) 2: 828-836.

18. Martinek, K., Goldmacher, V.S., Klibanov, A.M., Torchilin, V.P., Smirnov, V.N., Chazov, E.I., Berezin, I.V. 1976. Thermal stabilization of α-chymotrypsin by covalent attachment to a complementary surface of a polymeric matrix. Dokl. Acad. Nauk SSSR 228: 1468-1471.

19. Poglazov, B.F., Samokhin, G.P., Klibanov, A.M., Levitsky, D.I., Martinek, K., Berezin, I.V. 1977. Mechanochemistry of the myosin molecule. Dokl. Acad. Nauk SSSR 234: 482-485.

20. Berezin, I.V., Klibanov, A.M., Samokhin, G.P., Goldmacher, V.S., Martinek, K. 1977. Mechanosensitive and sound-sensitive systems as chemical amplifiers of weak signals. In: Biomedical Applications of Immobilized Enzymes and Proteins (T.M.S. Chang, ed.), vol. 2, pp. 237-251, Plenum Press, New York.

21. Klibanov, A.M., Samokhin, G.P., Martinek, K., Berezin, I.V. 1977. A new mechanochemical method of enzyme immobilization. Biotechnol. Bioeng. 19: 211-218.

22. Martinek, K., Klibanov, A.M., Samokhin, G.P., Semenov, A.M., Berezin, I.V. 1977. Preparative enzymatic synthesis in biphasic water-organic systems. J. Bioorg. Chem. (Russ.) 3: 696-702.

23. Klibanov, A.M., Samokhin, G.P., Martinek, K., Berezin, I.V. 1977. A new approach to preparative enzymatic synthesis. Biotechnol. Bioeng. 19: 1351-1361.

24. Martinek, K., Klibanov, A.M., Goldmacher, V.S., Berezin, I.V. 1977. The principles of enzyme stabilization. I. Increase in thermostability of enzymes covalently bound to a complementary surface of a polymeric support in a multipoint fashion. Biochim. Biophys. Acta 485: 1-12.

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25. Martinek, K., Klibanov, A.M., Goldmacher, V.S., Tchernysheva, A.V., Mozhaev, V.V., Berezin, I.V., Glotov, B.O. 1977. The principles of enzyme stabilization. II. Increase in thermostability of enzymes as a result of multipoint non-covalent interactions with a polymeric support. Biochim. Biophys. Acta 485: 13-28.

26. Torchilin, V.P., Maksimenko, A.V., Smirnov, V.N., Martinek, K., Klibanov, A.M., Berezin, I.V. 1978. The principles of enzymes stabilization. III. The effect of the length of intramolecular cross-linking agents on the thermostability of enzymes. Biochim. Biophys. Acta 522: 277-283.

27. Samokhin, G.P., Klibanov, A.M., Martinek, K. 1978. Photochemical immobilization of enzymes. Bull. Moscow Univ. 19: 433-436.

28. Poglazov, B.F., Samokhin, G.P., Klibanov, A.M., Levitsky, D.I., Martinek, K., Berezin, I.V. 1978. The effect of mechanical stretching of myosin rod part (fragment LMM + HMM S-2) on the ATPase activity of myosin. Biochim. Biophys. Acta 524: 245-253.

29. Klibanov, A.M., Semenov, A.N., Samokhin, G.P., Martinek, K. 1978. Enzymatic reactions in water-organic solutions. The criterion for selecting optimal organic co-solvents. J. Bioorg. Chem. (Russ.) 4: 236-242.

30. Sinitsyn, A.P., Klibanov, A.M., Klesov, A.A., Martinek, K. 1978. The dependence of stability of immobilized glycoamylase on the method of immobilization. Appl. Biochem. Microbiol. (Russ.) 14: 236-242.

31. Klibanov, A.M., Mozhaev, V.V. 1978. On the mechanism of irreversible thermoinactivation of enzymes and possibilities for reactivation of "irreversibly" inactivated enzymes. Biochem. Biophys. Res. Commun. 83: 1012-1017.

32. Klibanov, A.M., Kaplan, N.O., Kamen, M.D. 1978. A rationale for stabilization of oxygen-labile enzymes: application to a clostridial hydrogenase. Proc. Natl. Acad. Sci. USA 75: 3640-3643.

33. Torchilin, V.P., Maksimenko, A.V., Smirnov, V.N., Berezin, I.V., Klibanov, A.M., Martinek, K. 1979. The principles of enzyme stabilization. IV. Modification of "key" functional groups in the tertiary structure of proteins. Biochim. Biophys. Acta 567: 1-11.

34. Klibanov, A.M., Kaplan, N.O., Kamen, M.D. 1979. Chelating agents protect hydrogenase against oxygen inactivation. Biochim. Biophys. Acta 547: 411-416.

35. Klibanov, A.M. 1979. Stabilization of enzymes by immobilization. Anal. Biochem. 93: 1-25.

36. Klibanov, A.M., Kaplan, N.O., Kamen, M.D. 1980. Thermal stabilities of membrane-bound, solubilized and artificially immobilized hydrogenase from *Chromatium vinosum*. Arch. Biochem. Biophys. 199: 545-549.

37. Klibanov, A.M., Puglisi, A.V. 1980. The regeneration of coenzymes using immobilized hydrogenase. Biotechnol. Lett. 2: 445-450.

38. Klibanov, A.M., Kaplan, N.O., Kamen, M.D. 1980. Approaches to stabilization of hydrogenase and nitrogenase against oxygen inactivation. In: Enzyme Engineering V (H.H. Weetall and G.P. Royer, eds.), pp.135-142, Plenum Press, New York.

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39. Klibanov, A.M., Alberti, B.N., Morris, E.D., Felshin, L.M. 1980. Enzymatic removal of toxic phenols and anilines from waste waters. J. Appl. Biochem. 2: 414-421.

40. Khan, S.M., Klibanov, A.M., Kaplan, N.O., Kamen, M.D. 1981. The effect of electron carriers and other ligands on oxygen stability of clostridial hydrogenase. Biochim. Biophys. Acta 659: 457-465.

41. Klibanov, A.M., Morris, E.D. 1981. Horseradish peroxidase for the removal of carcinogenic aromatic amines from water. Enzyme Microb. Technol. 3: 119-122.

42. Klibanov, A.M., Huber, J. 1981. Application of immobilized hydrogenase for the de-tritiation of water. Biotechnol. Bioeng. 23: 1537-1551.

43. Klibanov, A.M., Barta, T.E. 1981. Protection of immobilized sulfhydryl groups against auto-oxidation by alterations in their microenvironment. Appl. Biochem. Biotechnol. 6: 201-207.

44. Alberti, B.N., Klibanov, A.M. 1981. Enzymatic removal of dissolved aromatics from industrial aqueous effluents. Biotechnol. Bioeng. Symp. 11: 373-379.

45. Klibanov, A.M., Berman, Z., Alberti, B.N. 1981. Preparative hydroxylation of aromatic compounds catalyzed by peroxidase. J. Am. Chem. Soc. 103: 6263-6264.

46. Klibanov, A.M., Alberti, B.N., Zale, S.E. 1982. Enzymatic synthesis of formic acid from H_2 and CO_2 and production of hydrogen from formic acid. Biotechnol. Bioeng. 24: 25-36.

47. Maneepun, S., Klibanov, A.M. 1982. Stabilization of microbial proteases against autolysis using acylation with dicarboxylic acid anhydrides. Biotechnol. Bioeng. 24: 483-486.

48. Klibanov, A.M., Giannousis, P.P. 1982. Geometrically specific oxidation of ß-arylacroleins catalyzed by xanthine oxidase: the preparative potential. Biotechnol. Lett. 4: 57-60.

49. Alberti, B.N., Klibanov, A.M. 1982. Preparative production of hydroquinone from benzoquinone catalyzed by immobilized glucose oxidase. Enzyme Microb. Technol. 4: 47-49.

50. Klibanov, A.M., Siegel, E.H. 1982. Geometric specificity of porcine liver carboxyl esterase and its application for the production of *cis* arylacrylic esters. Enzyme Microb. Technol. 4: 172-174.

51. Klibanov, A.M., Giannousis, P.P. 1982. Geometric specificity of alcohol dehydrogenases and its potential for separation of *trans* and *cis* isomers of unsaturated aldehydes. Proc. Natl. Acad. Sci. USA 79: 3462-3465.

52. Klibanov, A.M., Weare, N.M. 1982. Oxygen inactivation and stabilization of *Azotobacter* nitrogenase. In: From Cyclotrones to Cytochromes (N.O. Kaplan and A.B. Robinson, eds.), pp. 509-516, Academic Press, New York.

53. Zale, S.E., Klibanov, A.M. 1982. Application of immobilized hydrogenase to H_2 storage in concentrated solutions of methyl viologen. Appl. Biochem. Biotechnol. 7: 317-323.

54. Klibanov, A.M., Alberti, B.N., Marletta, M.A. 1982. Stereospecific oxidation of aliphatic alcohols catalyzed by galactose oxidase. Biochem. Biophys. Res. Commun. 108: 804-808.

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55. Pungor, E., Klibanov, A.M., Cooney, C.L., Weaver, J.C. 1982. Continuous ^{2}HHO determination in H_2O solutions by a computer-controlled mass spectrometer. Biomed. Mass Spectrom. 9: 181--185.

56. Klibanov, A.M. 1983. Unconventional catalytic properties of conventional enzymes: applications in organic chemistry. In: The Biological Basis of New Developments in Biotechnology (A. Hollaender, A.I. Laskin, and P. Rogers, eds.), pp. 497-518, Plenum Press, New York.

57. Klibanov, A.M. 1983. Biotechnological potential of the enzyme hydrogenase. Process Biochem. 18: 13-16.

58. Pelsy, G., Klibanov, A.M. 1983. Remarkable positional (regio-) specificity of xanthine oxidase and some dehydrogenases in the reactions with substituted benzaldehydes. Biochim. Biophys. Acta 742: 352-357.

59. Klibanov, A.M. 1983. Stabilization of enzymes against thermal inactivation. Adv. Appl. Microbiol. 29: 1-28.

60. Klibanov, A.M., Chendrasekhar, A., Alberti, B.N. 1983. Enzymatic separation of *cis* and *trans* isomers of alicyclic alcohols. Enzyme Microb. Technol. 5: 265-268.

61. Klibanov, A.M. 1983. Immobilized enzymes and cells as practical catalysts. Science 219: 722-727.

62. Pelsy, G., Klibanov, A.M. 1983. Preparative separation of α- and ß- naphthols catalyzed by immobilized sulfatase. Biotechnol. Bioeng. 25: 919-928.

63. Zale, S.E., Klibanov, A.M. 1983. On the role of reversible denaturation (unfolding) in the irreversible thermal inactivation of enzymes. Biotechnol. Bioeng. 25: 2221-2230.

64. Klibanov, A.M., Tu, T.-M., Scott, K.P. 1983. Peroxidase-catalyzed removal of phenols from coal conversion wastewaters. Science 221: 259-261.

65. Atlow, S.C., Bonadonna-Aparo, L., Klibanov, A.M. 1984. Dephenolization of industrial wastewaters catalyzed by polyphenol oxidase. Biotechnol. Bioeng. 26: 599-603.

66. Cambou, B., Klibanov, A.M. 1984. Preparative production of optically active esters and alcohols using esterase-catalyzed stereospecific transesterifications in organic media. J. Am. Chem. Soc. 106: 2687-2692.

67. Dropsy, E.P., Klibanov, A.M. 1984. Cholinesterase-catalyzed resolution of D,L-carnitine. Biotechnol. Bioeng. 26: 911-915.

68. Cambou, B., Klibanov, A.M. 1984. Lipase-catalyzed production of optically active acids via asymmetric hydrolysis of esters: effect of the alcohol moiety. Appl. Biochem. Biotechnol. 9: 255-260.

69. Zaks, A., Klibanov, A.M. 1984. Enzymatic catalysis in organic media at 100°C. Science 224: 1249-1251.

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70. Cambou, B., Klibanov, A.M. 1985. Comparison of different strategies for the lipase- catalyzed preparative resolution of racemic acids and alcohols: asymmetric hydrolysis, esterification, and transesterification. Biotechnol. Bioeng. 26: 1449-1454.

71. Scollar, M.P., Sigal, G., Klibanov, A.M. 1985. Preparative resolution of D,L-threonine catalyzed by immobilized phosphatase. Biotechnol. Bioeng. 27: 247-252.

72. Ahern, T.J., Klibanov, A.M. 1985. The mechanism of irreversible enzyme inactivation at 100°C. Science 228: 1280-1284.

73. Cesti, P., Zaks, A., Klibanov, A.M. 1985. Preparative regioselective acylation of glycols by enzymatic transesterification in organic solvents. Appl. Biochem. Biotechnol. 11: 401-407.

74. Zaks, A., Klibanov, A.M. 1985. Enzyme-catalyzed processes in organic solvents. Proc. Natl. Acad. Sci. USA 82: 3192-3196.

75. Kazandjian, R.Z., Klibanov, A.M. 1985. Regioselective oxidation of phenols catalyzed by polyphenol oxidase in chloroform. J. Am. Chem. Soc. 107: 5448-5450.

76. Kirchner, G., Scollar, M.P., Klibanov, A.M. 1985. Resolution of racemic mixtures via lipase catalysis in organic solvents. J. Am. Chem. Soc. 107: 7072-7076.

77. Lavin, A., Sung, C., Klibanov, A.M., Langer, R.S. 1985. Enzymatic removal of bilirubin from the blood: a potential treatment of neonatal jaundice. Science 230: 543-545.

78. Hammond, D.A., Karel, M., Klibanov, A.M., Krukonis, V.J. 1985. Enzymatic reactions in supercritical gases. Appl. Biochem. Biotechnol. 11: 393-400.

79. Sung, C., Lavin, A., Klibanov, A.M., Langer, R. 1986. An immobilized enzyme reactor for the treatment of severe jaundice. Trans. Am. Soc. Artif. Intern. Organs. 31: 264-269.

80. Kazandjian, R.Z., Dordick, J.S., Klibanov, A.M. 1986. Enzymatic analyses in organic solvents. Biotechnol. Bioeng. 28: 417-421.

81. Klibanov, A.M. 1986. Enzymes that work in organic solvents. CHEMTECH 16: 354-359.

82. Dordick, J.S., Klibanov, A.M., Marletta, M.A. 1986. Horseradish peroxidase-catalyzed hydroxylations: mechanistic studies. Biochemistry. 25: 2946-2951.

83. Sung, C., Lavin, A., Klibanov, A.M., Langer, R. 1986. An immobilized enzyme reactor for the detoxification of bilirubin. Biotechnol. Bioeng. 28: 1531-1539.

84. Zaks, A., Klibanov, A.M. 1986. Substrate specificity of enzymes in organic solvents *vs.* water is reversed. J. Am. Chem. Soc. 108: 2767-2768.

85. Dordick, J.S., Marletta, M.A., Klibanov, A.M. 1986. Peroxidases depolymerize lignin in organic media but not in water. Proc. Natl. Acad. Sci. USA 83: 6255-6257.

86. Zale, S.E., Klibanov, A.M. 1986. Why does ribonuclease irreversibly inactivate at high temperatures? Biochemistry 25: 5432-5444.

Momenta Pharm. v. BMS, IPR215-01537
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87. Grunwald, J., Wirz, B., Scollar, M.P., Klibanov, A.M. 1986. Asymmetric oxidoreductions catalyzed by alcohol dehydrogenase in organic solvents. J. Am. Chem. Soc. 108: 6732-6734.

88. Therisod, M., Klibanov, A.M. 1986. Facile enzymatic preparation of monoacylated sugars in pyridine. J. Am. Chem. Soc. 108: 5638-5640.

89. Boeriu, C.G., Dordick, J.S., Klibanov, A.M. 1986. Enzymatic reactions in liquid and solid paraffins: application for enzyme-based temperature abuse sensors. Bio/Technol. 4: 997-999.

90. Klibanov, A.M., Cambou, B. 1987. Enzymatic production of optically active compounds in biphasic aqueous-organic systems. Meth. Enzymol. 136C: 117-137.

91. Ahern, T.J., Casal, J.I., Petsko, G.A., Klibanov, A.M. 1987. Control of oligomeric enzyme thermostability by protein engineering. Proc. Natl. Acad. Sci. USA 84: 675-679.

92. Shaw, J.F., Klibanov, A.M. 1987. Preparation of various glucose esters via lipase-catalyzed hydrolysis of glucose pentaacetate. Biotechnol. Bioeng. 29: 648-651.

93. Casal, J.I., Ahern, T.J., Davenport, R.C., Petsko, G.A., Klibanov, A.M. 1987. Subunit interface of triosephosphate isomerase: site-directed mutagenesis and characterization of the altered enzyme. Biochemistry 26: 1258-1264.

94. Dordick, J.S., Marletta, M.A., Klibanov, A.M. 1987. Polymerization of phenols catalyzed by peroxidase in non-aqueous media. Biotechnol. Bioeng. 30: 31-36.

95. Visuri, K., Klibanov, A.M. 1987. Enzymatic production of high fructose corn syrup (HFCS) containing 55% fructose in aqueous ethanol. Biotechnol. Bioeng. 30: 917-920.

96. Margolin, A.L., Crenne, J.-Y., Klibanov, A.M. 1987. Stereoselective oligomerizations catalyzed by lipase in organic solvents. Tetrahedron Lett. 28: 1607-1610.

97. Volkin, D.B., Klibanov, A.M. 1987. Thermal destruction processes in proteins involving cystine residues. J. Biol. Chem. 262: 2945-2950.

98. Therisod, M., Klibanov, A.M. 1987. Regioselective acylation of secondary hydroxyl groups in sugars catalyzed by lipases in organic solvents. J. Am. Chem. Soc. 109: 3977-3981.

99. Ahern, T.J., Klibanov, A.M. 1987. Analysis of processes causing thermal inactivation of enzymes. Meth. Biochem. Analysis 33: 91-127.

100. Margolin, A.L., Klibanov, A.M. 1987. Peptide synthesis catalyzed by lipases in anhydrous organic solvents. J. Am. Chem. Soc. 109: 3802-3804.

101. Barzana, E., Klibanov, A.M., Karel, M. 1987. Enzyme-catalyzed gas phase reactions. Appl. Biochem. Biotechnol. 15: 25-34.

102. Margolin, A.L., Tai, D.-F., Klibanov, A.M. 1987. Incorporation of D-amino acids into peptides via enzymatic condensation in organic solvents. J. Am. Chem. Soc. 109: 7885-7887.

Momenta Pharm. v. BMS, IPR215-01537
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Samsung Bioepis Exhibit No. 1002
Page 7505

103. Chopineau, J., McCafferty, F.D., Therisod, M., Klibanov, A.M. 1988. Production of biosurfactants from sugar alcohols and vegetable oils catalyzed by lipases in a non-aqueous medium. Biotechnol. Bioeng. 31: 208-214.

104. Mullon, C.J.P., Klibanov, A.M., Langer, R. 1988. Kinetics of soluble bilirubin oxidase and modelling of an immobilized bilirubin oxidase reactor for bilirubin detoxification. Biotechnol. Bioeng. 31: 536-546.

105. Riva, S., Chopineau, J., Kieboom, A.P.G., Klibanov, A.M. 1988. Protease-catalyzed regioselective esterification of sugars and related compounds in anhydrous dimethylformamide. J. Am. Chem. Soc. 110: 584-589.

106. Tomazic, S.J., Klibanov, A.M. 1988. Mechanisms of irreversible thermal inactivation of *Bacillus* α-amylases. J. Biol. Chem. 263: 3086-3091.

107. Tomazic, S.J., Klibanov, A.M. 1988. Why is one *Bacillus* α-amylase more resistant against irreversible thermoinactivation than another? J. Biol. Chem. 263: 3092-3096.

108. Zaks, A., Klibanov, A.M. 1988. Enzymatic catalysis in non-aqueous solvents. J. Biol. Chem. 263: 3194-3201.

109. Riva, S., Klibanov, A.M. 1988. Enzymo-chemical regioselective oxidation of steroids without oxidoreductases. J. Am. Chem. Soc. 110: 3291-3295.

110. Zaks, A., Klibanov, A.M. 1988. The effect of water on enzyme action in organic media. J. Biol. Chem. 263: 8017-8021.

111. Russell, A.J., Klibanov, A.M. 1988. Inhibitor-induced enzyme activation in organic solvents. J. Biol. Chem. 263: 11624-11626.

112. Weaver, J.C., Williams, G.B., Klibanov, A.M., Demain, A.L. 1988. Gel microdroplets: rapid detection and enumeration of individual microorganisms by their metabolic activity. Bio/Technology 6: 1084-1089.

113. Kitaguchi, H., Tai, D.-F., Klibanov, A.M. 1988. Enzymatic formation of an isopeptide bond involving the ε-amino group of lysine. Tetrahedron Lett. 29: 5487-5488.

114. Sakurai, T., Margolin, A.L., Russell, A.J., Klibanov, A.M. 1988. Control of enzyme enantio-selectivity by the reaction medium. J. Am. Chem. Soc. 110: 7236-7237.

115. Volkin, D.B., Klibanov, A.M. 1989. Mechanism of thermoinactivation of immobilized glucose isomerase. Biotechnol. Bioeng. 33: 1104-1111.

116. Chinsky, N., Margolin, A.L., Klibanov, A.M. 1989. Chemoselective enzymatic monoacylation of bifunctional compounds. J. Am. Chem. Soc. 111: 386-388.

117. Russell, A.J., Trudel, L.J., Skipper, P.L., Groopman, J.D., Tannenbaum, S.R., Klibanov, A.M. 1989. Antibody-antigen binding in organic solvents. Biochem. Biophys. Res. Commun. 158: 80-85.

Momenta Pharm. v. BMS, IPR215-01537
10/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7506

118. Barzana, E., Karel, M., Klibanov, A.M. 1989. Enzymatic oxidation of ethanol in the gaseous phase. Biotechnol. Bioeng. 34: 1178-1185.

119. Kitaguchi, H., Fitzpatrick, P.A., Huber, J.E., Klibanov, A.M. 1989. Enzymatic resolution of racemic amines: crucial role of the solvent. J. Am. Chem. Soc. 111: 3094-3095.

120. Dorval, B.L., Chow, M., Klibanov, A.M. 1989. Stabilization of poliovirus against heat inactivation. Biochem. Biophys. Res. Commun. 159: 1177-1183.

121. Barzana, E., Klibanov, A.M., Karel, M. 1989. A colorimetric method for the enzymatic analysis of gases: the determination of ethanol and formaldehyde vapors using solid alcohol oxidase. Anal. Biochem. 182: 109-115.

122. Volkin, D.B., Klibanov, A.M. 1989. Minimizing protein inactivation. *In:* Protein Function - A Practical Approach (T.E. Creighton, ed.), pp. 1-24, IRL Press, Oxford.

123. Klibanov, A.M. 1989. Enzymatic catalysis in anhydrous organic solvents. Trends Biochem. Sci. 14: 141-144.

124. Kanerva, L.T., Klibanov, A.M. 1989. Hammett analysis of enzyme action in organic solvents. J. Am. Chem. Soc. 111: 6864-6865.

125. Burke, P.A., Smith, S.O., Bachovchin, W.W., Klibanov, A.M. 1989. Demonstration of structural integrity of an enzyme in organic solvents by solid-state NMR. J. Am. Chem. Soc. 111: 8290-8291.

126. Kitaguchi, H., Klibanov, A.M. 1989. Enzymatic peptide synthesis via segment condensation in the presence of water mimics. J. Am. Chem. Soc. 111: 9272-9273.

127. Braco, L., Dabulis, K., Klibanov, A.M. 1990. Design of novel receptors by molecular imprinting of proteins. Proc. Natl. Acad. Sci. USA 87: 274-277.

128. Margolin, A.L., Klibanov, A.M. 1990. Enzymatic synthesis of peptides in anhydrous organic solvents. In: Peptides. Chemistry, Structure and Biology (J.E. Rivier and G.R. Marshall, eds.), pp. 355-359, ESCOM Science Publishers, Leiden.

129. Dorval, B.L., Chow, M., Klibanov, A.M. 1990. Lysine and other diamines dramatically stabilize poliovirus against thermoinactivation. Biotechnol. Bioeng. 35: 1051-1054.

130. Klibanov, A.M. 1990. Asymmetric transformations catalyzed by enzymes in organic solvents. Acc. Chem. Res. 23: 114-120.

131. Adams, K.A.H., Chung, S.-H., Klibanov, A.M. 1990. Kinetic isotope effect investigation of enzyme mechanism in organic solvents. J. Am. Chem. Soc. 112: 9418-9419.

132. Liu, W.R., Langer, R., Klibanov, A.M. 1991. Moisture-induced aggregation of lyophilized proteins in the solid state. Biotechnol. Bioeng. 37: 177-184.

133. Rubio, E., Fernandez-Mayorales, A., Klibanov, A.M. 1991. Effect of the solvent on enzyme regioselectivity. J. Am. Chem. Soc. 113: 695-696.

Momenta Pharm. v. BMS, IPR215-01537
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Samsung Bioepis Exhibit No. 1002
Page 7507

134. Chang, N., Hen, S.J., Klibanov, A.M. 1991. Protein separation and purification in neat dimethyl sulfoxide (DMSO). Biochem. Biophys. Res. Commun. 176: 1462-1468.

135. Volkin, D.B., Staubli, A., Langer, R., Klibanov, A.M. 1991. Enzyme thermoinactivation in anhydrous organic solvents. Biotechnol. Bioeng. 37: 843-853.

136. Schulze, B., Klibanov, A.M. 1991. Inactivation and stabilization of subtilisins in neat organic solvents. Biotech. Bioeng. 38: 1001-1006.

137. Fitzpatrick, P.A., Klibanov, A.M. 1991. How can the solvent affect enzyme enantioselectivity? J. Am. Chem. Soc. 113: 3166-3171.

138. Margolin, A.L., Fitzpatrick, P.A., Dubin, P.L., Klibanov, A.M. 1991. Chemo-enzymatic synthesis of optically active (meth)acrylic polymers. J. Am. Chem. Soc. 113: 4693-4694.

139. Sluzky, V., Tamada, J.A., Klibanov, A.M., Langer, R. 1991. Kinetics of insulin aggregation in aqueous solutions upon agitation in the presence of hydrophobic surfaces. Proc. Natl. Acad. Sci. USA 88: 9377-9381.

140. Gardossi, L., Bianchi, D., Klibanov, A.M. 1991. Selective acylation of peptides catalyzed by lipases in organic solvents. J. Am. Chem. Soc. 113: 6328-6329.

141. Kitaguchi, H., Ono, M., Itoh, I., Klibanov, A.M. 1991. Regioselective modification of lysine's amino groups by proteases. Agric. Biol. Chem. 55: 3067-3073.

142. Dabulis, K., Klibanov, A.M. 1992. Molecular imprinting of proteins and other macromolecules resulting in new adsorbents. Biotechnol. Bioeng. 39: 176-185.

143. Chang, N., Klibanov, A.M. 1992. Chromatographic separation of proteins in neat organic solvents. Biotechnol. Bioeng. 39: 575-578.

144. Tawaki, S., Klibanov, A.M. 1992. Inversion of enzyme enantioselectivity mediated by the solvent. J. Am. Chem. Soc. 114: 1882-1884.

145. Fitzpatrick, P.A., Ringe, D., Klibanov, A.M. 1992. Computer-assisted modeling of subtilisin enantioselectivity in organic solvents. Biotechnol. Bioeng. 40: 735-742.

146. Sluzky, V., Klibanov, A.M., Langer, R. 1992. Mechanism of insulin aggregation and stabilization in agitated aqueous solutions. Biotechnol. Bioeng. 40: 895-903.

147. Burke, P.A., Griffin, R.G., Klibanov, A.M. 1992. Solid-state NMR assessment of enzyme active center structure under nonaqueous conditions. J. Biol. Chem. 267: 20057-20064.

148. Klyachko, N.L., Klibanov, A.M. 1992. Oxidation of dibenzothiophene catalyzed by hemoglobin and other hemoproteins in various aqueous-organic media. Appl. Biochem. Biotechnol. 37: 53-68.

149. Narayan, V.S., Klibanov, A.M. 1993. Are water-immiscibility and apolarity of the solvent relevant to enzyme efficiency? Biotechnol. Bioeng. 41: 390-393.

Momenta Pharm. v. BMS, IPR215-01537
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Samsung Bioepis Exhibit No. 1002
Page 7508

150. Dabulis, K., Klibanov, A.M. 1993. Dramatic enhancement of enzymatic activity in organic solvents by lyoprotectants. Biotechnol. Bioeng. 41: 566-571.

151. Terradas, F., Teston-Henry, M., Fitzpatrick, P.A., Klibanov, A.M. 1993. Marked dependence of enzyme prochiral selectivity on the solvent. J. Am. Chem. Soc. 115: 390-396.

152. Burke, P.A., Griffin, R.G., Klibanov, A.M. 1993. Solid-state NMR investigation of the solvent dependence of tyrosyl ring motion in an enzyme. Biotechnol. Bioeng. 42: 87-94.

153. Wescott, C.R., Klibanov, A.M. 1993. Solvent variation inverts substrate specificity of an enzyme. J. Am. Chem. Soc. 115: 1629-1631.

154. Fitzpatrick, P.A., Steinmetz, A.C.U., Ringe, D., Klibanov, A.M. 1993. Enzyme crystal structure in a neat organic solvent. Proc. Natl. Acad. Sci. USA 90: 8653-8657.

155. Tawaki, S., Klibanov, A.M. 1993. Chemoselectivity of enzymes in anhydrous media is strongly solvent dependent. Biocatalysis 8: 3-19.

156. Klibanov, A.M. 1993. Enzyme catalysis without water. R&D Innovator 2(4): 1-7.

157. Ikeda, I., Klibanov, A.M. 1993. Lipase-catalyzed acylation of sugars solubilized in hydrophobic solvents by complexation. Biotechnol. Bioeng. 42: 788-791.

158. Wescott, C.R., Klibanov, A.M. 1993. Predicting the solvent dependence of enzymatic substrate specificity using semi-empirical thermodynamic calculations. J. Am. Chem. Soc. 115: 10362-10363.

159. Costantino, H.R., Langer, R., Klibanov, A.M. 1994. Moisture-induced aggregation of lyophilized insulin. Pharm. Res. 11: 21-29.

160. Bromberg, L.E., Klibanov, A.M. 1994. Detergent-enabled transport of proteins and nucleic acids through hydrophobic solvents. Proc. Natl. Acad. Sci. USA 91: 143-147.

161. Chin, J.T., Wheeler, S.L., Klibanov, A.M. 1994. On protein solubility in organic solvents. Biotechnol. Bioeng. 44: 140-145.

162. Fitzpatrick, P.A., Ringe, D., Klibanov, A.M. 1994. X-Ray crystal structure of cross-linked subtilisin Carlsberg in water *vs.* acetonitrile. Biochem. Biophys. Res. Commun. 198: 675-681.

163. Wescott, C.R., Klibanov, A.M. 1994. The solvent dependence of enzyme specificity. Biochim. Biophys. Acta 1206: 1-9.

164. Orsat, B., Drtina, G.J., Williams, M.G., Klibanov, A.M. 1994. Effect of support material and enzyme pretreatment on enantioselectivity of immobilized subtilisin in organic solvents. Biotechnol. Bioeng. 44: 1265-1269.

165. Desai, U.R., Osterhout, J.J., Klibanov, A.M. 1994. Protein structure in the lyophilized state: a hydrogen isotope exchange/NMR study with bovine pancreatic trypsin inhibitor. J. Am. Chem. Soc. 116: 9420-9422.

Momenta Pharm. v. BMS, IPR215-01537
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Samsung Bioepis Exhibit No. 1002
Page 7509

166. Costantino, H.R., Langer, R., Klibanov, A.M. 1994. Solid-phase aggregation of proteins under pharmaceutically relevant conditions. J. Pharm. Sci. 83: 1662-1669.

167. Bromberg, L.E., Klibanov, A.M. 1995. Transport of proteins dissolved in organic solvents across biomimetic membranes. Proc. Natl. Acad. Sci. USA 92: 1262-1266.

168. Magner, E., Klibanov, A.M. 1995. The oxidation of chiral alcohols catalyzed by catalase in organic solvents. Biotechnol. Bioeng. 46: 175-179.

169. Desai, U.R., Klibanov, A.M. 1995. Assessing the structural integrity of a lyophilized protein in organic solvents. J. Am. Chem. Soc. 117: 3940-3945.

170. Ferrante, A.A., Augliera, J., Lewis, K., Klibanov, A.M. 1995. Cloning of an organic solvent resistance gene in *E. coli*: the unexpected role of alkylhydroperoxide reductase. Proc. Natl. Acad. Sci. USA 92: 7617-7621.

171. Costantino, H.R., Langer, R., Klibanov, A.M. 1995. Aggregation of a lyophilized pharmaceutical protein, recombinant human albumin: effect of moisture and stabilization by excipients. Bio/Technology 13: 493-496.

172. Klibanov, A.M. 1995. Enzyme memory – What is remembered and why? Nature 374: 596.

173. Griebenow, K., Klibanov, A.M. 1995. Lyophilization-induced changes in the secondary structure of proteins. Proc. Natl. Acad. Sci. USA 92: 10969-10976.

174. Costantino, H.R., Griebenow, K., Mishra, P., Langer, R., Klibanov, A.M. 1995. Fourier-transform infrared (FTIR) spectroscopic investigation of protein stability in the lyophilized form. Biochim. Biophys. Acta 1253: 69-74.

175. Schwendeman, S.P., Costantino, H.R., Gupta, R.K., Siber, G.R., Klibanov, A.M., Langer, R. 1995. Stabilization of tetanus and diphtheria toxoids against moisture-induced aggregation. Proc. Natl. Acad. Sci. USA 92: 11234-11238.

176. Almarsson, O., Klibanov, A.M. 1996. Remarkable activation of enzymes in nonaqueous media by denaturing organic cosolvents. Biotechnol. Bioeng. 49: 87-92.

177. Schwendeman, S.P., Cardamone, M., Klibanov, A.M., Langer, R., Brandon, M.R. 1996. Stability of proteins and their delivery from biodegradable polymer microspheres. In: Microparticulate Systems for the Delivery of Proteins and Vaccine (S. Cohen and H. Bernstein, eds.), pp. 1-49, M. Dekker, New York.

178. Louwrier, A., Drtina, G.J., Klibanov, A.M. 1996. On the issue of interfacial activation of lipase in nonaqueous media. Biotechnol. Bioeng. 50: 1-5.

179. Schmitke, J.L., Wescott, C.R., Klibanov, A.M. 1996. The mechanistic dissection of the plunge in enzymatic activity upon transition from water to anhydrous solvents. J. Am. Chem. Soc. 118: 3360-3365.

180. Noritomi, H., Almarsson, O., Barletta, G.L., Klibanov, A.M. 1996. The influence of the mode of enzyme preparation on enzymatic enantioselectivity in organic solvents and its temperature dependence. Biotechnol. Bioeng. 51: 95-99.

Momenta Pharm. v. BMS, IPR215-01537
14/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7510

181. Ke, T., Wescott, C.R., Klibanov, A.M. 1996. Prediction of the solvent dependence of enzymatic prochiral selectivity by means of structure-based thermodynamic calculations. J. Am. Chem. Soc. 118: 3366-3374.

182. Mishra, P., Griebenow, K., Klibanov, A.M. 1996. Structural basis for the molecular memory of imprinted proteins in anhydrous media. Biotechnol. Bioeng. 52: 609-614.

183. Xu, K., Klibanov, A.M. 1996. pH Control of the catalytic activity of cross-linked enzyme crystals in organic solvents. J. Am. Chem. Soc. 118: 9815-9819.

184. Koskinen, A.M.P., Klibanov, A.M., eds. 1996. Enzymatic Reactions in Organic Media, Blackie Academic & Professional, London.

185. Wescott, C.R., Noritomi, H., Klibanov, A.M. 1996. Rational control of enzymatic enantio-selectivity by solvation thermodynamics. J. Am. Chem. Soc. 118: 10365-10370.

186. Costantino, H.R., Schwendeman, S.P., Griebenow, K., Klibanov, A.M., Langer, R. 1996. The secondary structure and aggregation of lyophilized tetanus toxoid. J. Pharm. Sci. 85: 1290-1293.

187. Griebenow, K., Klibanov, A.M. 1996. On protein denaturation in aqueous-organic mixtures but not in pure organic solvents. J. Am. Chem. Soc. 118: 11695-11700.

188. Costantino, H.R., Shieh, L., Klibanov, A.M., Langer, R. 1997. Heterogeneity of serum albumin samples with respect to solid-state aggregation via thiol-disulfide interchange – implications for sustained release from polymers. J. Controlled Release 44: 255-261.

189. Costantino, H.R., Griebenow, K., Langer, R., Klibanov, A.M. 1997. On the "pH memory" of lyophilized compounds containing protein functional groups. Biotechnol. Bioeng. 53: 345-348.

190. Schmitke, J.L., Stern, L.J., Klibanov, A.M. 1997. Crystal structure of subtilisin Carlsberg in anhydrous dioxane and its comparison with those in water and acetonitrile. Proc. Natl. Acad. Sci. USA 94: 4250-4255.

191. Griebenow, K., Klibanov, A.M. 1997. Can conformational changes be responsible for solvent and excipient effects on the catalytic behavior of subtilisin Carlsberg in organic solvents? Biotechnol. Bioeng. 53: 351-362.

192. Klibanov, A.M. 1997. Why are enzymes less active in organic solvents than in water? Trends Biotechnol. 15: 97-101.

193. Wescott, C.R., Klibanov, A.M. 1997. Thermodynamic analysis of the solvent effect on substrate specificity of lyophilized enzymes suspended in organic media. Biotechnol. Bioeng. 56: 340-344.

194. Costantino, H.R., Liauw, S., Mitragotri, S., Langer, R., Klibanov, A.M., Sluzky, V. 1997. The pharmaceutical development of insulin: historical perspectives and future directions. ACS Symp. Ser. 675: 29-66.

195. Xu, K., Griebenow, K., Klibanov, A.M. 1997. Correlation between catalytic activity and secondary structure of subtilisin dissolved in organic solvents. Biotechnol. Bioeng. 56: 485-491.

Momenta Pharm. v. BMS, IPR215-01537
15/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7511

196. Rariy, R.V., Klibanov, A.M. 1997. Correct protein folding in glycerol. Proc. Natl. Acad. Sci. USA 94: 13520-13523.

197. Ke, T., Tidor, B., Klibanov, A.M. 1998. Molecular-modeling calculations of enzymatic enantioselectivity taking hydration into account. Biotechnol. Bioeng. 57: 741-745.

198. Costantino, H.R., Schwendeman, S.P., Langer, R., Klibanov, A.M. 1998. Deterioration of lyophilized pharmaceutical proteins. Biochemistry (Moscow) 63: 422-429.

199. Ke, T., Klibanov, A.M. 1998. On enzymatic activity in organic solvents as a function of enzyme history. Biotechnol. Bioeng. 57: 746-750.

200. Luque, S., Ke, T., Klibanov, A.M. 1998. On the role of transition-state substrate desolvation in enzymatic enantioselectivity in aqueous-organic mixtures. Biocatalysis Biotrans. 16: 233-248.

201. Schmitke, J.L., Stern, L.J., Klibanov, A.M. 1998. Comparison of X-ray crystal structures of an acyl-enzyme intermediate of subtilisin Carlsberg formed in water and in anhydrous acetonitrile. Proc. Natl. Acad. Sci. USA 95: 12918-12923.

202. Ke, T., Klibanov, A.M. 1998. Insights into the solvent dependence of chymotryptic prochiral selectivity. J. Am. Chem. Soc. 120: 4259-4263.

203. Schmitke, J.L., Stern, L.J., Klibanov, A.M. 1998. Organic solvent binding to crystalline subtilisin in mostly aqueous media and in the neat organic solvents. Biochem. Biophys. Res. Commun. 248: 273-277.

204. Knubovets, T., Osterhout, J.J., Connolly, P.J., Klibanov, A.M. 1999. Structure, thermostability, and conformational flexibility of hen egg-white lysozyme dissolved in glycerol. Proc. Natl. Acad. Sci. USA 96: 1262-1267.

205. Ke, T., Rariy, R.V., Schmitke, J.L., Klibanov, A.M. 1999. Computational and experimental examination of the effect of inorganic salts on chymotryptic enantioselectivity in water. Biocatalysis Biotrans.17: 81-93.

206. Rariy, R.V., Klibanov, A.M. 1999. Protein refolding in predominantly organic media markedly enhanced by common salts. Biotechnol. Bioeng. 62: 704-710.

207. Fu, K., Griebenow, K., Hsieh, L., Klibanov, A.M., Langer, R. 1999. FTIR characterization of the secondary structure of proteins encapsulated within PLGA microspheres. J. Controlled Release 58: 357-366.

208. Knubovets, T., Osterhout, J.J., Klibanov, A.M. 1999. Structure of lysozyme dissolved in neat organic solvent as assessed by NMR and CD spectroscopies. Biotechnol. Bioeng. 63: 242-248.

209. Ke, T., Klibanov, A.M. 1999. Markedly enhancing enzymatic enantioselectivity in organic solvents by forming substrate salts. J. Am. Chem. Soc. 121: 3334-3340.

210. Dai, L., Klibanov, A.M. 1999. Striking activation of oxidative enzymes suspended in nonaqueous media. Proc. Natl. Acad. Sci. USA 96: 9475-9478.

Momenta Pharm. v. BMS, IPR215-01537
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Samsung Bioepis Exhibit No. 1002
Page 7512

211. Fu, K., Pack, D.W., Klibanov, A.M., Langer, R. 2000. Visual evidence of acidic environment within degrading poly(lactic-*co*-glycolic acid) (PLGA) microspheres. Pharm. Res. 17: 100-106.

212. Arredondo, A.R., Dorval, B.L., Klibanov, A.M., Lewis, K. 2000. Rapid immunodetection of *Escherichia coli.* Biotechnol. Lett. 22: 547-550.

213. Shin, J.-S., Luque, S., Klibanov, A.M. 2000. Improving lipase enantioselectivity in organic solvents by forming substrate salts with chiral agents. Biotechnol. Bioeng. 69: 577-583.

214. Rariy, R.V., Klibanov, A.M. 2000. On the relationship between enzymatic enantioselectivity in organic solvents and enzyme flexibility. Biocatalysis Biotrans. 18: 401-407.

215. Bonner, G., Klibanov, A.M. 2000. Structural stability of DNA in nonaqueous solvents. Biotechnol. Bioeng. 68: 339-344.

216. Klibanov, A.M. 2000. Answering the question "Why did biocatalysis in organic media not take off in the 1930s?". Trends Biotechnol. 18: 85-86.

217. Burova, T.V., Grinberg, N.V., Grinberg, V. Ya., Rariy, R.V., Klibanov, A.M. 2000. Calorimetric evidence for a native-like conformation of hen egg-white lysozyme dissolved in glycerol. Biochim. Biophys. Acta 1478: 309-317.

218. Fu, K., Klibanov, A.M., Langer, R. 2000. Protein stability in controlled release systems. Nature Biotechnol. 18: 24-25.

219. Arntzen, C.J., Dale, B.E., Beachy, R.N., Bemiller, J.N., Burgess, R.R., Gallagher, P., Hardy, R.W.F., Johnson, D.L., Kirk, T.K., Kishore, G.M., Klibanov, A.M., Pierce, J., Shanks, J.V., Wang, D.I.C., Westpheling, J., Zeikus, J.G. 2000. Biobased Industrial Products. National Academy Press, Washington, D.C.

220. Dai, L., Klibanov, A.M. 2000. Peroxidase-catalyzed asymmetric sulfoxidation in organic solvents *vs.* in water. Biotechnol. Bioeng. 70: 353-357.

221. Ozawa, S., Klibanov, A.M. 2000. Myoglobin-catalyzed epoxidation of styrene in organic solvents accelerated by bioimprinting. Biotechnol. Lett. 22: 1269-1272.

222. Wise, D.L., Klibanov, A.M., Langer, R., Mikos, A., Brannon-Peppas, L., Peppas, N.A., Trantolo, D.J., Wnek, G.E., Yaszemski, M.J., Eds. 2000. Handbook of Pharmaceutical Controlled Release Technology. M. Dekker, New York.

223. Tiller, J.C., Bonner, G., Pan, L.-C., Klibanov, A.M. 2001. Improving biomaterial properties of collagen films by chemical modification. Biotechnol. Bioeng. 73: 246-252.

224. Tiller, J., Liao, C.-J., Lewis, K., Klibanov, A.M. 2001. Designing surfaces that kill bacteria on contact. Proc. Natl. Acad. Sci. USA 98: 5981-5985.

225. Mathiowitz, E., Jacob, J.S., Jong, Y.S., Hekal, T.M., Spano, W., Guemonprez, R., Klibanov, A.M., Langer, R. 2001. Novel desiccants based on designed polymeric blends. J. Appl. Polymer Sci. 80: 317-327.

Momenta Pharm. v. BMS, IPR215-01537
17/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7513

226. Klibanov, A.M. 2001. Improving enzymes by using them in organic solvents. Nature 409: 241-246.

227. Choi, W.S., Krishna Murthy, G.G., Edwards, D.A., Langer, R., Klibanov, A.M. 2001. Inhalation delivery of proteins from ethanol suspensions. Proc. Natl. Acad. Sci. USA 98: 11103-11107.

228. Xie, Y., Das, P.K., Klibanov, A.M. 2001. Excipients activate peroxidases in specific but not in nonspecific reactions in organic solvents. Biotechnol. Lett. 23: 1451-1454.

229. Das, P.K., Caaveiro, J.M.M., Luque, S., Klibanov, A.M. 2002. Binding of hydrophobic hydroxamic acids enhances peroxidase's stereoselectivity in nonaqueous sulfoxidations. J. Am. Chem. Soc. 124: 782-787.

230. Das, P.K., Caaveiro, J.M.M., Luque, S., Klibanov, A.M. 2002. Asymmetric sulfoxidations mediated by α-chymotrypsin. Biotechnol. Bioeng. 78: 104-109.

231. Xie, Y., Das, P.K., Caaveiro, J.M.M., Klibanov, A.M. 2002. Unexpectedly enhanced stereoselectivity of peroxidase-catalyzed sulfoxidation in branched alcohols. Biotechnol. Bioeng. 79: 105-111.

232. Lin, J., Tiller, J.C., Lee, S.B., Lewis, K., Klibanov, A.M. 2002. Insights into bactericidal action of surface-attached poly(vinyl-*N*-hexylpyridinium) chains. Biotechnol. Lett. 24: 801-805.

233. Tiller, J.C., Lee, S.B., Lewis, K., Klibanov, A.M. 2002. Polymer surfaces derivatized with poly(vinyl-*N*-hexylpyridinium) kill airborne and waterborne bacteria. Biotechnol. Bioeng. 79: 465-471.

234. Thomas, M., Klibanov, A.M. 2002. Enhancing polyethylenimine's delivery of plasmid DNA into mammalian cells. Proc. Natl. Acad. Sci. USA 99: 14640-14645.

235. Lin, J., Shuyi, Q., Lewis, K., Klibanov, A.M. 2002. Bactericidal properties of flat surfaces and nanoparticles derivatized with alkylated polyethylenimines. Biotechnol. Progr. 18: 1082-1086.

236. Fu, K., Harrell, R., Zinski, K., Um, C., Jaklenec, A., Frazier, J., Lotan, N., Burke, P., Klibanov, A.M., Langer, R. 2003. A potential approach for decreasing the burst effect of protein from PLGA microspheres. J. Pharm. Sci. 92: 1582-1591.

237. Kamiya, N., Klibanov, A.M. 2003. Controlling the rate of protein release from polyelectrolyte complexes. Biotechnol. Bioeng. 82: 590-594.

238. Lin, J., Shuyi, Q., Lewis, K., Klibanov, A.M. 2003. The mechanism of bactericidal and fungicidal activities of textiles covalently modified with alkylated polyethylenimine. Biotechnol. Bioeng. 83: 168-172.

239. Thomas, M., Klibanov, A.M. 2003. Non-viral gene therapy: polycation-mediated DNA delivery. Appl. Microbiol. Biotechnol. 62: 27-34.

240. Klibanov, A.M. 2003. Asymmetric enzymatic oxidoreductions in organic solvents. Curr. Opinion Biotechnol. 14: 427-431.

Momenta Pharm. v. BMS, IPR215-01537
18/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7514

241. Chakrabarti, R., Klibanov, A.M. 2003. Nanocrystals modified by peptide nucleic acids (PNAs) for selective self-assembly and DNA detection. J. Am. Chem. Soc. 125: 12531-12540.

242. Thomas, M., Klibanov, A.M. 2003. Conjugation to gold nanoparticles enhances polyethylenimine's transfer of plasmid DNA into mammalian cells. Proc. Natl. Acad. Sci. USA 100: 9138-9143.

243. Lin, J., Murthy, S.K., Olsen, B.D., Gleason, K.K., Klibanov, A.M. 2003. Making thin polymeric materials including fabrics microbicidal and also water-repellent. Biotechnol. Lett. 25: 1661-1665.

244. Dzyuba, S.V., Klibanov, A.M. 2003. Asymmetric thiosulfinations catalyzed by bovine serum albumin and horseradish peroxidase. Biotechnol. Lett. 25: 1961-1965.

245. Klibanov, A.M., Schefiliti, J.A. 2004. On the relationship between conformation and stability in solid pharmaceutical protein formulations. Biotechnol. Lett. 26: 1103-1106.

246. Dzyuba, S.V., Klibanov, A.M. 2004. Stereoselective oxidations and reductions catalyzed by non-redox proteins. Tetrahedron Asymmetry 15: 2771-2777.

247. Gelman, F., Lewis, K., Klibanov, A.M. 2004. Drastically lowering the titer of waterborne bacteriophage PRD1 by exposure to immobilized hydrophobic polycations. Biotechnol. Lett. 26: 1695-1700.

248. Thomas, M., Ge, Q., Lu, J.J., Chen, J., Klibanov, A.M. 2005. Cross-linked small polyethylenimines: while still non-toxic, deliver DNA efficiently to mammalian cells *in vitro* and *in vivo*. Pharm. Res. 22: 373-380.

249. Sharma, V., Thomas, M., Klibanov, A.M. 2005. Mechanistic studies on aggregation of polyethylenimine-DNA complexes and its prevention. Biotechnol. Bioeng. 90: 614-620.

250. Varga, C.M., Tedford, N.C., Thomas, M., Klibanov, A.M., Griffith, L.G., Lauffenburger, D.A. 2005. Quantitative comparison of polyethylenimine formulations and adenoviral vectors in terms of intracellular gene delivery processes. Gene Therapy 12: 1023-1032.

251. Akinc, A., Thomas, M., Klibanov, A.M., Langer, R. 2005. Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. J. Gene Medicine 7: 657-663.

252. Milovic, N., Wang, J., Lewis, K., Klibanov, A.M. 2005. Immobilized *N*-alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed. Biotechnol. Bioeng. 90: 715-722.

253. Thomas, M., Ge, Q., Lu, J.J., Klibanov, A.M., Chen, J. 2005. Polycation-mediated delivery of short interfering RNAs for prophylaxis and treatment of influenza virus infection. Expert Opinion Biol. Therapy 5: 495-505.

254. Lewis, K., Klibanov, A.M. 2005. Surpassing nature: rational design of sterile-surface materials. Trends Biotechnol. 23: 343-348.

255. Thomas, M., Lu, J.J., Ge, Q., Zhang, C., Chen, J., Klibanov, A.M. 2005. Full deacylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung.

Momenta Pharm. v. BMS, IPR215-01537
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Samsung Bioepis Exhibit No. 1002
Page 7515

Proc. Natl. Acad. Sci. USA 102: 5679-5684.

256. Chakrabarti, R., Klibanov, A.M., Friesner, R.A. 2005. Computational prediction of native protein ligand-binding and enzyme active site sequences. Proc. Natl. Acad. Sci. USA 102: 10153-10158.

257. Chakrabarti, R., Klibanov, A.M., Friesner, R.A. 2005. Sequence optimization and designability of enzyme active sites. Proc. Natl. Acad. Sci. USA 102: 12035-12040.

258. Park, D., Wang, J., Klibanov, A.M. 2006. One-step, painting-like coating procedures to make surfaces highly and permanently bactericidal. Biotechnol. Progr. 22: 584-589.

259. Yu, J.-H., Klibanov, A.M. 2006. Co-lyophilization with D-proline greatly enhances peroxidase's stereoselectivity in a nonaqueous medium. Biotechnol. Lett. 28: 555-558.

260. Haldar, J., An, D., Alvarez de Cienfuegos, L., Chen, J., Klibanov, A.M. 2006. Polymeric coatings that inactivate both influenza virus and pathogenic bacteria. Proc. Natl. Acad. Sci. USA 103: 17667-17671.

261. Sharma, V.K., Klibanov, A.M. 2007. Moisture-induced aggregation of lyophilized DNA and its prevention. Pharm. Res. 24: 168-175.

262. Rojas, A.M., Gonzalez, P.A., Antipov, E., Klibanov, A.M. 2007. Specificity of a DNA-based (DNAzyme) peroxidative biocatalyst. Biotechnol. Lett. 29: 227-232.

263. Thomas, M., Lu, J.J., Chen, J., Klibanov, A.M. 2007. Non-viral siRNA delivery to the lung. Adv. Drug Delivery Revs. 59: 124-133.

264. Watanabe, W., Thomas, M., Clarke, R., Klibanov, A.M., Langer, R., Katstra, J., Fuller, G.G., Griel, L.C., Fiegel, J., Edwards, D. 2007. Why inhaling salt water changes what we exhale. J. Colloid Interface Sci. 307: 71-78.

265. Lipovšek, D., Antipov, E., Armstrong, K.A., Olsen, M.J., Tidor, B., Wittrup, K.D. 2007. Selection of horseradish peroxidase variants with enhanced enantioselectivity by yeast surface display. Chem. Biol. 14: 1176-1185.

266. Thomas, M., Lu, J.J., Zhang, C.C., Chen, J., Klibanov, A.M. 2007. Novel superior polycationic vectors for gene delivery prepared by high-throughput synthesis and screening of a combinatorial library. Pharm. Res. 24: 1564-1571.

267. Klibanov, A.M. 2007. Permanently microbicidal materials coatings. J. Mater. Chem. 17: 2479-2482.

268. Haldar, J., Weight, A.K., Klibanov, A.M. 2007. Preparation, application, and testing of permanently antibacterial and antiviral coatings. Nature Protocols 2: 2412-2417.

269. Devitt, G., Thomas, M., Klibanov, A.M., Pfeiffer, T., Bosch, V. 2007. Optimized protocol for the large-scale production of HIV pseudovirions by transient transfection of HEK293T cells with linear fully deacylated polyethylenimine. J. Virol. Meth. 146: 298-304.

Momenta Pharm. v. BMS, IPR215-01537
20/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7516

270. Haldar, J., Chen, J., Tumpey, T.M., Gubareva, L.V., Klibanov, A.M. 2008. Hydrophobic polycationic coatings inactivate wild-type and zanamivir- and/or oseltamivir- resistant human and avian influenza viruses. Biotechnol. Lett. 30: 475-479.

271. Mukherjee, K., Rivera, J.J., Klibanov, A.M. 2008. Practical aspects of hydrophobic polycationic "bactericidal paints". Appl. Biochem. Biotechnol. 151: 61-70.

272. Antipov, E., Cho, A.E., Wittrup, K.D., Klibanov, A.M. 2008. Highly L- and D- enantioselective variants of horseradish peroxidase discovered by an ultra high-throughput selection method. Proc. Natl. Acad. Sci. USA 105: 17694-17699.

273. Singleton, P.A., Chatchavalvanich, S., Fu, P., Xing, J., Birukova, A.A., Fortune, L., Klibanov, A.M., Garcia, J.G.N., Birukov, K.G. 2009. Akt-mediated transactivation of the $S1P_1$ receptor in caveolin-enriched microdomains regulates endothelial barrier enhancement by oxidized phospholipids. Circulation Res. 104: 978-986.

274. Antipov, E., Cho, A.E., Klibanov, A.M. 2009. How a single-point mutation in horseradish peroxidase markedly enhances enantioselectivity. J. Am. Chem. Soc. 131: 11155-11160.

275. Haldar, J., Álvarez de Cienfuegos, L., Tumpey, T.M., Gubareva, L.V., Chen, J., Klibanov, A.M. Bifunctional polymeric inhibitors of human influenza A viruses. 2010. Pharm. Res. 27: 259-263.

276. Agresti, J.J., Antipov, E., Abate, A.R., Ahn, K., Rowat, A.C., Baret, J.-C., Marquez, M., Klibanov, A.M., Griffiths, A.D., Weitz, D.A. 2010. Ultrahigh-throughput screening in drop-based microfluidics for directed evolution. Proc. Natl. Acad. Sci. USA 107: 4004-4009.

277. Fortune, J.A., Wu, B.-I., Klibanov, A.M. 2010. Radio frequency radiation (RFR) causes no non-thermal damage in enzymes and living cells. Biotechnol. Progr. 26: 1772-1776.

278. Wong, S.Y., Li, Q., Veselinovic, J., Kim, B.-S., Klibanov, A.M., Hammond, P.T. 2010. Bactericidal and virucidal ultrathin films assembled layer by layer from polycationic *N*-alkylated polyethylenimines and polyanions. Biomaterials 31: 4079-4087.

279. Birukova, A.A., Xing, J., Fu, P., Yakubov, B., Dubrovskyi, O., Fortune, J.A., Klibanov, A.M., Birukov, K.G. 2010. Atrial natriuretic peptide attenuates LPS-induced lung vascular leak: role of PAK1. Am. J. Physiol. 299: L652-L663.

280. Wong, S.Y., Moskowitz, J.S., Veselinovic, J., Rosario, R.A., Timachova, K., Blaisse, M.R., Smith, R.C., Klibanov, A.M., Hammond, P.T. 2010. Dual functional polyelectrolyte multilayer coatings for implants: permanent microbicidal base with controlled release of therapeutic agents. J. Am. Chem. Soc. 132: 17840-17848.

281. Du, W., Klibanov, A.M. 2011. Hydrophobic salts markedly diminish viscosity of concentrated protein solutions. Biotechnol. Bioeng. 108: 632-636.

282. Fortune, J.A., Novobrantseva, T., Klibanov, A.M. 2011. Highly effective gene transfection *in vivo* by alkylated polyethylenimine. J. Drug Delivery 2011: 6 pages, doi:10.1155/2011/204058.

283. Larson, A.M., Hsu, B.B., Rautaray, D., Haldar, J., Chen, J., Klibanov, A.M. 2011. Hydrophobic polycationic coatings disinfect poliovirus and rotavirus. Biotechnol. Bioeng. 108: 720-723.

Momenta Pharm. v. BMS, IPR215-01537
21/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7517

284. Weight, A.K., Haldar, J., Álvarez de Cienfuegos, L., Tumpey, T.M., Gubareva, L.V., Chen, J., Klibanov, A.M. 2011. Attachment to a polymer markedly enhances activity of zanamivir against drug-resistant strains of influenza A virus. J. Pharm. Sci. 100: 831-835.

285. Hsu, B.B., Klibanov, A.M. 2011. Light-activated covalent coating of cotton with hydrophobic polycations. Biomacromolecules 12: 6-9.

286. Hsu, B.B., Ouyang, J., Wong, S.Y., Hammond, P.T., Klibanov, A.M. 2011. On structural damage incurred by bacteria upon exposure to hydrophobic polycationic coatings. Biotechnol. Lett. 33: 411-416.

287. Fortune, J.A., Klibanov, A.M. 2011. Attaching anti-ACE monoclonal antibody to polyethylenimine (PEI) does not enhance specificity of *in vivo* PEI-mediated gene delivery. Open J. Pharmacol. **1**-**2**: 5 pages.

288. Onnis-Hayden, A., Hsu, B.B., Klibanov, A.M., Gu, A.Z. 2011. An antimicrobial polycationic sand filter for water disinfection. Water Sci. Technol. 63: 1997-2003.

289. Sharma, A., Tandon, A., Tovey, J.C.K., Gupta, R.R., Robertson, J.D., Fortune, J.A., Klibanov, A.M., Cowden, J.W., Rieger, F.G., Mohan, R.R. 2011. Polyethylenimine-conjugated gold nanoparticles: gene transfer potential and low toxicity in the cornea. Nanomedicine 7: 505-513.

290. Hsu, B.B., Wong, S.Y., Hammond, P.T., Chen, J., Klibanov, A.M. 2011. On the mechanism of influenza virus inactivation by immobilized hydrophobic polycations. Proc. Natl. Acad. Sci. USA 108: 61-66.

291. Long, F., Gao, C., Shi, H.C., He, M., Zhu, A.N., Klibanov, A.M., Gu, A.Z. 2011. Reusable evanescent-wave DNA biosensor for a rapid, highly sensitive, and selective detection of mercury ions. Biosensors Bioelectron. 26: 4018-4023.

292. Behlau, I., Mukherjee, K., Todani, A., Tisdale, A.S., Cade, F., Wang, L., Leonard, E.M., Zakka, F.R., Gilmore, M.S., Jakobiec, F.A., Dohlman, C.H., Klibanov, A.M. 2011. Biocompatibility and biofilm inhibition of *N,N*-hexyl,methyl-polyethylenimine bonded to Boston Keratoprosthesis artificial cornea materials. Biomaterials 32: 8783-8796.

293. Wong, S.Y., Han, L., Timachova, K., Veselinovic, J., Hyder, M.N., Ortiz, C., Klibanov, A.M., Hammond, P.T. 2012. Drastically lowered protein adsorption on microbicidal hydrophobic/hydrophilic polyelectrolyte multilayers. Biomacromolecules: 13: 719-726.

294. Schaer, T.P., Stewart, S., Hsu, B.B., Klibanov, A.M. 2012. Hydrophobic polycationic coatings that inhibit biofilms and support bone healing during infection. Biomaterials 33: 1245-1254.

295. Nassar, R.A., Brown, E.P., Chen, J., Klibanov, A.M. 2012. Removing human immunodeficiency virus (HIV) from the blood using immobilized heparin. Biotechnol. Lett. 34: 853-856.

296. Larson, A.M., Wang, H., Cao, Y., Jiang, T., Chen, J., Klibanov, A.M. 2012. Conjugating drug candidates to polymeric chains does not necessarily enhance anti-influenza activity. J. Pharm. Sci. 101: 3896-3905.

297. Sharma, A., Rodier, J.T., Tandon, A., Klibanov, A.M., Mohan, R.R. 2012. Attenuation of corneal myofibroblast development through nanoparticle-mediated soluble transforming growth factor-β

Momenta Pharm. v. BMS, IPR215-01537
22/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7518

type II receptor (sTGFβRII) gene transfer. Molecular Vision 18: 2598-2607.

298. Guo, Z., Chen, A., Nassar, R.A., Helk, B., Mueller, C., Tang, Y., Gupta, K., Klibanov, A.M. 2012. Structure-activity relationship for hydrophobic salts as viscosity-lowering excipients for concentrated aqueous solutions of monoclonal antibodies. Pharm. Res. 29: 3102-3109.

299. Grant, E.V., Thomas, M., Fortune, J., Klibanov, A.M., Letvin, N.L. 2012. Enhancement of plasmid DNA immunogenicity with linear polyethylenimine. Eur. J. Immunol. 42: 2937-2948.

300. Lee, C.M., Weight, A.K., Haldar, J., Wang, L., Klibanov, A.M., Chen, J. 2012. Polymer-attached zanamivir inhibits synergistically both early and late stages of influenza virus infection. Proc. Natl. Acad. Sci. USA 109: 20385-20390.

301. Larson, A.M., Oh, H.S., Knipe, D.M., Klibanov, A.M. 2013. Decreasing herpes simplex viral infectivity in solution by surface-immobilized and suspended *N*,*N*-dodecyl,methyl-polyethylenimine. Pharm. Res. 30: 25-31.

302. Park, D., Larson, A.M., Klibanov, A.M., Wang, Y. 2013. Antiviral and antibacterial polyurethanes of various modalities. Appl. Biochem. Biotechnol. 169: 1134-1146.

303. Srinivasan, C., Weight, A.K., Bussemer, T., Klibanov, A.M. 2013. Non-aqueous suspensions of antibodies are much less viscous than equally concentrated aqueous solutions. Pharm. Res. 30: 1749-1757.

304. Larson, A.M., Klibanov, A.M. 2013. Biocidal packaging of pharmaceuticals, foods, and other perishables. Ann. Rev. Chem. Biomolec. Eng. 4: 171-186.

305. Gerrard, S.E., Larson, A.M., Klibanov, A.M., Slater, N.K.H., Hanson, C.V., Abrams, B.F., Morris, M.K. 2013. Reducing infectivity of HIV upon exposure to surfaces coated with *N*,*N*-dodecyl,methyl-polyethylenimine. Biotechnol. Bioeng. 110: 2058-2062.

306. Larson, A.M., Chen, J., Klibanov, A.M. 2013. Conjugation to polymeric chains of influenza drugs targeting M2 ion channels partially restores inhibition of drug-resistant mutants. J. Pharm. Sci. 102: 2450-2459.

307. Weight, A.K., Belser, J.A., Tumpey, T.M., Chen, J., Klibanov, A.M. 2013. Zanamivir conjugated to poly-L-glutamine is much more active against influenza viruses in mice and ferrets than the drug itself. Pharm. Res. 31: 466-474.

308. Tandon, A., Sharma, A., Rodier, J.T., Klibanov, A.M., Rieger, F.G., Mohan, R.R. 2013. BMP7 gene transfer via gold nanoparticles into stroma inhibits corneal fibrosis *in vivo*. PLOS ONE 8(6): e66434, 1-9.

309. Liu, H., Kim, Y., Mello, K., Lovaasen, J., Shah, A., Rice, N., Yim, J.H., Pappas, D., Klibanov, A.M. 2014. Aerosol-assisted plasma deposition of hydrophobic polycations makes surfaces highly antimicrobial. Appl. Biochem. Biotechnol. 172: 1254-1264.

310. Zhu, A., Liu, H.K., Long, F., Su, E., Klibanov, A.M. 2015. Biocidal action of electric current applied to composite membranes containing carbon nanotubes. Appl. Biochem. Biotechnol. 175: 666-676.

Momenta Pharm. v. BMS, IPR215-01537
23/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7519

311. Liu, K., Elkin, I., Chen, J., Klibanov, A.M. 2015. Why do some immobilized *N*-alkylated polyethylenimines far surpass others in inactivating influenza viruses? Biomacromolecules 16: 351-356.

312. Su, E., Klibanov, A.M. 2015. Low-transition-temperature mixtures (LTTMs) for dissolving proteins and for drug formulation. Appl. Biochem. Biotechnol. 177: 753-758.

313. Elkin, I., Weight, A.K., Klibanov, A.M. 2015. Markedly lowering the viscosity of aqueous solutions of DNA by additives. International J. Pharmaceutics 494: 66-72.

314. Kumar, A., Klibanov, A.M. 2015. Bulky organic salts drastically lower viscosities of concentrated suspensions of bacterial cells. Biotechnol. Bioeng., submitted.

Patents:

1. Klibanov, A.M., Langer, R.S. Methods of decreasing the hydrophobicity of fibroblast and other interferons. U.S. Patent No. 4,414,147; November 8, 1983.

2. Klibanov, A.M., Kirchner, G. Enzymatic production of optical isomers of 2-halopropionic acids. U.S. Patent No. 4,601,987; July 22, 1986.

3. Klibanov, A.M. Removal of combined organic substances from aqueous solutions. U.S. Patent No. 4,623,465; November 18, 1986.

4. Klibanov, A.M. Enzymatic resolution of racemic mixtures of hydroxy compounds. U.S. Patent No. 4,659,671; 1987.

5. Klibanov, A.M., Dordick, J.S. Enzymatic temperature change indicator. U.S. Patent No. 4,826,762; May 2, 1989.

6. Dorval, B.L., Denham, L., Klibanov, A.M. Radial flow assay, delivering membrane, test kit, and methods. U.S. Patent No. 5,547,833; August 20, 1996.

7. Dorval, B.L., Denham, L., Klibanov, A.M. Detection reagent, particle, and immunoassay method. U.S. Patent No. 5,561,045; October 1, 1996.

8. Dorval, B.L., Chow, M., Klibanov, A.M. Stabilized vaccine compositions. U.S. Patent No. 5,618,539; April 8, 1997. European Patent No. 0 487 632; March 4, 1998.

9. Klibanov, A.M., Lewis, K., Ferrante, A.A., Coyle, C.L., Zylstra, G., Logan, M.S.P., Grossman, M.J. Solvent-resistant microorganisms. U.S. Patent No. 5,807,735; September 15, 1998.

10. Gresser, J.D., Trantolo, D.J., Langer, R., Klibanov, A.M., Wise, D.L. Material for buffered resorbable internal fixation devices and method for making the same. U.S. Patent No. 5,817,328; October 6, 1998.

11. Hekal, I.M., Langer, R.S., Klibanov, A.M., Mathiowitz, E. Monolithic polymer composition having a water absorption material. U.S. Patent No. 6,174,952; January 16, 2001.

Momenta Pharm. v. BMS, IPR215-01537
24/41 BMS EXHIBIT 2016

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Samsung Bioepis Exhibit No. 1002
Page 7520

12. Gresser, J.D., Trantolo, D.J., Langer, R., Lewandrowski, K.-U., Klibanov, A.M., Wise, D.L. Resorbable interbody spinal fusion devices. U.S. Patent No. 6,241,771; June 5, 2001.

13. Trantolo, D.J., Langer, R., Klibanov, A.M., Wise, D.L. Buffered resorbable internal fixation devices and methods for making material therefor. U.S. Patent No. 6,419,945; July 16, 2002.

14. Gresser, J.D., Trantolo, D.J., Langer, R., Lewandrowski, K.-U., Klibanov, A.M., Wise, D.L. Method of making biodegradable interbody spinal fusion devices. U.S. Patent No. 6,548,002; April 15, 2003.

15. Klibanov, A.M. Nonaqueous solutions and suspensions of macromolecules for pulmonary delivery. U.S. Patent No. 6,660,715; December 9, 2003.

16. Tiller, J.C., Lewis, K., Liao, C.-J., Klibanov, A.M. Antimicrobial polymeric surfaces. U.S. Patent No. 7,151,139; December 19, 2006.

17. Hirsh, J.C., Fleming, A.B., Rariy, R.V., Klibanov, A.M. Abuse-deterrent pharmaceutical formulations. U.S. Patent No. 8,449,909; May 28, 2013.

18. Rariy, R.V., Fleming, A.B., Hirsh, J.C., Klibanov, A.M. Abuse-deterrent pharmaceutical compositions of opioids and other drugs. U.S. Patent No. 8,557,291; October 15, 2013.

19. Hirsh, J.C., Klibanov, A.M., Swager, T.M., Buchwald, S.L., Lo, W.Y., Fleming, Rariy, R.V. Abuse-deterrent pharmaceutical compositions of opioids and other drugs. U.S. Patent No. 9,044,398; June 2, 2015.

20. Schaer, T.P., Stewart, S., Klibanov, A.M. Antibacterial coatings that inhibit biofilm formation on implants. U.S. Patent No. 9,089,407; July 28, 2015.

21. Schaer, P.T., Hsu, B.B., Stewart, S., Klibanov, A.M. Hydrophobic polycationic coatings that inhibit biofilm formation on orthopedic implants and support healing during infection, pending.

22. Haldar, J., Chen, J., Klibanov, A.M. Non-leaching virucidal coatings, pending.

23. Haldar, J., An, D., Álvarez de Cienfuegos, L., Chen, J., Klibanov, A.M. Polymeric coatings that inactivate viruses and bacteria. U.S. Patent Appl. Publ. No. 2010/0136072; June 3, 2010.

24. Pedersen, M., Stanley, M., Gouin, S., Klibanov, A.M, Hsu, B.B. Antimicrobial quat coating on fabrics. PCT Intl. Appl. WO 2012/06561, 2012.

25. Haldar, J., Alvarez de Cienfuegos, L., Klibanov, A.M., Chen, J. Bi-functional polymer-attached inhibitors of influenza virus. PCT Intl. Appl. WO 2009/032605, 2012.

Invited Lectures:

1. Gordon Conference on Immobilized Enzymes, Complexes, and Cells (Plymouth, NH). August 1978.

2. 5th International Conference on Enzyme Engineering (Henniker, NH). August 1978.

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3. Miles Laboratories (Elkhart, IN). February 1980.
4. Gordon Conference on Immobilized Species (Henniker, NH). August 1980.
5. Novo Laboratories (Wilton, CT). October 1980.
6. Corning Glass Works Co. (Corning, NY). October 1980.
7. Stauffer Chemical Co. (Dobbs Ferry, NY). December 1980.
8. DuPont Experimental Station (Wilmington, DE). January 1981.
9. Dow Chemical Co. (Wayland, MA). April 1981.
10. Cetus Corp. (Berkeley, CA). April 1981.
11. Exxon Research & Engineering Co. (Linden, NJ). April 1981.
12. Meeting of the American Solar Energy Society (Philadelphia, PA). May 1981.
13. 6th International Conference on Enzyme Engineering (Kashikojima, Japan). September 1981.
14. Genex Corp. (Rockville, MD). October 1981.
15. ILP Symposium for Senior Executives (London, England). November 1981.
16. California Institute of Technology (Pasadena, CA). December 1981.
17. Symposium of the American Society for Microbiology (Atlanta, GA). March 1982.
18. Kodak Research Center (Rochester, NY). March 1982.
19. Repligen Corp. (Cambridge, MA). April 1982.
20. Symposium on the Biological Basis of New Developments in Biotechnology (Minneapolis, MN). May 1982.
21. Meeting of the Biochemical Society (Oxford, England). July 1982.
22. Meeting of the American Chemical Society (Kansas City, MO). September 1982.
23. Rotenburger Symposium on Enzyme Technology (Kassel, Germany). September 1982.
24. Genetics Institute (Boston, MA). September 1982.
25. Meeting of the American Institute of Chemical Engineers (Los Angeles, CA). November 1982.
26. G.D. Searle Co. (Chicago, IL). October 1982.
27. Halcon International Research Center (Montvale, NJ). October 1982.

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28. International Workshop on Immobilized Enzymes and Cells (Bangkok, Thailand). December 1982.

29. Amoco Research Center (Naperville, IL). January 1983.

30. University College London (London, England). May 1983.

31. International Conference on Biotechnology Biotech '83 (London, England). May 1983.

32. Tufts University Workshop on Immobilized Enzymes and Proteins (Medford, MA). June 1983.

33. Gordon Conference on Organic Reactions and Processes (New Hampton, NH). July 1983.

34. Symposium "Biocatalysis: New Discoveries and Uses", Meeting of the American Society for Industrial Microbiology (Sarasota, FL). August 1983.

35. Monsanto Co. (St. Louis, MO). September 1983.

36. Pasteur Biosciences '83 Conference (Paris, France). September 1983.

37. 7th International Conference on Enzyme Engineering (White Haven, PA). September 1983.

38. University of Iowa (Iowa City, IA). October 1983.

39. CPC Moffett Technical Center (Summitt-Argo, IL). December 1983.

40. General Foods Co. (Dobbs Ferry, NY). December 1983.

41. U.S. National Bureau of Standards (Gaithersburg, MD). April 1984.

42. Millipore Corp. (Bedford, MA). April 1984.

43. Technical University of Munich (Munich, F.R.G.). May 1984.

44. Danish Academy of Technical Sciences (Copenhagen, Denmark). May 1984.

45. Novo International Biotechnology Symposium (Copenhagen, Denmark). May 1984.

46. CRA Scientific Conference (St. Charles, IL). June 1984.

47. Gordon Conference on Immobilized Species in Biotechnology (Plymouth, NH). August 1984.

48. Meeting of the American Chemical Society (Philadelphia, PA). August 1984.

49. International Biotechnology Conference Biotech '84 (Washington, DC). September 1984.

50. Staley Manufacturing Co. (Decatur, IL). October 1984.

51. Case Western Reserve University (Cleveland, OH). October 1984.

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52. IMC Corp. (Terre Haute, IN). October 1984.
53. Syracuse University (Syracuse, NY). November 1984.
54. National University of Mexico (Mexico City, Mexico). November 1984.
55. National Starch & Chemical Corp. (Bridgewater, NJ). November 1984.
56. Mead Corp. (Chillicothe, OH). November 1984.
57. Hebrew University of Jerusalem (Jerusalem, Israel). November 1984.
58. U.S. Army Chemical R&D Center (Aberdeen Proving Ground, MD). December 1984.
59. Allied Corp. (Morristown, NJ). December 1984.
60. Bristol Laboratories (Syracuse, NY). January 1985.
61. University of Rochester (Rochester, NY). January 1985.
62. G.D. Searle Co. (Chicago, IL). February 1985.
63. Wellcome Research Laboratories (Research Triangle Park, NC). February 1985.
64. UCLA Symposium on Protein Structure, Folding and Design (Keystone, CO). April 1985.
65. International Conference on Application of Biocatalysts in Organic Synthesis (Noordwijkerhout, Holland). April 1985.
66. Guido Donegani Research Institute (Novara, Italy). April 1985.
67. Hoffmann-LaRoche Co. (Basel, Switzerland). April 1985.
68. Pittsburgh-Cleveland Catalysis Society Meeting (Pittsburgh, PA). May 1985.
69. Ethyl Corp. (Baton Rouge, LA). May 1985.
70. Stony Brook Symposium on Protein Engineering (Stony Brook, NY). May 1985.
71. Amgen Corp. (Thousand Oaks, CA). June 1985.
72. Gordon Conference on Applied Microbiology (New London, NH). August 1985.
73. International Congress of Biochemistry (Amsterdam, Holland). August 1985.
74. IUPAC Congress (Manchester, England). September 1985.
75. International Enzyme Engineering Conference (Helsingor, Denmark). September 1985.
76. Biotechnica '85 (Hannover, West Germany). October 1985.

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77. Biotec 85 (Dusseldorf, West Germany). October 1985.

78. Smith Kline & French Co. (Philadelphia, PA). November 1985.

79. Exxon Corporate Research (Anandale, NJ). December 1985.

80. Pennsylvania State University (University Park, PA). December 1985.

81. Georgetown University (Washington, DC). February 1986.

82. Columbia University (New York, NY). February 1986.

83. Upjohn Co. (Kalamazoo, MI). February 1986.

84. UNIDO Workshop on Biotechnology (Trieste, Italy). March 1986.

85. Enericherche (Monterotondo, Italy). March 1986.

86. EEC Biomolecular Engineering Meeting (Compiegne, France). April 1986.

87. ACS Conference on Chemical Aspects of Biotechnology (Gaithersburg, MD). May 1986.

88. Biotech '86 Europe International Conference (London, England). May 1986.

89. IUPAC International Symposium on Organic Chemistry in Technological Perspective (Jerusalem, Israel). June 1986.

90. University of Tel-Aviv (Ramat-Aviv, Israel). June 1986.

91. Annual Meeting of the Canadian Biochemical Society (Guelph, Canada). June 1986.

92. International Symposium on Biologically Engineered Polymers (Cambridge, England). July 1986.

93. TNO Institute of Applied Chemistry (Zeist, The Netherlands). August 1986.

94. IUPAC International Symposium on the Chemistry of Natural Products (The Hague, The Netherlands). August 1986.

95. American Chemical Society National Meeting (Anaheim, CA). September 1986.

96. Catalytica Annual Science and Technology Symposium (San Jose, CA). September 1986.

97. International Conference on Enzyme Engineering (Cambridge, England). September 1986.

98. Ohio State University (Columbus, OH). October 1986.

99. Michigan State University (Lansing, MI). October 1986.

100. Biotechnology Process Engineering Symposium (Cambridge, MA). October 1986.

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101. University of Delaware (Newark, DE). November 1986.

102. American Red Cross Blood Research Institute (Bethesda, MD). November 1986.

103. International Symposium on Biocatalysis in Non-Aqueous Solvents (Wageningen, The Netherlands). December 1986.

104. University of Trondheim (Trondheim, Norway). December 1986.

105. Colgate-Palmolive Research Center (Piscataway, NJ). January 1987.

106. International Paper Co. (Tuxedo Park, NY). January 1987.

107. University of Massachusetts (Amherst, MA). February 1987.

108. American Society for Microbiology Biocatalysis Symposium (Atlanta, GA). March 1987.

109. Hope College (Holland, MI). March 1987.

110. Pfizer Corporate Research Center (Groton, CT). April 1987.

111. OBBS Symposium on Protein Engineering (Ottawa, Canada). April 1987.

112. University of Texas (Austin, TX). April 1987.

113. Florida Catalysis Conference (Palm Coast, FL). May 1987.

114. PPG Industries (Barberton, OH). May 1987.

115. Abbott Laboratories (North Chicago, IL). May 1987.

116. American Oil Chemists Society Symposium on Lipases (New Orleans, LA). May 1987.

117. Argonne National Laboratory (Argonne, IL). June 1987.

118. Canadian Chemical Conference (Quebec City, Canada). June 1987.

119. Squibb Co. (New Brunswick, NJ). June 1987.

120. FEBS Annual Meeting (Ljubljana, Yugoslavia). July 1987.

121. L'Oreal Research Institute (Paris, France). July 1987.

122. International Symposium on Biointeractions (Cambridge, England). July 1987.

123. Eli Lilly Co. (Indianapolis, IN). August 1987.

124. Upjohn Co. (Kalamazoo, MI). September 1987.

125. International Enzyme Engineering Conference (Santa Barbara, CA). October 1987.

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126. Shell Development Co. (Houston, TX). October 1987.

127. DuPont Experimental Station (Wilmington, DE). October 1987.

128. University of Helsinki (Helsinki, Finland). November 1987.

129. International Conference on Bioreactors and Biotransformations (Gleneagles, Scotland). November 1987.

130. Sandoz Co. (Basel, Switzerland). November 1987.

131. University of Waterloo (Waterloo, Canada). December 1987.

132. Norwegian Biochemical Society Annual Meeting (Bejito, Norway). January 1988.

133. Rutgers University (New Brunswick, NJ). February 1988.

134. University of Ottawa (Ottawa, Canada). February 1988.

135. Boston Biomedical Research Institute (Boston, MA). March 1988.

136. Princeton University (Princeton, NJ). March 1988.

137. Royal Society of Chemistry Meeting (Kent, England). April 1988.

138. Stanford University (Stanford, CA). May 1988.

139. University of Graz (Graz, Austria). May 1988.

140. Belgian Organic Chemistry Symposium (Ghent, Belgium). May 1988.

141. Dow Chemical Co. (Midland, MI). May 1988.

142. Michigan State University (Lansing, MI). May 1988.

143. American Chemical Society Meeting (Toronto, Canada). June 1988.

144. Proctor & Gamble Co. (Cincinnati, OH). June 1988.

145. International Congress of Biochemistry (Prague, Czechoslovakia). July 1988.

146. International Biotechnology Symposium (Paris, France). July 1988.

147. Gordon Conference on Catalysis (Newport, RI). August 1988.

148. Conference on Biocatalytic Synthesis of Organic Compounds (Saratoga, NY). August 1988.

149. International Symposium on Thermodynamics Applied to Biological Systems (Santa Margherita, Italy). September 1988.

150. Cornell University (Ithaca, NY). September 1988.

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151. Eastman Kodak Co. (Kingsman, TN). September 1988.

152. Symposium on Biotechnology Challenges in the Food Industry (Williamsburg, VA). October 1988.

153. University of California at Irvine (Irvine, CA). October 1988.

154. UCSF Symposium on Protein and Drug Design (San Francisco, CA). October 1988.

155. Boston University (Boston, MA). October 1988.

156. Wayne State University (Detroit, MI). November 1988.

157. Kyoto Symposium on Frontiers in Biocatalysis (Kyoto, Japan). November 1988.

158. Rohm & Haas Co. (Spring House, PA). January 1989.

159. Block Drug Co. (Jersey City, NJ). January 1989.

160. State University of New York (Albany, NY). March 1989.

161. Scripps Clinic and Research Foundation (La Jolla, CA). April 1989.

162. Texas A&M University (College Station, TX). May 1989.

163. University of Texas (Austin, TX). May 1989.

164. International Congress of Food Engineering (Cologne, F.R.G.). May 1989.

165. University of Groningen (Groningen, The Netherlands). June 1989.

166. Gordon Conference on Enzymes, Coenzymes, and Metabolic Pathways (Meridan, NH). July 1989.

167. American Peptide Symposium (La Jolla, CA). July 1989.

168. International Symposium on Prospects in Protein Engineering (Haren, The Netherlands). August 1989.

169. Connaught Laboratories (Toronto, Canada). September 1989.

170. University of Georgia (Athens, GA). October 1989.

171. Spanish National Congress of Biochemistry (Alicante, Spain). October 1989.

172. Symposium on Receptor- and Enzyme-Based Drug Design (Chapel Hill, NC). October 1989.

173. Wesleyan University (Middletown, CT). November 1989.

174. University of Wisconsin (Madison, WI). February 1990.

175. Merck Sharp & Dohme Research Laboratories (Rahway, NJ). February 1990.

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176. International Symposium on Biochemical Engineering (Stuttgart, F.R.G.). March 1990.

177. University of Geneva (Geneva, Switzerland). March 1990.

178. University of Lausanne (Lausanne, Switzerland). March 1990.

179. University of Bern (Bern, Switzerland). March 1990.

180. University of Fribourg (Fribourg, Switzerland). March 1990.

181. International Conference on Protein Stability (Cambridge, England). March 1990.

182. AFRC Institute of Food Research (Reading, England). March 1990.

183. Lehigh University (Bethlehem, PA). April 1990.

184. Biotechnology Research Institute (Montreal, Canada). April 1990.

185. Ohio State University (Columbus, OH). April 1990.

186. Pharmaceutical Manufacturers Association Annual Meeting (St. Louis, MO). April 1990.

187. International Conference on Industrial Use of Enzymes (Chicago, IL). May 1990.

188. Korea Advanced Institute of Science and Technology (Seoul, Korea). June 1990.

189. Academia Sinica and National Taiwan University (Taipei, Taiwan). June 1990.

190. Gordon Conference on Biocatalysis (Plymouth, NH). June 1990.

191. IUPAC Conference on Organic Synthesis (Helsinki, Finland). July 1990.

192. International Conference on Solution Chemistry (Ottawa, Canada). August 1990.

193. Gordon Conference on Biomolecular Recognition (Plymouth, NH). August 1990.

194. University of Naples (Naples, Italy). September 1990.

195. International Conference on Water and Life (Crans-sur-Sierre, Switzerland). September 1990.

196. International Symposium on Thermodynamic Basis of Protein Structure and Function (Kansas City, MO). October 1990.

197. Johns Hopkins University (Baltimore, MD). October 1990.

198. Institute of Medicine Workshop on Microheterogeneity of Biological Macromolecules (Arlington, VA). November 1990.

199. U.S.-Japan Biotechnology Conference (Honolulu, HI). January 1991.

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200. Ciba-Geigy Corp. (Summit, NJ). February 1991.

201. City University of New York (New York, NY). March 1991.

202. Rensselaer Polytechnic Institute (Troy, NY). March 1991.

203. Bristol-Myers Squibb Co. (Wallingford, CT). April 1991.

204. British Biochemical Society Meeting (Reading, England). April 1991.

205. University of California at Berkeley (Berkeley, CA). April 1991.

206. California Institute of Technology (Pasadena, CA). April 1991.

207. Merck, Sharp & Dohme Research Laboratories (West Point, PA). May 1991.

208. Bristol-Myers Squibb Research Institute (Princeton, NJ). May 1991.

209. International Conference on Biocatalysis (Orlando, FL). June 1991.

210. U.S. Army Research, Development and Engineering Center (Natick, MA). July 1991.

211. Gordon Conference on Natural Products (Plymouth, NH). July 1991.

212. Lederle-Praxis Biologicals Co. (Sanford, NC). July 1991.

213. International Biochemistry Congress (Jerusalem, Israel). August 1991.

214. American Chemical Society Meeting (New York, NY). August 1991.

215. International Enzyme Engineering Conference (Kona, HI). September 1991.

216. University of Lowell (Lowell, MA). October 1991.

217. Rutgers University (Piscataway, NJ). November 1991.

218. International Symposium on Enzymes in Organic Synthesis (New Delhi, India). January 1992.

219. International BioSymposium Nagoya '92 (Nagoya, Japan). January 1992.

220. International Symposium on Biomolecules in Organic Solvents (Taxco, Mexico). February 1992.

221. Rockefeller University (New York, NY). March 1992.

222. Symposium on Molecular Chirality of the Pharmaceutical Society of Japan (Tokyo, Japan). April 1992.

223. Conference on Enzymes in Organic Chemistry of the Dutch Chemical Society (Wageningen, The Netherlands). April 1992.

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224. International Symposium on Bioprocessing of Coal (Clearwater Beach, FL). May 1992.

225. Exxon Workshop on New Leads in Coal Depolymerization (Montgomery, TX). May 1992.

226. Italian Symposium on Biochemical Biotechnology (Capri, Italy). June 1992.

227. Gordon Conference on Biocatalysis (Meriden, NH). July 1992.

228. FEBS Meeting (Dublin, Ireland). August 1992.

229. International Biotechnology Symposium (Washington, DC). August 1992.

230. International Symposium Bio Japan '92 (Yokohama, Japan). August 1992.

231. Texas College of Osteopathic Medicine (Fort Worth, TX). October 1992.

232. Rutgers University (Newark, NJ). October 1992.

233. Schering-Plough Co. (Bloomfield, NJ). November 1992.

234. International Symposium ISOPOW-V: *Properties of Water in Foods* (Peniscola, Spain). November 1992.

235. Carlsberg Laboratory (Copenhagen, Denmark). November 1992.

236. International Food Technology Conference (The Hague, The Netherlands). November 1992.

237. Singapore Institute of Standards and Industrial Research (Singapore). November 1992.

238. Drew University (Madison, NJ). December 1992.

239. Northeastern University (Boston, MA). January 1993.

240. Ciba Corning Diagnostics Corp. (Irvine, CA). January 1993.

241. University of Kansas (Lawrence, KS). January 1993.

242. 6th International Symposium on Recent Advances in Drug Delivery Systems (Salt Lake City, UT). February 1993.

243. Pennsylvania State University (University Park, PA). March 1993.

244. Northwestern University (Evanston, IL). March 1993.

245. International Symposium on Technologies for the Production of Enantiomerically Pure Chemicals (Amelia Island, FL). March 1993.

246. Gordon Conference on Bioanalytical Sensors (Ventura, CA). March 1993.

247. Pharmaceutical Manufacturers Association Analytical R&D/Pharmaceutical Development Subsections Joint Annual Meeting (Lake Buena Vista, FL). April 1993.

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248. University of Massachusetts Symposium on Breakthrough Technologies: *Paradigms for a New Century* (Amherst, MA). May 1993.

249. Parke-Davis Corp. (Ann Arbor, MI). May 1993.

250. NutraSweet Co. (Mt. Prospect, IL). June 1993.

251. IGT/GRI Workshop on Novel Concepts for the Production of Regio- and Stereospecific Compounds (Chicago, IL). June 1993.

252. Northeast Regional Meeting of the American Chemical Society (Boston, MA). June 1993.

253. Institute of Food Technologists' Biotechnology Meeting (Chicago, IL). July 1993.

254. Annual Meeting of Department of Energy's Biological and Chemical Technology Research Program Principal Investigators (Tiburon, CA). July 1993.

255. Allied-Signal Corp. (Des Plains, IL). July 1993.

256. American Chemical Society Meeting (Chicago, IL). August 1993.

257. University of Illinois at Urbana-Champaign (Urbana, IL). September 1993.

258. International Enzyme Engineering Conference (Deauville, France). September 1993.

259. Queens College (Flushing, NY). November 1993.

260. Exxon Chemical Research Meeting (Galveston, TX). November 1993.

261. Hoffmann-LaRoche Co. (Nutley, NJ). December 1993.

262. U.S.-Japan Symposium on Drug Delivery (Maui, HI). December 1993.

263. North Dakota State University (Fargo, ND). February 1994.

264. Annual Meeting of the American Institute for Medical and Biological Engineering (Washington, DC). March 1994.

265. Rhone-Poulenc Rorer Symposium on Visions in Chemistry (Collegeville, PA). May 1994.

266. International Flavors & Fragrances R&D (Union Beach, NJ). May 1994.

267. Istituto degli Ormoni (Milan, Italy). June 1994.

268. Gordon Conference on Enzymes, Coenzymes, and Metabolic Pathways (Meriden, NH). July 1994.

269. Annual Meeting of Department of Energy's Biological and Chemical Technology Research Program Principal Investigators (Denver, CO). July 1994.

270. William S. Johnson Symposium in Organic Chemistry (Stanford, CA). October 1994.

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271. R.W. Johnson Pharmaceutical Research Institute (Spring House, PA). October 1994.

272. International Congress of Biochemical Engineering (Mexico City, Mexico). October 1994.

273. National University of Mexico (Mexico City, Mexico). October 1994.

274. DuPont Merck Pharmaceutical Co. (Deepwater, NJ). December 1994.

275. Symposium on Therapeutic Protein Production and Processing (Cambridge, MA). January 1995.

276 International Symposium on Biocatalysts for Flavor Production, BioFlavour '95 (Dijon, France). February 1995.

277. Institut Pasteur (Paris, France). February 1995.

278. Florida State University (Tallahassee, FL). March 1995.

279. Abbott Laboratories (North Chicago, IL). March 1995.

280. University of Maryland, Baltimore County (Baltimore, MD). March 1995.

281 University of California at San Diego (La Jolla, CA). April 1995.

282. Great Lakes Regional Meeting of the American Chemical Society (LaCrosse, WI). June 1995.

283. Gordon Conference on Bioorganic Chemistry (Andover, NH). June 1995.

284. Annual Meeting of Department of Energy's Biochemical and Chemical Technology Research Principal Investigators (Albuquerque, NM). July 1995.

285. International Symposium "Biocatalysis-95" (Suzdal, Russia). August 1995.

286. International Enzyme Engineering Conference (San Diego, CA). October 1995.

287. Symposium on Biomacromolecules: *From 3-D Structure to Applications* (Pasco, WA). October 1995.

288. Enzymol International Inc. (Columbus, OH). December 1995.

289. International Symposium Pacifichem '95 (Honolulu, HI). December 1995.

290. 3rd U.S.-Japan Symposium on Drug Delivery (Maui, HI). December 1995.

291. New England Section of the Society for Industrial Microbiology (Cambridge, MA). February 1996.

292. Brown University (Providence, RI). February 1996.

293. University of Pittsburgh (Pittsburgh, PA). February 1996.

294. Food Research Institute (Reading, England). March 1996.

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295. International Symposium Chiral USA '96 (Boston, MA). May 1996.

296. BP Catalyst Colloquium (Uxbridge, England). June 1996.

297. International Symposium on Homogeneous Catalysis (Princeton, NJ). August 1996.

298. Juan March Foundation's International Workshop on Novel Biocatalysts (Madrid, Spain). March 1997.

299. University of Kentucky (Lexington, KY). April 1997.

300. Gordon Conference on Organic Reactions and Processes (Henniker, NH). August 1997.

301. Novo Nordisk Symposium on Protein Stability (Klampenborg, Denmark). August 1997.

302. International Enzyme Engineering Conference (Beijing, China). October 1997.

303. Biogen Inc. (Cambridge, MA). December 1997.

304. INBIO Europe'98 Conference on Advances in Industrial Biocatalysis (Amsterdam, The Netherlands). February 1998.

305. International Symposium on Stability and Stabilization of Biocatalysts (Córdoba, Spain). April 1998.

306. International Symposium on Protein Structure, Stability and Folding: *Fundamental and Medical Aspects* (Moscow, Russia). June 1998.

307. International Conference on Protein Stabilization (Leeds, England). June 1998.

308. University of Virginia (Charlottesville, VA). September 1998.

309. Danish Biotechnology Conference (Vejle, Denmark). May 1999.

310. International Conference on Biocatalysis and Biotransformation Biotrans '99 (Giardini Naxos, Italy). September 1999.

311. Annual Meeting of the American Association of Pharmaceutical Scientists (New Orleans, LA). November 1999.

312. Brandeis University (Waltham, MA). November 1999.

313. Rutgers University (Piscataway, NJ). March 2000.

314. Chiral USA 2000 Conference (Boston, MA). May 2000.

315. Land O'Lakes Conference on Solid-State Reactivity in Pharmaceutical Systems (Merrimac, WI). June 2000.

316. Agouron Pharmaceuticals Inc. (San Diego, CA). July 2000.

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317. U.S. Patent and Trademark Office Technology Fair (Arlington, VA). July 2000.

318. 23rd Gulf Coast Chemistry Conference (Pensacola, FL). September 2000.

319. Avon Products (Suffern, NY). November 2000.

320. Procter & Gamble Co. (Cincinnati, OH). November 2000.

321. Flamel Technologies (Venissieux, France). December 2000.

322. BioVision 2001 International Conference (Lyon, France). February 2001.

323. 9th Baxter Science and Technology Symposium (Hyland, IL). May 2001.

324. International Symposium on Applications of Enzymes in Chemical and Biological Defense (Orlando, FL). May 2001.

325. International Conference on Applied Biocatalysis (Trondheim, Norway). June 2001.

326. PPG Industries (Allison Park, PA). August 2001.

327 University of Kansas (Lawrence, KS). August 2001.

328. Alkermes Inc. (Cambridge, MA). October 2001.

329. 6th U.S.-Japan Symposium on Drug Delivery Systems (Maui, HI). December 2001.

330. Acambis Inc. (Cambridge, MA). January 2002.

331. Baxter BioScience Co. (Duarte, CA). January 2002.

332. International Symposium on Challenges in Drug Discovery and Development in the 21st Century (Kolkata, India). March 2002.

333. National Institute of Standards and Technology (Gaithersburg, MD). April 2002.

334. Albany Molecular Research, Inc. (Mt. Prospect, IL). April 2002.

335. Institute for International Research Conference on Protein and Peptide Formulation Strategies for Drug Development and Delivery (San Francisco, CA). August 2002.

336. R&D Management Council Meeting of the Technical Association of the Pulp and Paper Industry (Cambridge, MA). November 2002.

337. DuPont Central R&D Center (Wilmington, DE). December 2002.

338. *ACTIVE*Pack 2003 Conference (Ponte Verde Beach, FL). February 2003.

339. International Symposium on Industrial Applications of Biocatalysis: *Chemical Development Issues* (Boston, MA). September 2003.

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340. U.S. Army Workshop on Self-Decontaminating Materials and Multifunctional Coatings (Arlington, VA). October 2003.

341. British Royal Society Meeting on Biocatalysis in Alternative Media (London, England). December 2003.

342. National Autonomous University of Mexico (Mexico City, Mexico). March 2004.

343. Healthcare Research Initiative Workshop on the Effect of Radiofrequency Radiation on Pharmaceutical Product Quality (Cambridge, MA). June 2004.

344. Rensselaer Biotechnology Symposium on Biological Discoveries That Will Change the World (Troy, NY). September 2004.

345. Barnett International Conference: *The Consequences of Aggregation in Drug Development, Immunology, Quality Control, and Disease Genesis* (Philadelphia, PA). September 2004.

346. 4th Annual IBC Conference on Formulation Strategies for Protein Therapeutics (Boston, MA). October 2004.

347. U.S. Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD). August 2005.

348. World Congress on Synthetic Receptors (Salzburg, Austria). September 2005.

349. International Conference "Celebrating 30 Years of Robert Langer's Science" (Cambridge, MA). July 2006.

350. University of Montreal (Montréal, Québec, Canada). October 2006.

351. The Boeing Company Research Center (Everett, WA). December 2006.

352. Instrumentation Laboratory (Lexington, MA). December 2006.

353. Acambis Inc. (Cambridge, MA). January 2007.

354. 6th International Symposium on Industrial Microbiology and Biotechnology (Cambridge, MA). August 2007.

355. Scientific Conference on Chemical & Biological Defense Research (Timonium, MD). November 2007.

356. MIT Research and Development Conference (Cambridge, MA). November 2007.

357. Nanotech '2008 International Conference (Boston, MA). June 2008.

358. Materials Research Society Symposium (Boston, MA). December 2008.

359. Johnson & Johnson Research Briefing at MIT (Cambridge, MA). January 2009.

360. 3rd Annual Lyophilization Conference: *Ensuring Optimum Formulations for Pharmaceuticals*

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and Biologics (Boston, MA). February 2010.

361. DSM Corporate Research Center (Geleen, The Netherlands). June 2010.

362. Merck Serono Pharmaceutical Co. (Darmstadt, Germany). June 2010.

363. NanoFormulations '2010 International Conference (Stockholm, Sweden). June 2010.

364. Covidien Surgical Devices and Vascular Therapies Divisions (Cambridge, MA). October 2010.

365. World Lyophilization Summit (Cambridge, MA). May 2011.

366. Alvogen (Cambridge, MA). March 2012.

367. Colgate-Palmolive Co. (Cambridge, MA). April 2012.

368. FDA Parenteral Drug Association Glass Quality Conference (Washington, DC). June 2012.

369. University of Puerto Rico (Humacao, Puerto Rico). January 2013.

370. International Conference on Creating and Leveraging Intellectual Property in Developing Countries (Durban, South Africa – *via teleconference*). November 2013.

371. Northwestern University (Evanston, IL). April 2015.

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