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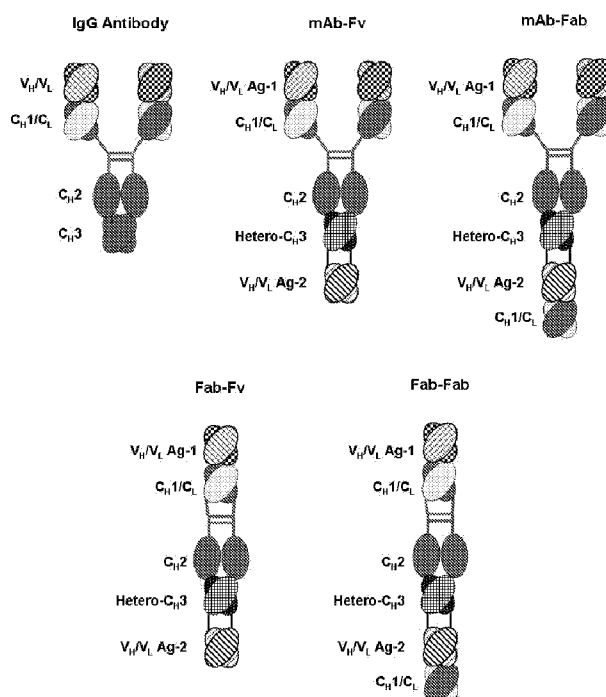
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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR SIMULTANEOUS BIVALENT AND MONOVALENT CO-ENGAGEMENT OF ANTIGENS

Figure 8



(57) Abstract: Immunoglobulin compositions that simultaneously co-engage antigens, where one of the antigens is bound bivalently and the other antigen is bound monovalently. The novel immunoglobulins described preferably utilize heterodimeric Fc regions.

**Declarations under Rule 4.17:**

- *as to the identity of the inventor (Rule 4.17(i))*
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COMPOSITIONS AND METHODS FOR SIMULTANEOUS BIVALENT AND MONOVALENT CO-ENGAGEMENT OF ANTIGENS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/239,316, filed September 2, 2009 under 35 U.S.C. §119(e) and incorporates the same in its entirety herein.

TECHNICAL FIELD

[1] The present disclosure provides novel antibody analog compositions that simultaneously co-engage antigens, where one of the antigens is bound bivalently and the other antigen is bound monovalently. The novel antibody analogs described preferably utilize heterodimeric Fc regions. Methods of using the novel antibody analog compositions, particularly for therapeutic purposes, are also described herein.

BACKGROUND

[2] Antibody-based therapeutics have been used successfully to treat a variety of diseases, including cancer and autoimmune/inflammatory disorders. Yet improvements to this class of drugs are still needed, particularly with respect to enhancing their clinical efficacy. One avenue being explored is the engineering of additional and novel antigen binding sites into antibody-based drugs such that a single immunoglobulin molecule co-engages two different antigens. Such non-native or alternate antibody formats that engage two different antigens are often referred to as bispecifics. Because the considerable diversity of the antibody variable region (Fv) makes it possible to produce an Fv that recognizes virtually any molecule, the typical approach to bispecific generation is the introduction of new variable regions into the antibody.

[3] A number of alternate antibody formats have been explored for bispecific targeting (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; expressly incorporated herein by reference). Initially, bispecific antibodies were made by fusing two cell lines that each produced a single monoclonal antibody (Milstein et al., 1983, Nature 305:537-540). Although the resulting hybrid hybridoma or quadroma did produce bispecific antibodies, they were only a minor population, and

extensive purification was required to isolate the desired antibody. An engineering solution to this was the use of antibody fragments to make bispecifics. Because such fragments lack the complex quaternary structure of a full length antibody, variable light and heavy chains can be linked in single genetic constructs. Antibody fragments of many different forms have been generated, including diabodies, single chain diabodies, tandem scFv's, and Fab₂ bispecifics (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; expressly incorporated herein by reference). While these formats can be expressed at high levels in bacteria and may have favorable penetration benefits due to their small size, they clear rapidly *in vivo* and can present manufacturing obstacles related to their production and stability. A principal cause of these drawbacks is that antibody fragments typically lack the constant region of the antibody with its associated functional properties, including larger size, high stability, and binding to various Fc receptors and ligands that maintain long half-life in serum (i.e. the neonatal Fc receptor FcRn) or serve as binding sites for purification (i.e. protein A and protein G).

[4] More recent work has attempted to address the shortcomings of fragment-based bispecifics by engineering dual binding into full length antibody -like formats (Wu et al., 2007, Nature Biotechnology 25[11]:1290-1297; USSN12/477,711; Michaelson et al., 2009, mAbs 1[2]:128-141; PCT/US2008/074693; Zuo et al., 2000, Protein Engineering 13[5]:361-367; USSN09/865,198; Shen et al., 2006, J Biol Chem 281[16]:10706-10714; Lu et al., 2005, J Biol Chem 280[20]:19665-19672; PCT/US2005/025472; expressly incorporated herein by reference). These formats overcome some of the obstacles of the antibody fragment bispecifics, principally because they contain an Fc region. One significant drawback of these formats is that, because they build new antigen binding sites on top of the homodimeric constant chains, binding to the new antigen is always bivalent.

[5] For many antigens that are attractive as co-targets in a therapeutic bispecific format, the desired binding is monovalent rather than bivalent. For many immune receptors, cellular activation is accomplished by cross-linking of a monovalent binding interaction. The mechanism of cross-linking is typically mediated by antibody/antigen immune complexes, or via effector cell to target cell engagement. For example, the low affinity Fc gamma receptors (FcγRs) such as FcγRIIa, FcγRIIb, and FcγRIIIa bind monovalently to the antibody Fc region. Monovalent binding does not activate cells expressing these FcγRs; however, upon immune complexation or cell-to-cell contact, receptors are cross-linked and clustered on the cell surface, leading to activation. For receptors responsible for mediating cellular killing, for example FcγRIIIa on natural killer (NK) cells, receptor cross-linking and cellular activation occurs when the effector cell engages the target cell in a highly avid format (Bowles & Weiner, 2005, J Immunol Methods 304:88-99, expressly incorporated by reference)..

Similarly, on B cells the inhibitory receptor FcγRIIb downregulates B cell activation only when it engages into an immune complex with the cell surface B-cell receptor (BCR), a mechanism that is mediated by immune complexation of soluble IgG's with the same antigen that is recognized by the BCR (Heyman 2003, Immunol Lett 88[2]:157-161; Smith and Clatworthy, 2010, Nature Reviews Immunology 10:328-343; expressly incorporated by reference). As another example, CD3 activation of T-cells occurs only when its associated T-cell receptor (TCR) engages antigen-loaded MHC on antigen presenting cells in a highly avid cell-to-cell synapse (Kuhns et al., 2006, Immunity 24:133-139). Indeed nonspecific bivalent cross-linking of CD3 using an anti-CD3 antibody elicits a cytokine storm and toxicity (Perruche et al., 2009, J Immunol 183[2]:953-61; Chatenoud & Bluestone, 2007, Nature Reviews Immunology 7:622-632; expressly incorporated by reference). Thus for practical clinical use, the preferred mode of CD3 co-engagement for redirected killing of targets cells is monovalent binding that results in activation only upon engagement with the co-engaged target.

[6] Thus while bispecifics generated from antibody fragments suffer biophysical and pharmacokinetic hurdles, a drawback of those built with full length antibody -like formats is that they engage co-target antigens multivalently in the absence of the primary target antigen, leading to nonspecific activation and potentially toxicity. The present invention solves this problem by introducing a novel set of bispecific formats that enable the simultaneous bivalent and monovalent co-engagement of distinct target antigens.

SUMMARY

[7] The present disclosure provides novel antibody analog compositions that co-engage a first and second antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[8] Figure 1. Heterodimeric Fc region proof-of-concept system. DNA constructs encoding two different immunoglobulin polypeptides were designed: (i) scFv-Fc (VH-linker-Vk-Hinge-CH2-CH3') and (ii) empty-Fc (Hinge-CH2-CH3''). As shown, three possible dimers can form from cotransfection and expression of these two polypeptides, each composed of a different number of immunoglobulin (Ig) domains and thus different molecular weights. The two different CH3 domains, CH3' (prime) and CH3'' (double prime), can be designed to promote heterodimer formation and discourage homodimer formation.

[9] Figure 2. Amino acid sequences of heterodimer constructs and native IgG1 and IgG2 constant heavy chains (SEQ ID NOs. 1-4).

[10] Figure 3. Electrophoretic visualization of protein species generated by simultaneous expression of empty-Fc and scFv-Fc polypeptides (each with wild-type IgG1 Fc sequence) using Agilent 2100 Bioanalyzer microfluidics-based platform. The molecular weights of the proteins in the ladder lane (designated L) are shown on the left in kilodaltons (KDa). The other lane assignments are as follows: Lanes 1-5: reducing environment, cotransfection DNA ratios of empty-Fc:scFv-Fc 1:1, 1:2, 1:4, 2:1, 4:1; Lanes 6-10: non-reducing environment, empty-Fc:scFv-Fc DNA ratios 1:1, 1:2, 1:4, 2:1, 4:1. In lanes 1-5, the top band (~70 KDa) is scFv-Fc monomer, and the bottom band (~38 kDa) is empty-Fc monomer. In lanes 6-10, the top band (~134 KDa) is scFv-Fc homodimer, the middle band (~105 KDa) is scFc-Fv/empty-Fc heterodimer, and the bottom band (~72 KDa) is empty-Fc homodimer.

[11] Figure 4. Electrophoretic visualization of protein species generated by simultaneous expression of variant empty-Fc and variant scFv-Fc (cotransfection DNA ratio of empty-Fc:scFv-Fc = 2:3) using the Agilent Bioanalyzer. Environment is non-reducing. The molecular weights of the proteins in the ladder lane (designated L) are shown on the left in kilodaltons (KDa). The other lane assignments at the top are as given in Figure 5, column 1.

[12] Figure 5. Protein species observed upon simultaneous expression of variant empty-Fc and variant scFv-Fc (cotransfection DNA ratio of empty-Fc:scFv-Fc = 2:3). Relative concentrations were determined using the Agilent Bioanalyzer (see Figure 4 for gel results). Empty-Fc refers to substitutions in the Empty-Fc chain and scFv-Fc refers to substitutions in the scFv-Fc chain. The last five columns list for each variant the molar percentage of Empty-Fc monomer (Empty-M), Empty-Fc dimer (Empty-D), Heterodimer (Hetero), scFv-Fc dimer (scFv-D), and scFv-Fc monomer (scFv-M).

[13] Figure 6. Protein species by molar % from simultaneous expression of variant empty-Fc and variant scFv-Fc. Empty-Fc refers to substitutions in the Empty-Fc chain and scFv-Fc refers to substitutions in the scFv-Fc chain. The last five columns list for each variant the molar percent of Empty-Fc monomer (Empty-M), Empty-Fc dimer (Empty-D), Heterodimer (Hetero), scFv-Fc dimer (scFv-D), and scFv-Fc monomer (scFv-M).

[14] Figure 7. Protein species by mass % from simultaneous expression of variant empty-Fc and variant scFv-Fc. Empty-Fc refers to substitutions in the Empty-Fc chain and scFv-Fc refers to substitutions in the scFv-Fc chain. The last five columns list for each variant the mass percent of Empty-Fc monomer (Empty-M), Empty-Fc dimer (Empty-D), Heterodimer (Hetero), scFv-Fc dimer (scFv-D), and scFv-Fc monomer (scFv-M).

[15] Figure 8. Illustration of mAb-Fv and mAb-Fab immunoglobulin formats. A native IgG1 antibody is included for reference. Although mAb-Fv and mAb-Fab immunoglobulins can be constructed with homodimeric CH3 domains, preferably they utilize heterodimeric CH3

domains, as depicted. Bivalent binding to antigen-1 (Ag-1) is mediated by the N-terminal VH/VL pairs (Fv-1), while monovalent binding to antigen-2 (Ag-2) is mediated by the C-terminal VH/VL pair (Fv-2). Also shown are the Fab-Fv and Fab-Fab analogs that bind both antigen-1 and antigen-2 monovalently.

[16] Figure 9. Sequences of mAb-Fv constructs with Fv-2 targeting FcγRIIIa. LC refers to light chain, and HC1 and HC2 refer to the two heavy chains that make up each mAb-Fv. Identification (ID) numbers correspond to those in Table 3 and the results. 9064 is a mAb-Fab, all other constructs are mAb-Fv's. For each construct the linker separating the Fc region with Fv-2 is underlined, and Fv-2 and any C-terminal linkers are shown in italics. In addition, the histidine tags in enumerated sequences are optional, and generally not included in therapeutic compositions (SEQ ID NOs. 5-21).

[17] Figure 10. Electrophoretic visualization of mAb-Fvs and mAb-Fab with Fv-2 targeting FcγRIIIa using the Agilent Bioanalyzer. Environment is non-reducing ("nr") or reducing ("red") as labeled. The molecular weights of the proteins in the ladder lane (L) are shown on the left in kilodaltons (KDa).

[18] Figure 11. Size exclusion chromatograms for αFcγRIIIa co-engaging mAb-Fv's 9516, 9517, and 9518.

[19] Figure 12. Biacore® sensorgrams depicting the binding of various FcγRs to mAb-Fv and antibodies captured by a protein A chip. Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals (response of reference channel and injection of running buffer).

[20] Figure 13. Biacore® sensorgrams depicting the binding of FcRn to mAb-Fv and antibodies captured by a HER2 chip. Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals (response of reference channel and injection of running buffer).

[21] Figure 14. Biacore® sensorgrams depicting the binding of various concentrations of FcγRIIIa(V158 isoform) to protein A-captured 9516 mAb-Fv. Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals (response of reference channel and injection of running buffer). The K_d of 111 nM was calculated by global fitting of binding data with a 1:1 Langmuir binding model using BIAevaluation software.

[22] Figure 15. Biacore® sensorgrams depicting the simultaneous co-engagement of Her2 and FcγRIIIa by mAb-Fv. 9516 (αHER2 x αFcγRIIIa) was captured to a HER2 chip

followed by binding of FcγRIIIa(158V). Data were processed by zeroing time and response before the injection of 9516.

[23] Figure 16. Biacore® sensorgrams depicting the binding of various concentrations of FcγRIIIa(158V) to HM1.24-captured 9518. Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals (response of reference channel and injection of running buffer). The K_d of 96 nM was calculated by global fitting of binding data with a 1:1 Langmuir binding model using BIAevaluation software.

[24] Figure 17. Biacore sensorgrams depicting the capture of 9518 (αHM1.24 x αFcγRIIIa) by a HM1.24 chip followed by dual binding of FcγRIIIa(158V). Data were processed by zeroing time and response before the injection of 9518.

[25] Figure 18. Cytotoxicity of various mAb-Fvs and mAbs against SK-BR-3 cells using human PBMCs. FcγRIIIa-dependent cytotoxicity was measured at multiple mAb-Fv or mAb concentrations by lactate dehydrogenase release (mean ± SE of triplicate wells).

[26] Figure 19. Sequences of mAb-Fv constructs with Fv-2 targeting FcγRIIb. In addition, the histidine tags in enumerated sequences are optional, and generally not included in therapeutic compositions (SEQ ID NOs. 22-26).

[27] Figure 20. Electrophoretic visualization of mAb-Fvs with Fv-2 targeting FcγRIIb using the Agilent Bioanalyzer. Environment is non-reducing ("nr") or reducing ("red") as labeled. The molecular weights of the proteins in the ladder lane (L) are shown on the left in kilodaltons (KDa).

[28] Figure 21. Biacore® sensorgrams depicting the binding of various concentrations of FcγRIIb to protein A-captured 9547 mAb-Fv. Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals (response of reference channel and injection of running buffer). The K_d of 44 nM was calculated by global fitting of binding data with a 1:1 Langmuir binding model using BIAevaluation software.

[29] Figure 22. Calcium response of CD40+ PBMCs after incubation with various concentrations of 9547 (αCD19 x αFcγRIIb) or 6187 (αCD19 FcKO). Calcium mobilization was induced with 10 μg/ml anti-CD79b (G236R/L328R) BCR-activating antibody. Intracellular free calcium concentration was measured by flow cytometry using a Fluo-4 NW calcium assay. Mean fluorescence intensity was based on a 30 second running average.

[30] Figure 23. Sequences of mAb-Fv constructs with Fv-2 targeting CD3. In addition, the histidine tags in enumerated sequences are optional, and generally not included in therapeutic compositions (SEQ ID NOs. 27-42).

[31] Figure 24. Electrophoretic visualization of mAb-Fvs with Fv-2 targeting CD3 using the Agilent Bioanalyzer. Environment is non-reducing ("nr") or reducing ("red") as labeled. The molecular weights of the proteins in the ladder lane (L) are shown on the left in kilodaltons (KDa).

[32] Figure 25. Size exclusion chromatograms for α CD3 co-engaging mAb-Fvs.

[33] Figure 26. Competition of unlabeled 9509 α HER2 x α CD3 mAb-Fv with FITC-labeled full-length OKT3 mAb for binding to CD3+ T cells in PBMCs.

[34] Figure 27. Cytotoxicity of mAb-Fvs against Her2+ SK-BR-3 cells using T cells isolated from human PBMCs. CD3-dependent cytotoxicity was measured at multiple mAb-Fv concentrations by lactate dehydrogenase release (mean $\pm$ SE of triplicate wells).

[35] Figure 28. Cytotoxicity of mAb-Fvs against Her2+ SKOV3 cells using T cells isolated from human PBMCs. CD3-dependent cytotoxicity was measured at multiple mAb-Fv concentrations by lactate dehydrogenase release (mean $\pm$ SE of triplicate wells).

[36] Figure 29. Cytotoxicity of various mAb-Fvs and mAbs against HM1.24+ RPMI 8224 cells using human PBMCs. CD3-dependent cytotoxicity was measured at multiple mAb-Fv or mAb concentrations by lactate dehydrogenase release (mean $\pm$ SE of triplicate wells).

DETAILED DESCRIPTION OF THE INVENTION

[37] Overview

[38] The present invention is directed to a variety of antibodies (generally referred to herein as "antibody analogs", as further described below) that co-engage at least two different antigens in novel ways. The two antigens of the invention are generally referred to as antigen-1 and antigen-2 (with some embodiments, described below, including antigen-3 as a third antigen). In general, there are two different types of antibody analogs that allow for co-engagement mechanisms, one that utilizes three antigen binding domains (e.g. one antigen is bound bivalently and the other is bound monovalently, although as is further described below, there can also be three different antigens that are bound or a single antigen), and one that relies on two antigen binding domains (e.g. each antigen is bound monovalently).

[39] In one embodiment, antibody analogs are made that have three antigen binding domains. This embodiment can be exemplified in a variety of ways. In an embodiment of

particular use, this is done by using a “traditional” antibody structure (two heavy chains with associated light chains that form antigen binding domains) that includes an additional antigen binding domain at the “opposite end” of the antibody. That is, in some embodiments, all three of the antigen binding domains of the antibody analog are antibody variable regions, comprising a variable heavy and variable light chain.

[40] Generally, in this embodiment, two of the antigen binding domains bind to antigen-1, e.g. bivalently, and the third antigen binding domain binds monovalently to antigen-2. As discussed below, there are particular therapeutic embodiments for which monovalent binding can be preferred, to avoid cross-linking and/or activation of certain pathways.

[41] As outlined in Figure 8, this “trimeric binding domain” embodiment can be accomplished using two different heavy chains; that is, the antibody analog is “heterodimeric”, as defined below. These heterodimeric heavy chains differ from one another in one or more ways. In general, one of the heavy chains has a C-terminal fusion (e.g. fused to the C-terminus of the CH3 domain of the first heavy chain) that comprises an “extra” (sometimes referred to herein as “exogenous”) variable heavy domain. The other heavy chain also has a C-terminal fusion that comprises an exogenous variable light domain. Together the two “extra” variable domains form an antigen binding domain. Thus, in addition to the two “traditional” binding domains, these antibody analogs have a third binding domain.

[42] Optionally, but preferred in some embodiments, the two heavy chains also differ from each other by the presence of amino acid substitutions in the Fc domain that favors heterodimeric formation over homodimers. That is, by changing amino acids in each heavy chain, different heavy chains are more likely to associate to form the antibody analog structure than to form homodimers with the same Fc amino acid sequences.

[43] In another embodiment, antibody analogs are made that have two different antigen binding domains (although as described below, the antigens could be identical). While these antibody analogs share some functional similarity to bispecific antibodies and some antibody fragment constructs, the bispecific antibody analogs of the present invention are easier to make and use than traditional bispecific antibodies.

[44] In this embodiment, the “dimeric binding domain” antibody analog also has components of traditional antibodies. The first heavy chain is identical to a “traditional” antibody construct, in that it contains the variable heavy and light regions, with the exception that it contains a C-terminal fusion at the CH3 domain of an exogenous variable domain (either heavy or light). The second heavy chain comprises the Fc region of the heavy chain and an N-terminal variable light chain instead of a N-terminal variable heavy chain, such that

the N-terminal portion of the analog forms a first antigen binding site (e.g. binds to antigen-1). The second heavy chain also contains a C-terminal fusion to the CH3 domain of an exogenous variable domain, either heavy or light, such that one chain contains the exogenous heavy chain and the other the exogenous light chain. This general scheme is shown in Figure 8.

[45] In some embodiments of the invention, the antigen binding regions of the antibody analog are antibody variable regions. In these embodiments, binding to antigens is mediated by variable regions, also referred to as Fv regions, each comprising a VH domain and a VL domain. The Fv region that binds antigen-1 is referred to as Fv-1, while the Fv region that binds antigen-2 is referred to as Fv-2.

[46] In some preferred embodiments of the invention, the antibody analog is a novel antibody format referred to as mAb-Fv or mAb-Fab. As described herein, mAb-Fv comprises an IgG antibody with a C-terminal Fv fusion. mAb-Fab comprises an IgG antibody with a C-terminal Fab fusion. The N-terminal Fab regions of the mAb-Fv and mAb-Fab contain Fv-1. Because there are two such Fv-1 regions, the mAb-Fv and mAb-Fab bind bivalently to antigen-1. That is, one immunoglobulin binds two of antigen-1 at the same time. In contrast, the C-terminal Fv region of the mAb-Fv or C-terminal Fab region of the mAb-Fab contain Fv-2, which is the binding site for antigen-2. Because there is only one such Fv-2 region, the mAb-Fv and mAb-Fab bind monovalently to antigen-2. That is, one immunoglobulin binds only one of antigen-2. The mAb-Fv and mAb-Fab immunoglobulins thus contain three variable regions, two of which are Fv-1 and one of which is Fv-2. The mAb-Fab differs from the mAb-Fv in that the former contains CH and CL constant domains C-terminal to the Fv-2 VH and VL domains respectively.

[47] As described herein, the mAb-Fv and mAb-Fab immunoglobulins comprise three distinct immunoglobulin chains. These chains include an antibody light chain and two antibody heavy chains. One of said heavy chains comprises a VH or VH-CH1 at its C-terminus, whereas the other of said heavy chains comprises a VL or VL-CL at its C-terminus.

[48] Also described herein are analogues of the mAb-Fv and mAb-Fab that bind both antigen-1 and antigen-2 monovalently. These immunoglobulins are referred to as Fab-Fv and Fab-Fab. The Fab-Fv and Fab-Fab immunoglobulins comprise an N-terminal Fab region that contains a single binding site for antigen-1, and comprise a C-terminal Fv or Fab region that contains a single binding site for antigen-2.

[49] mAb-Fv and mAb-Fab immunoglobulins of the invention may also comprise single domain VHH regions as Fv-1. That is, the N-terminal binding portion of the mAb-Fv and mAb-Fab may lack a light chain and a CH1 domain.

[50] In some preferred embodiment of the invention, the first and second antigens are distinct. That is, Fv-1 and Fv-2 bind different antigens. However, in some embodiments, antigen-1 and antigen-2 may be the same, i.e. Fv-1 and Fv-2 have specificity for the same antigen.

[51] Also contemplated herein are an immunoadhesin-Fv and immunoadhesin-Fab. In this format, Fv-1 is replaced with a fusion protein, for example a receptor, that binds antigen-1. Thus antigen-1 is bound by a fusion protein, while antigen-2 is bound by Fv-2.

[52] The novel antibody analogs described preferably comprise a heterodimeric Fc region. A heterodimer Fc region, as described herein, is a pair of variant Fc regions that comprise at least one amino acid modification in each Fc region. In a preferred embodiment, said amino acid modifications stabilize a heterodimeric Fc region while destabilizing homodimeric Fc regions. In one embodiment of the invention, said variant Fc regions comprise at least one substitution at a position selected from the group consisting of 349, 351, 354, 356, 357, 364, 366, 368, 370, 392, 394, 395, 396, 397, 399, 401, 405, 407, 409, 411, and 439, wherein numbering is according to the EU index as in Kabat. In a preferred embodiment, said variant Fc regions comprise at least one substitution selected from the group consisting of 349A, 349C, 349E, 349I, 349K, 349S, 349T, 349W, 351E, 351K, 354C, 356K, 357K, 364C, 364D, 364E, 364F, 364G, 364H, 364R, 364T, 364Y, 366D, 366K, 366S, 366W, 366Y, 368A, 368E, 368K, 368S, 370C, 370D, 370E, 370G, 370R, 370S, 370V, 392D, 392E, 394F, 394S, 394W, 394Y, 395T, 395V, 396T, 397E, 397S, 397T, 399K, 401K, 405A, 405S, 407T, 407V, 409D, 409E, 411D, 411E, 411K, and 439D.

[53] The present invention describes methods for generating the novel compositions of the invention. The present invention describes purification methods for the immunoglobulins herein, particularly methods for separating heterodimeric and homodimeric protein species. Also described are methods of testing the immunoglobulins herein, including *in vitro* and *in vivo* experiments.

[54] The present invention provides isolated nucleic acids encoding the novel antibody analog compositions described herein. The present invention provides vectors comprising said nucleic acids, optionally, operably linked to control sequences. The present invention provides host cells containing the vectors, and methods for producing and optionally recovering the antibody analog compositions.

[55] The present invention provides compositions comprising antibody analog polypeptides described herein, and a physiologically or pharmaceutically acceptable carrier or diluent.

[56] The present invention contemplates therapeutic and diagnostic uses for the antibody analog polypeptides disclosed herein.

[57]

[58] Definitions

[59] In order that the invention may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[60] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids or any non-natural analogues that may be present at a specific, defined position. Thus "amino acid" as used herein means both naturally occurring and synthetic amino acids. For example, homophenylalanine, citrulline and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. The side chain may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard *in vivo* degradation.

[61]

[62] By "constant region" of an antibody as defined herein is meant the region of the antibody that is encoded by one of the light or heavy chain immunoglobulin constant region genes. By "constant light chain" or "light chain constant region" or "CL" as used herein is meant the region of an antibody encoded by the kappa ($C\kappa$) or lambda ($C\lambda$) light chains. The constant light chain typically comprises a single domain, and as defined herein refers to positions 108-214 of $C\kappa$ or $C\lambda$, wherein numbering is according to the EU index. By "constant heavy chain" or "heavy chain constant region" as used herein is meant the region of an antibody encoded by the mu, delta, gamma, alpha, or epsilon genes to define the antibody's isotype as IgM, IgD, IgG, IgA, or IgE, respectively. For full length IgG antibodies, the constant heavy chain, as defined herein, refers to the N-terminus of the CH1 domain to the C-terminus of the CH3 domain, thus comprising positions 118-447, wherein numbering is according to the EU index.

[63] By "Fab" or "Fab region" as used herein is meant the polypeptides that comprise the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody or antibody fragment.

[64] By "Fc" or "Fc region", as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG,

and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (C γ 2 and C γ 3) and the hinge between Cgamma1 (C γ 1) and Cgamma2 (C γ 2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, for example an antibody. By "Fc polypeptide" as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides include antibodies, Fc fusions, isolated Fcs, and Fc fragments.

[65] By "Fc fusion" or "immunoadhesin" as used herein is meant a protein wherein one or more polypeptides is operably linked to an Fc domain. Fc fusion is herein meant to be synonymous with the terms "immunoadhesin", "Ig fusion", "Ig chimera", and "receptor globulin" as used in the prior art (Huang, 2009, Curr Opin Biotechnology 20:692-699; Chamow et al., 1996, Trends Biotechnol 14:52-60; Ashkenazi et al., 1997, Curr Opin Immunol 9:195-200, both hereby entirely incorporated by reference). An Fc fusion combines the Fc region of an immunoglobulin with a fusion partner, which in general may be any protein, polypeptide or small molecule. The role of the non-Fc part of an Fc fusion, i.e., the fusion partner, is to mediate target binding, and thus it is functionally analogous to the variable regions of an antibody. Virtually any protein or small molecule may be linked to Fc to generate an Fc fusion. Protein fusion partners may include, but are not limited to, the target-binding region of a receptor, an adhesion molecule, a ligand, an enzyme, a cytokine, a chemokine, or some other protein or protein domain. Small molecule fusion partners may include any therapeutic agent that directs the Fc fusion to a therapeutic target. The role of the latter is to mediate target recognition, and thus it is functionally analogous to the antibody variable region. Because of the structural and functional overlap of Fc fusions with antibodies, the discussion on antibodies in the present disclosure extends also to Fc fusions.

[66] Virtually any protein or small molecule may be linked to Fc to generate an Fc fusion. Protein fusion partners may include, but are not limited to, the variable region of any antibody, the target-binding region of a receptor, an adhesion molecule, a ligand, an enzyme, a cytokine, a chemokine, or some other protein or protein domain. Small molecule fusion partners may include any agent that directs the Fc fusion to a target antigen. Such target antigen may be any molecule, e.g., an extracellular receptor, that is implicated in disease. Fusion partners may be linked to any region of an Fc region, including at the N- or C- termini, or at some residue in-between the termini. In one embodiment, a fusion partner is linked at the N- or C-terminus of the Fc region.

[67] By "Fv region" or "variable region" when used herein refers to the minimum fragment of an antibody that retains both antigen-recognition and antigen-binding sites.

[68] By "heterodimeric Fc region" as used herein is meant an Fc region composed of two different heavy constant chains, e.g, having different sequences . As described more fully below, the changes can comprise one or more amino acid changes.

[69] By "immunoglobulin (Ig)" as used herein is meant a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including but not limited to full length antibodies, antibody fragments, and individual immunoglobulin domains. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin that exists as a distinct structural entity as ascertained by one skilled in the art of protein structure. Ig domains typically have a characteristic β -sandwich folding topology. By "immunoglobulin isotype" or "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE.

[70] By "IgG" as used herein is meant a polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises IgG1, IgG2, IgG3, and IgG4. In mice this class comprises IgG1, IgG2a, IgG2b, IgG3. As described herein and in the references below, IgG molecules also include hybrid IgG molecules, such as hybrids of IgG1/G2 described in U.S. Publication No. 2006/0134150, and IgG molecules that have amino acid substitutions as compared to wild-type IgG molecules. Thus, IgG molecules can include naturally occurring molecules or those with sequence modifications.

[71] By "parent polypeptide", "parent protein", "precursor polypeptide", or "precursor protein" as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. Said parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by "parent Fc polypeptide" as used herein is meant an Fc polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an antibody that is modified to generate a variant antibody.

[72] By "protein" or "polypeptide" as used herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein

may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e. “analogs”, such as peptoids.

[73] By “position” as used herein is meant a location in the sequence of a protein or nucleic acid. Protein positions may be numbered sequentially, or according to an established format, for example the Kabat index for antibody variable regions or the EU index for antibody constant regions. For example, position 297 is a position in the human antibody IgG1. Corresponding positions are determined as outlined above, generally through alignment with other parent sequences.

[74] By “residue” as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297, also referred to as N297) is a residue in the human antibody IgG1. In some embodiments it can also refer to nucleic acid bases.

[75] By “target antigen” or “target” or “antigen” as used herein is meant the molecule that is bound specifically by the variable region of an antibody, or by the fusion protein of an Fc fusion. A target antigen may be a protein, carbohydrate, lipid, or other chemical compound. A wide variety of suitable targets are described below.

[76] By “target cell” as used herein is meant a cell that expresses a target antigen.

[77] By “variant polypeptide”, “polypeptide variant”, or “variant” as used herein is meant a polypeptide sequence that differs from that of a parent polypeptide sequence by virtue of at least one amino acid modification. The parent polypeptide may be a naturally occurring or wild-type (WT) polypeptide, or may be a modified version of a WT polypeptide. Variant polypeptide may refer to the polypeptide itself, a composition comprising the polypeptide, or the amino sequence that encodes it. Preferably, the variant polypeptide has at least one amino acid modification compared to the parent polypeptide, e.g. from about one to about ten amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. The variant polypeptide sequence herein will preferably possess at least about 80% homology with a parent polypeptide sequence, and most preferably at least about 90% homology, more preferably at least about 95% homology. Accordingly, by “Fc variant” or “variant Fc” as used herein is meant an Fc sequence that differs from that of a parent Fc sequence by virtue of at least one amino acid modification. An Fc variant may only encompass an Fc region, or may exist in the context of an antibody, Fc fusion, isolated Fc, Fc fragment, or other polypeptide that is substantially encoded by Fc. Fc variant may refer to the Fc polypeptide itself, compositions comprising the Fc variant polypeptide, or the amino acid sequence that encodes it. By “Fc polypeptide variant” or “variant Fc polypeptide” as used herein is meant an Fc polypeptide that differs

from a parent Fc polypeptide by virtue of at least one amino acid modification. By "Fc variant antibody" or "antibody Fc variant" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification in the Fc region. By "protein variant" or "variant protein" as used herein is meant a protein that differs from a parent protein by virtue of at least one amino acid modification. By "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification. By "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG by virtue of at least one amino acid modification. By "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification.

[78] By "wild type" or "WT" or "native" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein, polypeptide, antibody, immunoglobulin, IgG, etc. has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

By "scFv" as used herein is meant a polypeptide consisting of two variable regions connected by a short linker sequence; e.g., V_H-linker-V_K, V_H-linker-V_λ, V_K-linker-V_H, or V_λ-linker-V_H. Examples of linker sequences consist of but are not limited to (Gly₄Ser)₃, (Gly₄Ser)₄, and (Gly₃Ser)₄.

By "scFv-Fc" as used herein is meant an scFv fused to a Fc region; e.g., V_H-linker-V_K-Hinge-CH₂-CH₃.

[79] Immunoglobulins and Antibodies

[80] "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins (although as described herein, engineered glycoforms including aglycosylated and afucosylated forms can be made) having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. Thus, an immunoglobulin is a protein consisting of one or more polypeptides substantially encoded by all or part of at least one immunoglobulin gene. Immunoglobulins may have a number of structural forms, including but not limited to full length antibodies, antibody fragments and analogs (including Fc fusions and immunoadhesins as described below), as well as individual immunoglobulin domains.

[81] Antibodies are immunoglobulins that bind a specific antigen. In most mammals, including humans and mice, antibodies are constructed from paired heavy and light

polypeptide chains. The light and heavy chain variable regions show significant sequence diversity between antibodies, and are responsible for binding the target antigen. Each chain is made up of individual immunoglobulin (Ig) domains, and thus the generic term immunoglobulin can be used for such proteins.

[82] Traditional natural antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. IgA has several subclasses, including but not limited to IgA1 and IgA2. Thus, "isotype" as used herein is meant any of the classes and subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, IgD, and IgE. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the variable region.

[83] Each of the light and heavy chains are made up of two distinct regions, referred to as the variable and constant regions. The IgG heavy chain is composed of four immunoglobulin domains linked from N- to C-terminus in the order VH-CH1-CH2-CH3, referring to the heavy chain variable domain, heavy chain constant domain 1, heavy chain constant domain 2, and heavy chain constant domain 3 respectively (also referred to as VH-C γ 1-C γ 2-C γ 3, referring to the heavy chain variable domain, constant gamma 1 domain, constant gamma 2 domain, and constant gamma 3 domain respectively). The IgG light chain is composed of two immunoglobulin domains linked from N- to C-terminus in the order VL-CL, referring to the light chain variable domain and the light chain constant domain respectively. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. The structure that constitutes the natural biological form of an antibody, including the variable and constant regions, is referred to herein as a "full length antibody". In most mammals, including humans and mice, the full length antibody of the IgG isotype is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy chain, each light chain comprising immunoglobulin domains VL and CL, and each heavy chain comprising immunoglobulin domains VH, C γ 1, C γ 2, and C γ 3. In some mammals, for example in camels

and llamas, IgG antibodies may consist of only two heavy chains, each heavy chain comprising a variable domain attached to the Fc region.

[84] The variable region of an antibody contains the antigen binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The variable region is so named because it is the most distinct in sequence from other antibodies within the same class. The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. There are 6 CDRs total, three each per heavy and light chain, designated VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3. The variable region outside of the CDRs is referred to as the framework (FR) region. Although not as diverse as the CDRs, sequence variability does occur in the FR region between different antibodies. Overall, this characteristic architecture of antibodies provides a stable scaffold (the FR region) upon which substantial antigen binding diversity (the CDRs) can be explored by the immune system to obtain specificity for a broad array of antigens. A number of high-resolution structures are available for a variety of variable region fragments from different organisms, some unbound and some in complex with antigen. Sequence and structural features of antibody variable regions are disclosed, for example, in Morea et al., 1997, *Biophys Chem* 68:9-16; Morea et al., 2000, *Methods* 20:267-279, entirely incorporated by reference, and the conserved features of antibodies are disclosed, for example, in Maynard et al., 2000, *Annu Rev Biomed Eng* 2:339-376, entirely incorporated by reference.

[85] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function, e.g. the Fc region. In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain referred to as heavy constant (CH) regions. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat.

[86] Another important region of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the

IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230 to 236.

[87] For all constant region positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference). For all variable region positions discussed in the present invention, numbering is according to the Kabat numbering scheme (Kabat *et al.*, 1991, *ibid*). Those skilled in the art of antibodies will appreciate that these conventions consist of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index or by the Kabat numbering scheme will not necessarily correspond to its sequential sequence.

[88] The present invention provides antibody analogs. By "antibody" herein is meant a protein consisting of one or more polypeptides substantially encoded by all or part of the recognized immunoglobulin genes. The recognized immunoglobulin genes, for example in humans, include the kappa (κ), lambda (λ), and heavy chain genetic loci, which together comprise the myriad variable region genes, and the constant region genes mu (μ), delta (δ), gamma (γ), sigma (σ), and alpha (α) which encode the IgM, IgD, IgG (IgG1, IgG2, IgG3, and IgG4), IgE, and IgA (IgA1 and IgA2) isotypes respectively. Antibody herein is meant to include full length antibodies and antibody fragments, and may refer to a natural antibody from any organism, an engineered antibody, or an antibody generated recombinantly for experimental, therapeutic, or other purposes.

[89] Of interest in embodiments described herein is the antibody Fc region. The Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. For IgG the Fc region, Fc comprises Ig domains C γ 2 and C γ 3 and the N-terminal hinge leading into C γ 2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (Fc γ Rs). These receptors mediate communication between antibodies and the cellular arm of the immune system (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290, both hereby entirely incorporated by reference). In humans this protein family includes Fc γ RI (CD64), including isoforms Fc γ RIa, Fc γ RIb, and Fc γ RIc;

Fc γ RII (CD32), including isoforms Fc γ RIIa (including allotypes H131 and R131), Fc γ RIIb (including Fc γ RIIb-1 and Fc γ RIIb-2), and Fc γ RIIc; and Fc γ RIII (CD16), including isoforms Fc γ RIIIa (including allotypes V158 and F158) and Fc γ RIIIb (including allotypes Fc γ RIIIb-NA1 and Fc γ RIIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, hereby entirely incorporated by reference). These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and $\gamma\delta$ T cells. Formation of the Fc/Fc γ R complex recruits these effector cells to sites of bound antigen, typically resulting in signaling events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack. The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan et al., 1996, Annu Rev Cell Dev Biol 12:181-220; Ghetie et al., 2000, Annu Rev Immunol 18:739-766; Ravetch et al., 2001, Annu Rev Immunol 19:275-290, both hereby entirely incorporated by reference). The cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP).

[90] The different IgG subclasses have different affinities for the Fc γ Rs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis et al., 2002, Immunol Lett 82:57-65, hereby entirely incorporated by reference). The Fc γ Rs bind the IgG Fc region with different affinities. The extracellular domains of Fc γ RIIIa and Fc γ RIIIb are 96% identical, however Fc γ RIIIb does not have an intracellular signaling domain. Furthermore, whereas Fc γ RI, Fc γ RIIa/c, and Fc γ RIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), Fc γ RIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and Fc γ RIIb is referred to as an inhibitory receptor. Despite these differences in affinities and activities, all Fc γ Rs bind the same region on Fc, at the N-terminal end of the C γ 2 domain and the preceding hinge.

[91] An overlapping but separate site on Fc serves as the interface for the complement protein C1q. In the same way that Fc/Fc γ R binding mediates ADCC, Fc/C1q binding mediates complement dependent cytotoxicity (CDC). A site on Fc between the C γ 2 and C γ 3 domains mediates interaction with the neonatal receptor FcRn, the binding of which recycles endocytosed antibody from the endosome back to the bloodstream (Raghavan et al., 1996, Annu Rev Cell Dev Biol 12:181-220; Ghetie et al., 2000, Annu Rev Immunol 18:739-766, both hereby entirely incorporated by reference). This process, coupled with preclusion of kidney filtration due to the large size of the full length molecule, results in favorable antibody serum half-lives ranging from one to three weeks. Binding of Fc to FcRn also plays a key role in antibody transport. The binding site for FcRn on Fc is also the site at which the bacterial proteins A and G bind. The tight binding by these proteins is typically exploited as a means to purify antibodies by employing protein A or protein G affinity chromatography during protein purification. The fidelity of these regions, the complement and FcRn/protein A binding regions are important for both the clinical properties of antibodies and their development.

[92] A key feature of the Fc region is the conserved N-linked glycosylation that occurs at N297. This carbohydrate, or oligosaccharide as it is sometimes referred, plays a critical structural and functional role for the antibody, and is one of the principle reasons that antibodies must be produced using mammalian expression systems. Efficient Fc binding to Fc γ R and C1q requires this modification, and alterations in the composition of the N297 carbohydrate or its elimination affect binding to these proteins.

[93] The antibodies of embodiments disclosed herein may be substantially encoded by immunoglobulin genes belonging to any of the antibody classes. In certain embodiments, the antibodies disclosed herein comprise sequences belonging to the IgG class of antibodies, including IgG1, IgG2, IgG3, or IgG4. In alternate embodiments, immunoglobulins disclosed herein comprise sequences belonging to the IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG, or IgM classes of antibodies. The immunoglobulins disclosed herein may comprise more than one protein chain, e.g., may be an antibody or Fc fusion that is a monomer or an oligomer, including a homo- or hetero-oligomer.

[94] Antibodies disclosed herein may be substantially encoded by genes from any organism, e.g., mammals (including, but not limited to humans, rodents (including but not limited to mice and rats), lagomorpha (including but not limited to rabbits and hares), camelidae (including but not limited to camels, llamas, and dromedaries), and non-human primates, including but not limited to Prosimians, Platyrrhini (New World monkeys), Cercopithecoidea (Old World monkeys), and Hominoidea including the Gibbons and Lesser

and Great Apes. In the most preferred embodiments, the immunoglobulins disclosed herein may be substantially human, with the modifications outlined herein.

[95] As is well known in the art, immunoglobulin polymorphisms exist in the human population. Gm polymorphism is determined by the IGHG1, IGHG2 and IGHG3 genes which have alleles encoding allotypic antigenic determinants referred to as G1m, G2m, and G3m allotypes for markers of the human IgG1, IgG2 and IgG3 molecules (no Gm allotypes have been found on the gamma 4 chain). Markers may be classified into `allotypes` and `isoallotypes`. These are distinguished on different serological bases dependent upon the strong sequence homologies between isotypes. Allotypes are antigenic determinants specified by allelic forms of the Ig genes. Allotypes represent slight differences in the amino acid sequences of heavy or light chains of different individuals. Even a single amino acid difference can give rise to an allotypic determinant, although in many cases there are several amino acid substitutions that have occurred. Allotypes are sequence differences between alleles of a subclass whereby the antisera recognize only the allelic differences. An isoallotype is an allele in one isotype which produces an epitope which is shared with a non-polymorphic homologous region of one or more other isotypes and because of this the antisera will react with both the relevant allotypes and the relevant homologous isotypes (Clark, 1997, IgG effector mechanisms, Chem Immunol. 65:88-110; Gorman & Clark, 1990, Semin Immunol 2(6):457-66, both hereby entirely incorporated by reference).

[96] Allelic forms of human immunoglobulins have been well-characterized (WHO Review of the notation for the allotypic and related markers of human immunoglobulins. J Immunogen 1976, 3: 357-362; WHO Review of the notation for the allotypic and related markers of human immunoglobulins. 1976, Eur. J. Immunol. 6, 599-601; Loghem E van, 1986, Allotypic markers, Monogr Allergy 19: 40-51, all hereby entirely incorporated by reference). Additionally, other polymorphisms have been characterized (Kim et al., 2001, J. Mol. Evol. 54:1-9, hereby entirely incorporated by reference). At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, et al., The human IgG subclasses: molecular analysis of structure, function and regulation. Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. et al., 1979, Hum. Genet.: 50, 199-211, both hereby entirely incorporated by reference). Allotypes that are inherited in fixed combinations are called Gm haplotypes. The immunoglobulins disclosed herein may be substantially encoded by any allotype, isoallotype, or haplotype of any immunoglobulin gene.

[97] Nonhuman, Chimeric, Humanized, and Fully Human Antibodies

[98] The variable region of an antibody, as is well known in the art, can compose sequences from a variety of species. In some embodiments, the antibody variable region can be from a nonhuman source, including but not limited to mice, rats, rabbits, camels, llamas, and monkeys. In some embodiments, the scaffold components can be a mixture from different species. As such, an antibody disclosed herein may be a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse or other nonhuman species and the constant region(s) from a human.

[99] "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally in a humanized antibody the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeven et al., 1988, Science 239:1534-1536. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (U.S. Pat. No. 5,693,762, incorporated entirely by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. A variety of techniques and methods for humanizing, reshaping, and resurfacing non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, herein expressly incorporated by reference). In certain variations, the immunogenicity of the antibody is reduced using a method described in Lazar et al., 2007, Mol Immunol 44:1986-1998 and USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof", filed on December 3, 2004, incorporated entirely by reference.

[100] In one embodiment, the antibody is a fully human antibody with at least one modification as outlined herein. "Fully human antibody" or "complete human antibody" refers to a human antibody having the gene sequence of an antibody derived from a human chromosome with the modifications outlined herein. Fully human antibodies may be obtained, for example, using transgenic mice (Lonberg, 2008, Handb Exp Pharmacol

181:69-97) or human antibody libraries coupled with selection methods (Mondon et al., 2008, Front Biosci 13:1117-29; Lonberg, 2008, Curr Opin Immunol. 20[4]:450-9).

[101] Antibody Analogs

[102] The present invention provides antibody analogs. As used herein, "antibodies" may comprise a variety of structures, including, but not limited to full length antibodies, antibody fragments, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), antibody fusions, antibody conjugates, and fragments of each, respectively.

[103] In one embodiment, the immunoglobulin comprises an antibody fragment. Specific antibody fragments include, but are not limited to (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment, which consists of a single variable, (v) isolated CDR regions, (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site, (viii) bispecific single chain Fv dimers, and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion. The antibody fragments may be modified. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains. Examples of antibody formats and architectures are described in Holliger & Hudson, 2006, Nature Biotechnology 23(9):1126-1136, and Carter 2006, Nature Reviews Immunology 6:343-357 and references cited therein, all expressly incorporated by reference.

[104] The present invention provides antibody analogs that are a novel set of formats that enable co-engaged antigens to be bound with different valencies. Specifically, as discussed herein, there are two general formats: an antibody analog that has three antigen binding domains, and one that has two.

[105] In one preferred embodiment, a first antigen can be bound bivalently while a second antigen is simultaneously bound monovalently. The novel immunoglobulin compositions herein co-engage a first and second antigen, referred to herein as antigen-1 and antigen-2 respectively. Binding to the antigens is mediated by first and second antigen binding regions such that antigen-1 is bound bivalently by the immunoglobulin, while antigen-2 is bound monovalently by the immunoglobulin. That is, the immunoglobulin of the invention contains two binding sites for antigen-1 but only one binding site for antigen-2.

[106] In preferred embodiments of the invention, the antigen binding regions of the immunoglobulin are antibody variable regions, also referred to as Fv regions, each

comprising a VH domain and a VL domain. The Fv region that binds antigen-1 is referred to as Fv-1, while the Fv region that binds antigen-2 is referred to as Fv-2.

[107] Preferred immunoglobulin formats of the invention for co-engaging antigens with different valencies comprise IgG antibodies with a C-terminal Fv region. Such immunoglobulins typically comprise a full length antibody that is linked C-terminally to a set of VH and VL domains that make up a novel Fv region (Fv-2) and thus a novel antigen binding site (for antigen-2). The C-terminal VH and VL domains may also be linked C-terminally to CH1 and CL domains, thereby resulting in a C-terminal Fab that makes up the novel antigen binding site. These immunoglobulins are referred to as mAb-Fv and mAb-Fab. The VH and VL domains are typically linked C-terminally to the Fc region via flexible linkers. A variety of linkers are described herein that may find use for linking the VH and VL domains C-terminally to the constant chain in order to generate the novel binding site Fv-2.

[108] The N-terminal Fab regions of the mAb-Fv and mAb-Fab contain the binding site for the primary or first antigen (antigen-1). Accordingly, this Fv region is referred to as Fv-1. Because there are two such Fv-1 regions, the mAb-Fv and mAb-Fab bind bivalently to antigen-1. That is, one immunoglobulin binds two of antigen-1 at the same time. In contrast, the C-terminal Fv region of the mAb-Fv or C-terminal Fab region of the mAb-Fab contain the binding site for the second antigen (antigen-2). Thus this Fv region is referred to as Fv-2. Because there is only one such Fv-2 region, the mAb-Fv and mAb-Fab bind monovalently to antigen-2. That is, one immunoglobulin binds only one of antigen-2. The mAb-Fv and mAb-Fab immunoglobulins thus contain three variable regions, two of which are Fv-1 and one of which is Fv-2.

[109] The mAb-Fv and mAb-Fab immunoglobulins comprise three distinct immunoglobulin chains. These chains include an antibody light chain and two antibody heavy chains. One of said heavy chains comprises a VH or VH-CH1 at its C-terminus, whereas the other of said heavy chains comprises a VL or VL-CL at its C-terminus. Thus the heavy chain dimer that is competent to bind the second antigen is a heterodimer, rather than a homodimer as is the case naturally for IgG's. The use of two distinct heavy chains is an important component of the mAb-Fv and mAb-Fab that enables the engineering of a second antigen binding site using a VH/VL pair.

[110] Because of the presence of this heterodimeric constant chain, the mAb-Fv and mAb-Fab formats are particularly suited for the use of variants that favor Fc heterodimerization and disfavor Fc homodimerization. Different variant heavy chains, for example containing CH3 substitutions, can be linked to the different heavy chains of the mAb-Fv and mAb-Fab. One variant heavy chain can be linked C-terminally to the VH of the mAb-Fv or VH-CH1 of

the mAb-Fab, while the other variant chain can be linked C-terminally to the VL of the mAb-Fv or VL-CL of the mAb-Fab, thereby generating the new monovalent Fv-2. Moreover, the additional Fv-2 VH/VL and VH-CH1/VL-CL pairings may add further specificity to the heterodimer relative to the homodimer.

[111] Heterodimeric Fc variants are not a necessity, however, for generation and use of the mAb-Fv and mAb-Fab. Protein purification methods may be employed to purify the desired heterodimeric heavy chains. In the absence of Fc variants that favor heterodimerization and disfavor homodimerization, transfection of the three different DNAs, one light chain and two heavy chains, would result in three separate species - 25% homodimer, 50% heterodimer, and 25% homodimer. The 50% heterodimer is the species that comprises both the C-terminal VH and C-terminal VL domains, and thus the species that comprises an Fv-2 that is competent to bind antigen-2. The two 25% homodimer species would comprise homodimeric heavy chains that contain two C-terminal VL's or two C-terminal VH's, and thus would typically not be able to bind antigen-2. Using protein purification methods described herein and generally known in the art, the desired 50% heterodimeric mAb-Fv or mAb-Fab species can be purified, yielding a composition that is competent of engaging antigen-2.

[112] The antibody analogs of the "trimeric" antigen binding domain constructs generally comprise two modified antibody heavy chains with associated light chains, where each heavy chain possesses two variable regions and a constant region. The first modified antibody heavy chain has the structure NH₂-Variable Heavy Domain-Constant Domain-Variable Domain-COOH, and the second modified antibody heavy chain has the structure NH₂-Variable Heavy Domain-Constant Domain-Variable Domain-COOH. Thus, the N-terminal variable heavy domains interact with the associated light chains to form an antigen binding site on each chain (as in a "traditional" antibody, providing two antigen binding domains). On the opposite "end" of the molecule, one of the variable domains at the C-terminus of one chain is a variable heavy domain, and the other C-terminal variable domain on the other chain is a variable light domain, such that a third antigen binding domain is formed. In most instances the two antigen binding domains at the N-terminal ends of the chains are to the same antigen, to bind the antigen bivalently. The third binding domain at the C-terminus is monovalent and generally directed to a second antigen as described herein.

[113] In some embodiments, as is depicted in Figure 8, the C-terminus of each chain may further comprise a CH1 domain as well. In this instance the first modified antibody heavy chain has the structure NH₂-Variable Heavy Domain-Constant Domain-Variable Domain-Constant Domain (CH1 and/or CL)-COOH, and the second modified antibody heavy chain

has the structure NH₂-Variable Heavy Domain-Constant Domain-Variable Domain-Constant Domain (CH₁ and/or CL)-COOH.

[114] Also described herein are analogues of the mAb-Fv and mAb-Fab that bind both antigen-1 and antigen-2 monovalently. These immunoglobulins are referred to as Fab-Fv and Fab-Fab. Rather than comprising three separate chains, these formats contain two different heavy chains, i.e. they lack a native light chain. For one of the heavy chains, the N-terminal Fv-1 VH-CH₁ half of the Fab region are replaced with the Fv-1 VL-CL region. Thus the heterodimer species of the Fab-Fv and Fab-Fab comprise an N-terminal Fab region that contains a single binding site for antigen-1, and comprise a C-terminal Fv or Fab region that contains a single binding site for antigen-2. For the Fab-Fv the first modified antibody heavy chain has the structure NH₂-Variable Heavy Domain-Constant Domain-Variable Heavy Domain-COOH, and the second modified antibody heavy chain has the structure NH₂-Variable Light Domain-Constant Domain-Variable Light Domain. For the Fab-Fab the first modified antibody heavy chain has the structure NH₂-Variable Heavy Domain-Constant Domain-Variable Heavy Domain-Constant Domain (e.g., CH₁)-COOH, and the second modified antibody heavy chain has the structure NH₂-Variable Light Domain-Constant Domain-Variable Light Domain-Constant Domain (e.g., CL)-COOH.

[115] The use of VH and VL domains to generate the novel binding site Fv-2 is a preferred embodiment. However, other antigen-1 binding modalities may also be useful when linked with a C-terminal monovalent Fv-2. For example, rather than using both VH and VL domains as Fv-1, mAb-Fv and mAb-Fab immunoglobulins may also comprise single domain VHH regions as Fv-1. Such VHH regions are described, for example, in Wesolowski et al., 2009, *Med Microbiol Immunol* 198[3]:157-174, and references therein, herein expressly incorporated by reference.

[116] In these constructs the N-terminal binding portion of the mAb-Fv and mAb-Fab may lack a light chain and a CH₁ domain. It is also possible to utilize the C-terminal monovalent Fv-2 in the context of an Fc fusion, also referred to as an immunoadhesin. In this format, Fv-1 is replaced with a fusion protein, for example a receptor that binds antigen-1. Thus antigen-1 is bound by a fusion protein, while antigen-2 is bound by Fv-2.

[117] Fc Modifications

[118] Antibody analogs disclosed herein may comprise an Fc variant. An Fc variant comprises one or more amino acid modifications relative to a parent Fc polypeptide, wherein the amino acid modification(s) provide one or more optimized properties. By "modification" herein is meant an alteration in the physical, chemical, or sequence properties of a protein, polypeptide, antibody, or immunoglobulin. An amino acid modification can be an amino acid

substitution, insertion, and/or deletion in a polypeptide sequence. By “amino acid substitution” or “substitution” herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. For example, the substitution Y349T refers to a variant polypeptide, in this case a constant heavy chain variant, in which the tyrosine at position 349 is replaced with threonine. By “amino acid insertion” or “insertion” as used herein is meant the addition of an amino acid at a particular position in a parent polypeptide sequence. By “amino acid deletion” or “deletion” as used herein is meant the removal of an amino acid at a particular position in a parent polypeptide sequence.

[119] An Fc variant disclosed herein differs in amino acid sequence from its parent by virtue of at least one amino acid modification. The immunoglobulins disclosed herein may have more than one amino acid modification as compared to the parent, for example from about one to fifty amino acid modifications, e.g., from about one to ten amino acid modifications, from about one to about five amino acid modifications, etc. compared to the parent. Thus the sequences of the Fc variants and those of the parent Fc polypeptide are substantially homologous. For example, the variant Fc variant sequences herein will possess about 80% homology with the parent Fc variant sequence, e.g., at least about 90% homology, at least about 95% homology, at least about 98% homology, at least about 99% homology, etc. Modifications disclosed herein also include glycoform modifications. Modifications may be made genetically using molecular biology, or may be made enzymatically or chemically.

[120] Fc variants disclosed herein are defined according to the amino acid modifications that compose them. Thus, for example, the substitution Y349T refers to a variant polypeptide, in this case a constant heavy chain variant, in which the tyrosine at position 349 is replaced with threonine. Likewise, Y349T/T394F defines an Fc variant with the substitutions Y349T and T394F relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 349T/394F. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 349T/394F is the same Fc variant as 394F/349T. Unless otherwise noted, constant region and Fc positions discussed herein are numbered according to the EU index or EU numbering scheme (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, hereby entirely incorporated by reference). The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference).

[121] In certain embodiments, the Fc variants disclosed herein are based on human IgG sequences, and thus human IgG sequences are used as the "base" sequences against which other sequences are compared, including but not limited to sequences from other organisms, for example rodent and primate sequences. Immunoglobulins may also comprise sequences from other immunoglobulin classes such as IgA, IgE, IgD, IgGM, and the like. It is contemplated that, although the Fc variants disclosed herein are engineered in the context of one parent IgG, the variants may be engineered in or "transferred" to the context of another, second parent IgG. This is done by determining the "equivalent" or "corresponding" residues and substitutions between the first and second IgG, typically based on sequence or structural homology between the sequences of the first and second IgGs. In order to establish homology, the amino acid sequence of a first IgG outlined herein is directly compared to the sequence of a second IgG. After aligning the sequences, using one or more of the homology alignment programs known in the art (for example using conserved residues as between species), allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of the first immunoglobulin are defined. Alignment of conserved residues may conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Equivalent residues may also be defined by determining structural homology between a first and second IgG that is at the level of tertiary structure for IgGs whose structures have been determined. In this case, equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the parent or precursor (N on N, CA on CA, C on C and O on O) are within about 0.13 nm, after alignment. In another embodiment, equivalent residues are within about 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the proteins. Regardless of how equivalent or corresponding residues are determined, and regardless of the identity of the parent IgG in which the IgGs are made, what is meant to be conveyed is that the Fc variants discovered as disclosed herein may be engineered into any second parent IgG that has significant sequence or structural homology with the Fc variant. Thus for example, if a variant antibody is generated wherein the parent antibody is human IgG1, by using the methods described above or other methods for determining equivalent residues, the variant antibody may be engineered in another IgG1 parent antibody that binds a different antigen, a human IgG2 parent antibody, a human IgA parent antibody, a mouse IgG2a or IgG2b parent antibody, and the like. Again, as described above, the context of the parent Fc variant does not affect the ability to transfer the Fc variants disclosed herein to other parent IgGs.

[122] Fc Modifications That Favor Heterodimerization

[123] Of particular benefit to the immunoglobulins of the invention are Fc variants that favor heterodimerization of the Fc region relative to homodimerization. That is, by modifying one or more amino acids within each heavy chain that forms the Fc region, such that each heavy chain comprises at least one amino acid variant as compared to the other chain, binding of the different heavy chains to form heterodimeric Fc regions is favored over the individual heavy chains forming homodimers.

[124] Such variants, referred to herein as hetero-Fc variants, comprise a pair of different variants such that one pair (referred to as variant') modifies one CH3 domain (referred to as CH3'), while the other pair (variant'') modifies another CH3 domain (CH3''). The variants are designed such that their interaction in a heterodimeric CH3 interface (variant'/variant'') is favorable, thus resulting in a stable heterodimeric Fc species (comprising CH3'/CH3''). In contrast, the pairing of each variant with itself across a homodimeric CH3 interface (variant'/variant' and variant''/variant'') is unfavorable, resulting in unstable homodimeric Fc species (comprising CH3'/CH3' and CH3''/CH3'').

[125] For two different protein chains with identical (i.e. native) CH3 domains, in the absence of hetero-Fc variants, transfection of two different DNAs encoding two different heavy chains would result in three separate species - 25% homodimer', 50% heterodimer, and 25% homodimer''. The hetero-Fc variants of the invention increase the percentage population of the heterodimer while reducing the population of the homodimers. For example, Fc variants that favor heterodimerization and disfavor homodimerization may alter the populations such that the heterodimer population increases to 70%, preferably 80%, more preferably 90%, and most preferably greater than 95%. For example, hetero-Fc variants may reduce the homodimer populations to 2.5% and 2.5% while increasing the heterodimer population to 95%. Populations of the different species may be measured using a variety of methods well-known in the art, including methods described herein, including for example gel electrophoresis and biochemical assays. Heterodimeric immunoglobulin species may optionally be isolated further from homodimeric species using any number of various protein purification methods well-known in the art.

[126] Hetero-Fc variants herein preferably comprise at least one substitution at a position in a CH3 domain selected from the group consisting of 349, 351, 354, 356, 357, 364, 366, 368, 370, 392, 394, 395, 396, 397, 399, 401, 405, 407, 409, 411, and 439, wherein numbering is according to the EU index as in Kabat. In a preferred embodiment, hetero-Fc variants comprise at least one CH3 domain substitution per heavy chain selected from the group consisting of 349A, 349C, 349E, 349I, 349K, 349S, 349T, 349W, 351E, 351K, 354C,

356K, 357K, 364C, 364D, 364E, 364F, 364G, 364H, 364R, 364T, 364Y, 366D, 366K, 366S, 366W, 366Y, 368A, 368E, 368K, 368S, 370C, 370D, 370E, 370G, 370R, 370S, 370V, 392D, 392E, 394F, 394S, 394W, 394Y, 395T, 395V, 396T, 397E, 397S, 397T, 399K, 401K, 405A, 405S, 407T, 407V, 409D, 409E, 411D, 411E, 411K, and 439D. Each of these variants can be used individually or in any combination for each heavy chain Fc region. As will be appreciated by those in the art, each heavy chain can comprise different numbers of substitutions. As shown in Tables 2 and 3, below, both heavy chains that make up the Fc region may comprise a single substitution, one chain may comprise a single substitution and the other two substitutions, both can contain two substitutions (although each chain will contain different substitutions), etc.

[127] In some embodiments, the hetero-Fc variants are made in combinations, that is, two or more variants per heavy chain Fc domain, selected from the group outlined above. Of use in particular embodiments are combination variants including, but not limited to, those shown in Tables 2 and 3 and in the Figures.

[128] Other Fc variants that favor heterodimerization that may find use in the creation of the antibody analogs of the invention have been described (Ridgeway et al., 1996, Protein Engineering 9[7]:617-621; US5,731,168; Xie et al., 2005, J Immunol Methods 296:95-101; Davis et al., 2010, Protein Engineering, Design & Selection 23[4]:195-202; Gunasekaran et al., 2010, J Biol Chem 285[25]:1937-19646; PCT/US2009/000071; all entirely incorporated herein by reference).

[129] Fc Modifications That Modulate Binding to Fc Receptors

[130] The Fc variants disclosed herein may be optimized for improved or reduced binding to Fc receptors or Fc ligands. By "Fc receptor" or "Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an antibody to form an Fc-ligand complex. Fc ligands include but are not limited to Fc γ Rs, (as described above, including but not limited to Fc γ RIIIa, Fc γ RIIa, Fc γ RIIb, Fc γ RI and FcRn), C1q, C3, mannan binding lectin, mannose receptor, *staphylococcal* protein A, *streptococcal* protein G, and viral Fc γ R. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the Fc γ Rs. Fc ligands may include undiscovered molecules that bind Fc.

[131] Immunoglobulins herein may be engineered to for optimized properties, including but are not limited to enhanced or reduced affinity for an Fc receptor. By "greater affinity" or "improved affinity" or "enhanced affinity" or "better affinity" than a parent Fc polypeptide, as used herein is meant that an Fc variant binds to an Fc receptor with a significantly higher equilibrium constant of association (K_A or K_a) or lower equilibrium constant of dissociation

(KD or K_d) than the parent Fc polypeptide when the amounts of variant and parent polypeptide in the binding assay are essentially the same. For example, the Fc variant with improved Fc receptor binding affinity may display from about 5 fold to about 1000 fold, e.g. from about 10 fold to about 500 fold improvement in Fc receptor binding affinity compared to the parent Fc polypeptide, where Fc receptor binding affinity is determined, for example, by the binding methods disclosed herein, including but not limited to Biacore, by one skilled in the art. Accordingly, by “reduced affinity” as compared to a parent Fc polypeptide as used herein is meant that an Fc variant binds an Fc receptor with significantly lower KA or higher KD than the parent Fc polypeptide. Greater or reduced affinity can also be defined relative to an absolute level of affinity.

[132] In one embodiment, particularly useful Fc modifications for the present invention are variants that reduce or ablate binding to one or more Fc γ Rs and/or complement proteins, thereby reducing or ablating Fc-mediated effector functions such as ADCC, ADCP, and CDC. Such variants are also referred to herein as “knockout variants” or “KO variants”. Variants that reduce binding to Fc γ Rs and complement are useful for reducing unwanted interactions mediated by the Fc region and for tuning the selectivity of the immunoglobulins. Preferred knockout variants are described in USSN11/981,606, filed 10/31/2007, entitled “Fc Variants with Optimized Properties,” herein expressly incorporated by reference. Preferred modifications include but are not limited substitutions, insertions, and deletions at positions 234, 235, 236, 237, 267, 269, 325, and 328, wherein numbering is according to the EU index. Preferred substitutions include but are not limited to 234G, 235G, 236R, 237K, 267R, 269R, 325L, and 328R, wherein numbering is according to the EU index. A preferred variant comprises 236R/328R. Variants may be used in the context of any IgG isotype or IgG isotype Fc region, including but not limited to human IgG1, IgG2, IgG3, and/or IgG4 and combinations thereof. Preferred IgG Fc regions for reducing Fc γ R and complement binding and reducing Fc-mediated effector functions are IgG2 and IgG4 Fc regions. Hybrid isotypes may also be useful, for example hybrid IgG1/IgG2 isotypes as described in USSN11/256,060, hereby incorporated by reference in its entirety. Other modifications for reducing Fc γ R and complement interactions include but are not limited to substitutions 297A, 234A, 235A, 237A, 318A, 228P, 236E, 268Q, 309L, 330S, 331S, 220S, 226S, 229S, 238S, 233P, and 234V, as well as removal of the glycosylation at position 297 by mutational or enzymatic means or by production in organisms such as bacteria that do not glycosylate proteins. These and other modifications are reviewed in Strohl, 2009, Current Opinion in Biotechnology 20:685-691, incorporated by reference in its entirety.

[133] Fc modifications that improve binding to Fc γ Rs and/or complement may also find use in the immunoglobulins herein. Such Fc variants may enhance Fc-mediated effector

functions such as ADCC, ADCP, and/or CDC. Preferred modifications for improving Fc γ R and complement binding are described in USSN11/124,620 (with Figure 41 being expressly incorporated by reference in its entirety for the modifications depicted therein) and USSN11/396,495, expressly incorporated herein by reference. Preferred modifications comprise a substitution at a position selected from the group consisting of 236, 239, 268, 324, and 332, wherein numbering is according to the EU index. Preferred substitutions include but are not limited to 236A, 239D, 239E, 268D, 267E, 268E, 268F, 324T, 332D, and 332E. Preferred variants include but are not limited to 239D/332E, 236A/332E, 236A/239D/332E, 268F/324T, 267E/268F, 267E/324T, and 267E/268F/324T. Other modifications for enhancing Fc γ R and complement interactions include but are not limited to substitutions 298A, 333A, 334A, 326A, 247I, 339D, 339Q, 280H, 290S, 298D, 298V, 243L, 292P, 300L, 396L, 305I, and 396L. These and other modifications are reviewed in Strohl, 2009, *ibid*.

[134] In one embodiment, the immunoglobulins disclosed herein may incorporate Fc variants that enhance affinity for an inhibitory receptor Fc γ RIIb. Such variants may provide the immunoglobulins herein with immunomodulatory activities related to Fc γ RIIb⁺ cells, including for example B cells and monocytes. In one embodiment, the Fc variants provide selectively enhanced affinity to Fc γ RIIb relative to one or more activating receptors. Modifications for altering binding to Fc γ RIIb are described in USSN 12/156,183, filed May 30, 2008, entitled "Methods and Compositions for Inhibiting CD32b Expressing Cells", herein expressly incorporated by reference. In particular, Fc variants that improve binding to Fc γ RIIb may include one or more modifications at a position selected from the group consisting of 234, 235, 236, 237, 239, 266, 267, 268, 325, 326, 327, 328, and 332, according to the EU index. Preferable substitutions for enhancing Fc γ RIIb affinity include but are not limited to 234D, 234E, 234W, 235D, 235F, 235R, 235Y, 236D, 236N, 237D, 237N, 239D, 239E, 266M, 267D, 267E, 268D, 268E, 327D, 327E, 328F, 328W, 328Y, and 332E. More preferably, substitutions include but are not limited to 235Y, 236D, 239D, 266M, 267E, 268D, 268E, 328F, 328W, and 328Y. Preferred Fc variants for enhancing binding to Fc γ RIIb include but are not limited to 235Y/267E, 236D/267E, 239D/268D, 239D/267E, 267E/268D, 267E/268E, and 267E/328F.

[135] In some embodiments, immunoglobulins disclosed herein may incorporate Fc variants that improve FcRn binding. Such variants may enhance the *in vivo* pharmacokinetic properties of the immunoglobulins. Preferred variants that increase binding to FcRn and/or improve pharmacokinetic properties include but are not limited to substitutions at positions 259, 308, 428, and 434, including but not limited to for example 259I, 308F, 428L, 428M,

434S, 434H, 434F, 434Y, 434M, 428L/434S, 259I/308F and 259I/308F/428L (and others described in USSN12/341,769, filed Dec. 22, 2008, entitled "Fc Variants with Altered Binding to FcRn", entirely incorporated by reference). Other variants that increase Fc binding to FcRn include but are not limited to: 250E, 250Q, 428L, 428F, 250Q/428L (Hinton et al., 2004, J. Biol. Chem. 279(8): 6213-6216, Hinton et al. 2006 Journal of Immunology 176:346-356), 256A, 272A, 286A, 305A, 307A, 307Q, 311A, 312A, 376A, 378Q, 380A, 382A, 434A (Shields et al, Journal of Biological Chemistry, 2001, 276(9):6591-6604, entirely incorporated by reference), 252F, 252T, 252Y, 252W, 254T, 256S, 256R, 256Q, 256E, 256D, 256T, 309P, 311S, 433R, 433S, 433I, 433P, 433Q, 434H, 434F, 434Y, 252Y/254T/256E, 433K/434F/436H, 308T/309P/311S (Dall'Acqua et al. Journal of Immunology, 2002, 169:5171-5180, Dall'Acqua et al., 2006, Journal of Biological Chemistry 281:23514-23524, entirely incorporated by reference). Other modifications for modulating FcRn binding are described in Yeung et al., 2010, J Immunol, 182:7663-7671.

[136] Immunoglobulins described herein can incorporate Fc modifications in the context of any IgG isotype or IgG isotype Fc region, including but not limited to human IgG1, IgG2, IgG3, and/or IgG4. The IgG isotype may be selected such as to alter FcγR- and/or complement- mediated effector function(s). Hybrid IgG isotypes may also be useful. For example, USSN11/256,060 describes a number of hybrid IgG1/IgG2 constant regions that may find use in the particular invention. In some embodiments of the invention, immunoglobulins may comprise means for isotypic modifications, that is, modifications in a parent IgG to the amino acid type in an alternate IgG. For example, an IgG1/IgG3 hybrid variant may be constructed by a substitutional means for substituting IgG1 positions in the CH2 and/or CH3 region with the amino acids from IgG3 at positions where the two isotypes differ. Thus a hybrid variant IgG antibody may be constructed that comprises one or more substitutional means, e.g., 274Q, 276K, 300F, 339T, 356E, 358M, 384S, 392N, 397M, 422I, 435R, and 436F. In other embodiments of the invention, an IgG1/IgG2 hybrid variant may be constructed by a substitutional means for substituting IgG2 positions in the CH2 and/or CH3 region with amino acids from IgG1 at positions where the two isotypes differ. Thus a hybrid variant IgG antibody may be constructed that comprises one or more substitutional means, e.g., one or more of the following amino acid substitutions: 233E, 234L, 235L, -236G (referring to an insertion of a glycine at position 236), and 327A.

[137] Target Antigens

[138] The immunoglobulins of the invention may target virtually any antigen or pair of antigens. The mAb-Fv and mAb-Fab are particularly beneficial for targeting distinct antigens, that is where antigen-1 and antigen-2 are different. Thus the immunoglobulins herein preferably co-engage two target antigens.

[139] Particular suitable applications of the immunoglobulins herein are co-target pairs for which it is beneficial or critical to engage a target antigen monovalently. Such antigens may be, for example, immune receptors that are activated upon immune complexation. Cellular activation of many immune receptors occurs only by cross-linking, achieved typically by antibody/antigen immune complexes, or via effector cell to target cell engagement. For some immune receptors, for example the CD3 signaling receptor on T cells, activation only upon engagement with co-engaged target is critical, as nonspecific cross-linking in a clinical setting can elicit a cytokine storm and toxicity. Therapeutically, by engaging such antigens monovalently rather than multivalently, using the immunoglobulins herein, such activation occurs only in response to cross-linking only in the microenvironment of the primary target antigen. The ability to target two different antigens with different valencies is a novel and useful aspect of the present invention. Examples of target antigens for which it may be therapeutically beneficial or necessary to co-engage monovalently include but are not limited to immune activating receptors such as CD3, FcγRs, toll-like receptors (TLRs) such as TLR4 and TLR9, cytokine, chemokine, cytokine receptors, and chemokine receptors.

[140] While the mAb-Fv and mAb-Fab immunoglobulins described herein are particularly beneficial for targeting distinct antigens, in some cases it may be beneficial to target only one antigen. That is, Fv-1 and Fv-2 may have specificity for the same antigen, thus binding the target antigen trivalently.

[141] Virtually any antigen may be targeted by the immunoglobulins herein, including but not limited to proteins, subunits, domains, motifs, and/or epitopes belonging to the following list of target antigens, which includes both soluble factors such as cytokines and membrane-bound factors, including transmembrane receptors: 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b-NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen,

Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCL, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Rel, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(1-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxin1, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor IIa, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF-8 (Myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GTR, Glucagon, Glut 4, glycoprotein IIb/IIIa (GP IIb/IIIa), GM-CSF, gp130, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha,

INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta1, integrin alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/beta1, integrin alpha5/beta3, integrin alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein L1, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bp1, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Muellierian-inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PIGF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta RIIB, TGF-beta RIII, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R1 Apo-2, DR4), TNFRSF10B (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcR1, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT),

TNFRSF1A (TNF RI CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEGFR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, and receptors for hormones and growth factors.

[142] Exemplary antigens that may be targeted specifically by the immunoglobulins of the invention include but are not limited to: CD20, CD19, Her2, EGFR, EpCAM, CD3, Fc γ RIIIa (CD16), Fc γ RIIa (CD32a), Fc γ RIIb (CD32b), Fc γ RI (CD64), Toll-like receptors (TLRs) such as TLR4 and TLR9, cytokines such as IL-2, IL-5, IL-13, IL-12, IL-23, and TNF α , cytokine receptors such as IL-2R, chemokines, chemokine receptors, growth factors such as VEGF and HGF, and the like.

[143] The choice of suitable target antigens and co-targets depends on the desired therapeutic application. Some targets that have proven especially amenable to antibody therapy are those with signaling functions. Other therapeutic antibodies exert their effects by blocking signaling of the receptor by inhibiting the binding between a receptor and its cognate ligand. Another mechanism of action of therapeutic antibodies is to cause receptor down regulation. Other antibodies do not work by signaling through their target antigen. The choice of co-targets will depend on the detailed biology underlying the pathology of the indication that is being treated.

[144] Monoclonal antibody therapy has emerged as an important therapeutic modality for cancer (Weiner et al., 2010, Nature Reviews Immunology 10:317-327; Reichert et al., 2005, Nature Biotechnology 23[9]:1073-1078; herein expressly incorporated by reference). For anti-cancer treatment it may be desirable to target one antigen (antigen-1) whose expression is restricted to the cancerous cells while co-targeting a second antigen (antigen-2) that mediates some immunological killing activity. For other treatments it may be beneficial to co-target two antigens, for example two angiogenic factors or two growth factors that are each known to play some role in proliferation of the tumor. Exemplary co-targets for oncology include but are not limited to HGF and VEGF, IGF-1R and VEGF, Her2 and VEGF, CD19 and CD3, CD20 and CD3, Her2 and CD3, CD19 and FcγRIIIa, CD20 and FcγRIIIa, Her2 and FcγRIIIa. An immunoglobulin of the invention may be capable of binding VEGF and phosphatidylserine; VEGF and ErbB3; VEGF and PLGF; VEGF and ROBO4; VEGF and BSG2; VEGF and CDCP1; VEGF and ANPEP; VEGF and c-MET; HER-2 and ERB3; HER-2 and BSG2; HER-2 and CDCP1; HER-2 and ANPEP; EGFR and CD64; EGFR and BSG2; EGFR and CDCP1; EGFR and ANPEP; IGF1R and PDGFR; IGF1R and VEGF; IGF1R and CD20; CD20 and CD74; CD20 and CD30; CD20 and DR4; CD20 and VEGFR2; CD20 and CD52; CD20 and CD4; HGF and c-MET; HGF and NRP1; HGF and phosphatidylserine; ErbB3 and IGF1R; ErbB3 and IGF1,2; c-Met and Her-2; c-Met and NRP1; c-Met and IGF1R; IGF1,2 and PDGFR; IGF1,2 and CD20; IGF1,2 and IGF1R; IGF2 and EGFR; IGF2 and HER2; IGF2 and CD20; IGF2 and VEGF; IGF2 and IGF1R; IGF1 and IGF2; PDGFRa and VEGFR2; PDGFRa and PLGF; PDGFRa and VEGF; PDGFRa and c-Met; PDGFRa and EGFR; PDGFRb and VEGFR2; PDGFRb and c-Met; PDGFRb and EGFR; RON and c-Met; RON and MTSP1; RON and MSP; RON and CDCP1; VGFR1 and PLGF; VGFR1 and RON; VGFR1 and EGFR; VEGFR2 and PLGF; VEGFR2 and NRP1; VEGFR2 and RON; VEGFR2 and DLL4; VEGFR2 and EGFR; VEGFR2 and ROBO4; VEGFR2 and CD55; LPA and S1P; EPHB2 and RON; CTLA4 and VEGF; CD3 and EPCAM; CD40 and IL6; CD40 and IGF; CD40 and CD56; CD40 and CD70; CD40 and VEGFR1; CD40 and DR5; CD40 and DR4; CD40 and APRIL; CD40 and BCMA; CD40 and RANKL; CD28 and MAPG; CD80 and CD40; CD80 and CD30; CD80 and CD33; CD80 and CD74; CD80 and CD2; CD80 and CD3; CD80 and CD19; CD80 and CD4; CD80 and CD52; CD80 and VEGF; CD80 and DR5; CD80 and VEGFR2; CD22 and CD20; CD22 and CD80; CD22 and CD40; CD22 and CD23; CD22 and CD33; CD22 and CD74; CD22 and CD19; CD22 and DR5; CD22 and DR4; CD22 and VEGF; CD22 and CD52; CD30 and CD20; CD30 and CD22; CD30 and CD23; CD30 and CD40; CD30 and VEGF; CD30 and CD74; CD30 and CD19; CD30 and DR5; CD30 and DR4; CD30 and VEGFR2; CD30 and CD52; CD30 and CD4; CD138 and RANKL; CD33 and FTL3; CD33 and VEGF; CD33 and VEGFR2; CD33 and CD44; CD33 and DR4; CD33 and

DR5; DR4 and CD137; DR4 and IGF1,2; DR4 and IGF1R; DR4 and DR5; DR5 and CD40; DR5 and CD137; DR5 and CD20; DR5 and EGFR; DR5 and IGF1,2; DR5 and IGFR, DR5 and HER-2, and EGFR and DLL4. Other target combinations include one or more members of the EGF/erb-2/erb-3 family.

[145] Other targets (one or more) involved in oncological diseases that the immunoglobulins herein may bind include, but are not limited to those selected from the group consisting of: CD52, CD20, CD19, CD3, CD4, CD8, BMP6, IL12A, IL1A, IL1B, 1L2, IL24, INHA, TNF, TNFSF10, BMP6, EGF, FGF1, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GRP, IGF1, IGF2, IL12A, IL1A, IL1B, 1L2, INHA, TGFA, TGFB1, TGFB2, TGFB3, VEGF, CDK2, FGF10, FGF18, FGF2, FGF4, FGF7, IGF1R, IL2, BCL2, CD164, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CDKN3, GNRH1, IGFBP6, IL1A, IL1B, ODZ1, PAWR, PLG, TGFB1I1, AR, BRCA1, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, E2F1, EGFR, ENO1, ERBB2, ESR1, ESR2, IGFBP3, IGFBP6, IL2, INSL4, MYC, NOX5, NR6A1, PAP, PCNA, PRKCQ, PRKD1, PRL, TP53, FGF22, FGF23, FGF9, IGFBP3, IL2, INHA, KLK6, TP53, CHGB, GNRH1, IGF1, IGF2, INHA, INSL3, INSL4, PRL, KLK6, SHBG, NR1D1, NR1H3, NR1I3, NR2F6, NR4A3, ESR1, ESR2, NR0B1, NR0B2, NR1D2, NR1H2, NR1H4, NR1I2, NR2C1, NR2C2, NR2E1, NR2E3, NR2F1, NR2F2, NR3C1, NR3C2, NR4A1, NR4A2, NR5A1, NR5A2, NR6 μ l, PGR, RARB, FGF1, FGF2, FGF6, KLK3, KRT1, APOC1, BRCA1, CHGA, CHGB, CLU, COL1A1, COL6A1, EGF, ERBB2, ERK8, FGF1, FGF10, FGF11, FGF13, FGF14, FGF16, FGF17, FGF18, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GNRH1, IGF1, IGF2, IGFBP3, IGFBP6, IL12A, IL1A, IL1B, 1L2, IL24, INHA, INSL3, INSL4, KLK10, KLK12, KLK13, KLK14, KLK15, KLK3, KLK4, KLK5, KLK6, KLK9, MMP2, MMP9, MSMB, NTN4, ODZ1, PAP, PLAUI, PRL, PSAP, SERPINA3, SHBG, TGFA, TIMP3, CD44, CDH1, CDH10, CDH19, CDH20, CDH7, CDH9, CDH1, CDH10, CDH13, CDH18, CDH19, CDH20, CDH7, CDH8, CDH9, ROBO2, CD44, ILK, ITGA1, APC, CD164, COL6A1, MTSS1, PAP, TGFB1I1, AGR2, AIG1, AKAP1, AKAP2, CANT1, CAV1, CDH12, CLDN3, CLN3, CYB5, CYC1, DAB2IP, DES, DNCL1, ELAC2, ENO2, ENO3, FASN, FLJ12584, FLJ25530, GAGEB1, GAGEC1, GGT1, GSTP1, HIP1, HUMCYT2A, IL29, K6HF, KAI1, KRT2A, MIB1, PART1, PATE, PCA3, PIAS2, PIK3CG, PPID, PR1, PSCA, SLC2A2, SLC33 μ l, SLC43 μ l, STEAP, STEAP2, TPM1, TPM2, TRPC6, ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB 1, VEGF, VEGFC, ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB 1, ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2, CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, EDG1, EFNA1, EFNA3,

EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFB1, CCL2, CDH5, COL1A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2, BAD, BAG1, BCL2, CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CDH1 (E-cadherin), CDKN1B (p27Kip1), CDKN2A (p16INK4a), COL6A1, CTNNB1 (b-catenin), CTSB (cathepsin B), ERBB2 (Her-2), ESR1, ESR2, F3 (TF), FOSL1 (FRA-1), GATA3, GSN (Gelsolin), IGFBP2, IL2RA, IL6, IL6R, IL6ST (glycoprotein 130), ITGA6 (a6 integrin), JUN, KLK5, KRT19, MAP2K7 (c-Jun), MKI67 (Ki-67), NGFB (GF), NGFR, NME1 (M23A), PGR, PLA1 (uPA), PTEN, SERPINB5 (maspin), SERPINE1 (PAI-1), TGFA, THBS1 (thrombospondin-1), TIE (Tie-1), TNFRSF6 (Fas), TNFSF6 (FasL), TOP2A (topoisomerase IIa), TP53, AZGP1 (zinc-a-glycoprotein), BPAG1 (plectin), CDKN1A (p21Wap1/Cip1), CLDN7 (claudin-7), CLU (clusterin), ERBB2 (Her-2), FGF1, FLRT1 (fibronectin), GABRP (GABAa), GNAS1, ID2, ITGA6 (a6 integrin), ITGB4 (b 4 integrin), KLF5 (GC Box BP), KRT19 (Keratin 19), KRTHB6 (hair-specific type II keratin), MACMARCKS, MT3 (metallothionein-III), MUC1 (mucin), PTGS2 (COX-2), RAC2 (p21Rac2), S100A2, SCGB1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SCGB2A2 (mammaglobin 1), SPRR1B (Spr1), THBS1, THBS2, THBS4, and TNFAIP2 (B94), RON, c-Met, CD64, DLL4, PLGF, CTLA4, phosphatidylserine, ROBO4, CD80, CD22, CD40, CD23, CD28, CD80, CD55, CD38, CD70, CD74, CD30, CD138, CD56, CD33, CD2, CD137, DR4, DR5, RANKL, VEGFR2, PDGFR, VEGFR1, MTSP1, MSP, EPHB2, EPHA1, EPHA2, EpCAM, PGE2, NKG2D, LPA, SIP, APRIL, BCMA, MAPG, FLT3, PDGFR alpha, PDGFR beta, ROR1, PSMA, PSCA, SCD1, and CD59.

[146] Monoclonal antibody therapy has become an important therapeutic modality for treating autoimmune and inflammatory disorders (Chan & Carter, 2010, Nature Reviews Immunology 10:301-316; Reichert et al., 2005, Nature Biotechnology 23[9]:1073-1078; herein expressly incorporated by reference). Many proteins have been implicated in general autoimmune and inflammatory responses, and thus may be targeted by the immunoglobulins of the invention. Autoimmune and inflammatory targets include but are not limited to C5, CCL1 (I-309), CCL11 (eotaxin), CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19, CCL2 (mcp-1), CCL20 (MIP-3a), CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8 (mcp-2), CXCL1, CXCL10 (IP-10), CXCL11 (1-TAC/IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL2, CXCL3, CXCL5 (ENA-78/LIX), CXCL6 (GCP-2), CXCL9, IL13, IL8, CCL13 (mcp-4), CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, IL8RA, XCR1 (CCXCR1), IFNA2, IL10, IL13, IL17C, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL22, IL5, IL8, IL9, LTA, LTB, MIF, SCYE1 (endothelial Monocyte-activating cytokine), SPP1, TNF, TNFSF5, IFNA2, IL10RA, IL10RB, IL13, IL13RA1, IL5RA, IL9, IL9R, ABCF1, BCL6, C3, C4A, CEBPB, CRP,

ICEBERG, IL1R1, IL1RN, IL8RB, LTB4R, TOLLIP, FADD, IRAK1, IRAK2, MYD88, NCK2, TNFAIP3, TRADD, TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, ACVR1, ACVR1B, ACVR2, ACVR2B, ACVRL1, CD28, CD3E, CD3G, CD3Z, CD69, CD80, CD86, CNR1, CTLA4, CYSLTR1, FCER1A, FCER2, FCGR3A, GPR44, HAVCR2, OPRD1, P2RX7, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, BLR1, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CL1, CX3CR1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL10, CXCL11, CXCL12, CXCL13, CXCR4, GPR2, SCYE1, SDF2, XCL1, XCL2, XCR1, AMH, AMHR2, BMPR1A, BMPR1B, BMPR2, C19orf10 (IL27w), CER1, CSF1, CSF2, CSF3, DKFZp451J0118, FGF2, GFI1, IFNA1, IFNB1, IFNG, IGF1, IL1A, IL1B, IL1R1, IL1R2, IL2, IL2RA, IL2RB, IL2RG, IL3, IL4, IL4R, IL5, IL5RA, IL6, IL6R, IL6ST, IL7, IL8, IL8RA, IL8RB, IL9, IL9R, IL10, IL10RA, IL10RB, IL11, IL12RA, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL13RA1, IL13RA2, IL15, IL15RA, IL16, IL17, IL17R, IL18, IL18R1, IL19, IL20, KITLG, LEP, LTA, LTB, LTB4R, LTB4R2, LTBR, MIF, NPPB, PDGFB, TBX21, TDGF1, TGFA, TGFB1, TGFB1I1, TGFB2, TGFB3, TGFB1, TGFB1R1, TGFB1R2, TGFB1R3, TH1L, TNF, TNFRSF1A, TNFRSF1B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF11A, TNFRSF21, TNFSF4, TNFSF5, TNFSF6, TNFSF11, VEGF, ZFPM2, and RNF110 (ZNF144).

[147] Exemplary co-targets for autoimmune and inflammatory disorders include but are not limited to IL-1 and TNFalpha, IL-6 and TNFalpha, IL-6 and IL-1, IgE and IL-13, IL-1 and IL-13, IL-4 and IL-13, IL-5 and IL-13, IL-9 and IL-13, CD19 and FcγRIIb, and CD79 and FcγRIIb.

[148] Immunglobulins of the invention with specificity for the following pairs of targets to treat inflammatory disease are contemplated: TNF and IL-17A; TNF and RANKL; TNF and VEGF; TNF and SOST; TNF and DKK; TNF and alphaVbeta3; TNF and NGF; TNF and IL-23p19; TNF and IL-6; TNF and SOST; TNF and IL-6R; TNF and CD-20; IgE and IL-13; IL-13 and IL23p19; IgE and IL-4; IgE and IL-9; IgE and IL-9; IgE and IL-13; IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-9; IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-23p19; IL-13 and IL-9; IL-6R and VEGF; IL-6R and IL-17A; IL-6R and RANKL; IL-17A and IL-1beta; IL-1beta and RANKL; IL-1beta and VEGF; RANKL and CD-20; IL-1alpha and IL-1beta; IL-1alpha and IL-1beta.

[149] Pairs of targets that the immunoglobulins described herein can bind and be useful to treat asthma may be determined. In an embodiment, such targets include, but are not limited to, IL-13 and IL-1beta, since IL-1beta is also implicated in inflammatory response in asthma; IL-13 and cytokines and chemokines that are involved in inflammation, such as IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-5; IL-13 and IL-25; IL-13 and TARC; IL-13 and MDC; IL-13

and MIF; IL-13 and TGF- β ; IL-13 and LHR agonist; IL-13 and CL25; IL-13 and SPRR2a; IL-13 and SPRR2b; and IL-13 and ADAM8. The immunoglobulins herein may have specificity for one or more targets involved in asthma selected from the group consisting of CSF1 (MCSF), CSF2 (GM-CSF), CSF3 (GCSF), FGF2, IFNA1, IFNB1, IFNG, histamine and histamine receptors, IL1A, IL1B, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12A, IL12B, IL13, IL14, IL15, IL16, IL17, IL18, IL19, KITLG, PDGFB, IL2RA, IL4R, IL5RA, IL8RA, IL8RB, IL12RB1, IL12RB2, IL13RA1, IL13RA2, IL18R1, TSLP, CCLi, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL13, CCL17, CCL18, CCL19, CCL20, CCL22, CCL24, CX3CL1, CXCL1, CXCL2, CXCL3, XCLi, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CX3CR1, GPR2, XCR1, FOS, GATA3, JAK1, JAK3, STAT6, TBX21, TGFB1, TNF, TNFSF6, YY1, CYSLTR1, FCER1A, FCER2, LTB4R, TB4R2, LTBR, and Chitinase.

[150] Pairs of targets involved in rheumatoid arthritis (RA) may be co-targeted by the invention, including but not limited to TNF and IL-18; TNF and IL-12; TNF and IL-23; TNF and IL-1beta; TNF and MIF; TNF and IL-17; and TNF and IL-15.

[151] Antigens that may be targeted in order to treat systemic lupus erythematosus (SLE) by the immunoglobulins herein include but are not limited to CD-20, CD-22, CD-19, CD28, CD4, CD80, HLA-DRA, IL10, IL2, IL4, TNFRSF5, TNFRSF6, TNFSF5, TNFSF6, BLR1, HDAC4, HDAC5, HDAC7A, HDAC9, ICOSL, IGBP1, MS4A1, RGS1, SLA2, CD81, IFNB1, IL10, TNFRSF5, TNFRSF7, TNFSF5, AICDA, BLNK, GALNAC4S-6ST, HDAC4, HDAC5, HDAC7A, HDAC9, IL10, IL11, IL4, INHA, INHBA, KLF6, TNFRSF7, CD28, CD38, CD69, CD80, CD83, CD86, DPP4, FCER2, IL2RA, TNFRSF8, TNFSF7, CD24, CD37, CD40, CD72, CD74, CD79A, CD79B, CR2, ILIR2, ITGA2, ITGA3, MS4A1, ST6GALI, CDIC, CHSTIO, HLA-A, HLA-DRA, and NT5E.; CTLA4, B7.1, B7.2, BlyS, BAFF, C5, IL-4, IL-6, IL-10, IFN- α , and TNF- α .

[152] The immunoglobulins herein may target antigens for the treatment of multiple sclerosis (MS), including but not limited to IL-12, TWEAK, IL-23, CXCL13, CD40, CD40L, IL-18, VEGF, VLA-4, TNF, CD45RB, CD200, IFNgamma, GM-CSF, FGF, C5, CD52, and CCR2. An embodiment includes co-engagement of anti-IL-12 and TWEAK for the treatment of MS.

[153] One aspect of the invention pertains to immunoglobulins capable of binding one or more targets involved in sepsis, in an embodiment two targets, selected from the group consisting TNF, IL-1, MIF, IL-6, IL-8, IL-18, IL-12, IL-23, FasL, LPS, Toll-like receptors, TLR-4, tissue factor, MIP-2, ADORA2A, CASP1, CASP4, IL-10, IL-1B, NF κ B1, PROC, TNFRSF1A, CSF3, CCR3, IL1RN, MIF, NF κ B1, PTAFR, TLR2, TLR4, GPR44, HMOX1, midkine, IRAK1, NF κ B2, SERPINA1, SERPINE1, and TREM1.

[154] In some cases, immunoglobulins herein may be directed against antigens for the treatment of infectious diseases.

[155] One skilled in the art will appreciate that any of the aforementioned target antigens, the ligands or receptors that bind them, or other members of their corresponding biochemical pathway, may be operably linked to the immunoglobulins of the present invention in order to generate an Fc fusion. Thus for example, an Fc fusion that targets TNF α could be constructed by operably linking an Fc region to TNFR1 or TNFR2. Thus virtually any polypeptide, whether a ligand, receptor, or some other protein or protein domain, including but not limited to the aforementioned targets and the proteins that compose their corresponding biochemical pathways, may be operably linked to the immunoglobulins of the present invention to develop an Fc fusion.

[156] Linkers

[157] The term “linker” is used to denote polypeptides comprising two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123). A variety of linkers may find use in some embodiments described herein to covalently link Fc regions to a fusion partner. “Linker” herein is also referred to as “linker sequence”, “spacer”, “tethering sequence” or grammatical equivalents thereof. Homo-or hetero-bifunctional linkers as are well known (see, 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated entirely by reference). A number of strategies may be used to covalently link molecules together. These include, but are not limited to polypeptide linkages between N- and C-termini of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant techniques or peptide synthesis. The linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 30 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used. Useful linkers include glycine-serine polymers, including for example (GS) n , (GSGGS) n , (GGGGS) n , and (GGGS) n , where n is an integer of at least one, glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of

polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use as linkers.

[158] Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains. Linkers can be derived from immunoglobulin light chain, for example C κ or C λ . Linkers can be derived from immunoglobulin heavy chains of any isotype, including for example C γ 1, C γ 2, C γ 3, C γ 4, C α 1, C α 2, C δ , C ϵ , and C μ . Linker sequences may also be derived from other proteins such as Ig-like proteins (e.g. TCR, FcR, KIR), hinge region-derived sequences, and other natural sequences from other proteins.

[159] Glycoform Modifications

[160] All antibodies contain carbohydrate at conserved positions in the constant regions of the heavy chain. Each antibody isotype has a distinct variety of N-linked carbohydrate structures. Aside from the carbohydrate attached to the heavy chain, up to 30% of human IgGs have a glycosylated Fab region. IgG has a single N-linked biantennary carbohydrate at Asn297 of the CH2 domain. For IgG from either serum or produced ex vivo in hybridomas or engineered cells, the IgG are heterogeneous with respect to the Asn297 linked carbohydrate. For human IgG, the core oligosaccharide normally consists of GlcNAc2Man3GlcNAc, with differing numbers of outer residues.

[161] The carbohydrate moieties of immunoglobulins disclosed herein will be described with reference to commonly used nomenclature for the description of oligosaccharides. A review of carbohydrate chemistry which uses this nomenclature is found in Hubbard et al. 1981, Ann. Rev. Biochem. 50:555-583. This nomenclature includes, for instance, Man, which represents mannose; GlcNAc, which represents 2-N-acetylglucosamine; Gal which represents galactose; Fuc for fucose; and Glc, which represents glucose. Sialic acids are described by the shorthand notation NeuNAc, for 5-N-acetylneuraminic acid, and NeuNGc for 5-glycolylneuraminic.

[162] The term "glycosylation" means the attachment of oligosaccharides (carbohydrates containing two or more simple sugars linked together e.g. from two to about twelve simple sugars linked together) to a glycoprotein. The oligosaccharide side chains are typically linked to the backbone of the glycoprotein through either N- or O-linkages. The oligosaccharides of immunoglobulins disclosed herein occur generally are attached to a CH2 domain of an Fc region as N-linked oligosaccharides. "N-linked glycosylation" refers to the attachment of the carbohydrate moiety to an asparagine residue in a glycoprotein chain. The skilled artisan will recognize that, for example, each of murine IgG1, IgG2a, IgG2b and IgG3 as well as human

IgG1, IgG2, IgG3, IgG4, IgA and IgD CH2 domains have a single site for N-linked glycosylation at residue 297.

[163] For the purposes herein, a "mature core carbohydrate structure" refers to a processed core carbohydrate structure attached to an Fc region which generally consists of the following carbohydrate structure GlcNAc(Fucose)-GlcNAc-Man-(Man-GlcNAc)₂ typical of biantennary oligosaccharides. The mature core carbohydrate structure is attached to the Fc region of the glycoprotein, generally via N-linkage to Asn297 of a CH2 domain of the Fc region. A "bisecting GlcNAc" is a GlcNAc residue attached to the α 1,4 mannose of the mature core carbohydrate structure. The bisecting GlcNAc can be enzymatically attached to the mature core carbohydrate structure by a α (1,4)-N-acetylglucosaminyltransferase III enzyme (GnTIII). CHO cells do not normally express GnTIII (Stanley et al., 1984, J. Biol. Chem. 261:13370-13378), but may be engineered to do so (Umana et al., 1999, Nature Biotech. 17:176-180).

[164] Described herein are immunoglobulins that comprise modified glycoforms or engineered glycoforms. By "modified glycoform" or "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to a protein, for example an antibody, wherein said carbohydrate composition differs chemically from that of a parent protein. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing Fc γ R-mediated effector function. In one embodiment, the immunoglobulins disclosed herein are modified to control the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region.

[165] A variety of methods are well known in the art for generating modified glycoforms (Umaña et al., 1999, Nat Biotechnol 17:176-180; Davies et al., 2001, Biotechnol Bioeng 74:288-294; Shields et al., 2002, J Biol Chem 277:26733-26740; Shinkawa et al., 2003, J Biol Chem 278:3466-3473; USSN12/434,533; all of which are expressly incorporated by reference). These techniques control the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells), by regulating enzymes involved in the glycosylation pathway (for example FUT8 [α 1,6-fucosyltransferase] and/or β 1-4- N-acetylglucosaminyltransferase III [GnTIII]), by modifying carbohydrate(s) after the IgG has been expressed, or by expressing antibody in the presence of fucose analogs as enzymatic inhibitors. Other methods for modifying glycoforms of the immunoglobulins disclosed herein include using glycoengineered strains of yeast (Li et al., 2006, Nature Biotechnology 24(2):210-215), moss (Nechansky et al., 2007, Mol Immunol 44(7):1826-8), and plants (Cox

et al., 2006, Nat Biotechnol 24(12):1591-7). The use of a particular method to generate a modified glycoform is not meant to constrain embodiments to that method. Rather, embodiments disclosed herein encompass immunoglobulins with modified glycoforms irrespective of how they are produced.

[166] In one embodiment, immunoglobulins disclosed herein are glycoengineered to alter the level of sialylation. Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality (Scallon et al., 2007, Mol Immunol. 44(7):1524-34), and differences in levels of Fc sialylation can result in modified anti-inflammatory activity (Kaneko et al., 2006, Science 313:670-673). Because antibodies may acquire anti-inflammatory properties upon sialylation of Fc core polysaccharide, it may be advantageous to glycoengineer the immunoglobulins disclosed herein for greater or reduced Fc sialic acid content.

[167] Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus for example an immunoglobulin may comprise an engineered glycoform. Alternatively, engineered glycoform may refer to the immunoglobulin that comprises the different carbohydrate or oligosaccharide. In one embodiment, a composition disclosed herein comprises a glycosylated immunoglobulin having an Fc region, wherein about 51-100% of the glycosylated antibody, e.g., 80-100%, 90-100%, 95-100%, etc. of the antibody in the composition comprises a mature core carbohydrate structure which lacks fucose. In another embodiment, the antibody in the composition both comprises a mature core carbohydrate structure that lacks fucose and additionally comprises at least one amino acid modification in the Fc region. In an alternative embodiment, a composition comprises a glycosylated immunoglobulin having an Fc region, wherein about 51-100% of the glycosylated antibody, 80-100%, or 90-100%, of the antibody in the composition comprises a mature core carbohydrate structure which lacks sialic acid. In another embodiment, the antibody in the composition both comprises a mature core carbohydrate structure that lacks sialic acid and additionally comprises at least one amino acid modification in the Fc region. In yet another embodiment, a composition comprises a glycosylated immunoglobulin having an Fc region, wherein about 51-100% of the glycosylated antibody, 80-100%, or 90-100%, of the antibody in the composition comprises a mature core carbohydrate structure which contains sialic acid. In another embodiment, the antibody in the composition both comprises a mature core carbohydrate structure that contains sialic acid and additionally comprises at least one amino acid modification in the Fc region. In another embodiment, the combination of engineered glycoform and amino acid modification provides optimal Fc receptor binding properties to the antibody.

[168] Other Modifications

[169] Immunoglobulins disclosed herein may comprise one or more modifications that provide optimized properties. Said modifications may be amino acid modifications, or may be modifications that are made enzymatically or chemically. Such modification(s) likely provide some improvement in the immunoglobulin, for example an enhancement in its stability, solubility, function, or clinical use. Disclosed herein are a variety of improvements that may be made by coupling the immunoglobulins disclosed herein with additional modifications.

[170] In one embodiment, the variable region of an immunoglobulin disclosed herein may be affinity matured, that is to say that amino acid modifications have been made in the VH and/or VL domains to enhance binding of the antibody to its target antigen. Such types of modifications may improve the association and/or the dissociation kinetics for binding to the target antigen. Other modifications include those that improve selectivity for target antigen vs. alternative targets. These include modifications that improve selectivity for antigen expressed on target vs. non-target cells. Immunoglobulins disclosed herein may comprise one or more modifications that provide reduced or enhanced internalization of an immunoglobulin.

[171] In one embodiment, modifications are made to improve biophysical properties of the immunoglobulins disclosed herein, including but not limited to stability, solubility, and oligomeric state. Modifications can include, for example, substitutions that provide more favorable intramolecular interactions in the immunoglobulin such as to provide greater stability, or substitution of exposed nonpolar amino acids with polar amino acids for higher solubility. Other modifications to the immunoglobulins disclosed herein include those that enable the specific formation of homodimeric or homomultimeric molecules. Such modifications include but are not limited to engineered disulfides, as well as chemical modifications or aggregation methods.

[172] In further embodiments, the immunoglobulins disclosed herein comprise modifications that remove proteolytic degradation sites. These may include, for example, protease sites that reduce production yields, as well as protease sites that degrade the administered protein in vivo. In one embodiment, additional modifications are made to remove covalent degradation sites such as deamidation (i.e. deamidation of glutamyl and asparaginy residues to the corresponding glutamyl and aspartyl residues), oxidation, and proteolytic degradation sites. Deamidation sites that are particularly useful to remove are those that have enhanced propensity for deamidation, including, but not limited to asparaginy and glutamyl residues followed by glycines (NG and QG motifs, respectively). In such cases, substitution of either residue can significantly reduce the tendency for deamidation. Common oxidation sites include methionine and cysteine residues. Other covalent modifications, that can either be introduced or removed, include hydroxylation of proline and lysine,

phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains, acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group. Additional modifications also may include but are not limited to posttranslational modifications such as N-linked or O-linked glycosylation and phosphorylation.

[173] Modifications may include those that improve expression and/or purification yields from hosts or host cells commonly used for production of biologics. These include, but are not limited to various mammalian cell lines (e.g. CHO), yeast cell lines, bacterial cell lines, and plants. Additional modifications include modifications that remove or reduce the ability of heavy chains to form inter-chain disulfide linkages. Additional modifications include modifications that remove or reduce the ability of heavy chains to form intra-chain disulfide linkages.

[174] The immunoglobulins disclosed herein may comprise modifications that include the use of unnatural amino acids incorporated using, including but not limited to methods described in Liu & Schultz, 2010, *Annu Rev Biochem* 79:413-444, herein expressly incorporated by reference. In some embodiments, these modifications enable manipulation of various functional, biophysical, immunological, or manufacturing properties discussed above. In additional embodiments, these modifications enable additional chemical modification for other purposes.

[175] Other modifications are contemplated herein. For example, the immunoglobulin may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Additional amino acid modifications may be made to enable specific or non-specific chemical or posttranslational modification of the immunoglobulins. Such modifications, include, but are not limited to PEGylation and glycosylation. Specific substitutions that can be utilized to enable PEGylation include, but are not limited to, introduction of novel cysteine residues or unnatural amino acids such that efficient and specific coupling chemistries can be used to attach a PEG or otherwise polymeric moiety. Introduction of specific glycosylation sites can be achieved by introducing novel N-X-T/S sequences into the immunoglobulins disclosed herein.

[176] Modifications to reduce immunogenicity may include modifications that reduce binding of processed peptides derived from the parent sequence to MHC proteins. For example, amino acid modifications would be engineered such that there are no or a minimal number of immune epitopes that are predicted to bind, with high affinity, to any prevalent

MHC alleles. Several methods of identifying MHC-binding epitopes in protein sequences are known in the art and may be used to score epitopes in an antibody disclosed herein.

[177] Covalent modifications are included within the scope of immunoglobulins disclosed herein, and are generally, but not always, done post-translationally. For example, several types of covalent modifications can be introduced into the molecule by reacting specific amino acid residues with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues. In some embodiments, the covalent modification of the immunoglobulins disclosed herein comprises the addition of one or more labels. The term "labeling group" means any detectable label. In some embodiments, the labeling group is coupled to the immunoglobulin via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labeling proteins are known in the art and may be used in generating immunoglobulins disclosed herein.

[178] Conjugates

[179] In one embodiment, the immunoglobulins disclosed herein are "fusion proteins", sometimes referred to herein as "conjugates". The fusion partner or conjugate partner can be proteinaceous or non-proteinaceous; the latter generally being generated using functional groups on the immunoglobulin and on the conjugate partner. Conjugate and fusion partners may be any molecule, including small molecule chemical compounds and polypeptides. For example, a variety of conjugates and methods are described in Trail et al., 1999, Curr. Opin. Immunol. 11:584-588, incorporated entirely by reference. Possible conjugate partners include but are not limited to cytokines, cytotoxic agents, toxins, radioisotopes, chemotherapeutic agent, anti-angiogenic agents, a tyrosine kinase inhibitors, and other therapeutically active agents. In some embodiments, conjugate partners may be thought of more as payloads, that is to say that the goal of a conjugate is targeted delivery of the conjugate partner to a targeted cell, for example a cancer cell or immune cell, by the immunoglobulin. Thus, for example, the conjugation of a toxin to an immunoglobulin targets the delivery of said toxin to cells expressing the target antigen. As will be appreciated by one skilled in the art, in reality the concepts and definitions of fusion and conjugate are overlapping. The designation of a fusion or conjugate is not meant to constrain it to any particular embodiment disclosed herein. Rather, these terms are used loosely to convey the broad concept that any immunoglobulin disclosed herein may be linked genetically, chemically, or otherwise, to one or more polypeptides or molecules to provide some desirable property.

[180] Suitable conjugates include, but are not limited to, labels as described below, drugs and cytotoxic agents including, but not limited to, cytotoxic drugs (e.g., chemotherapeutic

agents) or toxins or active fragments of such toxins. Suitable toxins and their corresponding fragments include diphtheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin and the like. Cytotoxic agents also include radiochemicals made by conjugating radioisotopes to immunoglobulin, or binding of a radionuclide to a chelating agent that has been covalently attached to the immunoglobulin. Additional embodiments utilize calicheamicin, auristatins, geldanamycin, maytansine, and duocarmycins and analogs. Antibody-drug conjugates are described in Alley et al., 2010, *Curr Opin Chem Biol* 14[4]:529-37, herein expressly incorporated by reference.

[181] In one embodiment, the immunoglobulins disclosed herein are fused or conjugated to a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. For example, as described in Penichet et al., 2001, *J. Immunol. Methods* 248:91-101, incorporated entirely by reference, cytokines may be fused to an immunoglobulin to provide an array of desirable properties. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; C5a; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

[182] In yet another embodiment, an immunoglobulins disclosed herein may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the immunoglobulin-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide). In an

alternate embodiment, the immunoglobulin is conjugated or operably linked to an enzyme in order to employ Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT). ADEPT may be used by conjugating or operably linking the immunoglobulin to a prodrug-activating enzyme that converts a prodrug (e.g. a peptidyl chemotherapeutic agent).

[183] Production of Immunoglobulins

[184] Also disclosed herein are methods for producing and experimentally testing immunoglobulins. The disclosed methods are not meant to constrain embodiments to any particular application or theory of operation. Rather, the provided methods are meant to illustrate generally that one or more immunoglobulins may be produced and experimentally tested to obtain immunoglobulins. General methods for antibody molecular biology, expression, purification, and screening are described in Antibody Engineering, edited by Kontermann & Dubel, Springer, Heidelberg, 2001; and Hayhurst & Georgiou, 2001, Curr Opin Chem Biol 5:683-689; Maynard & Georgiou, 2000, Annu Rev Biomed Eng 2:339-76;

[185] In one embodiment disclosed herein, nucleic acids are created that encode the immunoglobulins, and that may then be cloned into host cells, expressed and assayed, if desired. Thus, nucleic acids, and particularly DNA, may be made that encode each protein sequence. These practices are carried out using well-known procedures. For example, a variety of methods that may find use in generating immunoglobulins disclosed herein are described in Molecular Cloning - A Laboratory Manual, 3rd Ed. (Maniatis, Cold Spring Harbor Laboratory Press, New York, 2001), and Current Protocols in Molecular Biology (John Wiley & Sons), both incorporated entirely by reference. There are a variety of techniques that may be used to efficiently generate DNA encoding immunoglobulins disclosed herein. Such methods include but are not limited to gene assembly methods, PCR-based method and methods which use variations of PCR, ligase chain reaction-based methods, pooled oligo methods such as those used in synthetic shuffling, error-prone amplification methods and methods which use oligos with random mutations, classical site-directed mutagenesis methods, cassette mutagenesis, and other amplification and gene synthesis methods. As is known in the art, there are a variety of commercially available kits and methods for gene assembly, mutagenesis, vector subcloning, and the like, and such commercial products find use in for generating nucleic acids that encode immunoglobulins.

[186] The immunoglobulin proteins disclosed herein may be produced by culturing a host cell transformed with nucleic acid, e.g., an expression vector, containing nucleic acid encoding the immunoglobulins, under the appropriate conditions to induce or cause expression of the protein. The conditions appropriate for expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the

art through routine experimentation. A wide variety of appropriate host cells may be used, including but not limited to mammalian cells, bacteria, insect cells, yeast, and plant cells. For example, a variety of cell lines that may find use in generating immunoglobulins disclosed herein are described in the ATCC® cell line catalog, available from the American Type Culture Collection.

[187] In one embodiment, the immunoglobulins are expressed in mammalian expression systems, including systems in which the expression constructs are introduced into the mammalian cells using virus such as retrovirus or adenovirus. Any mammalian cells may be used, e.g., human, mouse, rat, hamster, and primate cells. Suitable cells also include known research cells, including but not limited to Jurkat T cells, NIH3T3, CHO, BHK, COS, HEK293, PER C.6, HeLa, Sp2/0, NS0 cells and variants thereof. In an alternate embodiment, library proteins are expressed in bacterial cells. Bacterial expression systems are well known in the art, and include *Escherichia coli* (*E. coli*), *Bacillus subtilis*, *Streptococcus cremoris*, and *Streptococcus lividans*. In alternate embodiments, immunoglobulins are produced in insect cells (e.g. Sf21/Sf9, *Trichoplusia ni* Bti-Tn5b1-4) or yeast cells (e.g. *S. cerevisiae*, *Pichia*, etc). In an alternate embodiment, immunoglobulins are expressed in vitro using cell free translation systems. In vitro translation systems derived from both prokaryotic (e.g. *E. coli*) and eukaryotic (e.g. wheat germ, rabbit reticulocytes) cells are available and may be chosen based on the expression levels and functional properties of the protein of interest. For example, as appreciated by those skilled in the art, in vitro translation is required for some display technologies, for example ribosome display. In addition, the immunoglobulins may be produced by chemical synthesis methods. Also transgenic expression systems both animal (e.g. cow, sheep or goat milk, embryonated hen's eggs, whole insect larvae, etc.) and plant (e.g. corn, tobacco, duckweed, etc.)

[188] The nucleic acids that encode the immunoglobulins disclosed herein may be incorporated into an expression vector in order to express the protein. A variety of expression vectors may be utilized for protein expression. Expression vectors may comprise self-replicating extra-chromosomal vectors or vectors which integrate into a host genome. Expression vectors are constructed to be compatible with the host cell type. Thus expression vectors which find use in generating immunoglobulins disclosed herein include but are not limited to those which enable protein expression in mammalian cells, bacteria, insect cells, yeast, and in in vitro systems. As is known in the art, a variety of expression vectors are available, commercially or otherwise, that may find use for expressing immunoglobulins disclosed herein.

[189] Expression vectors typically comprise a protein operably linked with control or regulatory sequences, selectable markers, any fusion partners, and/or additional elements.

By “operably linked” herein is meant that the nucleic acid is placed into a functional relationship with another nucleic acid sequence. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the immunoglobulin, and are typically appropriate to the host cell used to express the protein. In general, the transcriptional and translational regulatory sequences may include promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. As is also known in the art, expression vectors typically contain a selection gene or marker to allow the selection of transformed host cells containing the expression vector. Selection genes are well known in the art and will vary with the host cell used.

[190] Immunoglobulins may be operably linked to a fusion partner to enable targeting of the expressed protein, purification, screening, display, and the like. Fusion partners may be linked to the immunoglobulin sequence via a linker sequences. The linker sequence will generally comprise a small number of amino acids, typically less than ten, although longer linkers may also be used. Typically, linker sequences are selected to be flexible and resistant to degradation. As will be appreciated by those skilled in the art, any of a wide variety of sequences may be used as linkers. For example, a common linker sequence comprises the amino acid sequence GGGGS. A fusion partner may be a targeting or signal sequence that directs immunoglobulin and any associated fusion partners to a desired cellular location or to the extracellular media. As is known in the art, certain signaling sequences may target a protein to be either secreted into the growth media, or into the periplasmic space, located between the inner and outer membrane of the cell. A fusion partner may also be a sequence that encodes a peptide or protein that enables purification and/or screening. Such fusion partners include but are not limited to polyhistidine tags (His-tags) (for example H6 and H10 or other tags for use with Immobilized Metal Affinity Chromatography (IMAC) systems (e.g. Ni⁺² affinity columns)), GST fusions, MBP fusions, Strep-tag, the BSP biotinylation target sequence of the bacterial enzyme BirA, and epitope tags which are targeted by antibodies (for example c-myc tags, flag-tags, and the like). As will be appreciated by those skilled in the art, such tags may be useful for purification, for screening, or both. For example, an immunoglobulin may be purified using a His-tag by immobilizing it to a Ni⁺² affinity column, and then after purification the same His-tag may be used to immobilize the antibody to a Ni⁺² coated plate to perform an ELISA or other binding assay (as described below). A fusion partner may enable the use of a selection method to screen immunoglobulins (see below). Fusion partners that enable a variety of selection methods are well-known in the art.

[191] For example, by fusing the members of an immunoglobulin library to the gene III protein, phage display can be employed. Fusion partners may enable immunoglobulins to be labeled. Alternatively, a fusion partner may bind to a specific sequence on the expression vector, enabling the fusion partner and associated immunoglobulin to be linked covalently or noncovalently with the nucleic acid that encodes them. The methods of introducing exogenous nucleic acid into host cells are well known in the art, and will vary with the host cell used. Techniques include but are not limited to dextran-mediated transfection, calcium phosphate precipitation, calcium chloride treatment, polybrene mediated transfection, protoplast fusion, electroporation, viral or phage infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In the case of mammalian cells, transfection may be either transient or stable.

[192] In one embodiment, immunoglobulins are purified or isolated after expression. Proteins may be isolated or purified in a variety of ways known to those skilled in the art. Purification may be particularly useful in the invention for separating heterodimeric heavy chain species from homodimeric heavy chain species, as described herein. Standard purification methods include chromatographic techniques, including ion exchange, hydrophobic interaction, affinity, sizing or gel filtration, and reversed-phase, carried out at atmospheric pressure or at high pressure using systems such as FPLC and HPLC. Purification methods also include electrophoretic, isoelectric focusing, immunological, precipitation, dialysis, and chromatofocusing techniques. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. As is well known in the art, a variety of natural proteins bind Fc and antibodies, and these proteins can find use for purification of immunoglobulins disclosed herein. For example, the bacterial proteins A and G bind to the Fc region. Likewise, the bacterial protein L binds to the Fab region of some antibodies, as of course does the antibody's target antigen. Purification can often be enabled by a particular fusion partner. For example, immunoglobulins may be purified using glutathione resin if a GST fusion is employed, Ni²⁺ affinity chromatography if a His-tag is employed, or immobilized anti-flag antibody if a flag-tag is used. For general guidance in suitable purification techniques, see, e.g. incorporated entirely by reference Protein Purification: Principles and Practice, 3rd Ed., Scopes, Springer-Verlag, NY, 1994, incorporated entirely by reference. The degree of purification necessary will vary depending on the screen or use of the immunoglobulins. In some instances no purification is necessary. For example in one embodiment, if the immunoglobulins are secreted, screening may take place directly from the media. As is well known in the art, some methods of selection do not involve purification of proteins.

[193] In Vitro Experimentation

[194] Immunoglobulins may be screened using a variety of *in vitro* methods, including but not limited to those that use binding assays, cell-based assays, and selection technologies. Automation and high-throughput screening technologies may be utilized in the screening procedures. Screening may employ the use of a fusion partner or label. The use of fusion partners has been discussed above. By "labeled" herein is meant that the immunoglobulins disclosed herein have one or more elements, isotopes, or chemical compounds attached to enable the detection in a screen. In general, labels fall into three classes: a) immune labels, which may be an epitope incorporated as a fusion partner that is recognized by an antibody, b) isotopic labels, which may be radioactive or heavy isotopes, and c) small molecule labels, which may include fluorescent and colorimetric dyes, or molecules such as biotin that enable other labeling methods. Labels may be incorporated into the compound at any position and may be incorporated *in vitro* or *in vivo* during protein expression.

[195] In one embodiment, the functional and/or biophysical properties of immunoglobulins are screened in an *in vitro* assay. *In vitro* assays may allow a broad dynamic range for screening properties of interest. Particularly relevant for the present invention, the immunoglobulins may be tested for their affinity for one or more antigens. Properties that may be screened include but are not limited to stability, solubility, and affinity for Fc ligands, for example FcγRs. Multiple properties may be screened simultaneously or individually. Proteins may be purified or unpurified, depending on the requirements of the assay. In one embodiment, the screen is a qualitative or quantitative binding assay for binding of immunoglobulins to a protein or nonprotein molecule that is known or thought to bind the immunoglobulin. In one embodiment, the screen is a binding assay for measuring binding to the target antigen. In an alternate embodiment, the screen is an assay for binding of immunoglobulins to an Fc ligand, including but are not limited to the family of FcγRs, the neonatal receptor FcRn, the complement protein C1q, and the bacterial proteins A and G. Said Fc ligands may be from any organism. In one embodiment, Fc ligands are from humans, mice, rats, rabbits, and/or monkeys. Binding assays can be carried out using a variety of methods known in the art, including but not limited to FRET (Fluorescence Resonance Energy Transfer) and BRET (Bioluminescence Resonance Energy Transfer) - based assays, AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay), Scintillation Proximity Assay, ELISA (Enzyme-Linked Immunosorbent Assay), SPR (Surface Plasmon Resonance, also known as BIACORE®), isothermal titration calorimetry, differential scanning calorimetry, gel electrophoresis, and chromatography including gel filtration. These and other methods may take advantage of some fusion partner or label of the immunoglobulin. Assays may employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels.

[196] The biophysical properties of immunoglobulins, for example stability and solubility, may be tested using a variety of methods known in the art. Protein stability may be determined by measuring the thermodynamic equilibrium between folded and unfolded states. For example, immunoglobulins disclosed herein may be unfolded using chemical denaturant, heat, or pH, and this transition may be monitored using methods including but not limited to circular dichroism spectroscopy, fluorescence spectroscopy, absorbance spectroscopy, NMR spectroscopy, calorimetry, and proteolysis. As will be appreciated by those skilled in the art, the kinetic parameters of the folding and unfolding transitions may also be monitored using these and other techniques. The solubility and overall structural integrity of an immunoglobulin may be quantitatively or qualitatively determined using a wide range of methods that are known in the art. Methods which may find use for characterizing the biophysical properties of immunoglobulins disclosed herein include gel electrophoresis, isoelectric focusing, capillary electrophoresis, chromatography such as size exclusion chromatography, ion-exchange chromatography, and reversed-phase high performance liquid chromatography, peptide mapping, oligosaccharide mapping, mass spectrometry, ultraviolet absorbance spectroscopy, fluorescence spectroscopy, circular dichroism spectroscopy, isothermal titration calorimetry, differential scanning calorimetry, analytical ultra-centrifugation, dynamic light scattering, proteolysis, and cross-linking, turbidity measurement, filter retardation assays, immunological assays, fluorescent dye binding assays, protein-staining assays, microscopy, and detection of aggregates via ELISA or other binding assay. Structural analysis employing X-ray crystallographic techniques and NMR spectroscopy may also find use. In one embodiment, stability and/or solubility may be measured by determining the amount of protein solution after some defined period of time. In this assay, the protein may or may not be exposed to some extreme condition, for example elevated temperature, low pH, or the presence of denaturant. Because function typically requires a stable, soluble, and/or well-folded/structured protein, the aforementioned functional and binding assays also provide ways to perform such a measurement. For example, a solution comprising an immunoglobulin could be assayed for its ability to bind target antigen, then exposed to elevated temperature for one or more defined periods of time, then assayed for antigen binding again. Because unfolded and aggregated protein is not expected to be capable of binding antigen, the amount of activity remaining provides a measure of the immunoglobulin's stability and solubility.

[197] In one embodiment, immunoglobulins may be tested using one or more cell-based or *in vitro* assays. For such assays, immunoglobulins, purified or unpurified, are typically added exogenously such that cells are exposed to immunoglobulins described herein. These assays are typically, but not always, based on the biology of the ability of the

immunoglobulin to bind to the target antigen and mediate some biochemical event, for example effector functions like cellular lysis, phagocytosis, ligand/receptor binding inhibition, inhibition of growth and/or proliferation, inhibition of calcium release and/or signaling, apoptosis and the like. Such assays often involve monitoring the response of cells to immunoglobulin, for example cell survival, cell death, cellular phagocytosis, cell lysis, change in cellular morphology, or transcriptional activation such as cellular expression of a natural gene or reporter gene. For example, such assays may measure the ability of immunoglobulins to elicit cell killing, for example ADCC, ADCP, and CDC. Assays that measure cellular killing that is mediated by co-engagement of antigens are particularly relevant for the invention. For some assays additional cells or components, that is in addition to the target cells, may need to be added, for example serum complement, or effector cells such as peripheral blood monocytes (PBMCs), NK cells, macrophages, T cells, and the like. Such additional cells may be from any organism, e.g., humans, mice, rat, rabbit, and monkey. Crosslinked or monomeric antibodies may cause apoptosis of certain cell lines expressing the antibody's target antigen, or they may mediate attack on target cells by immune cells which have been added to the assay. Methods for monitoring cell death or viability are known in the art, and include the use of dyes, fluorophores, immunochemical, cytochemical, and radioactive reagents. For example, caspase assays or annexin-fluorconjugates may enable apoptosis to be measured, and uptake or release of radioactive substrates (e.g. Chromium-51 release assays) or the metabolic reduction of fluorescent dyes such as alamar blue may enable cell growth, proliferation or activation to be monitored. In one embodiment, the DELFIA EuTDA-based cytotoxicity assay (Perkin Elmer, MA) is used. Alternatively, dead or damaged target cells may be monitored by measuring the release of one or more natural intracellular proteins, for example lactate dehydrogenase. Transcriptional activation may also serve as a method for assaying function in cell-based assays. In this case, response may be monitored by assaying for natural genes or proteins which may be upregulated or down-regulated, for example the release of certain interleukins may be measured, or alternatively readout may be via a luciferase or GFP-reporter construct. Cell-based assays may also involve the measure of morphological changes of cells as a response to the presence of an immunoglobulin. Cell types for such assays may be prokaryotic or eukaryotic, and a variety of cell lines that are known in the art may be employed. Alternatively, cell-based screens are performed using cells that have been transformed or transfected with nucleic acids encoding the immunoglobulins.

[198] In Vivo Experimentation

[199] The biological properties of the immunoglobulins disclosed herein may be characterized in cell, tissue, and whole organism experiments. As is known in the art, drugs

are often tested in animals, including but not limited to mice, rats, rabbits, dogs, cats, pigs, and monkeys, in order to measure a drug's efficacy for treatment against a disease or disease model, or to measure a drug's pharmacokinetics, toxicity, and other properties. Said animals may be referred to as disease models. With respect to the immunoglobulins disclosed herein, a particular challenge arises when using animal models to evaluate the potential for in-human efficacy of candidate polypeptides – this is due, at least in part, to the fact that immunoglobulins that have a specific effect on the affinity for a human Fc receptor may not have a similar affinity effect with the orthologous animal receptor. These problems can be further exacerbated by the inevitable ambiguities associated with correct assignment of true orthologues (Mechetina et al., 2002, Immunogenetics 54:463–468, incorporated entirely by reference), and the fact that some orthologues simply do not exist in the animal. Therapeutics are often tested in mice, including but not limited to nude mice, Rag-deficient mice, SCID mice, xenograft mice, and transgenic mice (including knockins and knockouts). For example, an immunoglobulin of the present invention that is intended as an anti-cancer therapeutic may be tested in a mouse cancer model, for example a xenograft mouse. In this method, a tumor or tumor cell line is grafted onto or injected into a mouse, and subsequently the mouse is treated with the therapeutic to determine the ability of the drug to reduce or inhibit cancer growth and metastasis. Therapeutic immunoglobulins herein can be tested in mouse strains NZB, NOD, BXSB, MRL/lpr, K/BxN and transgenics (including knockins and knockouts). Such mice can develop various autoimmune conditions that resemble human organ specific, systemic autoimmune or inflammatory disease pathologies such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). For example, an immunoglobulin disclosed herein intended for autoimmune diseases may be tested in such mouse models by treating the mice to determine the ability of the immunoglobulin to reduce or inhibit the development of the disease pathology. Because of the incompatibility between the mouse and human Fc γ receptor system, an alternative approach is to use a murine SCID model in which immune deficient mice are engrafted with human PBLs or PBMCs (huPBL-SCID, huPBMC-SCID) providing a semi-functional human immune system with human effector cells and Fc receptors. Other organisms, e.g., mammals, may also be used for testing. For example, because of their genetic similarity to humans, monkeys can be suitable therapeutic models, and thus may be used to test the efficacy, toxicity, pharmacokinetics, or other property of the immunoglobulins disclosed herein. Tests of the immunoglobulins disclosed herein in humans are ultimately required for approval as drugs, and thus of course these experiments are contemplated. Thus the immunoglobulins disclosed herein may be tested in humans to determine their therapeutic efficacy, toxicity, pharmacokinetics, and/or other clinical properties.

[200] In some embodiments, immunoglobulins disclosed herein may be assessed for efficacy in clinically relevant animal models of various human diseases. In many cases, relevant models include various transgenic animals for specific antigens and receptors.

[201] In one embodiment, the testing of immunoglobulins may include study of efficacy in primates (e.g. cynomolgus monkey model) to facilitate the evaluation of depletion of specific target cells harboring the target antigen. Additional primate models include but are not limited to use of the rhesus monkey to assess immunoglobulins in therapeutic studies of autoimmune, transplantation and cancer.

[202] Toxicity studies are performed to determine drug related-effects that cannot be evaluated in standard pharmacology profiles, or occur only after repeated administration of the agent. Most toxicity tests are performed in two species – a rodent and a non-rodent – to ensure that any unexpected adverse effects are not overlooked before new therapeutic entities are introduced into man. In general, these models may measure a variety of toxicities including genotoxicity, chronic toxicity, immunogenicity, reproductive/developmental toxicity and carcinogenicity. Included within the aforementioned parameters are standard measurement of food consumption, bodyweight, antibody formation, clinical chemistry, and macro- and microscopic examination of standard organs/tissues (e.g. cardiotoxicity). Additional parameters of measurement are injection site trauma and the measurement of neutralizing antibodies, if any. Traditionally, monoclonal antibody therapeutics, naked or conjugated, is evaluated for cross-reactivity with normal tissues, immunogenicity/antibody production, conjugate or linker toxicity and “bystander” toxicity of radiolabelled species. Nonetheless, such studies may have to be individualized to address specific concerns and following the guidance set by ICH S6 (Safety studies for biotechnological products, also noted above). As such, the general principles are that the products are sufficiently well characterized, impurities/contaminants have been removed, that the test material is comparable throughout development, and that GLP compliance is maintained.

[203] The pharmacokinetics (PK) of the immunoglobulins disclosed herein may be studied in a variety of animal systems, with the most relevant being non-human primates such as the cynomolgus and rhesus monkeys. Single or repeated i.v./s.c. administrations over a dose range of 6000-fold (0.05-300 mg/kg) can be evaluated for half-life (days to weeks) using plasma concentration and clearance. Volume of distribution at a steady state and level of systemic absorbance can also be measured. Examples of such parameters of measurement generally include maximum observed plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), the area under the plasma concentration-time curve from time 0 to infinity [AUC(0-inf)] and apparent elimination half-life (T_{1/2}). Additional measured parameters could include

compartmental analysis of concentration-time data obtained following i.v. administration and bioavailability.

[204] Pharmacodynamic studies may include, but are not limited to, targeting specific cells or blocking signaling mechanisms, measuring inhibition of antigen-specific antibodies etc. The immunoglobulins disclosed herein may target particular effector cell populations and thereby be direct drugs to induce certain activities to improve potency or to increase penetration into a particularly favorable physiological compartment. Such pharmacodynamic effects may be demonstrated in animal models or in humans.

[205] Clinical Use

[206] The immunoglobulins disclosed herein may find use in a wide range of products. In one embodiment an immunoglobulin disclosed herein is a therapeutic, a diagnostic, or a research reagent. The immunoglobulins may find use in a composition that is monoclonal or polyclonal. The immunoglobulins disclosed herein may be used for therapeutic purposes. As will be appreciated by those in the art, the immunoglobulins disclosed herein may be used for any therapeutic purpose that antibodies, Fc fusions, and the like may be used for. The immunoglobulins may be administered to a patient to treat disorders including but not limited to cancer, infectious diseases, autoimmune and inflammatory diseases.

[207] A “patient” for the purposes disclosed herein includes both humans and other animals, e.g., other mammals. Thus the immunoglobulins disclosed herein have both human therapy and veterinary applications. The term “treatment” or “treating” as disclosed herein is meant to include therapeutic treatment, as well as prophylactic, or suppressive measures for a disease or disorder. Thus, for example, successful administration of an immunoglobulin prior to onset of the disease results in treatment of the disease. As another example, successful administration of an optimized immunoglobulin after clinical manifestation of the disease to combat the symptoms of the disease comprises treatment of the disease. “Treatment” and “treating” also encompasses administration of an optimized immunoglobulin after the appearance of the disease in order to eradicate the disease. Successful administration of an agent after onset and after clinical symptoms have developed, with possible abatement of clinical symptoms and perhaps amelioration of the disease, comprises treatment of the disease. Those “in need of treatment” include mammals already having the disease or disorder, as well as those prone to having the disease or disorder, including those in which the disease or disorder is to be prevented.

[208] In one embodiment, an immunoglobulin disclosed herein is administered to a patient having a disease involving inappropriate expression of a protein or other molecule. Within the scope disclosed herein this is meant to include diseases and disorders characterized by

aberrant proteins, due for example to alterations in the amount of a protein present, protein localization, posttranslational modification, conformational state, the presence of a mutant or pathogen protein, etc. Similarly, the disease or disorder may be characterized by alterations molecules including but not limited to polysaccharides and gangliosides. An overabundance may be due to any cause, including but not limited to overexpression at the molecular level, prolonged or accumulated appearance at the site of action, or increased activity of a protein relative to normal. Included within this definition are diseases and disorders characterized by a reduction of a protein. This reduction may be due to any cause, including but not limited to reduced expression at the molecular level, shortened or reduced appearance at the site of action, mutant forms of a protein, or decreased activity of a protein relative to normal. Such an overabundance or reduction of a protein can be measured relative to normal expression, appearance, or activity of a protein, and said measurement may play an important role in the development and/or clinical testing of the immunoglobulins disclosed herein.

[209] The immunoglobulins herein may be used to treat cancer. By "cancer" and "cancerous" herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma), neuroendocrine tumors, mesothelioma, schwannoma, meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies.

[210] More particular examples of such cancers include hematologic malignancies, such as Hodgkin's lymphoma; non-Hodgkin's lymphomas (Burkitt's lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, mycosis fungoides, mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma, hairy cell leukemia and lymphoplasmacytic leukemia), tumors of lymphocyte precursor cells, including B-cell acute lymphoblastic leukemia/lymphoma, and T-cell acute lymphoblastic leukemia/lymphoma, thymoma, tumors of the mature T and NK cells, including peripheral T-cell leukemias, adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia, Langerhans cell histiocytosis, myeloid neoplasias such as acute myelogenous leukemias, including AML with maturation, AML without differentiation, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemias, myelodysplastic syndromes, and chronic myeloproliferative disorders, including chronic myelogenous leukemia; tumors of the central nervous system such as glioma, glioblastoma, neuroblastoma, astrocytoma, medulloblastoma, ependymoma, and retinoblastoma; solid tumors of the head and neck (eg. nasopharyngeal cancer, salivary gland carcinoma, and esophagael cancer), lung (eg. small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung), digestive system (eg.

gastric or stomach cancer including gastrointestinal cancer, cancer of the bile duct or biliary tract, colon cancer, rectal cancer, colorectal cancer, and anal carcinoma), reproductive system (eg. testicular, penile, or prostate cancer, uterine, vaginal, vulval, cervical, ovarian, and endometrial cancer), skin (eg. melanoma, basal cell carcinoma, squamous cell cancer, actinic keratosis), liver (eg. liver cancer, hepatic carcinoma, hepatocellular cancer, and hepatoma), bone (eg. osteoclastoma, and osteolytic bone cancers) additional tissues and organs (eg. pancreatic cancer, bladder cancer, kidney or renal cancer, thyroid cancer, breast cancer, cancer of the peritoneum, and Kaposi's sarcoma), and tumors of the vascular system (eg. angiosarcoma and hemangiopericytoma).

[211] The immunoglobulins herein may be used to treat autoimmune diseases. By "autoimmune diseases" herein include allogenic islet graft rejection, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, antineutrophil cytoplasmic autoantibodies (ANCA), autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune myocarditis, autoimmune neutropenia, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, autoimmune urticaria, Behcet's disease, bullous pemphigoid, cardiomyopathy, Castleman's syndrome, celiac spruce-dermatitis, chronic fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, factor VIII deficiency, fibromyalgia-fibromyositis, glomerulonephritis, Grave's disease, Guillain-Barre, Goodpasture's syndrome, graft-versus-host disease (GVHD), Hashimoto's thyroiditis, hemophilia A, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, IgM polyneuropathies, immune mediated thrombocytopenia, juvenile arthritis, Kawasaki's disease, lichen plantus, lupus erthematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, type 1 diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Reynauld's phenomenon, Reiter's syndrome, rheumatoid arthritis, sarcoidosis, scleroderma, Sjorgen's syndrome, solid organ transplant rejection, stiff-man syndrome, systemic lupus erythematosus, takayasu arteritis, temporal arteritis / giant cell arteritis, thrombotic thrombocytopenia purpura, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegner's granulomatosis.

[212] The immunoglobulins herein may be used to treat inflammatory disorders. By "inflammatory disorders" herein include acute respiratory distress syndrome (ARDS), acute

septic arthritis, adjuvant arthritis, juvenile idiopathic arthritis, allergic encephalomyelitis, allergic rhinitis, allergic vasculitis, allergy, asthma, atherosclerosis, chronic inflammation due to chronic bacterial or viral infection, chronic obstructive pulmonary disease (COPD), coronary artery disease, encephalitis, inflammatory bowel disease, inflammatory osteolysis, inflammation associated with acute and delayed hypersensitivity reactions, inflammation associated with tumors, peripheral nerve injury or demyelinating diseases, inflammation associated with tissue trauma such as burns and ischemia, inflammation due to meningitis, multiple organ injury syndrome, pulmonary fibrosis, sepsis and septic shock, Stevens-Johnson syndrome, undifferentiated arthropathy, and undifferentiated spondyloarthropathy.

[213] Some autoimmune and inflammatory diseases that may be targeted by the immunoglobulins disclosed herein include Systemic Lupus Erythematosus, Rheumatoid arthritis, Sjogren's syndrome, Multiple sclerosis, Idiopathic thrombocytopenic purpura (ITP), Graves disease, Inflammatory bowel disease, Psoriasis, Type I diabetes, and Asthma.

[214] The immunoglobulins herein may be used to treat infectious diseases. By "infectious diseases" herein include diseases caused by pathogens such as viruses, bacteria, fungi, protozoa, and parasites. Infectious diseases may be caused by viruses including adenovirus, cytomegalovirus, dengue, Epstein-Barr, hanta, hepatitis A, hepatitis B, hepatitis C, herpes simplex type I, herpes simplex type II, human immunodeficiency virus, (HIV), human papilloma virus (HPV), influenza, measles, mumps, papova virus, polio, respiratory syncytial virus, rinderpest, rhinovirus, rotavirus, rubella, SARS virus, smallpox, viral meningitis, and the like. Infectious diseases may also be caused by bacteria including *Bacillus anthracis*, *Borrelia burgdorferi*, *Campylobacter jejuni*, *Chlamydia trachomatis*, *Clostridium botulinum*, *Clostridium tetani*, Diphtheria, *E. coli*, *Legionella*, *Helicobacter pylori*, *Mycobacterium rickettsia*, *Mycoplasma nesissaria*, Pertussis, *Pseudomonas aeruginosa*, *S. pneumonia*, *Streptococcus*, *Staphylococcus*, *Vibria cholerae*, *Yersinia pestis*, and the like. Infectious diseases may also be caused by fungi such as *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Candida albicans*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Penicillium marneffeii*, and the like. Infectious diseases may also be caused by protozoa and parasites such as chlamydia, kokzidioa, leishmania, malaria, rickettsia, trypanosoma, and the like.

[215] Furthermore, immunoglobulins disclosed herein may be used to prevent or treat additional conditions including but not limited to heart conditions such as congestive heart failure (CHF), myocarditis and other conditions of the myocardium; skin conditions such as rosecea, acne, and eczema; bone and tooth conditions such as bone loss, osteoporosis, Paget's disease, Langerhans' cell histiocytosis, periodontal disease, disuse osteopenia, osteomalacia, monostotic fibrous dysplasia, polyostotic fibrous dysplasia, bone metastasis,

bone pain management, humoral malignant hypercalcemia, periodontal reconstruction, spinal cord injury, and bone fractures; metabolic conditions such as Gaucher's disease; endocrine conditions such as Cushing's syndrome; and neurological and neurodegenerative conditions such as Alzheimer's disease.

[216] Formulation

[217] Pharmaceutical compositions are contemplated wherein an immunoglobulin disclosed herein and one or more therapeutically active agents are formulated. Formulations of the immunoglobulins disclosed herein are prepared for storage by mixing said immunoglobulin having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980, incorporated entirely by reference), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; sweeteners and other flavoring agents; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; additives; coloring agents; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). In one embodiment, the pharmaceutical composition that comprises the immunoglobulin disclosed herein may be in a water-soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

"Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Some embodiments include at least one of the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. The formulations to be used for in vivo administration may be sterile. This is readily accomplished by filtration through sterile filtration membranes or other methods.

[218] The immunoglobulins disclosed herein may also be formulated as immunoliposomes. A liposome is a small vesicle comprising various types of lipids, phospholipids and/or surfactant that is useful for delivery of a therapeutic agent to a mammal. Liposomes containing the immunoglobulin are prepared by methods known in the art. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[219] The immunoglobulin and other therapeutically active agents may also be entrapped in microcapsules prepared by methods including but not limited to coacervation techniques, interfacial polymerization (for example using hydroxymethylcellulose or gelatin-microcapsules, or poly-(methylmethacrylate) microcapsules), colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), and macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980, incorporated entirely by reference. Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymer, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron Depot® (which are injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), poly-D-(-)-3-hydroxybutyric acid, and ProLease® (commercially available from Alkermes), which

is a microsphere-based delivery system composed of the desired bioactive molecule incorporated into a matrix of poly-DL-lactide-co-glycolide (PLG).

[220] Administration

[221] Administration of the pharmaceutical composition comprising an immunoglobulin disclosed herein, e.g., in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to orally, subcutaneously, intravenously, intranasally, intraotically, transdermally, topically (e.g., gels, salves, lotions, creams, etc.), intraperitoneally, intramuscularly, intrapulmonary, vaginally, parenterally, rectally, or intraocularly. In some instances, for example for the treatment of wounds, inflammation, etc., the immunoglobulin may be directly applied as a solution or spray. As is known in the art, the pharmaceutical composition may be formulated accordingly depending upon the manner of introduction.

[222] Subcutaneous administration may be used in circumstances where the patient may self-administer the pharmaceutical composition. Many protein therapeutics are not sufficiently potent to allow for formulation of a therapeutically effective dose in the maximum acceptable volume for subcutaneous administration. This problem may be addressed in part by the use of protein formulations comprising arginine-HCl, histidine, and polysorbate. Immunoglobulins disclosed herein may be more amenable to subcutaneous administration due to, for example, increased potency, improved serum half-life, or enhanced solubility. As is known in the art, protein therapeutics are often delivered by IV infusion or bolus. The immunoglobulins disclosed herein may also be delivered using such methods. For example, administration may be by intravenous infusion with 0.9% sodium chloride as an infusion vehicle.

[223] Pulmonary delivery may be accomplished using an inhaler or nebulizer and a formulation comprising an aerosolizing agent. For example, AERx® inhalable technology commercially available from Aradigm, or Inhance™ pulmonary delivery system commercially available from Nektar Therapeutics may be used. Furthermore, immunoglobulins disclosed herein may be amenable to oral delivery.

[224] In addition, any of a number of delivery systems are known in the art and may be used to administer the immunoglobulins disclosed herein. Examples include, but are not limited to, encapsulation in liposomes, microparticles, microspheres (e.g., PLA/PGA microspheres), and the like. Alternatively, an implant of a porous, non-porous, or gelatinous material, including membranes or fibers, may be used. Sustained release systems may comprise a polymeric material or matrix such as polyesters, hydrogels, poly(vinylalcohol), polylactides, copolymers of L-glutamic acid and ethyl-L-gutamate,

ethylene-vinyl acetate, lactic acid-glycolic acid copolymers such as the Lupron Depot®, and poly-D-(-)-3-hydroxybutyric acid. It is also possible to administer a nucleic acid encoding an immunoglobulin disclosed herein, for example by retroviral infection, direct injection, or coating with lipids, cell surface receptors, or other transfection agents. In all cases, controlled release systems may be used to release the immunoglobulin at or close to the desired location of action.

[225] Dosing

[226] The dosing amounts and frequencies of administration are, in one embodiment, selected to be therapeutically or prophylactically effective. As is known in the art, adjustments for protein 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.

[227] The concentration of the therapeutically active immunoglobulin in the formulation may vary from about 0.1 to 100 weight %. In one embodiment, the concentration of the immunoglobulin is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically effective dose of the immunoglobulin disclosed herein may be administered. By “therapeutically effective dose” herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. Dosages may range from 0.0001 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight. In one embodiment, dosages range from 1 to 10mg/kg.

[228] In some embodiments, only a single dose of the immunoglobulin is used. In other embodiments, multiple doses of the immunoglobulin are administered. The elapsed time between administrations may be less than 1 hour, about 1 hour, about 1-2 hours, about 2-3 hours, about 3-4 hours, about 6 hours, about 12 hours, about 24 hours, about 48 hours, about 2-4 days, about 4-6 days, about 1 week, about 2 weeks, or more than 2 weeks.

[229] In other embodiments the immunoglobulins disclosed herein are administered in metronomic dosing regimes, either by continuous infusion or frequent administration without extended rest periods. Such metronomic administration may involve dosing at constant intervals without rest periods. Typically such regimens encompass chronic low-dose or continuous infusion for an extended period of time, for example 1-2 days, 1-2 weeks, 1-2 months, or up to 6 months or more. The use of lower doses may minimize side effects and the need for rest periods.

[230] In certain embodiments the immunoglobulins disclosed herein and one or more other prophylactic or therapeutic agents are cyclically administered to the patient. Cycling therapy involves administration of a first agent at one time, a second agent at a second time, optionally additional agents at additional times, optionally a rest period, and then repeating this sequence of administration one or more times. The number of cycles is typically from 2 – 10. Cycling therapy may reduce the development of resistance to one or more agents, may minimize side effects, or may improve treatment efficacy.

[231] Combination Therapies

[232] The immunoglobulins disclosed herein may be administered concomitantly with one or more other therapeutic regimens or agents. The additional therapeutic regimes or agents may be used to improve the efficacy or safety of the immunoglobulin. Also, the additional therapeutic regimes or agents may be used to treat the same disease or a comorbidity rather than to alter the action of the immunoglobulin. For example, an immunoglobulin disclosed herein may be administered to the patient along with chemotherapy, radiation therapy, or both chemotherapy and radiation therapy.

[233] The terms “in combination with” and “co-administration” are not limited to the administration of said prophylactic or therapeutic agents at exactly the same time. Instead, it is meant that the immunoglobulin disclosed herein and the other agent or agents are administered in a sequence and within a time interval such that they may act together to provide a benefit that is increased versus treatment with only either the immunoglobulin disclosed herein or the other agent or agents. In some embodiments, immunoglobulins disclosed herein and the other agent or agents act additively, and sometimes synergistically. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The skilled medical practitioner can determine empirically, or by considering the pharmacokinetics and modes of action of the agents, the appropriate dose or doses of each therapeutic agent, as well as the appropriate timings and methods of administration.

[234] The immunoglobulin disclosed herein may be administered in combination with one or more other prophylactic or therapeutic agents, including but not limited to cytotoxic agents, chemotherapeutic agents, antibiotics, antifungal agents, antiviral agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, immunostimulatory agents, immunosuppressive agents, agents that promote proliferation of hematological cells, angiogenesis inhibitors, protein tyrosine kinase (PTK) inhibitors, other antibodies, Fc fusions, or immunoglobulins, or other therapeutic agents. The therapies of the invention may be combined with other immunotherapies. The

therapies of the invention may be combined with antagonists of chemokines or cytokines, including but not limited to antibodies and Fc fusions.

[235] The immunoglobulins disclosed herein may be combined with other therapeutic regimens. For example, in one embodiment, the patient to be treated with an immunoglobulin disclosed herein may also receive radiation therapy. Radiation therapy can be administered according to protocols commonly employed in the art and known to the skilled artisan. Such therapy includes but is not limited to cesium, iridium, iodine, or cobalt radiation. The radiation therapy may be whole body irradiation, or may be directed locally to a specific site or tissue in or on the body, such as the lung, bladder, or prostate. Optionally, the radiation therapy may be administered as a single dose or as multiple, sequential doses. The skilled medical practitioner can determine empirically the appropriate dose or doses of radiation therapy useful herein. In accordance with another, an immunoglobulin disclosed herein and one or more other anti-cancer therapies are employed to treat cancer cells *ex vivo*. It is contemplated that such *ex vivo* treatment may be useful in bone marrow transplantation and particularly, autologous bone marrow transplantation. For instance, treatment of cells or tissue(s) containing cancer cells with immunoglobulin and one or more other anti-cancer therapies, such as described above, can be employed to deplete or substantially deplete the cancer cells prior to transplantation in a recipient patient. It is of course contemplated that the immunoglobulins disclosed herein may employ in combination with still other therapeutic techniques such as surgery.

[236] All cited references are herein expressly incorporated by reference in their entirety.

[237] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

EXAMPLES

[238] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation.

[239]

[240] Example 1. Design of Heterodimeric Fc Regions

[241] Variants that promote Fc heterodimerization and disfavor homodimerization were designed using a combination of computational structure-based and rational protein

engineering approaches. Computational structure-based methods (PDA® technology) were used to evaluate possible amino acid substitutions in the CH3 region for their ability to stabilize Fc heterodimers and destabilize Fc homodimers. Amino acid energies for selected pairs of positions were calculated in the context of the heterodimer (both variant amino acids) and the two homodimers (variant amino acid at one position and the wild-type amino acid at the other). Energies were normalized against the calculated energy of the wild-type amino acid pair. The preference for the heterodimeric species was calculated using the following two equations:

[242] Heterodimer Preference 1 =

$$E(\text{heterodimer}) - \max [E(\text{homodimer 1}), E(\text{homodimer 2})]$$

[243] Heterodimer Preference 2 =

$$E(\text{heterodimer}) - \min [E(\text{homodimer 1}), E(\text{homodimer 2})]$$

[244] Of particular interest are those variants for which one or both of the heterodimer preferences are negative. This indicates that the heterodimer is predicted to be more stable than one or both of the homodimers.

[245] Example 2. Screening of Variant Fc Regions for Fc Heterodimerization

[246] In order to screen for Fc variants that favor heterodimerization, an empty-Fc/scFv-Fc system was developed as a test system, illustrated in Figure 1. DNA constructs were designed encoding two different immunoglobulin polypeptides: (i) scFv-Fc (VH-linker-Vk-Hinge-CH2-CH3') and (ii) empty-Fc (Hinge-CH2-CH3"). Here the two different CH3 domains, CH3' (prime) and CH3" (double prime), represent the different variant versions that were designed to promote heterodimer formation and discourage homodimer formation. As shown in Figure 1, cotransfection and expression of these two polypeptides results in three possible dimers. Each of the three dimers is composed of a unique number of immunoglobulin (Ig) domains and thus unique molecular weights. Consequently, the population of each species can be visualized using gel electrophoresis. Figure 2 provides amino acid sequences of the scFv-Fc and empty-Fc constructs, along with sequences of native IgG's.

[247] The scFv-Fc/empty-Fc system was tested using wild-type IgG1 Fc regions and an scFv derived from a murine anti-CD3 antibody OKT3 (Kjer-Nielsen et al., 2004, Proc Natl Acad Sci USA 101:7675-7680). DNA encoding the OKT3 scFv was generated by gene synthesis (Integrated DNA Technologies). All constructs were subcloned into the expression vector pTT5 (Durocher et al., 2002, Nucleic acids research 30: E9) using standard molecular biology techniques. scFv-Fc and empty-Fc constructs with native IgG1 Fc regions were

cotransfected into HEK293E cells (Durocher et al., 2002, Nucleic Acids Research 30: E9) for expression, and resulting protein was purified from the supernatant using protein A affinity chromatography. The protein species generated were visualized electrophoretically using an Agilent 2100 Bioanalyzer microfluidics-based platform (Agilent Technologies). Agilent chips for SDS-PAGE were run on the BioAnalyzer using kit# 5067-1517, lot# NA06BP01 containing gels, chips, and buffers, carried out using the manufacturer's instructions. Samples were run in duplicate, one under reducing and the other under non-reducing conditions. Results are shown in Figure 3. The 240 kilodalton (KDa) band in all of the gel lanes represents a protein in the loading buffer used as a normalization standard. Under reducing conditions (lanes 1-5), two bands were observed, corresponding to scFv-Fc monomer and empty-Fc monomer. Under non-reducing conditions (lanes 6-10), three bands were observed, corresponding to scFv-Fc homodimer, scFc-Fv/empty-Fc heterodimer, and empty-Fc homodimer. This result confirmed the ability to detect each of the different protein species, indicating the scFv-Fc/empty-Fc system provided a good system for quantification of the degree of heterodimerization from designed Fc variants.

[248] A DNA library encoding variant immunoglobulin polypeptides, designed as described above, was constructed in the scFv-Fc and empty-Fc constructs. Pairs of scFv-Fc and empty-Fc constructs were cotransfected into HEK293E cells for expression, and resulting proteins were purified using protein A affinity chromatography. Relative concentrations of the expressed protein species were determined using the Agilent 2100 Bioanalyzer microfluidics-based platform, as described above. Resulting gels are shown in Figure 4. A quantitative summary of the results from this library is provided in Figure 5. A number of designed variants increased the content of heterodimer relative to that of the native parent Fc region.

[249] Using the information obtained from the previous library, a new library was designed and screened as described above. Quantitative electrophoretic results are shown in Figures 6 and 7. A number of designed variants increased the content of heterodimer relative to that of the native parent Fc region. Preferred and most preferred variants from this screen are listed in Tables 1 and 2 respectively.

Table 1. Preferred CH3 domain variants that favor Fc heterodimerization.

Variant 1	Variant 2
F405A	T394F
S364D	Y349K
S364E	L368K
S364E	Y349K
S364F	K370G
S364H	Y349K

S364H	Y349T
S364Y	K370G
T411K	K370E
V397S/F405A	T394F
K370R/T411K	K370E/T411E
L351E/S364D	Y349K/L351K
L351E/S364E	Y349K/L351K
L351E/T366D	L351K/T366K
P395T/V397S/F405A	T394F
S364D/K370G	S364Y/K370R
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364E/F405S	Y349K/T394Y
S364E/T411E	Y349K/D401K
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
Y349C/S364E	Y349K/S354C
L351E/S364D/F405A	Y349K/L351K/T394F
L351K/S364H/D401K	Y349T/L351E/T411E
S364E/T411E/F405A	Y349K/T394F/D401K
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K

Table 2. Most preferred CH3 domain variants that favor Fc heterodimerization.

Variant 1	Variant 2
F405A	T394F
S364D	Y349K
S364E	Y349K
S364H	Y349T
L351K	L351E
D401K	T411E
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
L351K/S364H/D401K	Y349T/L351E/T411E
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K

[250] Example 3. Description of mAb-Fv and mAb-Fab immunoglobulin formats

[251] Heterodimerization of distinct immunoglobulin heavy chains provides a novel beneficial approach to bispecific co-targeting of antigens. The capacity to co-engage multiple antigens, particularly the capacity to link binding of a target antigen to activation of a cellular receptor, offers the potential to enhance the clinical efficacy of antibody therapeutics, as well as engineer novel mechanisms of action. Yet an important property of bispecifics that engage many immune receptors is activation only in proximity to the primary or first target antigen. Biologically, the immune system accomplishes this naturally via monovalent binding

of an immune receptor to its cognate ligand, often at low affinity. Cellular activation occurs only by cross-linking, achieved typically by antibody/antigen immune complexes, or via effector cell to target cell engagement. For some immune receptors, for example the CD3 signaling receptor on T cells, activation only upon engagement with co-engaged target is critical, as nonspecific cross-linking can elicit a cytokine storm and toxicity (Perruche et al., 2009, J Immunol 183[2]:953-61). An engineering solution, as described here, is to engage such immune receptors monovalently rather than multivalently such that activation occurs in response to cross-linking only in the presence of the primary target antigen.

[252] Example 4. mAb-Fv and mAb-Fab immunoglobulins that monovalently co-engage FcγRIIIa

[253] The low affinity Fc gamma receptors (FcγRs) such as FcγRIIa, FcγRIIb, and FcγRIIIa bind monovalently to the antibody Fc region. Monovalent binding does not activate cells expressing these FcγRs; however, cell-to-cell contact leads to receptor cross-linking and clustering on the cell surface, leading to activation. For FcγRIIIa (CD16) expressed on natural killer (NK) cells, target cell killing is mediated by receptor cross-linking and cellular activation, which occurs when the effector cell engages the target cell in a highly avid format (Bowles & Weiner, 2005, J Immunol Methods 304:88-99, Márquez et al., 2010, Cell Immunol 264[1]:86-92; expressly incorporated by reference). In this regard, FcγRIIIa co-targeting is a particularly good application of the mAb-Fv format, which is capable of engaging target antigen bivalently while co-engaging FcγRIIIa monovalently.

[254] mAb-Fv and mAb-Fab immunoglobulins that co-target FcγRIIIa were constructed using the variable region of the anti-human FcγRIIIa antibody 3G8 as Fv-2. A variety of constructs were generated that target various primary antigens (antigen-1), including the solid tumor antigens Her2 and EGFR, and the myeloma antigen HM1.24. Antibody variable regions targeting these antigens were obtained from the anti-Her2 antibody trastuzumab (Carter et al., 1992, Proc Natl Acad Sci USA 89:4285-4289), a humanized version of the anti-EGFR antibody C225 (USSN11/004,054), and a humanized version of the anti-HM1.24 antibody HM1.24 (USSN11/857,310). The different constructs incorporated a variety of linker compositions and lengths, and a number of them incorporated hetero-Fc variants that favor heterodimerization. In addition, in order to engineer selectivity to FcγRIIIa relative to other Fc receptors, several constructs utilized knockout Fc variants that ablate binding to FcγRs and complement. In addition, some constructs incorporated detection/purification tags on the C-termini, including His-tags (HHHHHH) and Flag-tags (DYKDDDDK). The anti-FcγRIIIa (αFcγRIIIa) mAb-Fv and mAb-Fab constructs are listed in Table 3 along with a description of their relevant components. Each immunoglobulin is given an identification number (ID). Amino acid sequences of these immunoglobulins are listed in Figure 9. Also included in

Table 3 are control antibodies used as controls and comparators in the anti-Her2 experiments. These include an IgG1 version of trastuzumab, as well as variants with ablated (G236R/L328R) and enhanced (S239D/I332E) binding to FcγR's, including FcγRIIIa.

Table 3. mAb-Fv and mAb-Fab constructs and control antibodies for FcγRIIIa co-targeting

ID	Ag-1 x Ag-2	Fv-1 ^a	FcR Binding ^b	Hetero-Fc ^c	Linker ^d	Fv-2
9064	HER2 x FcγRIIIa	trastuzumab	Native IgG1	Homo	Linker 1	3G8
9074	HER2 x FcγRIIIa	trastuzumab	Native IgG1	Homo	Linker 2	3G8
9375	HER2 x FcγRIIIa	trastuzumab	Native IgG1	Hetero TF-HA	Linker 2	3G8
9387	EGFR x FcγRIIIa	humanized 225	Native IgG1	Hetero TF-HA	Linker 2	3G8
9516	HER2 x FcγRIIIa	trastuzumab	G236R/L328R	Hetero TF-HA	Linker 2	3G8
9517	EGFR x FcγRIIIa	hu 225	G236R/L328R	Hetero TF-HA	Linker 2	3G8
9518	HM1.24 x FcγRIIIa	hu αHM1.24	G236R/L328R	Hetero TF-HA	Linker 2	3G8
8427	HER2 only	trastuzumab	Native IgG1	Homo	None	None
8428	HER2 only	trastuzumab	S239D/I332E	Homo	None	None
8429	HER2 only	trastuzumab	G236R/L328R	Homo	None	None
4614	RSV	motavizumab	S239D/I332E	Homo	None	None

^a hu = humanized

^b G236R/L328R is an Fc knockout variant that ablates binding to all FcγRs and complement, and ablates FcγR- and complement- mediated effector function. S239D/I332E enhances affinity to activating FcγRs, and improves FcγR- mediated effector function.

^c Homo = Homodimeric native IgG Fc region. Hetero TF-HA = Y349T/T394F and S364H/F405A variant CH3 domains that favor heterodimerization and disfavor homodimerization.

^d Linker 1 = SSDKTHTSPSPGGGGSGGGG (SEQ ID NO. 43)
Linker 2 = SSDKTHTSPSPGGGGSGGGGSGGGGSGGGG (SEQ ID NO. 44)

[255] The immunoglobulins in Table 3 were constructed using a combination of gene synthesis (Blue Heron Biotechnology, Bothell, WA), PCR subcloning, quick-change mutagenesis, and other standard molecular biology methods. All DNA was constructed in the context of the expression vector pTT5 (Durocher et al., 2002, Nucleic acids research 30: E9) using standard molecular biology techniques. Heavy and light chains for each immunoglobulin were cotransfected into HEK293E cells (Durocher et al., 2002, Nucleic Acids Research 30: E9) for expression, and resulting protein was purified from the supernatant using protein A affinity chromatography.

[256] The proteins were visualized electrophoretically using the Agilent 2100 Bioanalyzer microfluidics-based platform (Agilent Technologies). Agilent chips for SDS-PAGE were run on the BioAnalyzer using kit# 5067-1517, lot# NA06BP01 containing gels, chips, and buffers,

carried out using the manufacturer's instructions. Samples were in duplicate, one under reducing and the other under non-reducing conditions. Representative gels are shown in Figure 10. As expected, under reducing conditions three bands were observed at the approximate calculated molecular weights, corresponding to the light chain (~25 KDa) and two heavy chains (~65 KDa) for each mAb-Fv. Under non-reducing conditions a single main band at ~180 KDa was observed for each mAb-Fv and ~200 KDa for the mAb-Fab, corresponding to the tetrameric immunoglobulin (two heavy chains, each complexed with a light chain). The results are consistent for the three anti-Fc γ R1IIa constructs co-targeting Her2, EGFR, and HM1.24, indicating the broad applicability of the mAb-Fv format for co-engaging targets.

[257] The immunoglobulins were analyzed using size-exclusion chromatography (SEC) to measure their size (more correctly, their hydrodynamic volume) and determine the native-like behavior of the purified samples. These experiments used an Agilent 1200 High-performance liquid chromatography (HPLC) system. Samples were injected onto a Superdex™ 200 10/300 GL column (model# 17-5175-01, lot# 10034171) (Amersham) at 0.5mLs/min using 1X PBS, pH 7.4 as the mobile phase. Results for select mAb-Fv samples are shown in Figure 11. A molecular weight standard was run (not shown). All of the mAb-Fv samples showed a main peak at an elution time that, based on the molecular weight standard, corresponded to the approximate molecular weight of the tetrameric mAb-Fv heterodimer heavy chain species (~180 KDa). The symmetry of the peaks and the relatively low populations of other species for all of the anti-Fc γ R1IIa mAb-Fv's (α Her2, α EGFR, and α HM1.24) indicated that the immunoglobulin format is robust.

[258] The 9516 anti-Her2 x anti-Fc γ R1IIa (α Her2 x α Fc γ R1IIa) mAb-Fv was constructed in the context of an IgG1 comprising the knockout Fc variant (Fc KO) G236R/L328R in order to engineer selective Fc γ R1IIa binding. In other words, binding of the Fc region to all of the Fc γ Rs and complement was ablated, and then only Fc γ R1IIa binding was built back as antigen-2 in the mAb-Fv format. Binding of 9516 and control antibodies (Native IgG1 and Fc KO) to the relevant human Fc γ R's was tested using surface plasmon resonance (Biacore®). Binding measurements were performed using a Biacore® 3000 instrument (Biacore®). Antibodies were captured onto an immobilized Protein A (Pierce) CM5 biosensor chip (Biacore®), generated using a standard primary amine coupling protocol. All measurements were performed in HBS-EP (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v surfactant P20), and glycine buffer (10 mM glycine-HCl, pH 1.5) was used for the Protein A surface regeneration. All antibodies (50 nM in HBS-EP) were immobilized on the Protein A surface. Fc receptors were injected over antibody bound surface, followed by a dissociation phase. After each cycle, the Protein A surface was regenerated by injecting glycine buffer

(pH 1.5). Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals (response of reference channel and injection of running buffer). The results in Figure 12 show that while native IgG1 binds to all of the Fc γ Rs, the Fc KO variant is ablated for binding to all of the Fc γ Rs. Similar to the Fc KO, the 9516 α Her2 x α Fc γ RIIIa mAb-Fv is ablated for binding to Fc γ RI, Fc γ RIIa, and Fc γ RIIb; however, in contrast to the knockout variant antibody, 9516 has strong affinity for Fc γ RIIIa, due to the engineered Fv-2 binding site. The kinetic off-rate of dissociation (k_{off}) for Fc γ RIIIa binding is slower than that of native IgG1, a consequence of the greater affinity of the 3G8 variable region for the receptor relative to the IgG1 Fc region.

[259] The neonatal Fc receptor (FcRn) protects IgG from degradation and is responsible in part for the long half-life (~21 days for IgG1) of antibodies in circulation. FcRn binds at a site on the Fc region separate from the binding site for Fc γ Rs, and the Fc KO variant G236R/L328R does not impact FcRn affinity. Binding to FcRn by the 9516 mAb-Fv and control antibodies was tested using a Biacore method similar to above, except that a Her2 antigen capture chip format was used (the protein A binding site on Fc overlaps with the site for FcRn). As can be seen in Figure 13, binding of the mAb-Fv to FcRn was uncompromised relative to native IgG1 and the Fc KO variant.

[260] Affinity of the mAb-Fv for Fc γ RIIIa was measured using Biacore®. The protein A capture format was used as described above, except that sensorgrams were obtained for a concentration series of receptor analyte in order to calculate an accurate affinity. Figure 14 shows Biacore® sensorgrams depicting the binding of various concentrations of the V158 isoform of Fc γ RIIIa to protein A-captured 9516 mAb-Fv. Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals (response of reference channel and injection of running buffer). The equilibrium constant was calculated by global fitting of binding data with a 1:1 Langmuir binding model using BIAevaluation software. The α Her2 x α Fc γ RIIIa mAb-Fv bound Fc γ RIIIa with an equilibrium constant of dissociation (K_d) of 110 nM, approximately 2.5-fold tighter than the 280 nM affinity of native IgG1 (Richards et al., 2008, Mol Cancer Ther 7[8]:2517-27), consistent with the slower dissociation observed relative to native IgG1 (Figure 12).

[261] Co-engagement of Her2 and Fc γ RIIIa by the 9516 mAb-Fv was confirmed using a Biacore experiment similar to as described above, but using Her2 capture instead of protein A. 100 nM concentration of the 9516 mAb-Fv was captured to a HER2 chip, followed by injection of 200 nM V158 Fc γ RIIIa analyte. Data were processed by zeroing time and response before the injection of 9516. The results in Figure 15 demonstrate the simultaneous co-engagement of Her2 by the Fv-1 region and Fc γ RIIIa by the Fv-2 region.

Engagement of Fc receptor is not due to binding by the Fc region, which due to the Fc KO variant is ablated for binding to all of the Fc receptors (Figure 12).

[262] Similar binding experiments were carried out for the α HM1.24 x α Fc γ R1IIa mAb-Fv. The protein A capture format was used with a receptor analyte concentration series to calculate the affinity of the 9518 mAb-Fv for V158 Fc γ R1IIa. Figure 16 shows Biacore® sensorgrams depicting the binding of receptor at various concentrations to protein A-captured 9518 mAb-Fv. Data were processed by zeroing time and response before the injection of receptor and by subtracting appropriate nonspecific signals. The equilibrium constant was calculated by global fitting of binding data with a 1:1 Langmuir binding model using BIAevaluation software. The α HM1.24 x α Fc γ R1IIa mAb-Fv bound V158 Fc γ R1IIa with an equilibrium constant of dissociation (Kd) of 96 nM, approximately 2.9-fold tighter than the 280 nM affinity of native IgG1 (Richards et al., 2008, Mol Cancer Ther 7[8]:2517-27).

[263] Co-engagement of HM1.24 and Fc γ R1IIa by the 9518 mAb-Fv was confirmed using the antigen capture Biacore® method as described above. 100 nM concentration of the 9518 mAb-Fv was captured to a HM1.24 chip, followed by injection of 200 nM V158 Fc γ R1IIa analyte. Data were processed by zeroing time and response before the injection of 9516. The results in Figure 17 demonstrate the simultaneous co-engagement of HM1.24 by the Fv-1 region and Fc γ R1IIa by the Fv-2 region.

[264] A cytotoxicity assay was carried out to test the capacity of the α Her2 x α Fc γ R1IIa mAb-Fv to mediate cellular killing against Her2-expressing cells. A native IgG1 version of the anti-Her2 antibody trastuzumab was run as a control, and an Fc variant version (S239D/I332E) with enhanced Fc γ R1IIa binding was also tested as a comparator. An anti-respiratory syncytial virus (RSV) antibody with the variable region of motavizumab, with the S239D/I332E Fc region served as a negative control for lack of binding to Her2. Human peripheral blood mononuclear cells (PBMC) were purified from leukopacks using a Ficoll™ gradient. Her2⁺ SK-BR-3 breast carcinoma cells were seeded into 96-well plates and opsonized with mAb-Fv or control/comparator antibodies at the indicated concentrations. Triton X-100 and PBMC alone were run as controls. Effector cells were added at 50:1 PBMC/target cells, and plates were incubated at 37 °C for 4 h. Cell lysis was measured by lactate dehydrogenase release using the Cytotox-ONE™ Homogeneous Membrane Integrity Assay (Promega). Cells were incubated with lactate dehydrogenase reaction mixture for 10 min., and fluorescence was measured using a Wallac Victor2 fluorometer (Perkin-Elmer). Fluorescence was normalized to minimal (PBMC alone) and maximal (Triton) lysis, and fit to a sigmoidal dose-response. The results are shown in Figure 18. The α Her2 x α Fc γ R1IIa mAb-Fv mediated a level of lysis substantially enhanced relative

to native IgG1 trastuzumab, and comparable to the Fc-enhanced variant S239D/I332E. The results confirm the high affinity FcγRIIIa (antigen-2) binding mediated by novel engineered Fv-2 binding site, and demonstrate the capacity of the mAb-Fv format for enabling selective and optimal functions.

[265] Example 5. mAb-Fv immunoglobulins that monovalently co-engage FcγRIIb

[266] The inhibitory receptor FcγRIIb downregulates cellular activation of B cells, monocytes, mast cells, and other immune cell types. On B cells, inhibitory activity is elicited only when FcγRIIb reengages into an immune complex with the cell surface B-cell receptor (BCR), a mechanism that is mediated by immune complexation of soluble IgG's with the same antigen that is recognized by the BCR (Heyman 2003, Immunol Lett 88[2]:157-161; Smith and Clatworthy, 2010, Nature Reviews Immunology 10:328-343; expressly incorporated by reference). We explored the applicability of the mAb-Fv format for engaging FcγRIIb monovalently while bivalently co-engaging an antigen in the BCR complex, specifically CD19. This immunomodulatory co-engagement mechanism is described in USSN 12/156,183, filed May 30, 2008, entitled "Methods and Compositions for Inhibiting CD32b Expressing Cells", herein expressly incorporated by reference. Other co-targets for the FcγRIIb-mediated immunomodulatory mechanism include other antigens involved with the BCR or components of its coreceptor complex, including for example CD21, CD22, CD72, CD79a, CD79b, and CD81, as described in USSN 12/156,183.

[267] mAb-Fv immunoglobulins that co-target FcγRIIb were constructed using as Fv-2 the variable region of 2B6, an antibody selective for the inhibitory receptor FcγRIIb as Fv-2 (Veri et al., 2007, Immunology 121:392-404). Targeting of CD19 antigen by Fv-1 utilized a humanized affinity matured version of the anti-CD19 antibody 4G7 described previously (USSN 12/156,183). The different constructs incorporated a gly-ser repeat linker, and knockout Fc variants in order to engineer selectivity to FcγRIIb relative to other Fc receptors. The mAb-Fv constructs utilized either native CH3 domains (ID 9506) or hetero-Fc variants ID 9547). The anti-FcγRIIb (αFcγRIIb) mAb-Fv and constructs are listed in Table 4 along with a description of their relevant components. Amino acid sequences of these immunoglobulins are listed in Figure 19. Also included in Table 4 is a control antibody (ID 6187) containing the knockout variant used in these experiments.

Table 4. mAb-Fv constructs and control antibodies for FcγRIIb co-targeting

ID	Ag-1 x Ag-2	Fv-1 ^a	FcR Binding ^b	Hetero-Fc ^c	Linker ^d	Fv-2
9506	CD19 x FcγRIIb	hu 4G7	G236R/L328R	Homo	Linker 3	2B6
9547	CD19 x FcγRIIb	hu 4G7	G236R/L328R	Hetero TF-HA	Linker 3	2B6
6187	CD19 only	hu 4G7	G236R/L328R	Homo	None	None

^a hu = humanized

^b G236R/L328R is an Fc knockout variant that ablates binding to all FcγRs and complement, and ablates FcγR- and complement- mediated effector function.

^c Homo = Homodimeric native IgG Fc region. Hetero TF-HA = Y349T/T394F and S364H/F405A variant CH3 domains that favor heterodimerization and disfavor homodimerization.

^d Linker 3 = GGGGSGGGGSGGGGSGGGGSGGGGSGGGG (SEQ ID NO. 45)

[268] The immunoglobulins in Table 4 were constructed as described above in the pTT5 expression vector. Heavy and light chains for each immunoglobulin were cotransfected into HEK293E cells for expression, and resulting protein was purified from the supernatant using protein A affinity chromatography, as described above.

[269] The proteins were analyzed using electrophoresis using the Agilent Bioanalyzer as described above. SDS-PAGE results are shown in Figure 20. Although the 9506 construct comprising the homodimeric Fc region expressed poorly, faint bands could nonetheless be observed approximately at the expected molecular weights of the light chain (~25 KDa) and two heavy chains (~65 KDa). Likewise, under non-reducing conditions a single main band was observed for at the expected molecular weight of the tetrameric protein (two heavy chains, each complexed with a light chain, ~180 KDa). Proper assembly of the mAb-Fv was supported by the SEC results (data not shown). The 9547 construct was identical 9506 except that it contained the Fc variants Y349T/T394F on one heavy chain and S364H/F405A on the other heavy chain to favor heterodimerization. Multiple bands were observed for 9547 in the SDS-PAGE gels. Bands were observed approximately at the expected molecular weights of the light chain and two heavy chains, and under non-reducing conditions a band was observed at the expected molecular weight of the tetrameric immunoglobulin. Assembly of the mAb-Fv was supported by the presence of an SEC peak that corresponded to the approximate molecular weight of the tetrameric mAb-Fv heterodimer species (data not shown). The size of the additional band in the non-reducing gel suggested a significant population of a monomeric heavy chain / light chain pair (~90 KDa). Thus due to the hetero-Fc variants, heavy chain monomers (complexed with light chain) are observed rather than homodimeric heavy chain species. Based on the intensity of the bands in the gel, this result appears to be due to differential expression between the two heavy chains. We suspect the poor expression of one of the heavy chains relative to the other is due to a glycosylation site in the 2B6 VL region (USSN12/349,876). Regardless, the results from the gels and SEC indicate a significant population of the designed heterodimeric mAb-Fv species.

[270] Affinity of the 9547 α CD19 x α Fc γ R11b mAb-Fv was measured using Biacore® as described above. Figure 21 shows Biacore sensorgrams depicting the binding of various concentrations of Fc γ R11b to protein A-captured 9547 mAb-Fv. Data were processed and fitted as described above. The α CD19 x α Fc γ R11b mAb-Fv bound Fc γ R11b with a K_d of 44 nM, approximately 52-fold tighter than the 2.3 μ M affinity of native IgG1 (Richards et al., 2008, Mol Cancer Ther 7[8]:2517-27). Because 9547 was constructed in the context of the knockout Fc variant G236R/L328R, binding to of the mAb-Fv is selective for Fc γ R11b, i.e. it binds the inhibitory receptor Fc γ R11b with high affinity but does not bind any of the activating Fc γ Rs.

[271] The 9547 mAb-Fv and 6187 control antibody were assayed for capacity to inhibit BCR-mediated B cell activation as a result of BCR/Fc γ R11b co-engagement. This mechanism of B cell inhibition has been described previously (USSN 12/156,183). Intracellular calcium mobilization was used as a quantitative measure of BCR-mediated B cell activation. Intracellular free calcium concentration ([Ca²⁺]) was measured by flow cytometry using a Fluo-4 NW calcium assay (Molecular Probes, Eugene, OR). PBMCs were resuspended in calcium assay buffer and pre-loaded with Fluo-4 dye for 30 min at room temperature. After incubation with immunoglobulin of interest, cells were stimulated by addition of 10 μ g/ml of anti-CD79b antibody. Calcium flux kinetics was recorded using a FACSCanto II flow cytometer and data were analyzed using FlowJo software (Tree Star, Ashland, OR). The data are plotted as the change of Mean Fluo-4 Intensity (MFI) over time. The results are shown in Figure 22. 9547 α CD19 x α Fc γ R11b mAb-Fv inhibited calcium mobilization induced by BCR crosslinking relative to the 6187 Fc KO α CD19 antibody. This activity is attributed to the high affinity (44 nM) Fc γ R11b co-engagement of the inhibitory receptor with BCR-associated CD19 antigen. The result further demonstrates the capacity of the mAb-Fv format for enabling selective and novel functional activities.

[272] Example 6. mAb-Fv immunoglobulins that monovalently co-engage CD3

[273] CD3 activation of T-cells occurs only when its associated T-cell receptor (TCR) engages antigen-loaded MHC on antigen presenting cells in a highly avid cell-to-cell synapse (Kuhns et al., 2006, Immunity 24:133-139). Because cross-linking of CD3 using an anti-CD3 antibody elicits a cytokine storm and toxicity (Perruche et al., 2009, J Immunol 183[2]:953-61; Chatenoud & Bluestone, 2007, Nature Reviews Immunology 7:622-632; expressly incorporated by reference), for practical clinical use, the preferred mode of CD3 co-engagement for redirected killing of targets cells is monovalent binding that results in activation only upon engagement with the co-engaged primary target. The mAb-Fv format is particularly well-suited for this mechanistic approach. We explored the applicability of the

mAb-Fv format for engaging CD3 monovalently while bivalently co-engaging tumor cell antigens.

[274] mAb-Fv immunoglobulins that co-target CD3 were constructed using as the Fv-2 variable region a humanized version of the anti-CD3 antibody OKT3. Fv-1 regions targeted the antigens Her2, HM1.24, and CD19 using the variable regions described above. A variety of constructs were generated co-targeting these primary antigens bivalently and CD3 monovalently that incorporated different linker compositions and lengths, hetero-Fc variants that favor heterodimerization, and knockout Fc variants to ablate binding to Fc γ Rs and complement. Constructs also incorporated His- and Flag- tags at the C-termini as potential detection/purification tags. The α CD3 mAb-Fv constructs are listed in Table 5 along with a description of their relevant components. Amino acid sequences of these immunoglobulins are listed in Figure 23.

Table 5. mAb-Fv constructs and control antibodies for CD3 co-targeting

ID	Ag-1 x Ag-2	Fv-1 ^a	FcR Binding ^b	Hetero-Fc ^c	Linker ^d	Fv-2 ^a
9344	HER2 x CD3	trastuzumab	Native IgG1	Homo	Linker 4	hu OKT3
9376	HER2 x CD3	trastuzumab	Native IgG1	Hetero TF-HA	Linker 4	hu OKT3
9509	HER2 x CD3	trastuzumab	G236R/L328R	Homo	Linker 3	hu OKT3
9510	HM1.24 x CD3	hu α HM1.24	G236R/L328R	Homo	Linker 3	hu OKT3
9511	CD19 x CD3	hu 4G7	G236R/L328R	Homo	Linker 3	hu OKT3
9550	HER2 x CD3	trastuzumab	G236R/L328R	Hetero TF-HA	Linker 3	hu OKT3
9551	HM1.24 x CD3	hu α HM1.24	G236R/L328R	Hetero TF-HA	Linker 3	hu OKT3
9552	CD19 x CD3	hu 4G7	G236R/L328R	Hetero TF-HA	Linker 3	hu OKT3
8427	HER2 only	trastuzumab	Native IgG1	Homo	None	None
5627	HM1.24 only	hu α HM1.24	Native IgG1	Homo	None	None
6166	HM1.24 only	hu α HM1.24	G236R/L328R	Homo	None	None

^a hu = humanized

^b G236R/L328R is an Fc knockout variant that ablates binding to all Fc γ Rs and complement, and ablates Fc γ R- and complement- mediated effector function.

^c Homo = Homodimeric native IgG Fc region. Hetero TF-HA = Y349T/T394F and S364H/F405A variant CH3 domains that favor heterodimerization and disfavor homodimerization.

^d Linker 3 = GGGGSGGGGSGGGGSGGGGSGGGGSGGGG (SEQ ID NO. 45)
Linker 4 = SSDKTHHTSPSPSG (SEQ ID NO. 46)

[275] The immunoglobulins in Table 5 were constructed as described above in the pTT5 expression vector. Heavy and light chains for each immunoglobulin were cotransfected into HEK293E cells for expression, and resulting protein was purified from the supernatant using protein A affinity chromatography, as described above.

[276] The proteins were visualized electrophoretically using the Agilent Bioanalyzer as described above. Representative gels are shown in Figure 24. As expected, under reducing conditions three bands were observed at the approximate calculated molecular weights, corresponding to the light chain (~25 KDa) and two heavy chains (~65 KDa) for each mAb-Fv. Under non-reducing conditions a main band was observed for each immunoglobulin (~180 KDa) corresponding to the tetrameric mAb-Fv species (two heavy chains, each complexed with a light chain). The additional band in the non-reducing gels for 9550, 9551, and 9552 is attributed to monomeric heavy chain / light chain pair (~90 KDa), similar to as was observed for the α CD19 x α Fc γ RIIb mAb-Fv. Nonetheless, based on the band intensities of the α CD3 mAb-Fv's the heterodimeric heavy chain complexes are the dominant species for the 9550, 9551, and 9552 constructs.

[277] The immunoglobulins were analyzed using SEC to measure their size and test the native-like behavior of the purified samples, as described above. Results for select mAb-Fv samples are shown in Figure 25. The mAb-Fv samples showed a main peak at an elution time that, based on a molecular weight standard (not shown), corresponded to the approximate molecular weight of the tetrameric mAb-Fv species (~180 KDa). The symmetry of the peaks and dominant population of the proper species for all of the α CD3 mAb-Fv's (α Her2, α CD19, and α HM1.24) indicated that the mAb-Fv format is robust for co-engagement of CD3 antigen. Consistent with the SDS-PAGE results, 9551 showed an additional smaller peak at a later elution time, attributed to the presence of monomeric heavy chain / light chain pairs.

[278] Affinity of the 9509 α Her2 x α CD3 mAb-Fv for CD3⁺ T cells was measured using a competition experiment with labeled OKT3 antibody. 9509 mAb-Fv at various concentrations was pre-incubated with ~0.8 million PBMC on ice for 1 hour. Commercial FITC-labeled OKT3 was added to 50 ng/ml for 45 minutes. The binding buffer was 3% FBS/PBS, and samples were washed twice before fixing with 1% Paraformaldehyde/PBS. Binding of OKT3-FITC to T cells was detected on a FACSCanto II flow cytometer (BD Biosciences) by analyzing CD16⁻CD20⁻ lymphocytes based on front scatter (FSS) and side scatter (SSC) scattering pattern that were negative for CD16 and CD20 using commercial labeled anti-CD16 and anti-CD20 antibody reagents. The results in Figure 26 demonstrate dose-dependent binding of the α Her2 x α CD3 mAb-Fv to T cells.

[279] Previous groups have demonstrated CD3-mediated cytotoxic killing by bispecific antibody fragments that co-engage CD3 and various target antigens (reviewed in Baeuerle & Reinhardt, 2009, Cancer Res 69[12]:4941–4). We tested the capacity of the CD3 co-engaging mAb-Fv's to mediate cytotoxic killing against tumor cells expressing Her2 and

HM1.24. Cytotoxicity assays were carried out as above, monitoring cell lysis by lactate dehydrogenase (LDH) release as described.

[280] α Her2 x α CD3 mAb-Fv's utilized purified T cells as effectors. T cells were purified using a T Cell Enrichment Kit from Stemcell Technologies (Vancouver, Canada). The 9509 α Her2 x α CD3 mAb-Fv was tested against Her2⁺ SK-BR-3 breast carcinoma cells at an effector to target ratio of 200,000 T cells to 10,000 target cells. Incubation time was 24 hrs. The 9551 α HM1.24 x α CD3 mAb-Fv was used as a negative control. The results in Figure 27 show strong CD3-mediated cytotoxicity against the Her2⁺ target cells mediated by the Her2-directed mAb-Fv, while the HM1.24-directed mAb-Fv showed no killing activity. The 9344 α Her2 x α CD3 mAb-Fv was tested against Her2⁺ SKOV3 ovarian carcinoma cells at an effector to target ratio of 250,000 T cells to 10,000 target cells. Incubation time was 24 hrs. Here the α Her2 IgG1 WT antibody trastuzumab was used as a negative control. The results in Figure 28 show strong CD3-mediated killing against the target cells by the mAb-Fv. In contrast, the WT IgG1 anti-Her2 antibody showed no capacity to mediate cytotoxic activity by the isolated T cells.

[281] The 9551 α HM1.24 x α CD3 mAb-Fv was tested for CD3-mediated cytotoxic activity using purified PBMCs. Because this mAb-Fv contains the knockout variant Fc region G236R/L328R, it does not have the capacity to mediate Fc γ R-mediated cytotoxic activity by other immune cells in the heterogeneous PBMC mixture. Both knockout variant (6166) and WT IgG1 (5627) anti-HM1.24 antibodies were tested as controls. In this experiment HM1.24⁺ RPMI 8224 myeloma cells were used as targets (seeded at 10,000 per well), and 500,000 PBMCs were added as effectors at a 50:1 effector/target cell ratio. Incubation time for this assay was 23 hrs. The results in Figure 29 show that the α HM1.24 x α CD3 mAb-Fv mediated strong cytotoxic activity against the tumor cells. The 6166 Fc knockout anti-HM1.24 antibody was completely devoid of activity, consistent with its lack of Fc γ R binding, and of course its inability as a native antibody to co-engage CD3. The 5627 WT IgG1 anti-HM1.24 antibody mediated very weak ADCC activity, if any at all, presumably due to Fc γ RIIIa-mediated killing by NK cells in the PBMC mixture. The dramatic CD3-mediated activity of the mAb-Fv relative to the Fc γ R-mediated activity of the normal IgG1 antibody targeting the same antigen demonstrates the potential of the novel formats herein for enabling powerful functional activities.

[282] All cited references are herein expressly incorporated by reference in their entirety.

[283] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous

variations of the details may be made without departing from the invention as described in the appended claims.

CLAIMS

We claim:

1. An antibody analog comprising:
 - a) a first heavy chain and an associated light chain, wherein said first heavy and light chains form a first antigen binding site, wherein the C-terminus of the CH3 domain of said first heavy chain is covalently attached to a C-terminal variable heavy domain;
 - b) a second heavy chain and an associated light chain, wherein said second heavy and light chains form a second antigen binding site, wherein the C-terminus of the CH3 domain of said second heavy chain is covalently attached to a C-terminal variable light domain;such that said C-terminal variable heavy and light domains form a third antigen binding site.
2. An antibody analog according to claim 1 wherein two of said antigen binding sites are the same and the third is different.
3. An antibody analog according to claim 1 wherein the antigen bound by said third antigen binding site is CD3.
4. An antibody analog according to claim 1 wherein the antigen bound by said third antigen binding site is FcγRIIb.
5. An antibody analog according to claim 1 wherein all three of said antigen binding sites are the same.
6. An antibody analog according to claim 1 wherein said the C-terminus of said C-terminal variable heavy domain is covalently attached to a CH1 domain and wherein said C-terminus of said C-terminal variable light domain is covalently attached to a CL domain.
7. An antibody analog according to claim 1 or 6 wherein each heavy chain comprises at least one amino acid substitution within the Fc region.
8. An antibody analog according to claim 7 wherein each heavy chain comprises a different amino acid substitution, such that a heterodimeric Fc region is more stable than a homodimeric Fc region.
9. An antibody analog according to claim 7 wherein said substitution reduces binding to at least one FcγR receptor.

10. An antibody analog according to claim 7 wherein said amino acid substitution increases binding to at least one FcγR receptor.

11. An antibody analog according to claim 7 wherein said amino acid substitution increases binding to the FcRn receptor.

12. An antibody analog according to claim 7 wherein said amino acid substitutions are selected from the group consisting of 349A, 349C, 349E, 349I, 349K, 349S, 349T, 349W, 351E, 351K, 354C, 356K, 357K, 364C, 364D, 364E, 364F, 364G, 364H, 364R, 364T, 364Y, 366D, 366K, 366S, 366W, 366Y, 368A, 368E, 368K, 368S, 370C, 370D, 370E, 370G, 370R, 370S, 370V, 392D, 392E, 394F, 394S, 394W, 394Y, 395T, 395V, 396T, 397E, 397S, 397T, 399K, 401K, 405A, 405S, 407T, 407V, 409D, 409E, 411D, 411E, 411K, and 439D.

13. An antibody analog comprising:

- a) a first heavy chain comprising a variable heavy domain;
- b) a second chain comprising the Fc domain of a heavy chain covalently linked to a light chain, wherein said first heavy chain and said second chain form a first antigen binding site; wherein the C-terminus of the CH3 domain of said first heavy chain is covalently attached to a variable heavy domain and the C-terminus of the CH3 domain of said second chain is covalently attached to a variable light domain, such that said variable heavy and light domains form a second antigen binding site.

14. An antibody analog according to claim 13 wherein a CH1 domain is covalently attached to said variable heavy domain covalently attached to the CH3 domain of said first heavy chain and wherein a CL domain is covalently attached to said variable light domain covalently attached to said CH3 domain of said second chain.

15. An antibody analog according to claim 13 or 14, wherein the wherein each of said protein chains comprises a variant CH3 domain relative to a native CH3 domain.

16. An antibody analog according to claim 15, wherein each of said variant CH3 domains comprise different amino acid substitutions, such that a heterodimeric protein pair is more stable than a homodimeric protein pair.

17. An antibody analog according to claim 16, wherein said substitution is selected from the group consisting of 349A, 349C, 349E, 349I, 349K, 349S, 349T, 349W, 351E, 351K, 354C, 356K, 357K, 364C, 364D, 364E, 364F, 364G, 364H, 364R, 364T, 364Y, 366D, 366K, 366S,

366W, 366Y, 368A, 368E, 368K, 368S, 370C, 370D, 370E, 370G, 370R, 370S, 370V, 392D, 392E, 394F, 394S, 394W, 394Y, 395T, 395V, 396T, 397E, 397S, 397T, 399K, 401K, 405A, 405S, 407T, 407V, 409D, 409E, 411D, 411E, 411K, and 439D, wherein numbering is according to the EU index as in Kabat.

Figure 1

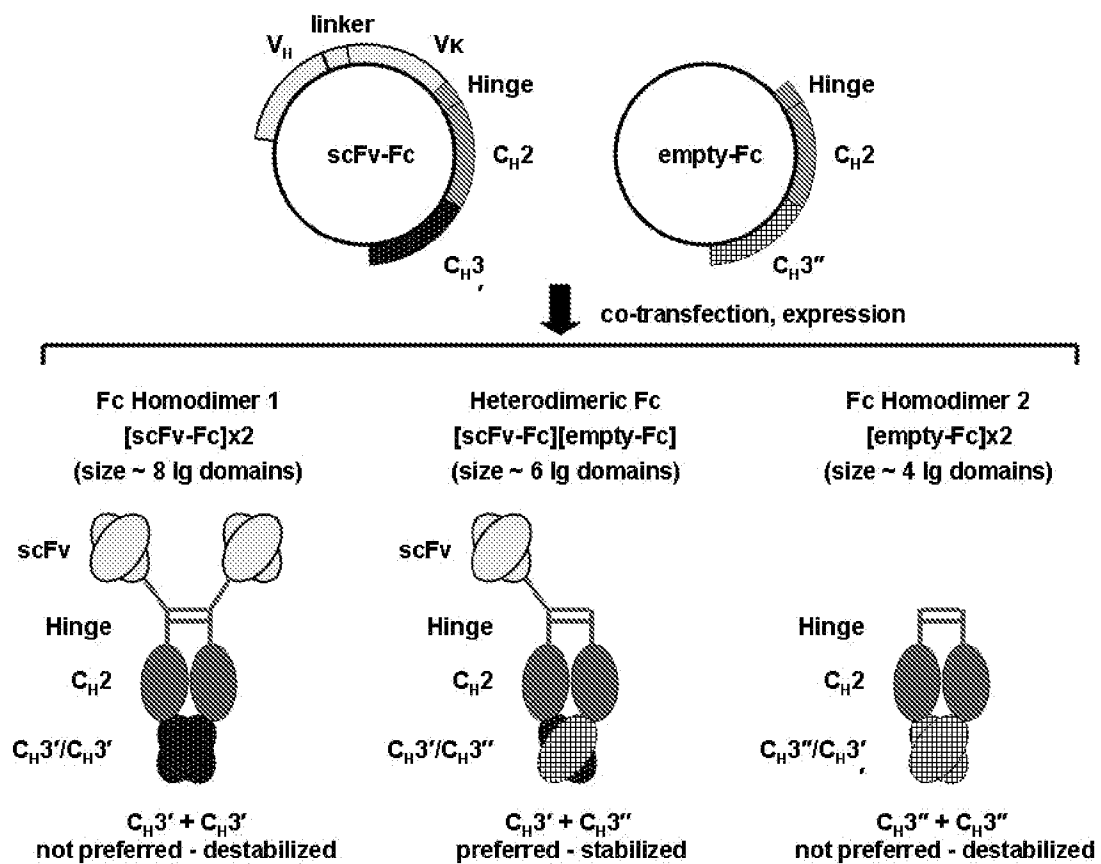


Figure 2**Native IgG1 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO. 1)**

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSSTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGN
 VFSCSVMHEALHNHYTQKSLSLSPGK

Native IgG2 constant heavy chain (CH1-hinge-CH2-CH3) (SEQ ID NO. 2)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSSTVTPSSNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPPCPAPPVAGPSVFLF
 PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
 SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQV
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSGDSFFLYSKLTVDKSRWQQGNVFS
 CSVMHEALHNHYTQKSLSLSPGK

scFv-Fc construct (SEQ ID NO. 3)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNY
 NQKFQDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSV
 EGGSGGSGGSGGSGGVDDIQLTQSPAISASPGKVTMTCRASSSVSYMNWYQQKSGT
 SPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGT
 KLELKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
 VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Empty-Fc construct (SEQ ID NO. 4)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
 KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV
 LDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 3

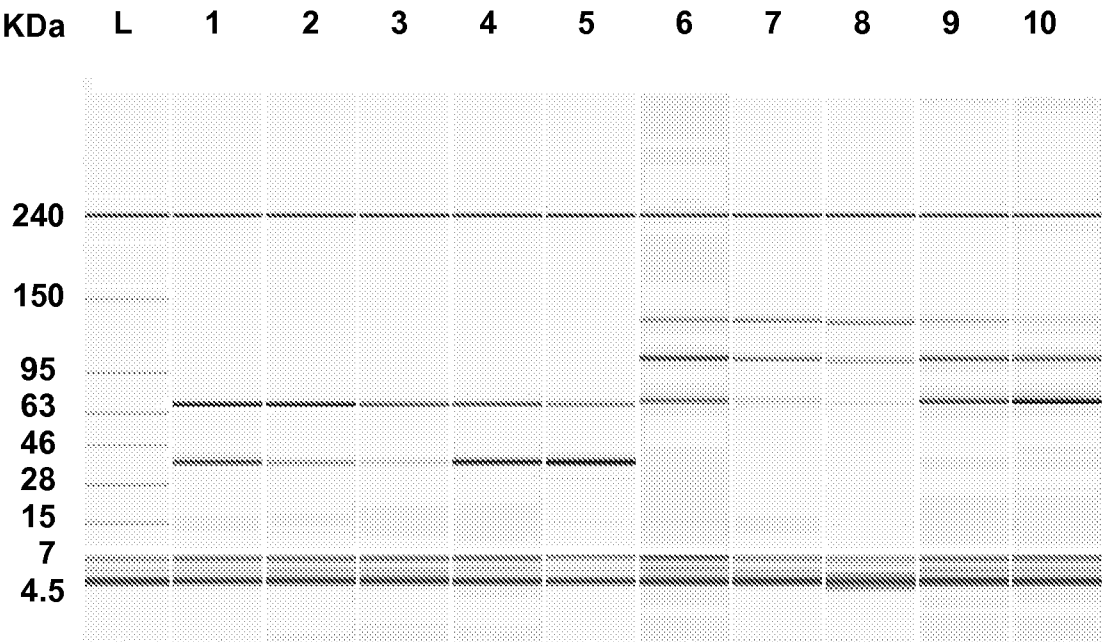


Figure 4

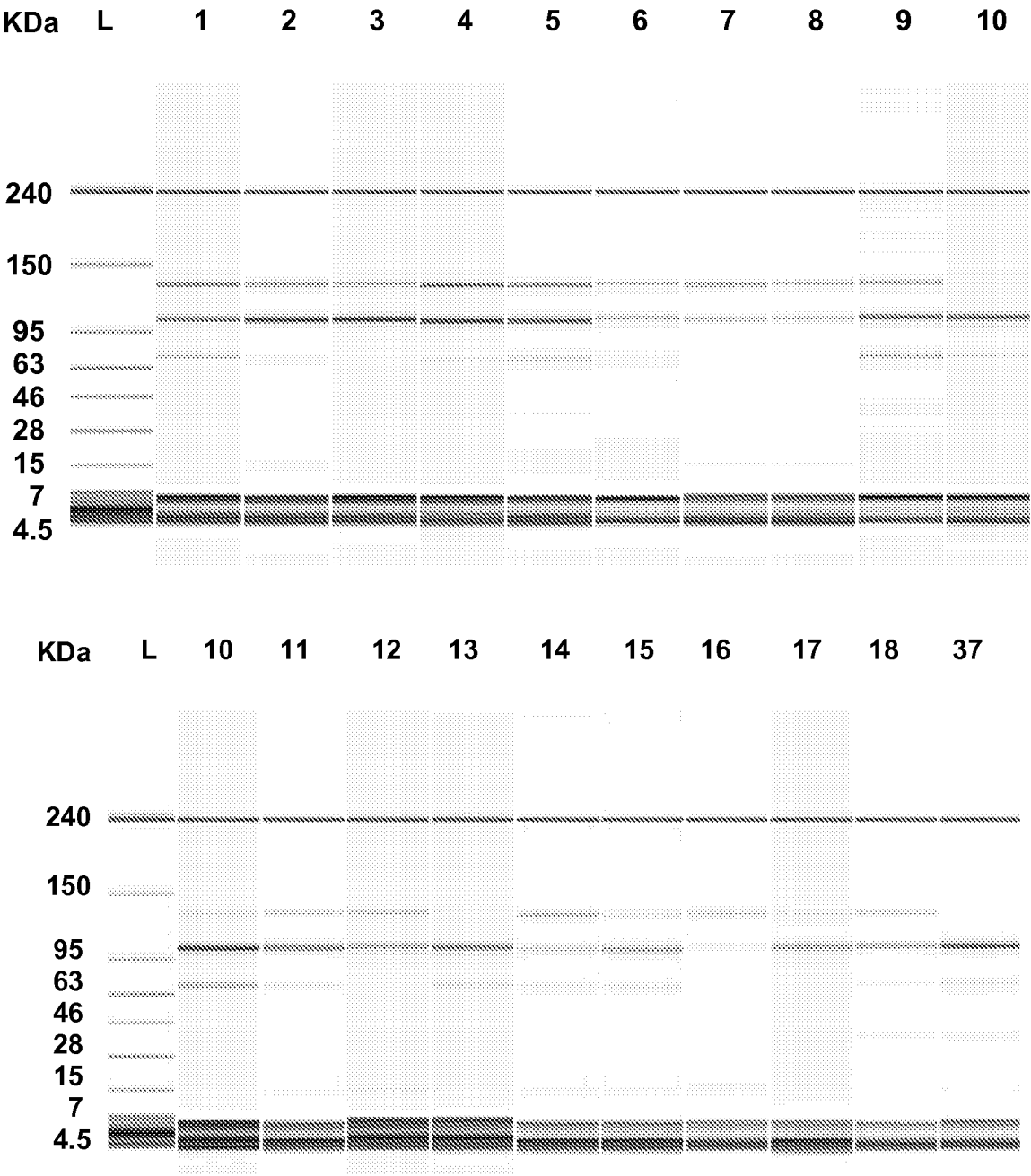


Figure 4 (continued)

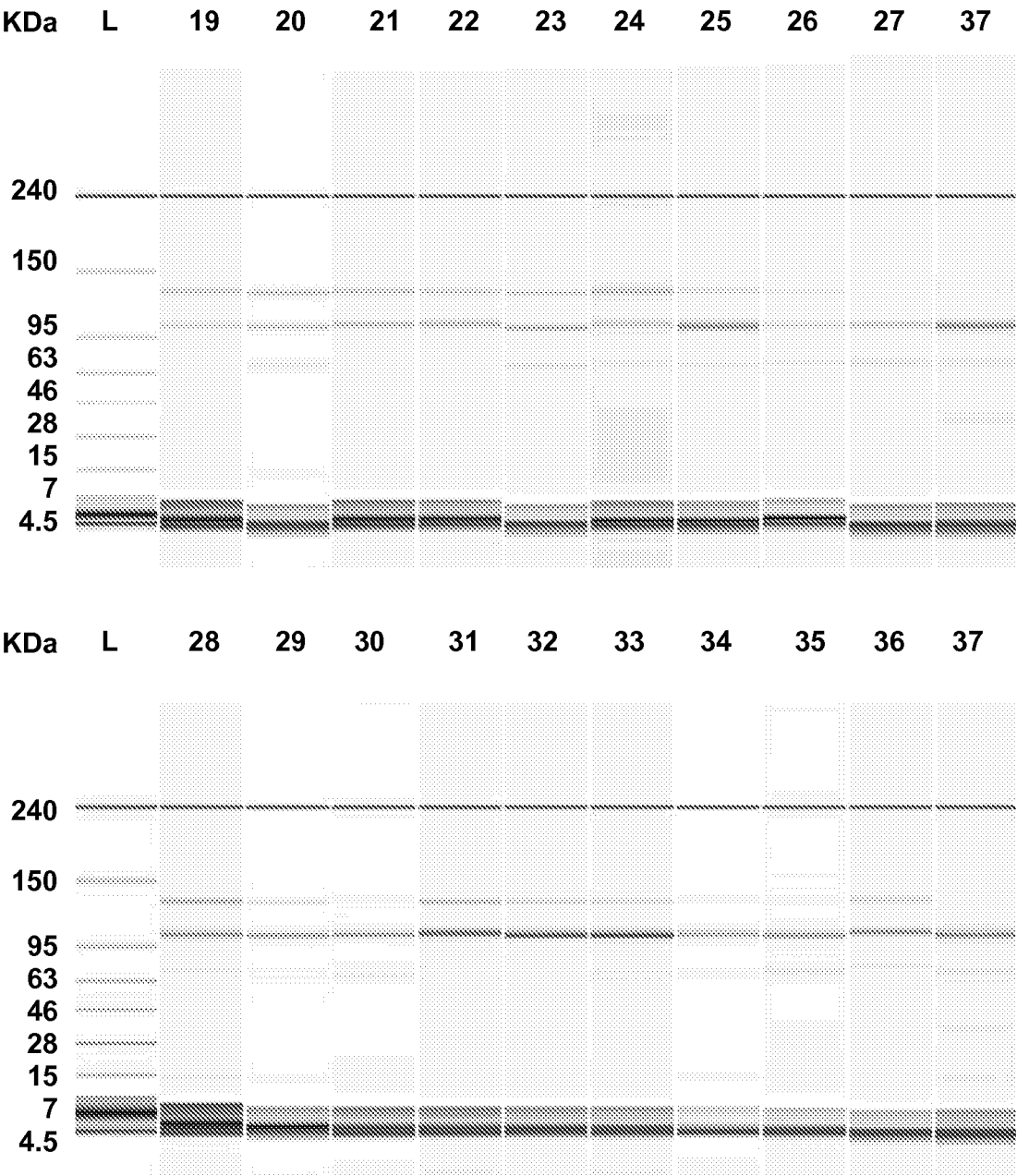


Figure 5

Lane	Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
1	IgG1 WT	IgG1 WT	2%	33%	42%	23%	0%
2	S364D	Y349K	0%	6%	73%	21%	0%
3	S364E	Y349K	0%	7%	75%	18%	0%
4	S364F	Y349A	0%	13%	59%	29%	0%
5	S364G	Y349W	0%	26%	52%	23%	0%
6	S364H	Y349T	0%	0%	65%	35%	0%
7	S364Y	Y349A	0%	2%	51%	47%	0%
8	S364Y	Y349S	0%	2%	56%	42%	0%
9	S364D	L368K	6%	31%	51%	12%	0%
10	S364E	L368S	4%	37%	54%	5%	0%
11	S364E	L368K	3%	19%	64%	14%	0%
12	K409D	L368K	0%	4%	73%	23%	0%
13	K409E	L368K	4%	24%	70%	1%	0%
14	S364D	K370R	0%	24%	38%	38%	0%
15	S364F	K370G	0%	22%	65%	13%	0%
16	S364H	K370S	0%	0%	35%	65%	0%
17	S364R	K370D	19%	11%	59%	11%	0%
18	S364R	K370E	22%	12%	50%	17%	0%
19	S364Y	K370G	0%	4%	50%	46%	0%
20	T411E	K370R	0%	29%	41%	29%	0%
21	T411K	K370D	0%	10%	53%	37%	0%
22	T411K	K370E	0%	12%	61%	27%	0%
23	Y349I/S364F	Y349E/S364D	0%	29%	48%	23%	0%
24	Y349W/S364H	Y349T/S364G	0%	12%	45%	43%	0%
25	S364D/K370G	S364Y/K370R	2%	16%	71%	11%	0%
26	S364Y/K370V	S364T/K370G	5%	35%	38%	22%	0%
27	L368E/K409E	L368K	6%	33%	61%	0%	0%
28	T411K	K370E/T411D	0%	11%	63%	26%	0%
29	K370R/T411K	K370E/T411E	0%	18%	69%	13%	0%
30	L351E/T366D	L351K/T366K	0%	25%	69%	7%	0%
31	K392E	D399K	0%	10%	73%	18%	0%
32	K409D	D399K	0%	9%	81%	10%	0%
33	K409E	D399K	0%	11%	81%	9%	0%
34	Y407T	T366Y	0%	21%	72%	7%	0%
35	F405A	T394W	3%	31%	64%	2%	0%
36	K439D	E356K	0%	17%	69%	14%	0%
37	T366S/L368A/Y407V	T366W	14%	17%	68%	1%	0%

Figure 6

Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
F405A	T394F	3%	26%	66%	5%	0%
F405A	T394W	3%	31%	64%	2%	0%
F405A	T394W	0%	5%	59%	36%	0%
F405S	T394Y	0%	6%	44%	50%	0%
K370C	S364C	0%	40%	0%	60%	0%
K392E	D399K	0%	10%	73%	18%	0%
K409D	D399K	0%	9%	81%	10%	0%
K409D	D399K	3%	13%	78%	7%	0%
K409D	D399K	2%	8%	77%	13%	0%
K409D	D399K	4%	24%	71%	1%	0%
K409D	D399K	0%	6%	52%	42%	0%
K409D	L368K	0%	4%	73%	23%	0%
K409E	D399K	0%	11%	81%	9%	0%
K409E	L368K	4%	24%	70%	1%	0%
K439D	E356K	0%	17%	69%	14%	0%
S364D	K370R	0%	24%	38%	38%	0%
S364D	L368K	6%	32%	51%	12%	0%
S364D	Y349K	0%	6%	73%	21%	0%
S364E	L368K	3%	19%	64%	14%	0%
S364E	L368S	4%	37%	54%	5%	0%
S364E	Y349K	0%	7%	75%	18%	0%
S364E	Y349K	0%	0%	0%	91%	9%
S364E	Y349K	0%	0%	63%	30%	7%
S364E	Y349K	5%	27%	68%	1%	0%
S364E	Y349K	7%	75%	18%	0%	0%
S364E	Y349K	7%	93%	0%	0%	0%
S364E	Y349T	4%	27%	56%	13%	0%
S364E	Y349T	6%	41%	47%	6%	0%
S364E	Y349T	7%	57%	35%	1%	0%
S364F	K370G	0%	22%	65%	13%	0%
S364F	Y349A	0%	13%	59%	29%	0%
S364G	Y349W	0%	26%	52%	23%	0%
S364H	K370S	0%	0%	35%	65%	0%
S364H	K370S	4%	30%	58%	8%	0%
S364H	K370S	5%	44%	49%	3%	0%
S364H	K370S	5%	42%	50%	4%	0%
S364H	L368S	3%	19%	61%	17%	0%
S364H	L368S	4%	35%	55%	6%	0%
S364H	L368S	4%	28%	59%	9%	0%
S364H	Y349K	2%	9%	61%	29%	0%
S364H	Y349K	3%	16%	72%	9%	0%
S364H	Y349K	4%	25%	68%	3%	0%
S364H	Y349T	0%	0%	64%	36%	0%
S364H	Y349T	0%	0%	0%	94%	6%
S364H	Y349T	0%	0%	77%	9%	14%
S364H	Y349T	3%	19%	60%	19%	0%
S364H	Y349T	7%	60%	32%	1%	0%
S364H	Y349T	8%	92%	0%	0%	0%

Figure 6 (continued)

Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
S364R	K370D	19%	11%	59%	11%	0%
S364R	K370E	22%	12%	50%	17%	0%
S364Y	K370G	0%	4%	50%	46%	0%
S364Y	K370G	0%	12%	60%	29%	0%
S364Y	K370G	4%	22%	64%	10%	0%
S364Y	K370G	6%	49%	44%	1%	0%
S364Y	Y349A	0%	2%	51%	47%	0%
S364Y	Y349S	0%	2%	56%	42%	0%
S364Y	Y349S	4%	36%	57%	3%	0%
S364Y	Y349S	4%	46%	49%	1%	0%
S364Y	Y349S	6%	77%	18%	0%	0%
T411E	K370R	0%	29%	41%	29%	0%
T411K	K370D	0%	10%	53%	37%	0%
T411K	K370E	0%	12%	61%	27%	0%
Y349C	S354C	34%	38%	26%	2%	0%
Y407T	T366Y	0%	21%	72%	7%	0%
IgG1 WT	IgG1 WT	2%	33%	42%	23%	0%
IgG1 WT	IgG1 WT	4%	37%	39%	20%	0%
L368E/K409E	L368K	6%	33%	61%	0%	0%
L368E/K409E	L368K	5%	24%	70%	1%	0%
L368E/K409E	L368K	6%	27%	66%	1%	0%
T411K	K370E/T411D	0%	11%	63%	26%	0%
V397S/F405A	T394F	5%	14%	73%	9%	0%
V397T/F405S	T394F	5%	30%	64%	1%	0%
K370R/T411K	K370E/T411E	0%	18%	69%	13%	0%
K392D/K409D	E356K/D399K	0%	14%	71%	15%	0%
K392D/K409D	E356K/D399K	0%	16%	64%	20%	0%
L351E/S364D	Y349K/L351K	3%	24%	69%	4%	0%
L351E/S364D	Y349K/L351K	3%	47%	50%	0%	0%
L351E/S364E	L351K/L368K	13%	57%	30%	0%	0%
L351E/S364E	Y349K/L351K	11%	51%	37%	0%	0%
L351E/S364E	Y349K/L351K	6%	24%	68%	2%	0%
L351E/S364F	K370G/L351K	0%	0%	0%	100%	0%
L351E/T366D	L351K/T366K	0%	25%	69%	7%	0%
L351E/T366D	L351K/T366K	0%	56%	30%	14%	0%
L351E/T366D	L351K/T366K	0%	31%	63%	6%	0%
L351E/T411E	L351K/D401K	0%	14%	56%	30%	0%
L351E/T411E	L351K/D401K	3%	55%	41%	0%	0%
L351K/S364H	Y349T/L351E	0%	10%	28%	62%	0%
L351K/S364H	Y349T/L351E	3%	36%	59%	1%	0%
P395T/V397S/F 405A	T394F	6%	14%	72%	9%	0%
S364D/F405A	Y349K/T394F	27%	35%	38%	0%	0%
S364D/K370G	S364Y/K370R	2%	16%	71%	11%	0%
S364D/T394F	Y349K/F405A	11%	23%	65%	1%	0%
S364D/T411E	Y349K/D401K	4%	47%	50%	0%	0%
S364D/T411E	Y349K/D401K	3%	58%	40%	0%	0%

Figure 6 (continued)

Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
S364E/F405A	Y349K/T394F	14%	12%	71%	2%	0%
S364E/F405A	Y349K/T394F	21%	28%	51%	0%	0%
S364E/F405S	Y349K/T394Y	11%	19%	65%	5%	0%
S364E/K370C	Y349K/S364C	27%	37%	36%	0%	0%
S364E/T394F	Y349K/F405A	35%	39%	26%	0%	0%
S364E/T394F	Y349K/F405A	21%	22%	57%	0%	0%
S364E/T411E	L368K/D401K	5%	55%	40%	0%	0%
S364E/T411E	Y349K/D401K	4%	47%	49%	0%	0%
S364E/T411E	Y349K/D401K	0%	31%	68%	1%	0%
S364F/D401K	K370G/T411E	0%	6%	46%	48%	0%
S364H/D401K	Y349T/T411E	0%	13%	77%	10%	0%
S364H/D401K	Y349T/T411E	0%	7%	79%	14%	0%
S364H/D401K	Y349T/T411E	5%	24%	66%	4%	0%
S364H/F405A	Y349T/T394F	32%	45%	23%	0%	0%
S364H/F405A	Y349T/T394F	5%	9%	81%	5%	0%
S364H/F405S	Y349T/T394Y	35%	48%	17%	0%	0%
S364H/K370G	Y349T/S364F	4%	84%	12%	0%	0%
S364H/T394F	Y349T/F405A	4%	12%	78%	6%	0%
S364Y/K370V	S364T/K370G	5%	35%	38%	22%	0%
T366S/L368A/Y407V	T366W	14%	17%	68%	1%	0%
T366S/L368A/Y407V	T366W	15%	18%	67%	0%	0%
Y349C/S364E	Y349K/S354C	9%	22%	68%	1%	0%
Y349I/S364F	Y349E/S364D	0%	29%	48%	23%	0%
Y349W/S364H	Y349T/S364G	0%	12%	45%	43%	0%
Y349W/S364H	Y349T/S364G	12%	39%	40%	9%	0%
Y349W/S364H	Y349T/S364G	9%	36%	45%	10%	0%
Y349W/S364H	Y349T/S364G	10%	50%	34%	5%	0%
K370D/K392D/K409D	E356K/E357K/D399K	0%	0%	100%	0%	0%
K370D/K392D/K409D	E356K/E357K/D399K	0%	0%	100%	0%	0%
L351E/S364D/F405A	Y349K/L351K/T394F	33%	16%	50%	2%	0%
L351E/S364D/T411E	Y349K/L351K/D401K	5%	46%	49%	0%	0%
L351E/S364H/F405A	Y349T/L351K/T394F	18%	21%	55%	6%	0%
L351K/S364H/D401K	Y349T/L351E/T411E	11%	11%	76%	2%	0%
L351K/S364H/F405A	Y349T/L351E/T394F	28%	25%	46%	1%	0%
S364E/T411E/F405A	Y349K/T394F/D401K	29%	14%	57%	0%	0%
S364H/D401K/F405A	Y349T/T394F/T411E	13%	10%	73%	4%	0%
S364H/F405A/T411E	Y349T/T394F/D401K	24%	15%	60%	1%	0%
S364H/T394F/D401K	Y349T/F405A/T411E	3%	21%	61%	15%	0%
T394S/P395V/P396T/ V397E/F405S	T394F	7%	20%	72%	1%	0%

Figure 7

Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
F405A	T394F	1%	19%	72%	8%	0%
F405S	T394Y	0%	3%	40%	57%	0%
K370C	S364C	0%	24%	0%	76%	0%
K409D	D399K	0%	4%	48%	49%	0%
Y349C	S354C	19%	41%	37%	4%	0%
IgG1 WT	IgG1 WT	2%	28%	43%	28%	0%
V397S/F405A	T394F	2%	10%	77%	12%	0%
V397T/F405S	T394F	2%	24%	73%	1%	0%
K392D/K409D	E356K/D399K	0%	9%	72%	19%	0%
K392D/K409D	E356K/D399K	0%	10%	65%	25%	0%
L351E/S364D	Y349K/L351K	1%	17%	76%	6%	0%
L351E/S364D	Y349K/L351K	2%	38%	60%	0%	0%
L351E/S364E	L351K/L368K	6%	53%	41%	0%	0%
L351E/S364E	Y349K/L351K	5%	46%	49%	0%	0%
L351E/S364E	Y349K/L351K	3%	19%	76%	3%	0%
L351E/S364F	K370G/L351K	0%	0%	0%	100%	0%
L351E/T411E	L351K/D401K	0%	9%	54%	37%	0%
L351E/T411E	L351K/D401K	2%	47%	51%	1%	0%
L351K/S364H	Y349T/L351E	0%	6%	24%	70%	0%
L351K/S364H	Y349T/L351E	1%	29%	68%	2%	0%
P395T/V397S/ F405A	T394F	2%	10%	76%	12%	0%
S364D/F405A	Y349K/T394F	14%	34%	53%	0%	0%
S364D/T394F	Y349K/F405A	4%	18%	77%	1%	0%
S364D/T411E	Y349K/D401K	2%	38%	60%	0%	0%
S364D/T411E	Y349K/D401K	1%	49%	50%	0%	0%
S364E/F405A	Y349K/T394F	6%	10%	81%	3%	0%
S364E/F405A	Y349K/T394F	10%	25%	66%	0%	0%
S364E/F405S	Y349K/T394Y	5%	14%	73%	7%	0%
S364E/K370C	Y349K/S364C	14%	35%	51%	0%	0%
S364E/T394F	Y349K/F405A	20%	40%	41%	0%	0%
S364E/T394F	Y349K/F405A	10%	19%	72%	0%	0%
S364E/T411E	L368K/D401K	2%	47%	51%	0%	0%
S364E/T411E	Y349K/D401K	2%	38%	60%	0%	0%
S364E/T411E	Y349K/D401K	0%	23%	75%	1%	0%
S364F/D401K	K370G/T411E	0%	3%	41%	55%	0%

Figure 7 (continued)

Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
S364H/D401K	Y349T/T411E	0%	9%	79%	13%	0%
S364H/D401K	Y349T/T411E	0%	5%	78%	17%	0%
S364H/D401K	Y349T/T411E	2%	19%	73%	6%	0%
S364H/F405A	Y349T/T394F	18%	46%	35%	0%	0%
S364H/F405A	Y349T/T394F	2%	6%	85%	7%	0%
S364H/F405S	Y349T/T394Y	20%	52%	27%	0%	0%
S364H/K370G	Y349T/S364F	2%	81%	17%	0%	0%
S364H/T394F	Y349T/F405A	2%	8%	83%	8%	0%
T366S/L368A/Y407V	T366W	6%	14%	79%	0%	0%
Y349C/S364E	Y349K/S354C	4%	18%	76%	2%	0%
K370D/K392D/K409D	E356K/E357K/D399K	0%	0%	100%	0%	0%
L351E/S364D/F405A	Y349K/L351K/T394F	16%	14%	67%	3%	0%
L351E/S364D/T411E	Y349K/L351K/D401K	2%	38%	60%	0%	0%
L351E/S364H/F405A	Y349T/L351K/T394F	8%	17%	67%	9%	0%
L351K/S364H/D401K	Y349T/L351E/T411E	4%	9%	84%	3%	0%
L351K/S364H/F405A	Y349T/L351E/T394F	14%	23%	61%	2%	0%
S364E/T411E/F405A	Y349K/T394F/D401K	14%	13%	73%	0%	0%
S364H/D401K/F405A	Y349T/T394F/T411E	5%	7%	82%	6%	0%
S364H/F405A/T411E	Y349T/T394F/D401K	11%	12%	76%	2%	0%
S364H/T394F/D401K	Y349T/F405A/T411E	1%	14%	65%	20%	0%
T394S/P395V/P396T/ V397E/F405S	T394F	3%	16%	80%	1%	0%

Figure 8

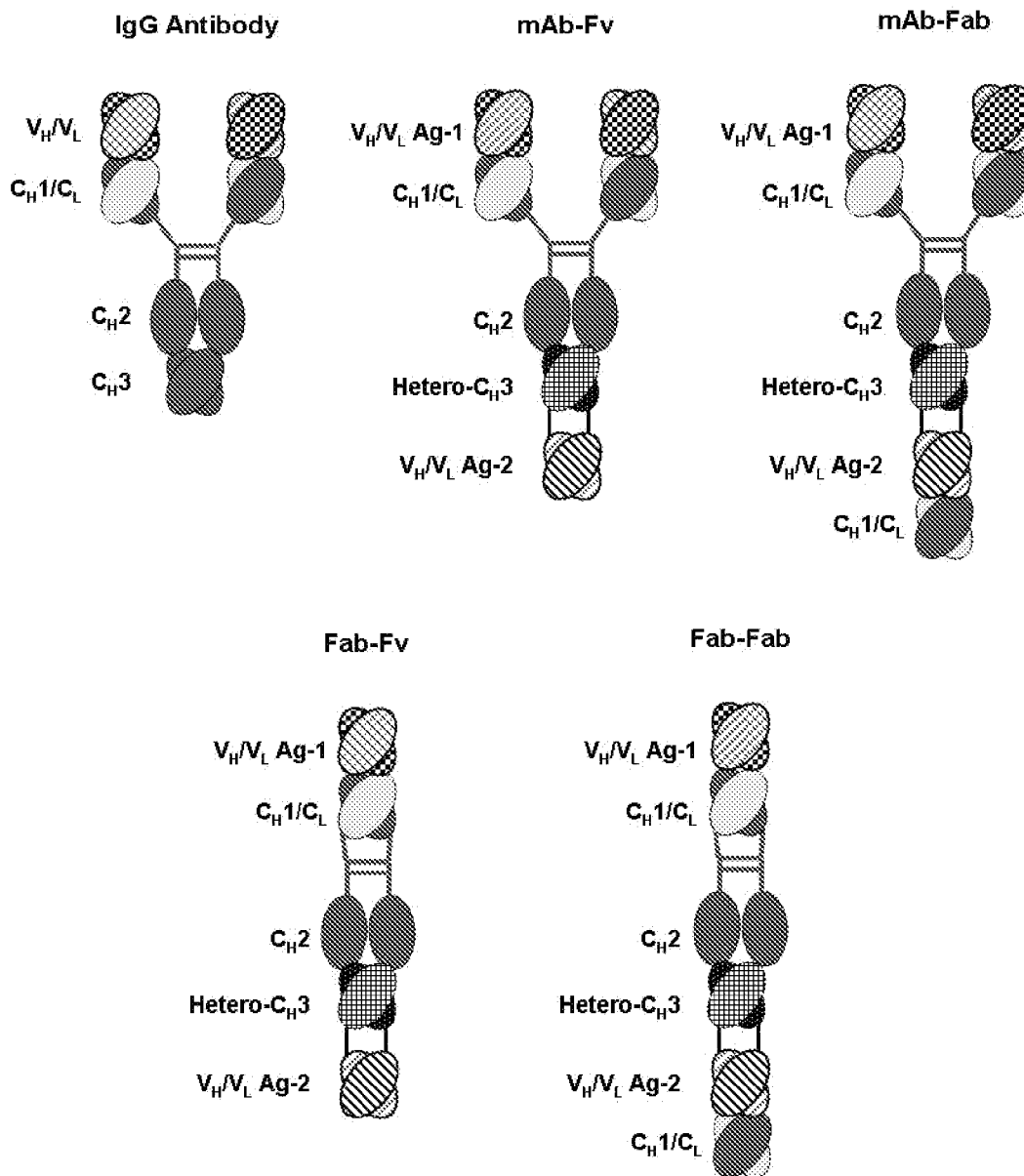


Figure 9

9064, 9074, 9375, 9516, 9344, 9376, 9509, 9550 α HER2 x α Fc γ RIIIa LC (SEQ ID NO. 5)

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSR
 FSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDE
 QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLTLSK
 ADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

9064 α HER2 x α Fc γ RIIIa HC1 (SEQ ID NO. 6)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSDDLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKSSDKTHTSPSPSGGGGSGGGGQVTLKESGPGIL
 QPSQTLSTCSFSGFSLRTSGMGVGVIRQPSGKGLEWLAHIWWDDDKRYNPALKSRLTIS
 KDTSSNQVFLKIASVDTADTATYYCAQINPAWFAYWGQGTLLTVSAASTKGPSVFPLAPSS
 KSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSDDL
 GTQTYICNVNHKPSNTKVDKKVEPKSC

9064 α HER2 x α Fc γ RIIIa HC2 (SEQ ID NO. 7)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSDDLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKSSDKTHTSPSPSGGGGSGGGGDIVLTQSPASLA
 VSLGQRATISCKASQSVDFDGDGFSMNWYQQKPGQPPKLLIYTTSNLESGIPARFSASGSGT
 DFTLNIHPVEEEDTATYYCQQSNEDPYTFGGGTGLELKRVAAPSVFIFPPSDEQLKSGTAS
 VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLTLSKADYEEKHKV
 YACEVTHQGLSSPVTKSFNRGEC

9074 α HER2 x α Fc γ RIIIa HC1 (SEQ ID NO. 8)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSDDLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKSSDKTHTSPSPSGGGGSGGGGSGGGGSGGGG
 QVTLKESGPGILQPSQTLSTCSFSGFSLRTSGMGVGVIRQPSGKGLEWLAHIWWDDDKR
 YNPALKSRLTISKDTSSNQVFLKIASVDTADTATYYCAQINPAWFAYWGQGTLLTVSA

Figure 9 (continued)

9074 α HER2 x α Fc γ RIIIa HC2 (SEQ ID NO. 9)

MGWSCIIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPG
KGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD
GFYAMDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG
SFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGG
GSGGGGSGGGGSGGGGDIVLTQSPASLAVSLGQRATISCKASQSVDFDGDSFMNWYQQ
KPGQPPKLLIYTTSNLESGIPARFSASGSGTDFTLNIHPVEEEDTATYYCQQSNEDPYTFGG
GTKLELK

9375 α HER2 x α Fc γ RIIIa HC1 (SEQ ID NO. 10)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTTLTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSLKLTVDKSRWQQG
NVFSCFSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGGGSGGGGSGGGGSGGGG
QVTLKESGPGILQPSQTLSTCSFSGFSLRTSGMGVGVIRQPSGKGLEWLAHIWWDDDKR
YNPALKSRLTISKDTSSNQVFLKIASVDTADTATYYCAQINPAWFAYWGQGTTLTVSASGHH
HHHH

9375 α HER2 x α Fc γ RIIIa HC2 (SEQ ID NO. 11)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTTLTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVHLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALYSLKLTVDKSRWQQG
NVFSCFSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGGGSGGGGSGGGGSGGGG
DIVLTQSPASLAVSLGQRATISCKASQSVDFDGDSFMNWYQQKPGQPPKLLIYTTSNLESGI
PARFSASGSGTDFTLNIHPVEEEDTATYYCQQSNEDPYTFGGGGTKLELKSGDYKDDDDK

9516 α HER2 x α Fc γ RIIIa HC1 (SEQ ID NO. 12)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTTLTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGP
SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSLKLTVDKSRWQQG
NVFSCFSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGGGSGGGGSGGGGSGGGG
QVTLKESGPGILQPSQTLSTCSFSGFSLRTSGMGVGVIRQPSGKGLEWLAHIWWDDDKR
YNPALKSRLTISKDTSSNQVFLKIASVDTADTATYYCAQINPAWFAYWGQGTTLTVSASGHH
HHHH

Figure 9 (continued)**9516 α HER2 x α Fc γ RIIIa HC2 (SEQ ID NO. 13)**

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVHLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFALYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGKSSDKTHTSPSPGGGGSGGGGSGGGGSGGGG
 DIVLTQSPASLAVSLGQRATISCKASQSVDFDGDGDSFMNWWYQQKPGQPPKLLIYTTSNLESGI
 PARFSASGSGTDFTLNIHPVEEEDTATYYCQQSNEDPYTFGGGTKELEKSGDYKDDDDK

9387, 9517 α EGFR x α Fc γ RIIIa LC (SEQ ID NO. 14)

DIQLTQSPSSLSASVGDRTITCRASQSISNNLHWYQQKPDQSPKLLIKYASESISGVPSRFS
 GSGSGTDFTLTISSLQAEDVAVYYCQQNNNWPPTTFGQGTKEIKRTVAAPSVFIFPPSDEQL
 KSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLKAD
 YEKHKVYACEVTHQGLSSPVTKSFNRGEC

9387 α EGFR x α Fc γ RIIIa HC1 (SEQ ID NO. 15)

QVQLQQSGPGLVKPSQTLSTCTVSGFSLSNYGVHWRQAPGKGLEWMGIIWSGGSTDY
 STSLKSRLTISKDTSKSQVLTMTNMDPVDATYYCARALTYDYEFAYWGQGTLLTVSSA
 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
 YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
 VVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
 VSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQGNV
 FSCSVMEALHNHYTQKSLSLSPGKSSDKTHTSPSPGGGGSGGGGSGGGGSGGGGQV
 TLKESGPGILQPSQTLSTCSFSGFSLRTSGMGVGVIRQPSGKGLEWLAHIWWDDDKRYN
 PALKSRLTISKDTSSNQVFLKIASVDTADTATYYCAQINPAWFAYWGQGTLLTVSASGHHH
 HHH

9387 α EGFR x α Fc γ RIIIa HC2 (SEQ ID NO. 16)

QVQLQQSGPGLVKPSQTLSTCTVSGFSLSNYGVHWRQAPGKGLEWMGIIWSGGSTDY
 STSLKSRLTISKDTSKSQVLTMTNMDPVDATYYCARALTYDYEFAYWGQGTLLTVSSA
 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
 YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
 VVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
 VHLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFALYSKLTVDKSRWQQGNV
 FSCSVMEALHNHYTQKSLSLSPGKSSDKTHTSPSPGGGGSGGGGSGGGGSGGGGDIV
 LTQSPASLAVSLGQRATISCKASQSVDFDGDGDSFMNWWYQQKPGQPPKLLIYTTSNLESGIPA
 RFSASGSGTDFTLNIHPVEEEDTATYYCQQSNEDPYTFGGGTKELEKSGDYKDDDDK

Figure 9 (continued)**9517 α EGFR x α Fc γ RIIIa HC1 (SEQ ID NO. 17)**

QVQLQQSGPGLVKPSQTLSTCTVSGFSLSNYGVHWRQAPGKGLEWMGIIWSGGSTDY
STSLKSRLTISKDTSKSQVLTMTNMDPVDATYYCARALTYDYEFAYWGQGTTLTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSFLTTLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVTTLPSSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTFFPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGGGSGGGGSGGGGSGGGGQV
TLKESGPGILQPSQTLSTCSFSGFSLRTSGMGVGVIRQPSGKGLEWLAHIWWDDDKRYN
PALKSRLTISKDTSSNQVFLKIASVDTADTATYYCAQINPAWFAYWGQGTTLTVSASGHHH
HHH

9517 α EGFR x α Fc γ RIIIa HC2 (SEQ ID NO. 18)

QVQLQQSGPGLVKPSQTLSTCTVSGFSLSNYGVHWRQAPGKGLEWMGIIWSGGSTDY
STSLKSRLTISKDTSKSQVLTMTNMDPVDATYYCARALTYDYEFAYWGQGTTLTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSFLTTLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VHLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGGGSGGGGSGGGGSGGGGDIV
LTQSPASLAVSLGQRATISCKASQSVDFDGSFMNWWYQQKPGQPPKLLIYTTSNLESGIPA
RFSASGSGTDFTLNIHPVEEEDTATYYCQQSNEDPYTFGGGTKLELKSGDYKDDDDK

Figure 9 (continued)**9518, 9510, 9551 α HM1.24 x α Fc γ RIIIa LC (SEQ ID NO. 19)**

DIVMTQSPLSLPVTLGQPASISCKASQDVNTAVAWYLQKPGKSPKLLIYSASNRYTGVPDRI
TGSGSGTDFTLKISRVEAEDVGVYYCQQHYSTPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKSTYSLSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

9518 α HM1.24 x α Fc γ RIIIa HC1 (SEQ ID NO. 20)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTPYWMQWVRQAPGGGLEWMGSIFPGSGDT
RYSQSFQGQVTISADKSISTAYMELSRRLSDDTAVYYCARGLRGGYYFDYWGGQGLTVT
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRG
PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVTTLPSSREEMT
KNQVSLTCLVKGFIYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGGGSGGGGSGGGGSGGGG
GQVTLKESGPGILQPSQTLSTLCSFSGFSLRTSGMGVGVIRQPSGKGLEWLAHIWWDDDK
RYNPALKSRLTISKDTSSNQVFLKIASVDTADTATYYCAQINPAWFAYWGQGLTVTSASGH
HHHHH

9518 α HM1.24 x α Fc γ RIIIa HC2 (SEQ ID NO. 21)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTPYWMQWVRQAPGGGLEWMGSIFPGSGDT
RYSQSFQGQVTISADKSISTAYMELSRRLSDDTAVYYCARGLRGGYYFDYWGGQGLTVT
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRG
PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVHLTCLVKGFIYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKSSDKTHTSPPSPGGGGSGGGGSGGGGSGGGG
GDIVLTQSPASLAVSLGQRATISCKASQSVDFDGSFMNWWYQQKPGQPPKLLIYTTSNLES
GIPARFSASGSGTDFTLNIHPVEEEDTATYYCQQSNEDPYTFGGGKLELKSGDYKDDDDK

Figure 10

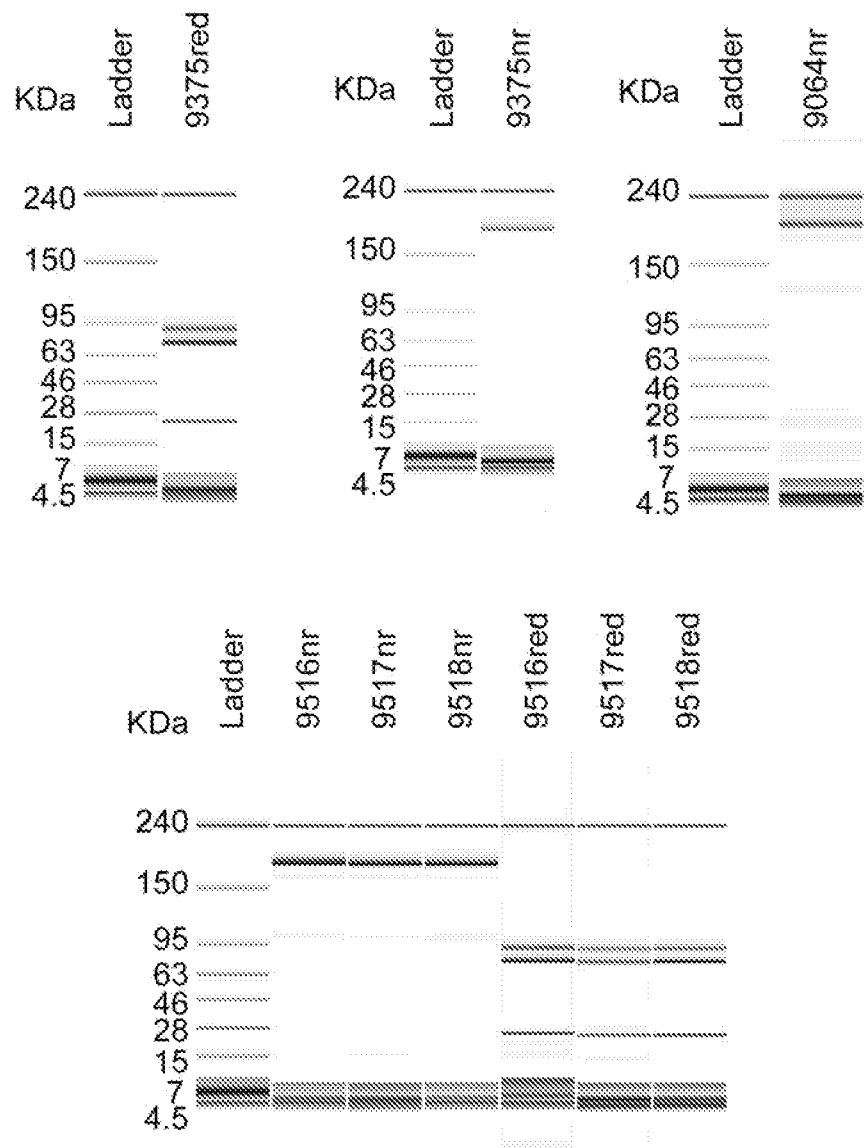


Figure 11

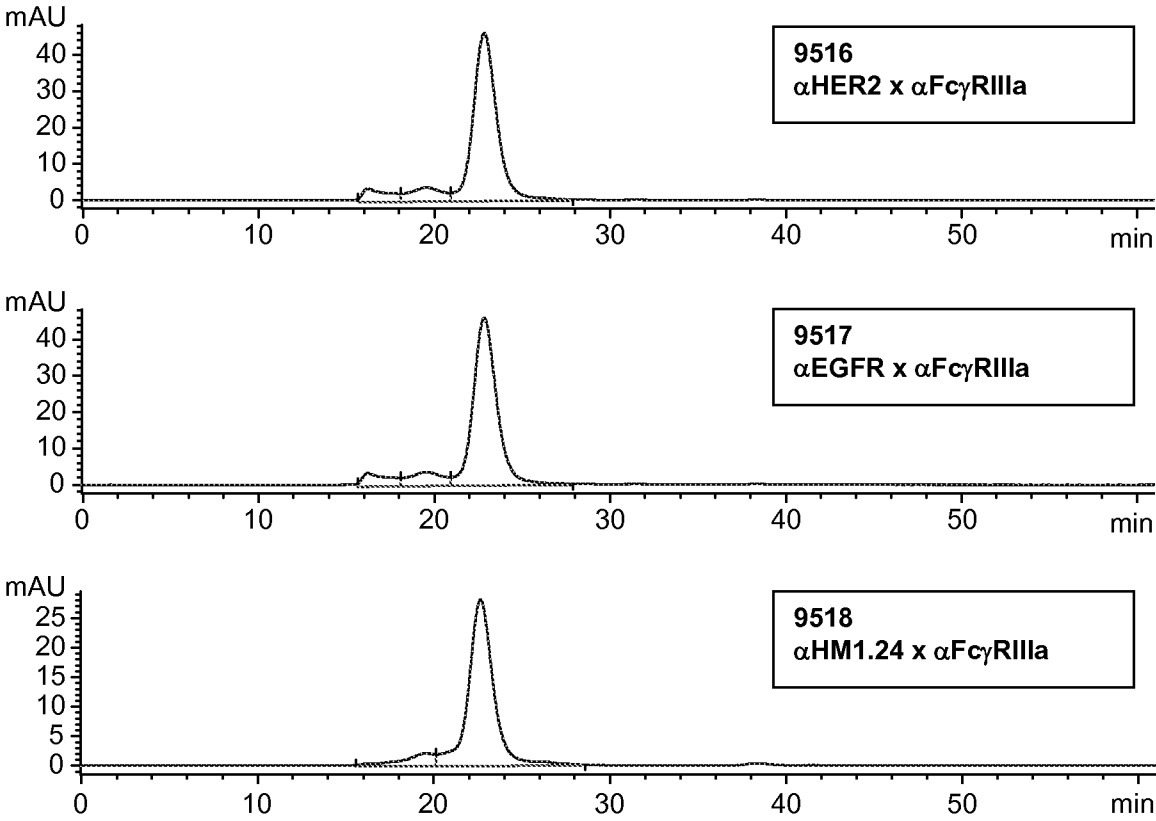


Figure 12

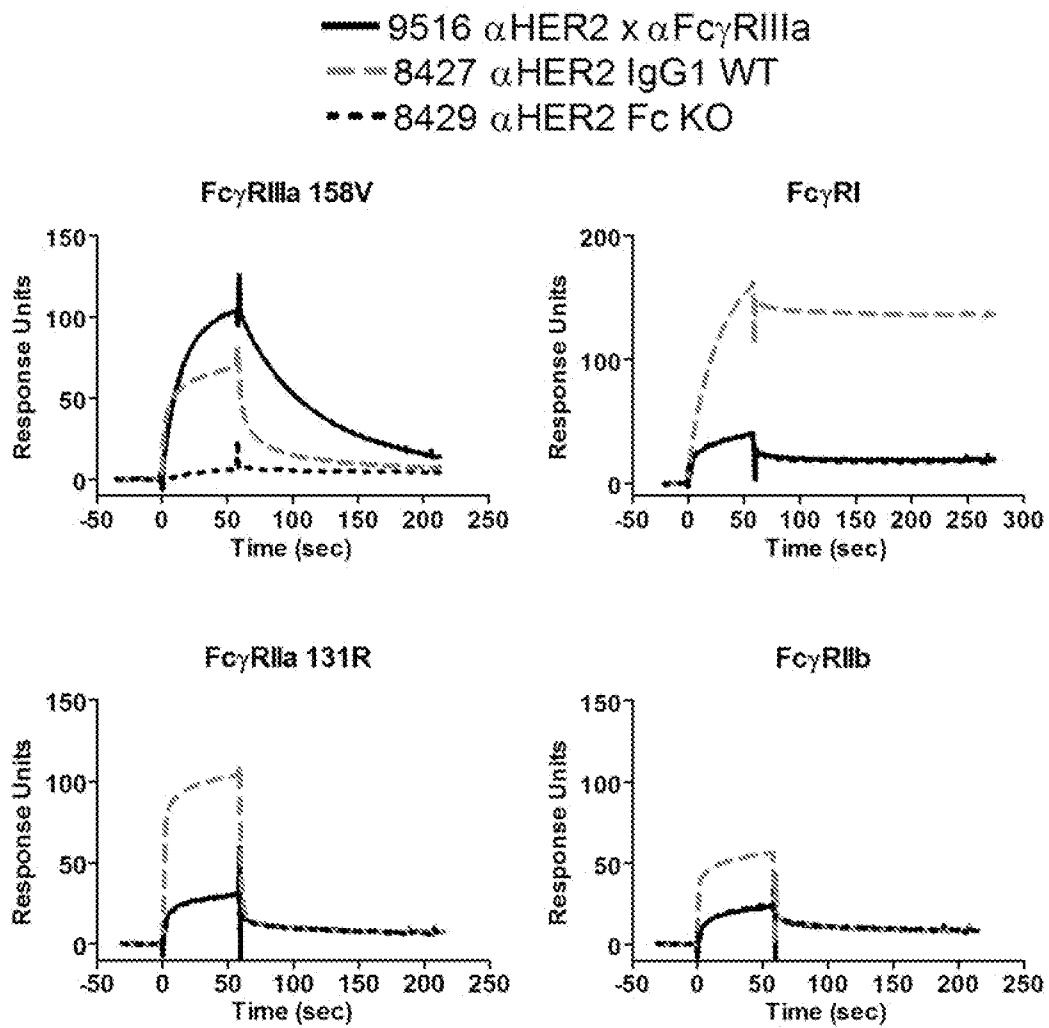


Figure 13

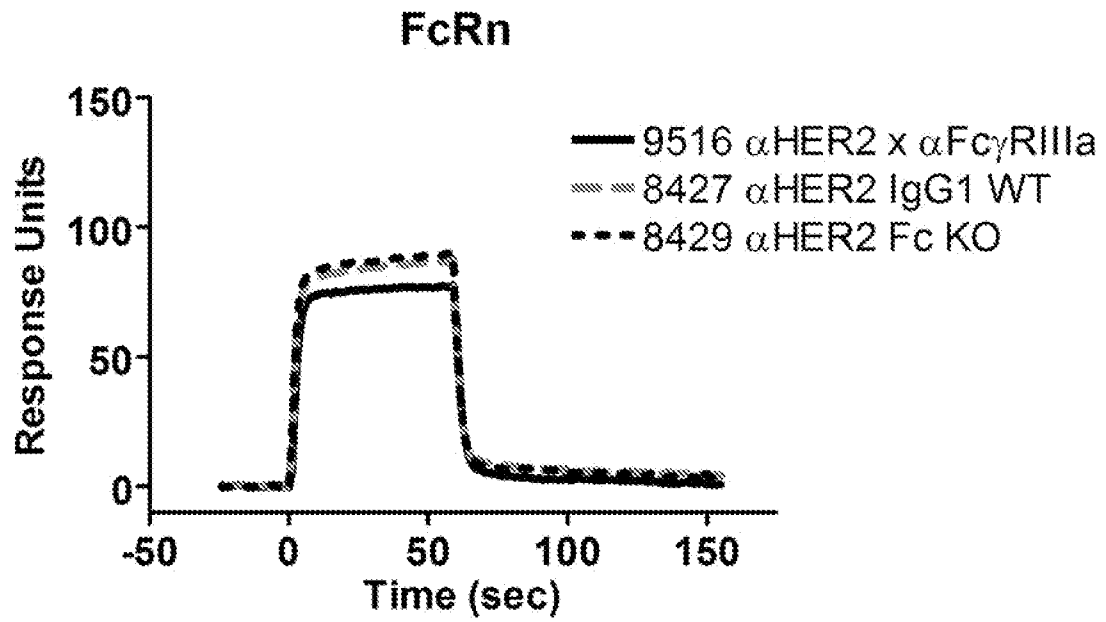


Figure 14

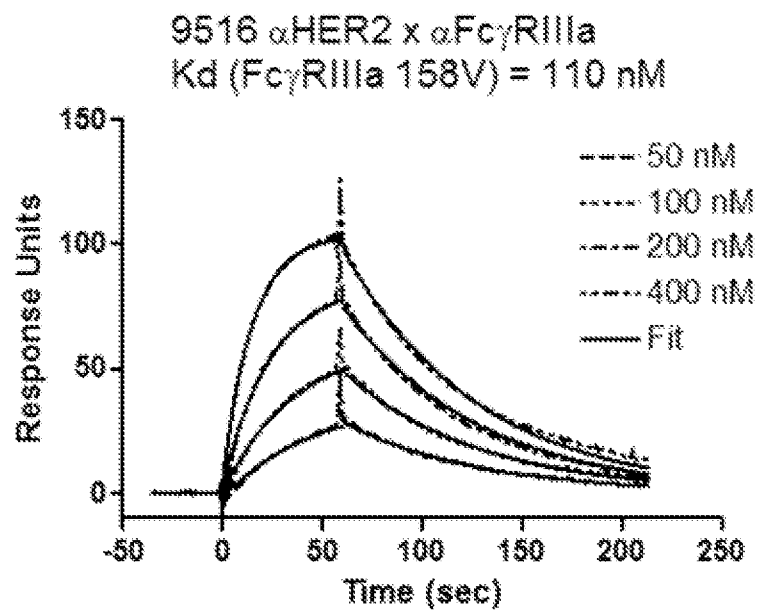


Figure 15

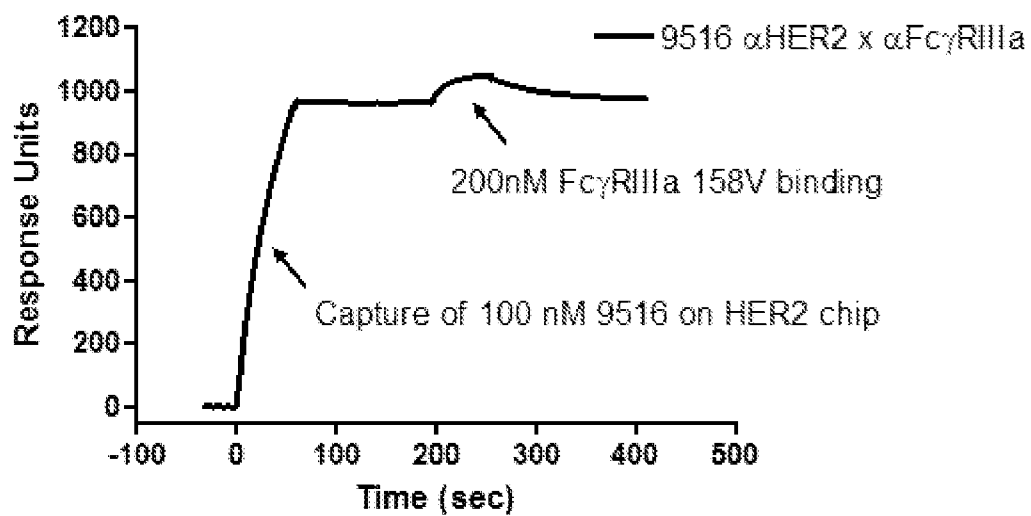


Figure 16

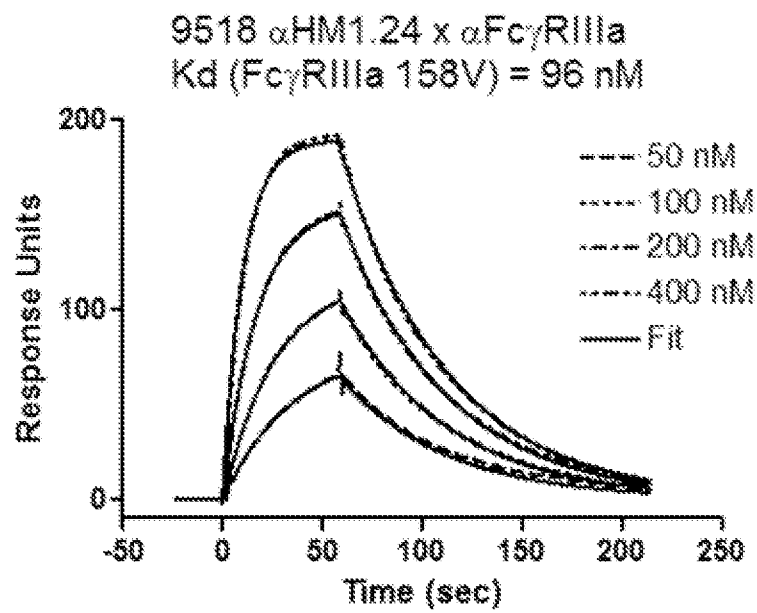


Figure 17

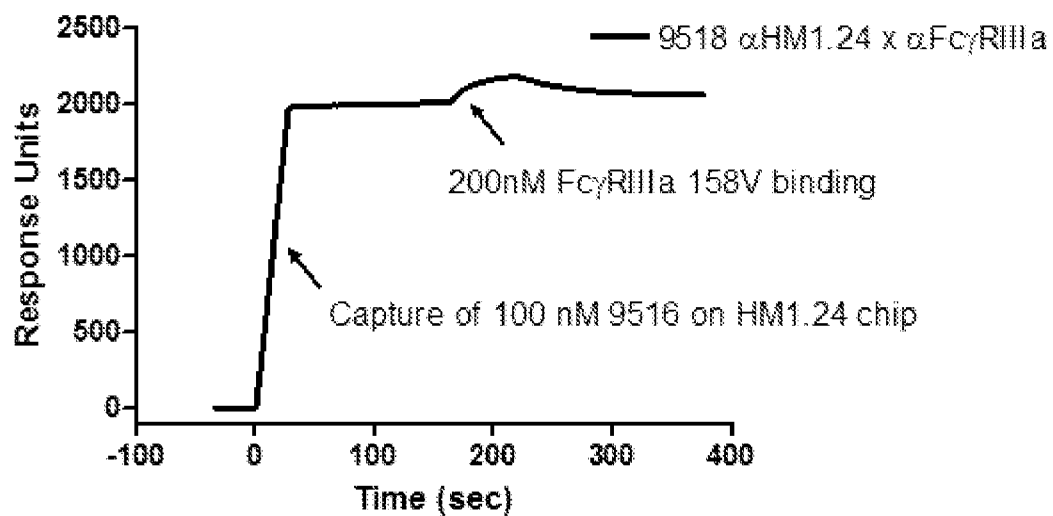


Figure 18

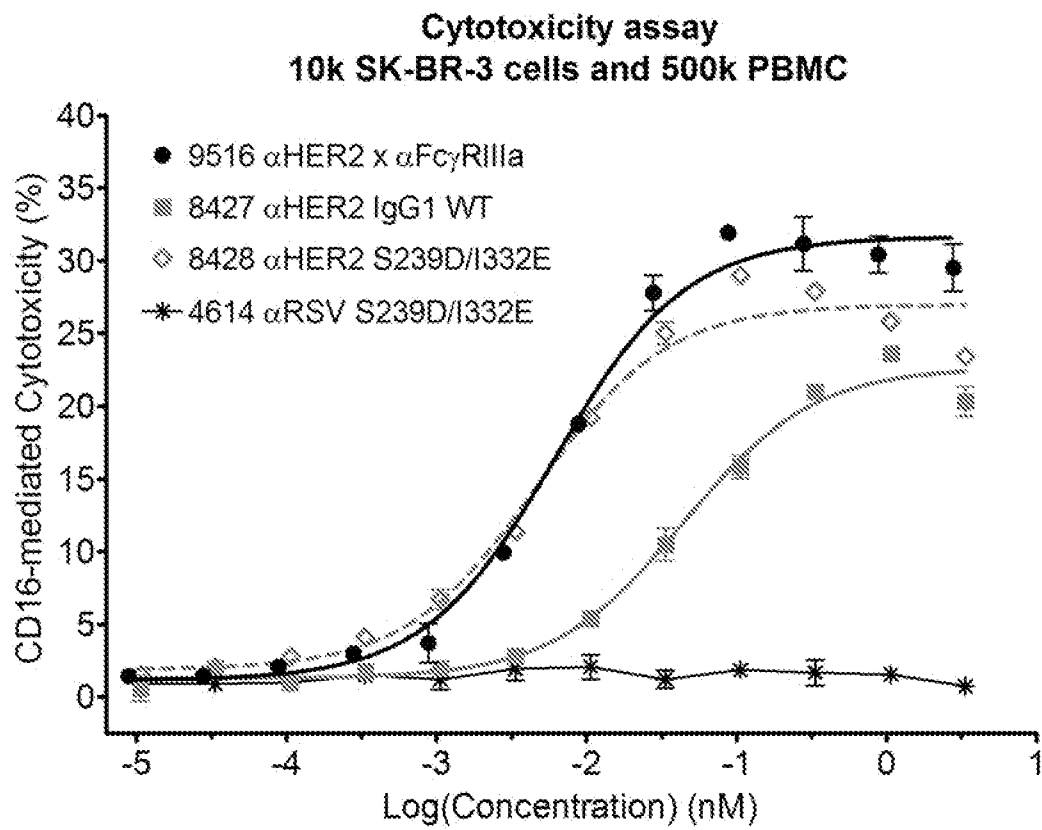


Figure 19

9506, 9547, 9511, 9552 α CD19 x α Fc γ RIIb LC (SEQ ID NO. 22)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
 GVPDRFSGSGSGTEFTLTISSELPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVAAPSVFIFP
 PSDEQLKSGTASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTL
 TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

9506 α CD19 x α Fc γ RIIb HC1 (SEQ ID NO. 23)

EVQLVESGGGLVKGPGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
 NEKFQGRVTISDDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLLTVSS
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGN
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQV
 QLQQPVTELVRPGASVMLSCKASDYPFTNYWIHWVKQRPGQGLEWIGVIDPSDTYPNYNK
 KFKGKATLTVVVSSTAYMQLSSLTSDDSAVYYCARNGDSYYSGMDYWGQGTSVTVSS
 GSHHHHHH

9506 α CD19 x α Fc γ RIIb HC2 (SEQ ID NO. 24)

EVQLVESGGGLVKGPGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
 NEKFQGRVTISDDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLLTVSS
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGN
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGDI
 LLTQSPAILSVSPGERVSFSCRTSQTGNIHWYQQRTNGFPRLLIKNVSEISIGIPSRFSGS
 GSGTDFILSINSVESEDIADYYCQQSNTWPFTFGGGTKLEIKGSHHHHHH

Figure 19 (continued)

9547 α CD19 x α Fc γ RIIb HC1 (SEQ ID NO. 25)

EVQLVESGGGLV~~K~~PGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGT~~L~~TVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSV~~V~~TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVTTLPSSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQV
QLQQPVTELVRPGASVMLSCKASDYPFTNYWIHWVKQRPGQGLEWIGVIDPSDTYPN~~Y~~NK
KFKGKATLTVVSSSTAYMQLSSLTSDDSAVYYCARNGDSDYYSGMDYWGQGTSVTVSS
GSHHHHHH

9547 α CD19 x α Fc γ RIIb HC2 (SEQ ID NO. 26)

EVQLVESGGGLV~~K~~PGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGT~~L~~TVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSV~~V~~TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVHLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGDI
LLTQSPAILSVSPGERVSFSCRTSQSIGTNIHWYQQRTNGFPRLLIKNVSE~~S~~ISGIPSRFSGS
GSGTDFILSINSVESEDIADYYCQQSNTWPFTFGGGTKLEIKGSHHHHHH

Figure 20

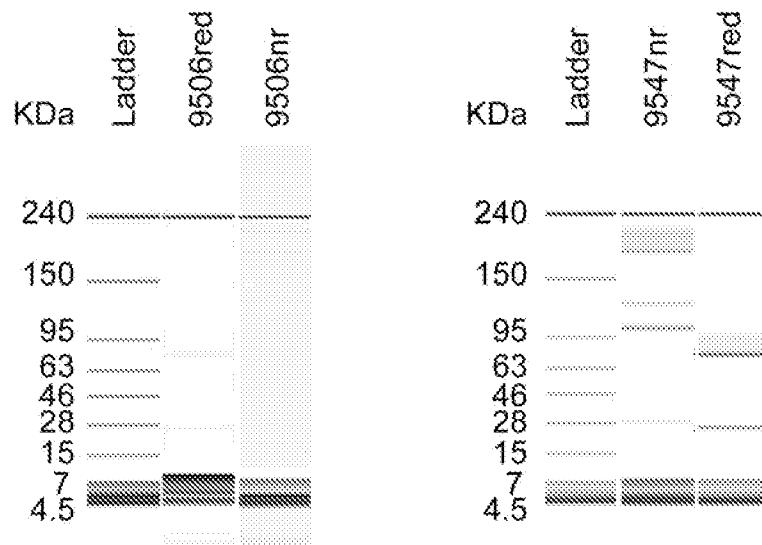


Figure 21

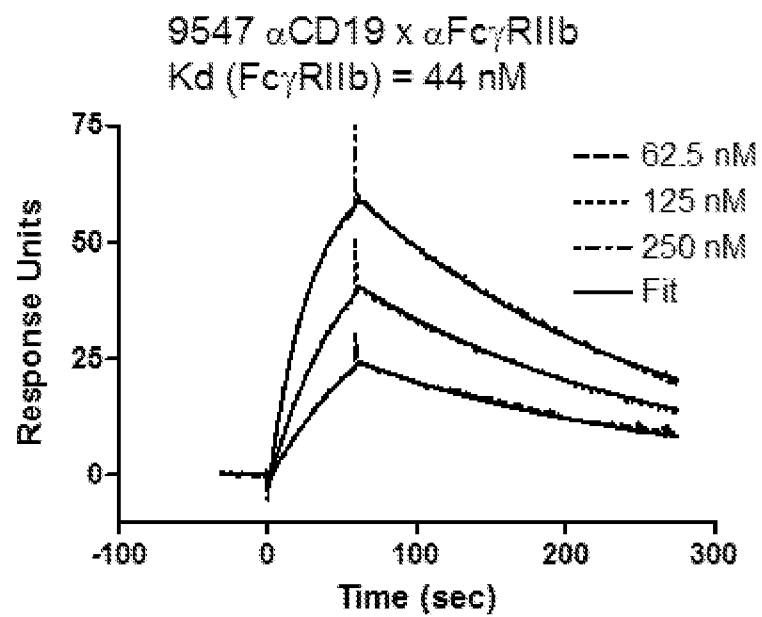


Figure 22

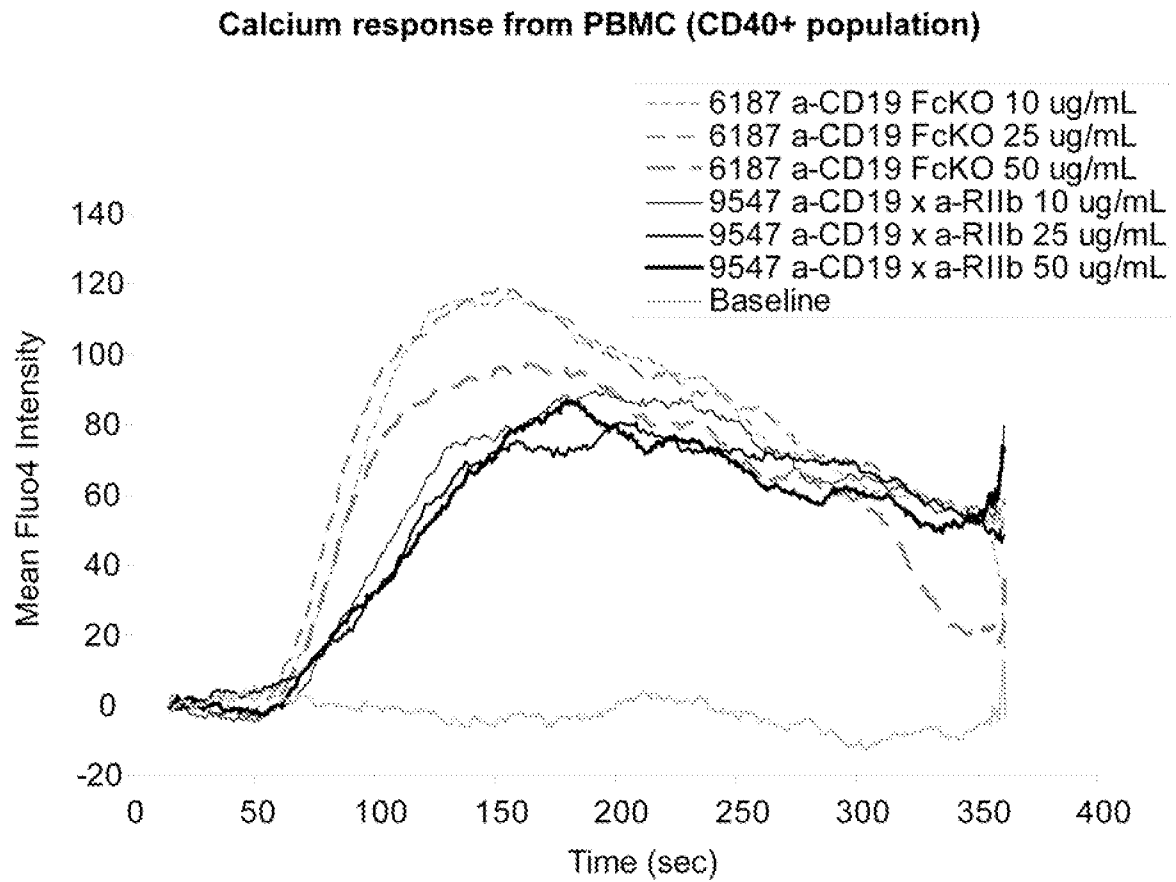


Figure 23

9344 α HER2 x α CD3 HC1 (SEQ ID NO. 27)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKSSDKTHTSPSPSGQVQLVQSGAEVKKPGASVK
 VSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYNQKFQGRVTMTTDKSTST
 AYMELSSLRSED TAVYYCARYYDDHYSLDYWGQGTTVTVSSSGHHHHHH

9344 α HER2 x α CD3 HC2 (SEQ ID NO. 28)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKSSDKTHTSPSPSGQIVLTQSPATLSLSPGERAT
 LSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPARFRGSGSGTDYTLTISSLEPED
 FAVYYCQQWSSNPFTFGSGTKLEIKSGDYKDDDDK

9376 α HER2 x α CD3 HC1 (SEQ ID NO. 29)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVTTLPSPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKSSDKTHTSPSPSGQVQLVQSGAEVKKPGASVK
 VSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYNQKFQGRVTMTTDKSTST
 AYMELSSLRSED TAVYYCARYYDDHYSLDYWGQGTTVTVSSSGHHHHHH

9376 α HER2 x α CD3 HC2 (SEQ ID NO. 30)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVHLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALYSKLTVDKSRWQQG
 NVFSCSVMHREALHNHYTQKSLSLSPGKSSDKTHTSPSPSGQIVLTQSPATLSLSPGERAT
 LSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPARFRGSGSGTDYTLTISSLEPED
 FAVYYCQQWSSNPFTFGSGTKLEIKSGDYKDDDDK

Figure 23 (continued)

9509 α HER2 x α CD3 HC1 (SEQ ID NO. 31)

EVQLVESGGGLVQPGGSLRLSCAASGFINIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYT
NYNQKFQGRVTMTTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYSLDYWGQGTTTVT
SSGSHHHHHH

9509 α HER2 x α CD3 HC2 (SEQ ID NO. 32)

EVQLVESGGGLVQPGGSLRLSCAASGFINIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
QIVLTQSPATLSLSPGERATLSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPARF
RGS GSGTDYTLTISSLEPEDFAVYYCQQWSSNPFTFGSGTKLEIKGSDYKDDDDK

9510 α HM1.24 x α CD3 HC1 (SEQ ID NO. 33)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTPYWMQWVRQAPGQGLEWMGSIFPGSGDT
 RYSQSFQGGVTISADKSISTAYMELSLRSDDTAVYYCARGLRGGYYFDYWGQGTLLTV
 SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 SGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRG
 PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMT
 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ
 GNVFSCSVMEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
GQVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGY
TNYNQKFQGRVTMTTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYSLDYWGQGTTTVT
SSGSHHHHHH

9510 α HM1.24 x α CD3 HC2 (SEQ ID NO. 34)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTPYWMQWVRQAPGQGLEWMGSIFPGSGDT
 RYSQSFQGGVTISADKSISTAYMELSLRSDDTAVYYCARGLRGGYYFDYWGQGTLLTV
 SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 SGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRG
 PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMT
 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ
 GNVFSCSVMEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
GQIVLTQSPATLSLSPGERATLSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPAR
FRGSGSGTDYTLTISSLEPEDFAVYYCQQWSSNPFTFGSGTKLEIKGSDYKDDDDK

Figure 23 (continued)

9511 α CD19 x α CD3 HC1 (SEQ ID NO. 35)

EVQLVESGGGLVKPGGSLKLSAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
 NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLLTVSS
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQV
 QLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYN
 QKFQGRVTMTTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYSLDYWGGQGTTVTVSSG
 SHHHHHH

9511 α CD19 x α CD3 HC2 (SEQ ID NO. 36)

EVQLVESGGGLVKPGGSLKLSAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
 NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLLTVSS
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQ/
 VLTQSPATLSLSPGERATLSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPARFR
 GSGSGTDYTLTISSELPEDFAVYYCQQWSSNPFTFGSGTKLEIKGSDYKDDDDK

9550 α HER2 x α CD3 HC1 (SEQ ID NO. 37)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVTTLPSPREEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYT
 NYNQKFQGRVTMTTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYSLDYWGGQGTTVTV
 SSGSHHHHHH

9550 α HER2 x α CD3 HC2 (SEQ ID NO. 38)

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY
 ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLLTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
 YRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
 NQVHLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALYSLTVDKSRWQQG
 NVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 QIVLTQSPATLSLSPGERATLSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPARF
 RGSGSGTDYTLTISSELPEDFAVYYCQQWSSNPFTFGSGTKLEIKGSDYKDDDDK

Figure 23 (continued)

9551 α HM1.24 x α CD3 HC1 (SEQ ID NO. 39)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTPYWMQWVRQAPGQGLEWMGSIFPGSGDT
 RYSQSFQGQVTISADKSISTAYMELSLRSDDTAVYYCARGLRGGYYFDYWGGGTLVTV
 SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
 SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRG
 PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVTTLPSSREEMT
 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQ
 GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
GQVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGY
TNYNQKFQGRVTMTTDKSTSTAYMELSSLRSEDAVYYCARYYDDHYSLDYWGGGTTVTV
 SSGSHHHHHH

9551 α HM1.24 x α CD3 HC2 (SEQ ID NO. 40)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTPYWMQWVRQAPGQGLEWMGSIFPGSGDT
 RYSQSFQGQVTISADKSISTAYMELSLRSDDTAVYYCARGLRGGYYFDYWGGGTLVTV
 SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
 SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRG
 PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMT
 KNQVHLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALYSKLTVDKSRWQQ
 GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
GQIVLTQSPATLSLSPGERATLSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPAR
FRGSGSGTDYTLTISSELEPEDFAVYYCQQWSSNPFTFGSGTKLEIKGSDYKDDDDK

9552 α CD19 x α CD3 HC1 (SEQ ID NO. 41)

EVQLVESGGGLVLPKGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
 NEKFQGRVTISSDKSISTAYMELSSLRSEDAMYYCARGTYYYGTRVFDYWGGGTLVTVSS
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
 VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVTTLPSSREEMTKN
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQGN
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQV
 QLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYN
 QKFQGRVTMTTDKSTSTAYMELSSLRSED

9552 α CD19 x α CD3 HC2 (SEQ ID NO. 42)

EVQLVESGGGLVLPKGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
 NEKFQGRVTISSDKSISTAYMELSSLRSEDAMYYCARGTYYYGTRVFDYWGGGTLVTVSS
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPS
 VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKARPAIEKTISKAKGQPREPQVYTLPPSREEMTKN
 QVHLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALYSKLTVDKSRWQQGN
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQ/
VLTQSPATLSLSPGERATLSCRASSSVSYMNWYQQKPGQSPRRLIYDTSKLASGVPARFR
GSGSGTDYTLTISSELEPEDFAVYYCQQWSSNPFTFGSGTKLEIKGSDYKDDDDK

Figure 24

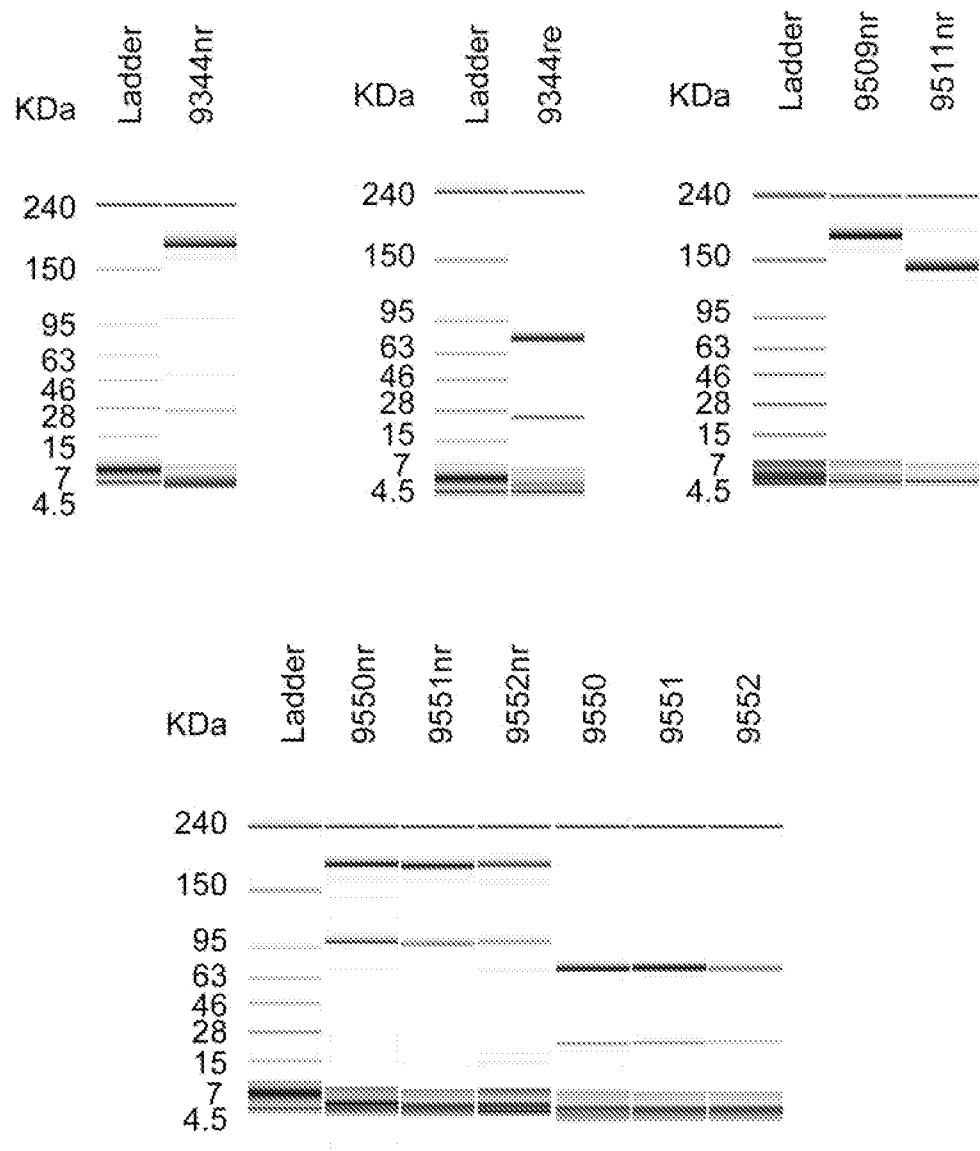


Figure 25

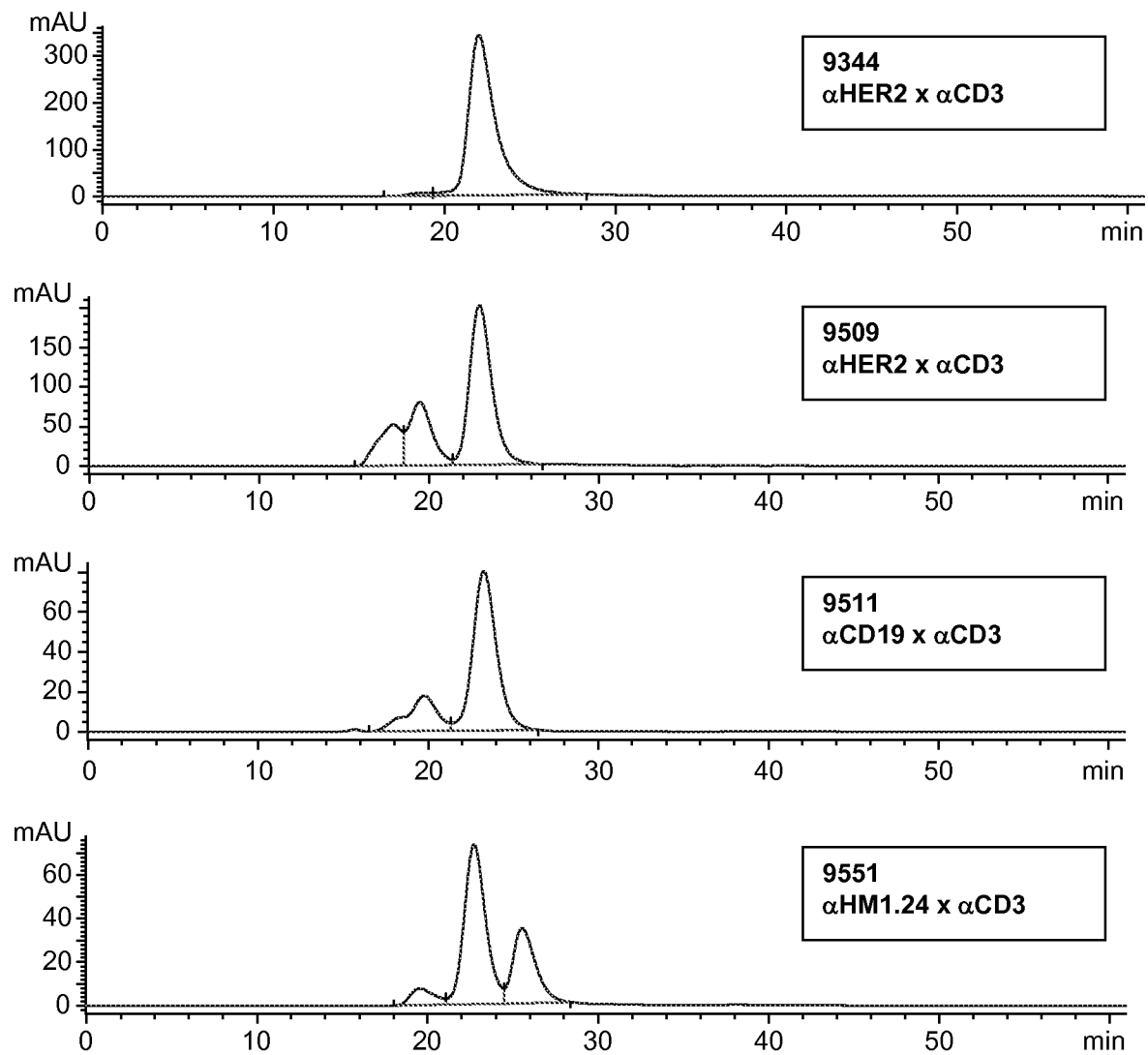


Figure 26

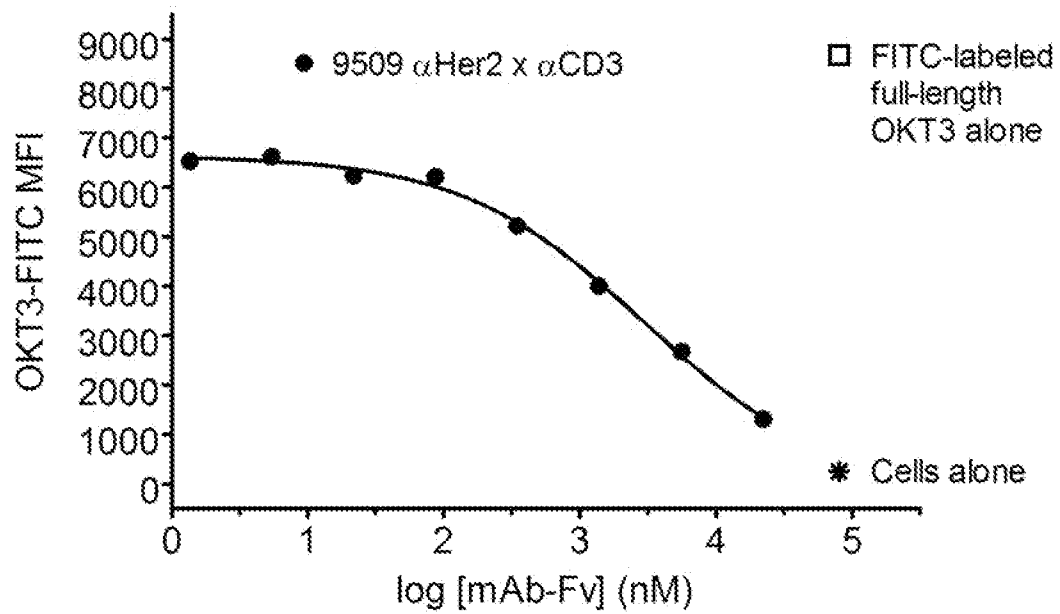


Figure 27

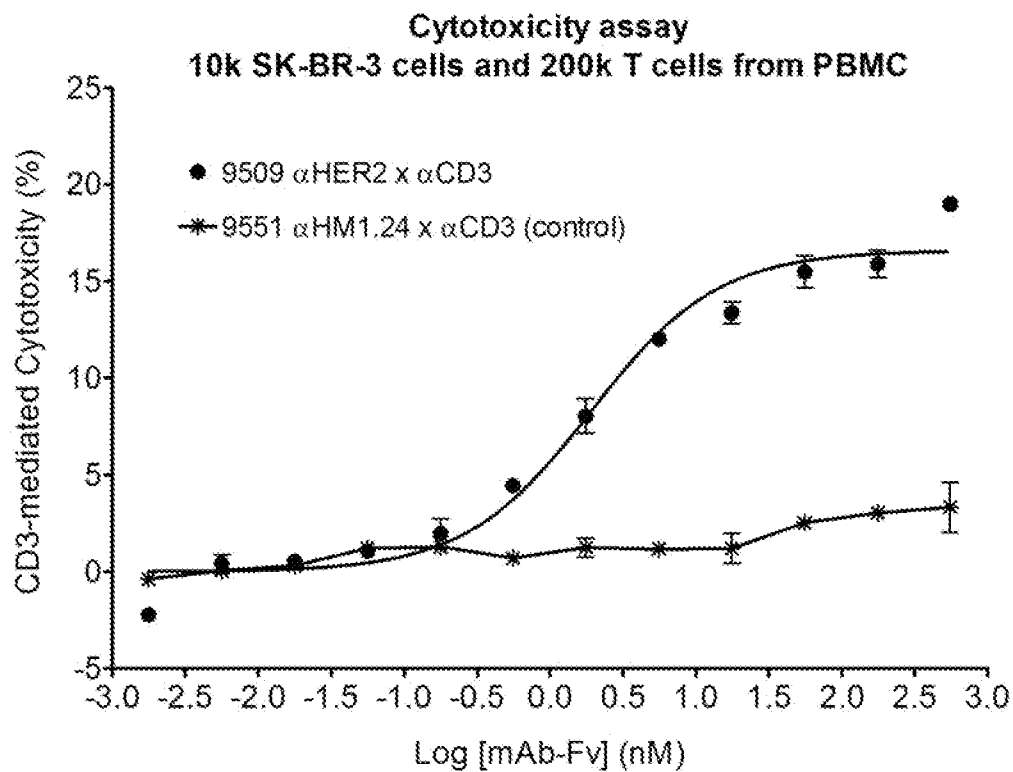


Figure 28

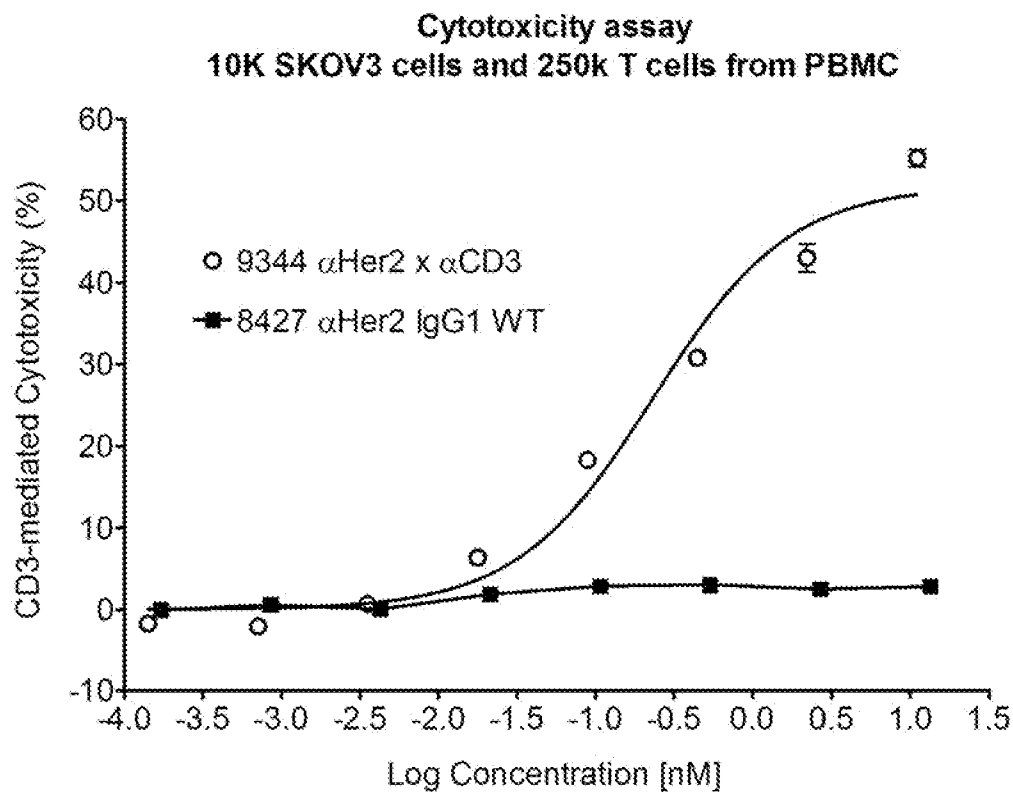
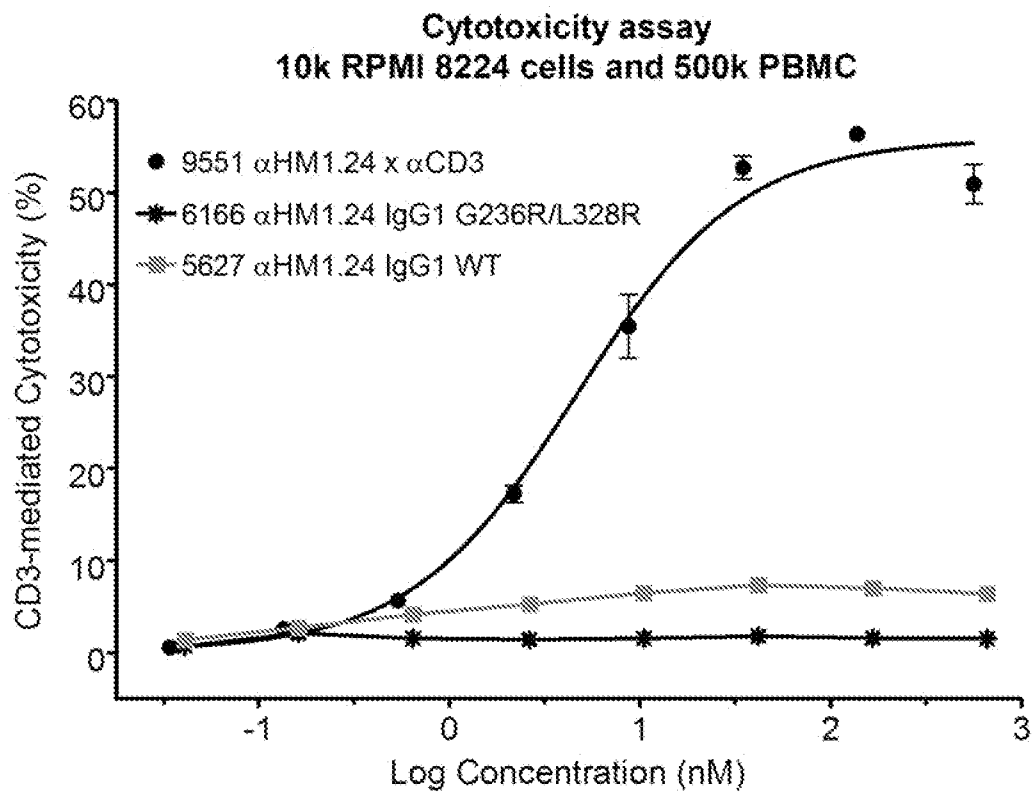


Figure 29



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/047741

A. CLASSIFICATION OF SUBJECT MATTERINV. C07K16/46 C07K16/28
ADD.

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)

C07K

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 practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZEIDLER R ET AL: "The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumour cells", BRITISH JOURNAL OF CANCER, vol. 83, no. 2, July 2000 (2000-07), pages 261-266, XP002610993, ISSN: 0007-0920 * abstract ----- -/--	1-12

☒ 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 document but 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

2 December 2010

Date of mailing of the international search report

14/12/2010

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Wagner, René

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2010/047741

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☒

 on paper

☐

 in electronic form
 - b. (time)

☒

 in the international application as filed

☒

 together with the international application in electronic form

☒

 subsequently to this Authority for the purpose of search
2.

☒

 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/047741

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KORTT A A ET AL: "Dimeric and trimeric antibodies: high avidity scFvs for cancer targeting", BIOMOLECULAR ENGINEERING, ELSEVIER, NEW YORK, NY, US, vol. 18, no. 3, 15 October 2001 (2001-10-15), pages 95-108, XP004305907, ISSN: 1389-0344, DOI: DOI:10.1016/S1389-0344(01)00090-9 figures 1,2	13-17
E	WO 2010/115589 A1 (ROCHE GLYCART AG [CH]; BRINKMANN ULRICH [DE]; CROASDALE REBECCA [DE];) 14 October 2010 (2010-10-14) figures 2,3 figure 3b	1,2,6-8, 13-16
X,P	WO 2010/028796 A1 (HOFFMANN LA ROCHE [CH]; ROCHE GLYCART AG [CH]; AUER JOHANNES [DE]; BRA) 18 March 2010 (2010-03-18) figure 3b	1,2,6-8, 13-16
X,P	WO 2010/034441 A1 (HOFFMANN LA ROCHE [CH]; ROCHE GLYCART AG [CH]; BRINKMANN ULRICH [DE];) 1 April 2010 (2010-04-01) figure 3b	1,2,6-8, 13-16
E	WO 2010/112193 A1 (ROCHE GLYCART AG [CH]; BRINKMANN ULRICH [DE]; BRUENKER PETER [CH]; CRO) 7 October 2010 (2010-10-07) figure 4	1,2
E	WO 2010/115553 A1 (ROCHE GLYCART AG [CH]; BOSSENMAIER BIRGIT [DE]; BRINKMANN ULRICH [DE];) 14 October 2010 (2010-10-14) figure 3b	1,2,6-8, 13-16
E	WO 2010/115551 A1 (ROCHE GLYCART AG [CH]; BOSSENMAIER BIRGIT [DE]; BRINKMANN ULRICH [DE];) 14 October 2010 (2010-10-14) figure 3b	1,2,6-8, 13-16
E	WO 2010/115552 A1 (ROCHE GLYCART AG [CH]; BOSSENMAIER BIRGIT [DE]; BRINKMAN ULRICH [DE];) 14 October 2010 (2010-10-14) figure 3b	1,2,6-8, 13-16
A	WO 95/09917 A1 (UNIV CALIFORNIA [US]) 13 April 1995 (1995-04-13) the whole document	1-17

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/047741

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	"A method for making multispecific antibodies having heteromultimeric and common components", EXPERT OPINION ON THERAPEUTIC PATENTS 1999 GB, vol. 9, no. 6, 1999, pages 785-790, XP002610994, ISSN: 1354-3776 the whole document -----	1-17
A	RIDGWAY J B ET AL: "'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization.", PROTEIN ENGINEERING JUL 1996 LNKD- PUBMED:8844834, vol. 9, no. 7, July 1996 (1996-07), pages 617-621, XP002610995, ISSN: 0269-2139 the whole document -----	1-17
A	FISCHER NICOLAS ET AL: "Bispecific antibodies: Molecules that enable novel therapeutic strategies", PATHOBIOLOGY, KARGER, BASEL, CH, vol. 74, no. 1, 1 May 2007 (2007-05-01), pages 3-14, XP008102855, ISSN: 1015-2008, DOI: DOI:10.1159/000101046 the whole document -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2010/047741

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010115589 A1	14-10-2010	US 2010256340 A1	07-10-2010
WO 2010028796 A1	18-03-2010	NONE	
WO 2010034441 A1	01-04-2010	US 2010081796 A1	01-04-2010
WO 2010112193 A1	07-10-2010	US 2010256338 A1	07-10-2010
WO 2010115553 A1	14-10-2010	WO 2010115551 A1	14-10-2010
		US 2010254988 A1	07-10-2010
		US 2010254989 A1	07-10-2010
WO 2010115551 A1	14-10-2010	WO 2010115553 A1	14-10-2010
		US 2010254988 A1	07-10-2010
		US 2010254989 A1	07-10-2010
WO 2010115552 A1	14-10-2010	US 2010256339 A1	07-10-2010
WO 9509917 A1	13-04-1995	NONE	