Safety Issues Specific to Clinical Development of Protein Therapeutics

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Discoveries in molecular sciences have advanced research into therapeutics that target an array of newly identified ligands and receptors with the hope of meeting critical needs of patients with chronic or life-threatening diseases. Protein or biologic therapeutics include peptides, endogenous proteins, monoclonal antibodies, hybrids such as Fc construct molecules (including peptibodies and soluble receptors), and avimers. Proteins may be purified animal derivatives, fully human, or have portions of animal and human proteins engineered together into chimeric and humanized monoclonal antibodies. Although some reports distinguish therapeutic proteins from monoclonal antibodies, for the purposes of this article the terms biologics and proteins are used interchangeably.

Although data are not available on other biologics, there are 21 monoclonal antibodies currently approved for use worldwide and more than 200 are in clinical development. The number of protein therapeutics entering clinical trials has increased nearly threefold in the past 10 years.[1] In clinical development, the average duration of time before a biologic product is approved is 7.6 years, comparable to the 7.5 years required for approval of small-molecule drugs. The success rate of humanized monoclonal antibodies entering clinical trials is 17% overall; 15% for anticancer candidates and 21% for immunological candidates, which is slighter higher than the approval rates for small-molecule drugs.

The safety of early clinical studies involving novel therapeutic proteins has come under increased scrutiny since the disastrous First-in-Human (FIH) trial of TGN1412 in 2006, the so-called TeGenero incident. Six volunteer subjects received a CD28 super agonist and experienced an unexpected, severe adverse reaction known as a "cytokine release storm" requiring intensive care management.[2] Species differences in potency, target affinity, and immune response may have been responsible for the discrepancies between the animal and the FIH-dosing experiences with this potent immune system agonist.[3] One outcome of the regulatory investigation that followed this tragedy was the convening of an expert scientific group that issued a number of recommendations to improve the safety of future clinical trials of similar investigational products.[4] In this article, we highlight key safety issues that are of relevance in the clinical development of protein therapeutics, from FIH safety observations to post-marketing surveillance. We focus on novel therapies that may be of potential high risk and necessitate special measures to ensure the well being of research study volunteers as well as patients.

PHARMACOKINETICS/PHARMACODYNAMICS OF THERAPEUTIC PROTEINS

Pharmacodynamic effects of biologics typically do not correlate with pharmacokinetic parameters such as peak plasma concentration (C_{max}) or time to maximum plasma concentration (T_{max}). In contrast to small molecules that tend to have concentration-dependent pharmacodynamic effects (both therapeutic and toxic), biologics tend to lag in clinical activity and frequently have a prolonged duration of action. Given that significant exposure to the biologic is often a requisite for attaining a therapeutic effect, area under the plasma concentration time curve (AUC) and trough concentrations are better predictors of pharmacodynamic response (as compared with C_{max}).[5] Certain adverse effects of biologics take time to manifest; for example, the time to onset of skin toxicity after panitumumab is administered averages ~12 days.[6] However, rapid adverse reactions to biologics, such as infusion reactions and cytokine release phenomena, are probably C_{max}-related events.

ON-TARGET TOXICITIES OF THERAPEUTIC PROTEINS

Because biologics are designed to act on highly specific targets and bind extracellularly, off-target activity is generally considered to be uncommon. In contrast, small molecules have direct intracellular effects and frequently exhibit off-target activity such as hepatotoxicity and other effects that are distinct from the desired target. With biologic agents, adverse reactions are most commonly a result of exaggerated pharmacology. Some well known on-target toxicities of selected biologics are listed in **Table 1**. For example, the cancer therapeutic bevacizumab

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Table 1 Known "on-target" adverse effects of selected protein therapeutics

Target	Generic name(s)	Molecule type	US-approved indication(s)	Observed toxicities
α4 Integrin	Natalizumab	Humanized mAb	Multiple sclerosis; Crohn's disease	Infections including PML; anaphylaxis
CD11a	Efalizumab	Humanized mAb	Psoriasis	Infection; malignancy; immune thrombocytopenia; hemolytic anemia
CD20	Rituximab	Chimeric mAb	NHL; RA	PML; tumor lysis syndrome; severe mucocutaneous reactions
EGFR	Cetuximab	Chimeric mAb	Cancer	Skin toxicity
	Panitumumab	Fully human mAb	Cancer	Skin toxicity
ESA	Darbopoetin-α	Modified growth factor	Anemia	Cardiovascular/thrombotic events
G-CSF	Pegfilgrastim	Pegylated growth factor	Leukopenia	Increased alkaline phosphatase; bone pain
HER-2	Trastuzumab	Humanized mAb	Cancer	Cardiac failure
IgE	Omalizumab	Humanized mAb	Asthma	Anaphylaxis, malignancy
TNF-α	Adalimumab	Fully human mAb	RA; AS; psoriatic arthritis; psoriasis; Crohn's disease	Serious infection
	Etanercept	Fc fusion (soluble receptor)	RA; AS; psoriatic arthritis; psoriasis	Serious infection
	Infliximab	Chimeric mAb	RA; Crohn's disease	Serious infection; hepatosplenic T-cell lymphoma
VEGF	Bevacizumab	Humanized mAb	Cancer	Thromboembolism; hemorrhage; GI perforation; hypertension; wound healing complications; RPLS

AS, ankylosing spondylitis; EGFR, epidermal growth factor receptor; ESA, erythropoiesis stimulating agent; G-CSF, granulocyte colony stimulating factor; GI, gastrointestinal; HER-2, human epidermal growth receptor 2; IgE, immunoglobulin E; mAb, monoclonal antibody; NHL, non-Hodgkins lymphoma; PML, progressive multifocal leukoencephalopathy; RA, rheumatoid arthritis; RPLS, reversible posterior leukoencephalopathy syndrome; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

targets vascular endothelial growth factor and inhibits angiogenesis. The pharmacology of vascular endothelial growth factor inhibition clinically translates into adverse reactions such as hypertension, hemorrhage, and arterial and venous thrombosis.[7] These effects would be considered to be on target. Similarly, skin reactions are common with epidermal growth factor receptor agents such as cetuximab[8] and panitumumab.[9] Dermal effects of these monoclonal antibody oncology products range from common mild rashes to rare, life-threatening skin infections with sepsis. Another example is an increase in unusual infections such as tuberculosis seen with tumor necrosis factor antagonists or sequestering agents; these effects relate to immune modulation activity and are generally considered on target for this class of agents. Some on-target toxicities are more common in patients with predisposing conditions. For example, cardiac toxicity was identified at an unexpectedly high rate in early clinical trials with trastuzumab.[10] Subsequent studies showed that concomitant use of anthracyclines and low baseline left ventricular ejection fraction were predictive of trastuzumab cardiac toxicity.[10]

OFF-TARGET TOXICITIES OF THERAPEUTIC PROTEINS

The most common toxicity associated with biologic agents that could reasonably be considered off target is the phenomenon of injection-site reactions. These occur with most large molecules when they are administered subcutaneously. The redness, swelling, itching, and pain at the site of injection are not generally considered as being on target but as being part of a reaction to a foreign protein. Similar effects are occasionally encountered at the site of an injection administered intravenously.

In addition to local effects at the site of administration, off-target systemic effects are also seen after intravenous administration. These infusion reactions may be related to cytokine release or formation of anti-drug antibodies. Signs and symptoms consist of fever, chills, nausea, fluctuations in heart rate and blood pressure, and skin rash and are most commonly seen after the first infusion. These reactions are more common with chimeric as opposed to humanized or fully human monoclonal antibodies. Generally, the greater the human sequence homology of the antibody, the less the acute reaction. For example, cetuximab (chimeric) is associated with a greater incidence and severity of infusion-related toxicity than is bevacizumab (humanized) or panitumumab (fully human).[12,13] In some cases it is unclear whether infusion reactions are on or off target, because cytokine release may be part of the desired targeted therapeutic response. Accordingly, whether a particular infusion reaction is to be considered on or off target relates to the particular mechanism of action of a given molecule.

Related to, but distinct from, these infusion reactions are anaphylactoid reactions that may be caused by biologic agents. Anaphylactoid reactions are clinically indistinguishable from immunoglobulin E–mediated anaphylactic reactions and may occur after administration of agents such as stem cell factor (Stemgen/Ancestim), when mast cells are released during the mobilization of stem cells from bone marrow.[14] The symptoms include urticaria, angioedema, bronchospasm, and hypotension. Anaphylactic or type 1 hypersensitivity reactions have a similar clinical presentation but a different time course (i.e., they occur most commonly not during the first infusion but after subsequent infusions) and can also occur after subcutaneous administration. Severe systemic immune reactions to biologic agents are less common today because of the use of highly purified recombinant products rather than the bacteria- and

animal-derived proteins that were formerly manufactured.[15] However, the occurrence of anaphylactic reactions to first-time use of cetuximab was recently linked to pre-existing immunoglobulin E antibodies to the drug in patients' sera.[16]

Another immunogenic reaction is the formation of neutralizing antibodies against the therapeutic protein. The most common effect of the formation of neutralizing antibodies is reduction in the therapeutic efficacy of the drug in the patient (such as human antibody against human antibody). Less often, neutralization of the native substrate occurs, resulting in the opposite of the intended therapeutic effect—shutting down endogenous target activity, sometimes permanently (e.g., pure red-cell aplasia, discussed later). This was also observed during clinical trials of megakaryocyte growth and development factor, a polyethylene glycol–conjugated N-terminal domain of thrombopoietin, which is no longer in clinical development. Subjects developed neutralizing antibodies against megakaryocyte growth and development factor, and these also neutralized endogenous thrombopoietin, rendering these individuals unable to generate platelets.[17,18] Although fully human proteins tend to be less immunogenic than nonhuman proteins, as mentioned earlier, on rare occasions close homology to endogenous proteins may elicit antibody formation and attacks on the host substrate, with devastating effects.

BIOSIMILARS/BIOGENERICS

Adverse effects of biologics that are first observed in the postmarketing period may be related to process or formulation variability due to manufacturing differences. In contrast to generic drugs, in which the active pharmaceutical ingredient is identical in composition to the patented small molecule of its type, so-called biosimilars or biogenerics can differ significantly from the innovator product. The production of protein therapeutics is a highly complex biotechnological process that requires superior sterility, handling, storage, and quality control. Improper formulation of the molecule can result in aggregation, conformational change, or oxidation, which could render the product more immunogenic or less efficacious.[19] Impurities or contaminants can themselves be immunogenic or may increase the immunogenicity of the therapeutic protein.

One example is the increased incidence of pure red-cell aplasia observed in the early 2000s associated with the use of a particular recombinant erythropoietin called Eprex (Johnson & Johnson, Bridgewater, NJ)[20] but not with other erythropoietin products marketed at the time. This outbreak involved approximately 200 patients with chronic kidney disease treated with human serum albumin-free epoetin-α via subcutaneous injection. Although the precise cause of this serious adverse reaction has not been clearly identified, the reports of such incidents decreased dramatically after stringent changes were made in manufacturing operations, handling, storage, and administration of the drug. The origin may have been multifaceted, with a number of variables contributing to a formulation change significant enough to induce an immune reaction in patients. Some cited variables include a switch to polysorbate 80 as a stabilizer (instead of human serum albumin), use of rubber-coated syringe stoppers, self-administration by subcutaneous injection (rather than intravenous administration), and inadequate cold storage.[21] Recent biophysical studies of two epoetin-α products, Epogen (Amgen, Thousand Oaks, CA) and Eprex, revealed that the proteins are not identical, providing solid evidence that biologics marketed as the same drug can be fundamentally different.[22]

LIFE CYCLE SAFETY MANAGEMENT

As with small molecules, with biologics rare serious adverse events may not be detected until a large number of patients have been exposed, frequently in the early postmarketing period. Although rare off-target, idiosyncratic-type reactions can occur with biologics, many serious adverse events are on-target effects such as progressive multifocal leukoencephalopathy associated with the use of the immune system modulators natalizumab[23] and rituximab.[24] Such events could be predicted based on known mechanisms and targets of these novel therapeutics, but they may not be detected in preclinical studies and clinical trials because of the small numbers of treated animals and subjects, respectively.

For products that pose an unusual type or high level of risk, a risk-minimization plan known as Risk Evaluation and Mitigation Strategy may be required for US regulatory filing of a new drug application to minimize risks to patients. This process utilizes several elements to ensure safe use and may include product labeling, targeted education and outreach, the requirement that prescriptions be issued only by trained specialists, informed patient consent, restricted distribution, and restricted-use programs such as performance-linked access systems.[25] In the European Union, all new drug candidates (not only products with identified risks) must have a risk-management plan prior to review.

THERAPEUTIC PROTEINS OF POTENTIALLY HIGH RISK

After the occurrence of adverse reactions in the TGN1412 trial, the Expert Scientific Group was established by the European Medicines Agency to review the lessons learned from this incident and to make recommendations to improve the safety of subjects in future FIH trials involving similar medicines. A key recommendation was to incorporate additional safety measures in the design of early clinical studies of therapeutics of potential high risk.[4] These include products that:

- Have a novel target or mechanism of action for which no previous human data exist;
- Are highly species-specific and for which animal studies may not be predictive or for which responses may be less than anticipated in humans because of differences in target distribution;
- Are highly potent and interact with cellular receptors with greater affinity or in a different way than native ligands; for instance, monoclonal antibodies may cross-link and bind to more than one cell or to other antibodies and produce exaggerated or unintended effects;
- Target the immune system or other highly regulated physiological processes such as endocrine-system cycles or the coagulation cascade.

The development of protein therapeutics in one or more of these high-risk categories necessitates a more cautious approach to first human exposure.

The Expert Scientific Group recommended several strategies to minimize possible harm from administering initial doses of potentially high-risk products to humans. These include exploring options other than the "no observed adverse effect level" method for dose calculation, such as the "minimal anticipated biological effect level" approach and the use of the lowest effective dose arrived at by various methods of calculation. The "sentinel subject" approach is a prudent strategy that involves sequential dose administration to the first few subjects with an interval of close observation between individuals to identify untoward effects. Selection of the FIH study population also requires careful consideration of the risk:benefit ratio. Healthy volunteers are frequently utilized in FIH trials because they are easier to recruit and have fewer confounding factors such as disease and concomitant medications. With biologics, however, there are instances when selecting a patient population is preferable. Proteins frequently have a long half-life and a prolonged effect lasting weeks or more that may be unacceptable in healthy volunteers. For example, biologics that modulate immune function may be better tolerated by patients with immune disorders such as lupus than by subjects with healthy immune responses. Finally, although clinical benefit is not an expected outcome in most FIH trials, some novel protein therapies may offer promise of relief to patients with grave illnesses for whom few or no treatment options exist.

No drug is without risk. However, most adverse effects of therapeutic proteins are extensions of the known pharmacological actions of the product and therefore can be anticipated and managed. Risks may be titrated to a low level with a thorough understanding of the disease and the molecular target of the therapeutic protein and by careful and prudent dose selection and close patient monitoring. New tools such as genetic screening may be useful in identifying patients who are likely to respond to treatment and in whom some risk is acceptable.[26] Risk evaluation and management should be planned and conducted for the entire life cycle of the medicine. A rational therapeutic approach that matches the right biologic to a suitable patient population minimizes the risk to all who may benefit from the therapeutic protein.

CONFLICT OF INTEREST

All of the authors are employed by Amgen, Inc., a company involved in the discovery, development, manufacture, and sales of protein therapeutics.

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