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Preclinical and clinical safety of monoclonal antibodies

Mohammad A. Tabrizi and Lorin K. Roskos

AstraZeneca Inc., 24500 Clawiter Road, Hayward, CA 94545, USA

Owing to their unique specificity, monoclonal antibodies have provided a novel approach to the treatment of human diseases. Several types of antibodies against a diverse array of pharmacological targets have been marketed and many more are currently in clinical trials. Factors related to antigen expression, target pharmacology, and antibody effector functions can contribute to the adverse event profiles observed with monoclonal antibodies. Effective translation of information gained from preclinical research and safety studies into clinical development is a crucial step for successful development of monoclonal antibodies.

Overview of antibody function and safety

Therapeutic monoclonal antibodies (mAbs) generally exhibit exclusive specificity for the target antigen. This unique characteristic discriminates mAbs from other therapeutic modalities such as small molecule drugs. Hence, it is not surprising that the pharmacology of the target antigen, mAb construct, and mAb isotype determine not only pharmacokinetics (PK) and pharmacodynamics (PD) but also the safety profiles. Factors influencing the PK and PD of mAbs have been discussed previously [1–3]. This article focuses on the assessment of antibody toxicity and cross-reactivity in preclinical safety studies.

A summary of the major clinical toxicities for the approved, non-conjugated mAbs for use in oncology, transplantation, and inflammatory diseases is given in Table 1. Monoclonal antibodies are generally safe and well tolerated, and the target-related toxicities, such as the skin toxicity observed with anti-EGF receptor antibodies, are usually predictable from preclinical studies and clinically manageable (Section 'Target-related toxicity'). Nonspecific (off-target) toxicities such as hypersensitivity reactions are among the common adverse reactions encountered with mAb therapeutics and other biotherapeutics (Section 'Nonspecific toxicity'; [4]).

In addition to the neonatal Fc receptor (FcRn) that has a protective role in maintaining IgG homeostasis, three families of Fc receptors (FcγRs) have been identified in humans (Box 1). FcγRs are expressed by various immune effector cells [11,16] and their expression profiles are heterogeneous and complicated by genetic polymorphism. Different IgG isotypes have different affinities for the FcγRs and hence have different abilities for engaging the immune effector cells. The effector functions (CDC & ADCC) not only enhance the pharmacological activity of the antibody but also influence the mAb toxicity profile. The adverse infusion reactions observed with rituximab were attributed to the effector functions of this chimeric IgG_1 antibody [17,18]. Mild-to-moderate infusion reactions and cytokine release syndrome have been observed in patients with non-Hodgkin's lymphoma (NHL), and severity of the reactions have been associated with elevated lymphocyte counts in patients with B-cell chronic lymphocytic leukemia [19]. The strongest experimental evidence for the contribution of CDC and ADCC to the pharmacology of rituximab is the lack of B-cell depletion in non-human primates by IgG_4 variant of this antibody that does not elicit the effector functions observed with the IgG_1 isotype [20].

In general, the concentration of drug at the biological receptor determines the magnitude of the pharmacological or toxicological responses. As shown in Figure 1, for drugs with a wide therapeutic index (TI), the separation of the concentration–effect curves for toxic and beneficial effects should generally allow efficacy while avoiding the toxic effects at clinically relevant doses. In instances where the target antigen is also expressed on normal tissues, a separation between pharmacological and the specific toxic effect

Corresponding author: Tabrizi, M.A. (mohammad.tabrizi@astrazeneca.com), and Roskos, L.K. (lorin.roskos@astrazeneca.com)

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TABLE 1

Safety profiles of approved, non-conjugated monoclonal antibodies for use in oncology, transplantation, and inflammatory diseases[a]

Trade® name (manufactured by)	Generic name	Technology	mAb isotype	Target	Indication	Adverse reactions
Avastin (Genetech, Inc.)	Bevacizumab	Humanized	IgG1	VEGF	Colorectal Cancer	Gastrointestinal perforations, wound healing complications, hemorrhage, hypertension, proteinuria, CHF, infusion-related reactions, immunogenicity
Campath (Millennium & ILex Partners)	Alemtuzumab	Humanized	IgG1	CD52	B-cell chronic lymphocytic leukemia	Immunosuppression, opportunistic infections, hematological toxicity, hypersensitivity, infusion-related reactions, immunogenicity
Erbitux (ImClone Systems, Inc.)	Cetuximab	Chimeric	IgG1	EGFr	Colorectal cancer	Infusion reactions, pulmonary toxicity, dermatological toxicity, immunogenicity
Herceptin (Genetech, Inc.)	Trastuzumab	Humanized	IgG1	HER2	Breast cancer	Cardiotoxicity, hypersensitivity, infusion reactions, immunogenicity
Humira (Abbott Laboratories)	Adalimumab	Human (Phage display)	IgG1	TNFα	Rheumatoid arthritis, Crohn's disease	Immunosuppression, infection (TB), neurological events, lymphoma (rare), hypersensitivity, infusion-related reactions, immunogenicity
Orthoclone-OKT3 (Ortho Biotech)	Muromonab-CD3	Murine	IgG2a	CD3	Organ transplant	Immunosuppression, infection, hypersensitivity, infusion-related reactions, immunogenicity
Raptiva (Genetech, Inc.)	Efalizumab	Humanized	IgG1	CD11a	Plaque psoriasis	Immunosuppression, infection, thrombocytopenia, hypersensitivity, infusion-related reactions, immunogenicity
Remicade (Centocor, Inc.)	Infliximab	Chimeric	IgG1	TNFα	Rheumatoid arthritis, Crohn's disease	Immunosuppression, infection (TB), neurological events, lymphoma (rare), hypersensitivity, infusion-related reactions, immunogenicity
Rituxan (Genetech, Inc.)	Rituximab	Chimeric	IgG1	CD20	B-cell non-Hodgkin's lymphoma	Severe infusion reactions, tumor lysis syndrome, renal toxicity, cardiac arrhythmias, severe mucocutaneous reactions, hypersensitivity, immunogenicity
Simulect (Novartis)	Basiliximab	Chimeric	IgG1	CD25	Organ transplant	Immunosuppression, hypersensitivity, immunogenicity
Tysabri (Biogen Idec Inc.)	Natalizumab	Humanized	IgG4	α4-Integrin	Relapsing forms of multiple sclerosis	Immunosuppression, infection (PML), hypersensitivity, infusion-related reactions
Vectibix (Amgen, Inc.)	Panitumumab	Human (Xenomouse®)	IgG2	EGFr	EGFr-expressing metastatic colorectal cancer	Infusion reactions, dermatological toxicity (90%), diarrhea (50–60%), hypomagnesemia (2%)
Xoliar (Genetech, Inc.)	Omalizumab	Humanized	IgG1	IgE	Asthma	Malignancy (rare), hypersensitivity, injection site reaction, immunogenicity
Zenapax (Roche Pharmaceuticals)	Daclizumab	Humanized	IgG1	CD25	Organ transplant	Immunosuppression, hypersensitivity, immunogenicity

[a] The data were extracted for each antibody from the prescribing information or the antibody's summary basis of approvals (SBA).

may not be achievable. As highlighted, interactions of mAbs with Fcγ receptors and cross-linking of immune effector cells could influence mAb safety (Section 'Preclinical safety studies of monoclonal antibodies'). Under certain circumstances where target expression is high in critical organs (e.g. heart, lung, and vasculature), effector functions might not be desirable and could be deleterious.

Mechanisms of monoclonal antibody toxicity

Target-related toxicity

Toxicity related to mAb pharmacology

Some of the toxicities observed with antibodies are primarily an extension of the pharmacological response. For example, immunosuppressive and anti-angiogenic mAbs exhibit clear mechanism-based toxicity profiles (Table 1). Bevacizumab is the first monoclonal antibody with the ability to inhibit angiogenesis by blocking the actions of VEGF. Angiogenesis is the process of formation of new blood vessels from pre-existing blood vessels and is of crucial importance in embryonic development, wound healing, as well as tumor growth and metastasis [21–24]. Because physiological processes such as wound healing cannot take place without the activity of VEGF, higher incidence of gastrointestinal perforations, wound healing complications, and an increased risk of hemorrhage have been reported after treatment with this antibody (bevacizumab prescribing information). The impaired wound healing observed with bevacizumab in patients has been

BOX 1

Antibody interaction with Fc receptors

For a detail discussion see Ref. [3]: IgG is the most abundant serum immunoglobulin, and serum IgG homeostasis is of particular importance in mediating humoral immunity [5]. The protective role of neonatal Fc receptor (FcRn), an MHC class-1-related receptor, in regulation of IgG homeostasis was postulated by Brambell [6]. Recent studies have further clarified the details of Brambell hypothesis and indicated that FcRn functions as a salvage receptor that regulates IgG catabolism [7–9]. In addition to FcRn, three classes of Fc gamma receptors (FcγRs) for IgG interactions have been identified in humans. These receptors are expressed by monocytes, macrophages, neutrophils, eosinophils, B and T cells, dendritic cells, natural killer cells, and platelets [10]. Human FcγRs bind IgG with various degrees of affinity ranging from low ($>10^{-7}$ M) for FcγRII (CD32), medium ($\leq 10^{-7}$ M) for FcγRIII (CD16), to high (10^{-8}–10^{-9} M) for FcγRI (CD64) [11,12]. Different IgG isotypes such as $IgG_{1, 2, 3}$, and IgG_4 bind differently to the various FcγRs [1,11–13] and hence have different abilities in mediating effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [14]. Polymorphisms of the FcγRs can differentially effect binding of different mAb isotypes, and some FcγR genotypes have been associated with clinical outcomes [15].

replicated in preclinical animal models (Summary Basis of Approval, SBA; Application Number: STN-125085/0). Additionally, a dose-dependent physeal dysplasia was observed in juvenile cynomolgus monkeys after administration of bevacizumab, which raises concern for the use of this antibody in the pediatric population (bevacizumab prescribing information).

Monoclonal antibodies that suppress the immune system have been utilized successfully in the management of various inflammatory diseases (Table 1) and a higher incidence of infection has been reported with the majority of the immunosuppressive antibodies. For example, because of the important role of TNFα in psoriasis, RA, and Crohn's disease, these diseases are successfully managed using anti-TNFα antibodies such as a chimeric antibody, infliximab, and a fully human antibody, adalimumab. Serious infections and a higher incidence of tuberculosis, sepsis, and invasive opportunistic infections have been reported with the use of these anti-TNFα antibodies [25,26]. Similarly, natalizumab is a humanized IgG4 antibody against α4-integrin indicated as monotherapy for treatment of patients with relapsing forms of multiple sclerosis (Table 1). The increasing risk of an opportunistic viral infection of the brain, progressive multifocal leukoencephalopathy (PML), was reported with natalizumab monotherapy in patients with an history or concomitant exposure to other immunosuppressive agents (natalizumab prescribing information). Natalizumab carries black box warning for PML and is only available under a special restricted distribution program in the United States.

Toxicity related to antigen expression on normal tissues

Toxicity of mAbs can arise from mAb interactions with the target antigen on tissues other than the intended target. The dermatological toxicity of cetuximab (anti-EGF, HER1 receptor) and the cardiotoxicity of trastuzumab (anti-HER2) have been attributed to the expression of the antigens in skin and cardiac muscle.

Growth factors and their transmembrane receptors play important roles in cell proliferation, survival, adhesion, migration, and differentiation [27]. The binding of epidermal growth factor (EGF) and transforming growth factor alpha (TGFα) to the receptor EGFr (HER1) triggers receptor dimerization, auto-phosphorylation of the intracellular domain of the receptor, and mitogenic signal transduction [27]. Cetuximab is a recombinant chimeric IgG1 mAb that binds specifically to the extra-cellular domain of EGFr, competitively inhibits the binding of EGFr ligands, and is effective in the treatment of metastatic colorectal cancer [28]. The principal toxicity reported with cetuximab and other anti-EGFr antagonists is the development of the dermatological toxicity, the acneiform rash that typically appears on the face, upper chest, and back but also occasionally extends to the extremities [28]. A similar toxicity profile was reported for a fully human anti-EGFr mAb, panitumumab [29]. The highest incidence of skin rash was observed at antibody doses that corresponded to the maximum saturation of the antigen [29]. The skin rash observed with anti-EGFr (HER1) mAb has been attributed to the EGFr expression in human keratinocytes.

Another example of toxicity related to the expression of antigen on normal tissue is the cardiac toxicity observed in some patients treated with trastuzumab for HER2-overexpressing metastatic breast cancer. HER2 is an internalizing transmembrane receptor that belongs to the EGF receptor family and is over-expressed in some types of human breast cancer [30]. Trastuzumab is a humanized IgG_1 monoclonal antibody targeting the HER2 receptor. The incidence of symptomatic heart failure in a pivotal clinical trial was 6–9% as monotherapy, 9–11% in combination with paclitaxel, and 26–28% in combination with anthracyclines [31]. Older age and combination of the mAb with anthracycline were highlighted as the important risk factors [32]. Although the mechanism of cardiotoxicity has not been fully elucidated, it might be related to the functional role of the antigen, HER2, in cardiomyocyte survival and mitochondrial function [33]. Also, contribution of the effector functions elicited by the antibody isotype (IgG1) to trastuzumab cardiotoxicity profile has not been ruled out.

Target-mediated infusion reactions due to release of cytokines have been also reported following infusion of monoclonal antibodies [34–37], The distinction between hypersensitivity reactions (see below) and target-mediated infusion reactions can be however difficult as some clinical symptoms (chest tightness, chills, shortness of breath, flushing, and fever) are similar [4,34]. The target-mediated infusion reactions observed with mAbs could be related directly to mAb binding to target cells or indirectly because of interaction of the mAb, generally of the IgG_1 isotypes, with immune effector cells (Box 1). Cytokine release after treatment with OKT-3 and rituximab is believed to be directly related to antibody binding to circulating T and B lymphocytes, respectively [19,38]. Additionally, cross-linking of effector cells are believed to cause the infusion reactions observed with bispecific antibodies targeting FcγRI and FcγRIII [39,40].

Nonspecific toxicity

Hypersensitivity reactions are among the common adverse reactions encountered with antibody therapeutics. The hypersensitivity reactions are often associated with immunogenicity of antibody therapeutics. All currently marketed antibodies have

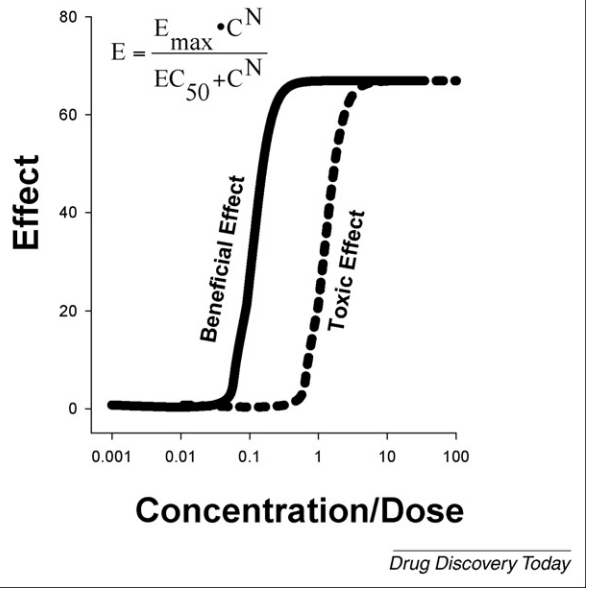

FIGURE 1

A theoretical relationship between drug dose or concentrations and the pharmacological and toxicological effects.

exhibited some level of immunogenicity (Table 1). The immunogenicity profiles of therapeutic mAbs have been addressed in a recent review [4].

Immunogenicity of biologic products can be a significant problem in therapeutic use and can adversely affect the product safety [4]. The primary cause for mAb immunogenicity is the extent of xenogeneic protein content. In general, murine antibodies are highly immunogenic; the development of chimeric, humanized, and fully human mAb technology has decreased the immunogenicity of therapeutic mAbs. Additionally, factors related to antibody structure, composition, patient's immune status, concomitant medications, dose, formulation, and antigen properties could contribute to mAb immunogenicity profiles [1,4].

Hypersensitivity reactions could be acute occurring immediately (up to six hours post mAb infusion) following administration of the first dose or occur following repeated dosing [34,41]. Anaphylactic shock caused by IgE sensitization has been described following treatment with the chimeric antibody, basiliximab [42] and in patients developing anti-antibody responses following treatment with infliximab [37,41]. A higher incidence of hypersensitivity reactions has been reported in patients positive for anti-infliximab antibodies [37].

Preclinical safety studies of monoclonal antibodies

Monoclonal antibodies are generally safe and well tolerated, and the toxicities observed with this class of biologics are usually clinically manageable. The antigen-related toxicities are often predictable from preclinical studies if the antibody cross-reacts with the target antigen in the relevant animal models.

Selection of the appropriate animal species for the conduct of mAb safety studies

The primary requirement for preclinical safety studies is that the mAb cross-reacts with and functionally modulates the target antigen in the species of interest in a manner comparable with that of the human antigen. This can be evaluated by analyzing sequence and structural properties of the antigen, critical residues in the binding region (epitope mapping), understanding of sequence conservation and cross-species homology, and characterization of affinity and functional potency. Comparable binding of antibody with antigen in relevant tissues in the animal species and man as assessed by immuohistochemistry (IHC) is another useful step in the determination of the toxicologically relevant species.

When toxicologically relevant animal models are selected, many of the antigen-related toxicities encountered in the clinic can be detected in preclinical safety studies. For example, the incidence of skin rash reported in clinical studies for anti-EGFr antibodies was observed dose-dependently in preclinical studies carried out in non-human primates. In a multiple dose study of cetuximab in monkeys, skin lesions, along with epidermal sloughing were reported in all mAb-treated animals (SBA; Application Number: STN/BLA 125084). The incidence and severity of the skin toxicities in monkeys were dose-dependent. Since cetuximab does not cross-react with the mouse or rat antigen, no toxicity in CD1-mice or Sprague–Dawley rats was reported. The effect of bevacizumab treatment on wound healing capacity, general growth, skeletal development, physeal dysplasia, fertility, and embryo–fetal development were predictable from single and multiple dose studies conducted in rabbits and non-human primates and are caused by pharmacological activity of the anti-angiogenic mAb (SBA; Application Number: STN-125085/0). The hypertensive effect of bevacizumab, which is related to the effect of VEGF on the potent vasomodulatory effects of nitric oxide, were reported following 26 weeks of treatment with the antibody in the female monkeys (SBA; Application Number: STN-125085/0).

Human toxicities are sometimes not accurately predicted by animal models, even when cross-reactivity and functional modulation of the animal target are comparable with that of human. Drug-induced adverse immune reactions, for example, are often poorly predicted by toxicity studies in animals [43]. The severe adverse events experienced by healthy subjects receiving the starting dose of TGN1412 in the first time in man clinical trial illustrate the potential limitations of animal models. TGN1412 is an agonistic antibody to CD28, the costimulatory receptor for the T-cell receptor/CD3 complex. In the Phase 1 study, all subjects receiving the starting dose (0.1 mg/kg) suffered life-threatening cytokine release syndrome [44]. The starting dose was chosen because no adverse effects occurred in cynomolgus monkeys receiving doses up to 50 mg/kg, and comparable cross-reactivity with cynomolgus CD28 was observed. Therefore, the starting dose provided a large safety margin when considering only the no observed adverse effect level (NOAEL). In a subsequent review of preclinical and clinical data available for TGN1412, the 'Expert Scientific Group on Phase 1 Clinical Trials' has recommended that the starting dose for the first time in human trials of high risk biopharmaceuticals be based on analyses of receptor occupancy, in vitro potency, *in vivo* dose–response data, and NOAEL [45]. For example, a theoretical calculation of receptor occupancy predicted that over 90% of CD28 molecules on T-cells would have been occupied by TGN1412 at the 0.1 mg/kg dose level. Receptor occupancy, potency, and dose–response data can be used to calculate a

minimum anticipated biologic effect level (MABEL); the starting dose would then be selected by the MABEL dose divided by a safety factor that considers the risk–benefit for the recipient population [45]. In addition to the MABEL calculations, the safety profile of biopharmaceuticals with similar mechanisms of action or similar target-mediated effects should be considered. For example, familiarity with the clinical safety profile of muromonab-CD3 (OKT-3), an agonist antibody to CD3, would have been valuable for justification of the starting dose of TGN1412.

Ordinarily, T-cell activation and cytokine release are triggered by simultaneous engagement of TCR and CD28 by the major histocompatibility complex and B7-1 or B7-2, respectively, on antigen presenting cells [46]. TGN1412 activates T-cells in absence of costimulation, thus having the potential to simultaneously activate all T-cells and trigger a cytokine storm. OKT-3 activates T-cells without CD28 costimulation and causes cytokine release syndrome in almost all patients receiving the 5 mg (~0.07 mg/kg) clinical dose [47]. HuM291 is an anti-CD3 antibody in clinical development that has been engineered to reduce Fcγ receptor binding and potentially decrease risk of cytokine release [48]. In preclinical safety assessment studies in chimpanzees, HuM291 caused detectable cytokine release at doses up to 10 mg, but no overt toxicity was observed [49]. Because of the recognized clinical risk of T-cell activation and cytokine release, the starting dose of HuM291 however was 0.015 μg/kg, or ~1/7000th the starting dose of TGN1412 [48]. No effects were observed at the starting dose level. The maximum dose tested was 0.015 mg/kg; this dose was associated with mild-to-moderate cytokine release syndrome. Collectively, these data demonstrate that T-cell activation and symptomatic cytokine release can be triggered by very low doses of agonist antibodies. Therefore, an evaluation of theoretical receptor occupancy and prior clinical experience with OKT-3 and HuM291 could have resulted in a recommendation of a much lower starting dose than was used in TGN1412 trial.

Restricted species cross-reactivity

If the mAb to be used in clinical studies does not cross-react with the antigen in non-human species, a generation of surrogate mAbs capable of recognizing the antigen in the species of interest might be required for the conduct of preclinical pharmacology and safety studies [50,51]. Moreover, in instances where the intended target is not expressed in animal species, development of relevant animal models (transgenic or diseased) will be warranted [43,52–54].

Lack of cross-reactivity of human antibodies (the lead mAb) to the target antigen in animal species generally denotes the absence of sequence conservation of the antigen epitope across species. When application of surrogate mAbs is necessary, comparative investigations of the antibody affinity, functional potency, eitope, exposure-response relationships, and effector functions for the lead and surrogate mAb are necessary [50,51].

Because the cross-reactivity of infliximab (a chimeric IgG1 antibody) is restricted to the antigen in humans and chimpanzee, a functionally comparable surrogate murine antibody of IgG2a isotype 'cV1Q' was employed to evaluate the pharmacology following TNFα suppression [50]. The surrogate antibody not only proved useful for assessing the effect of antigen suppression in a murine colitis model, but it further provided the opportunity for evaluating chronic toxicity and reproductive toxicity in a murine model [50]. Similarly, a surrogate anti-CD11a antibody, a chimeric mouse/rat anti-mouse CD11a (muM17), was used to validate surrogate approach in the preclinical development of efalizumab [51]. Using this surrogate antibody, various toxicology studies such as acute, subchronic, and placental transfer studies were undertaken following the conduct of a comparative *in vitro* tissue cross-reactivity studies in both mouse and human tissues using surrogate and the lead mAb, respectively [51].

Transgenic animals expressing the human antigen have been used previously as 'proof of principle' to demonstrate the efficacy of the mAb in a pharmacologically relevant system. Transgenic animals expressing human tumor necrosis factor have been crucial for the development of anti-TNFα biologics [54]. Additionally, a human CD4 transgenic mouse model was used for the assessment of chronic and reproductive toxicity studies during preclinical development of the anti-CD4 antibody, keliximab [52]. However, extensive studies had to be conducted to characterize this transgenic animal model with respect to the expression, distribution, and functionality of human CD4 expressed in the mice [52]. Although transgenic animals and the surrogate approach allow examination of pharmaco/toxicodynamic properties of the mAb in other non-human species, these approaches increase the complexities and challenges encountered during the course of mAb development. Effective translation of preclinical information, when transgenic animal models or surrogate mAbs are used, requires a detailed understanding of the underlying mechanisms governing mAb exposure and pharmacology in the model of interest.

Antibody immunogenicity in preclinical studies

Antibody responses against administered mAb products can alter pharmacokinetics and biodistribution [4,35,55]. In a mouse model, an anti-idiotypic mAb against the administered antibody produced dose-dependent increases in mAb clearance [55]. The accelerated clearance of mAb was attributed to formation of tertameric, hexameric, or octameric rings composed of the mAb and the anti-idiotypic antibody. Moreover, it was observed that the large immune complexes were rapidly cleared by the reticuloendothelial system [55]. A similar alteration in mAb PK has been reported following detection of human anti-antibody responses to chimeric, and human mAbs in patients (infliximab and adalimumab prescribing information). Formation, distribution, and elimination of immune complexes composed of infliximab and anti-infliximab antibodies were recently examined in cynomolgus monkeys [35]. The formation of immune complexes resulted in an accelerated (3–4 times faster) elimination of infliximab [35].

Estimating of the safe starting dose in clinical trials for therapeutic mAbs

The primary objectives of the Phase 1 clinical trails are the assessment of safety, pharmacokinetics, and pharmacodynamics [56]. In general, the selection of the first-human dose is guided by considerations of preclinical pharmacology, pharmacodynamics, pharmacokinetics, and toxicology. When feasible, attempts are made to scale up all the preclinical information with inclusion of translational assumptions in order to select an initial clinical dose that avoids toxicity.

As highlighted previously, successful translation of preclinical information during the course of mAb development requires

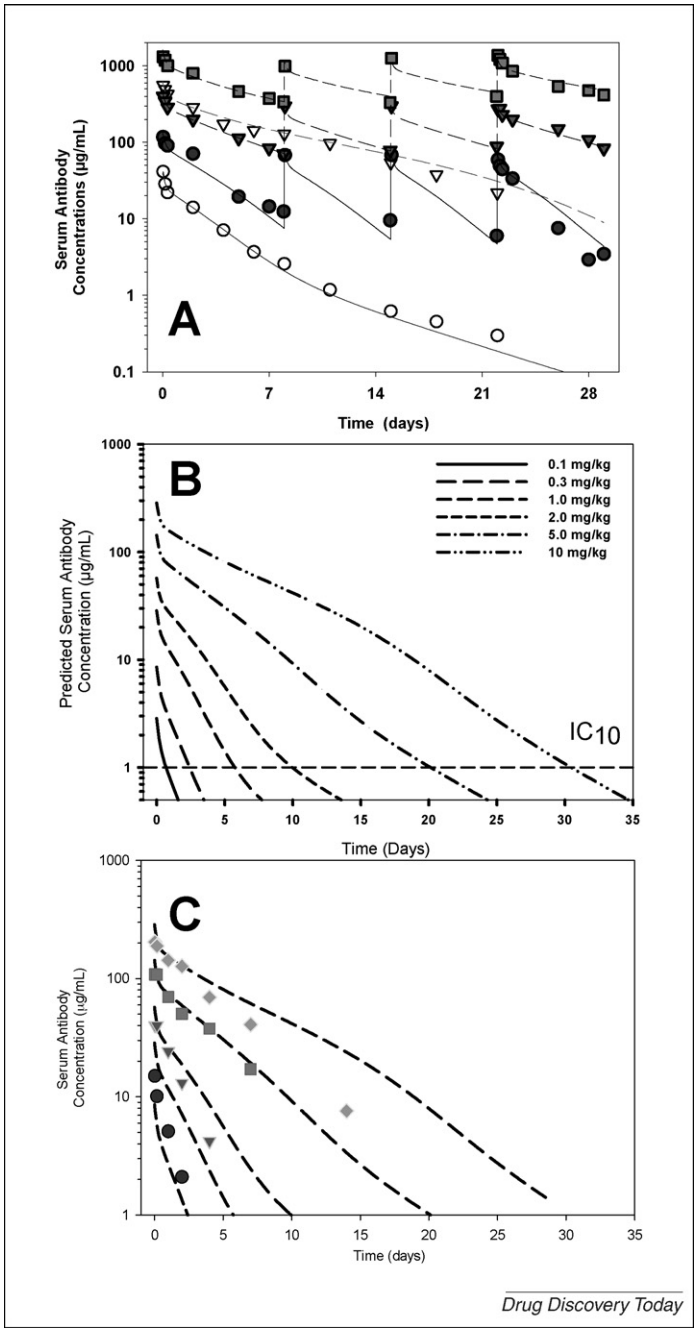

FIGURE 2

(A) Serum concentration–time profiles for anti-Muc18 human antibody in cynomolgus monkeys. Single (open circle: 2 mg/kg and open triangle: 20 mg/kg) or multiple intravenous weekly doses (close circle: 2 mg/kg, close triangle: 6 mg/kg, and close square: 20 mg/kg, in each case the first dose was a loading dose twice the maintenance dose) were administered to monkeys. A nonlinear pharmacokinetic model was used to capture the features of the nonlinear antibody PK in monkeys. The PK model provided a good description of single and multiple doses of the antibody. The Michaelis–Menten constant (k_m) describing saturation of the antigen sink was around 9.7 µg/mL. The maximum intrinsic clearance of the antigen sink (V_{max}/k_m) was 39 mL/day/kg, suggesting that at non-saturating levels of the antibody (serum concentration $\ll k_m$), the antigen sink accounted for greater than 90% of the clearance of the antibody. The lines represent the model fit to the mean data when all data were modeled simultaneously. **(B)** The serum concentration–time profiles following doses of 0.1–10 mg/kg in patients were predicted using the parameters obtained in monkeys and following inclusion of allometric adjustment of clearance in man. The IC_{10} value represent the predicted concentrations at which 10% of antigen is occupied

particular understanding of the antibody and antigen properties as well as the pharmacology in various species. In general, attempts to predict mAb pharmacokinetics from preclinical studies for the antibodies of IgG isotypes against soluble antigens such as IL-8, or VEGF have been successful [3,4]. In contrast to soluble antigens, membrane-associated internalizing antigens can greatly enhance the antibody clearance through a target-mediated, specific process [3]. The contribution of the target to mAb clearance depends on various antigen-related factors such as antigen concentration, distribution, and antigen internalization and turnover rates. Hence, it is not surprising that the differences in antigen properties and mAb affinity and potency across species are critical considerations for successful translation of preclinical pharmacokinetic data.

A strong *in vitro–in vivo* correlation for some marketed mAbs has allowed successful translation of PK/PD information from early development phases in support of clinical study design. Effective translation of PK/PD information during the development of efalizumab has been reported [57–61]. Internalization of the anti-CD11a antibodies was characterized in purified mouse and human T-cells [58,59]. In line with these observations, target-mediated clearance of efalizumab following administration of single intravenous doses in human and chimpanzee was also determined [57,61]. *In vitro* half-maximal binding of efalizumab to lymphocytes was achieved at an EC_{50} of 0.1 µg/mL (similar to the observed K_m for saturation of the antigen sink in chimpanzees), with saturation requiring concentrations around 10 µg/mL [58,59]. When corrected for differences in the PK across species, similar steady-state serum trough concentrations were achieved in man at the therapeutically effective doses [57,60,61].

In our experience, human pharmacokinetics of mAbs in relevant animal species—with inclusion of translational assumptions regarding the allometric adjustment of the clearance rates, the antigen properties, and the antigen-mediated elimination and efficiency across species—are usually highly predictive of human pharmacokinetics. For example, the pharmacokinetic parameters obtained from preclinical studies of a mAb to MUC18 were predictive of the actual exposure in Phase 1 clinical study in patients with malignant melanoma (Figure 2). As shown in Figure 2A, important features of the anti-Muc18 antibody PK in monkeys were captured using a nonlinear PK model. Because biomarker and pharmacodynamic data were not available, the nonlinear elimination of the anti-MUC18 antibody was used to approximate the saturation of antigen over a range of doses. Simulation of the concentration–time profile in patients predicted a MABEL dose with approximately 10% antigen saturation in patients at 0.1 mg/kg (Figure 2B). Given the severity of disease in the Phase 1 population, and that additional safety factor was not applied, a starting dose of 0.1 mg/kg was recommended. The simulations were also used to justify the dose escalation strategy. Escalation to the next dose level was conducted four weeks after the enrollment of the last patient in the preceding cohort because the simulations predicted washout of drug by four weeks after dosing. In Figure 2C the observed clinical PK is overlayed on the simulations. Concentra-

(C) The serum concentrations–time profiles (actual data) for the antibody in melanoma patients following a single dose administration of 1 mg/kg (circle), 2 mg/kg (inverted triangle), 5 mg/kg (square) and 10 mg/kg (diamond) along with model predictions (lines) are presented [62].

tions were overpredicted at the two lowest dose levels; this probably occurred because of a high level of soluble MUC18 subsequently found to be present in human serum that was not present in monkey serum. A slightly faster nonlinear clearance (body weight normalized) of the anti-Muc18 antibody was observed in humans than in monkeys; this might have been due to species differences or to additional clearance of the antibody by tumor antigen. Overall the clinical PK was reasonably predicted by the monkey data, and the simulations successfully accomplished their objectives in the design of the Phase 1 study.

Future prospects

Therapeutic monoclonal antibodies provide a unique and novel opportunity for the treatment of many human diseases. With the advancement of new technologies and the diversity in the novel therapeutic targets, many new opportunities for the clinical development of mAb-based therapeutics should arise. Many examples exist of successful transitions from preclinical to clinical development that have been greatly facilitated by preclinical safety and pharmacology data. As the unfortunate incidence with TGN1412 highlighted, the preclinical evaluation of clinical safety risk will require a case-by-case evaluation and understanding of many factors affecting the preclinical PK, PD, and safety of monoclonal antibodies.

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