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Translational strategies for development of monoclonal antibodies from discovery to the clinic

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Successful strategies for the development of monoclonal antibodies require integration of knowledge with respect to target antigen properties, antibody design criteria such as affinity, isotype selection, Fc domain engineering, PK/PD properties and antibody cross-reactivity across species from the early stages of antibody development. Biophysical measurements are one of the critical components necessary for the design of effective translational strategies for lead selection and evaluation of relevant animal species for preclinical safety and efficacy studies. Incorporation of effective translational strategies from the early stages of the antibody development process is a necessity; when considered it not only reduces development time and cost, but also fosters implementation of rational decision-making throughout all phases of antibody development.

Introduction

The biologics market continues to witness an impressive rate of growth and the monoclonal antibody market, in particular, has contributed remarkably to the expansion of this segment within the pharmaceutical industry. In 2006, close to 80% of the annual biologics growth rate in the United States (US) was attributed to cancer and anti-TNF antibodies, with increases in growth of 56% and 25%, respectively, compared to those in the previous year [1]. Additionally, the monoclonal antibody sector is anticipated to achieve a growth rate of approximately 14% by 2012, easily outstripping the predicted 0.6% growth rate in the small molecules market [2]. The robust late-stage antibody pipeline within the biotech sector has drawn an increasing amount of interest from the large pharmaceutical industry and has triggered the largest product and platform deals in 2006, with values more than $2.1 and $5.1 billion in partnering and mergers and acquisitions, respectively [3].

The advancement in science and confluence of technologies has made it possible to generate, rapidly and effectively, highly tailored fully human antibodies against a diverse array of targets. The number of approved antibodies in the US further underscores the clinical effectiveness of this class of therapeutics in oncology and inflammatory diseases [4,5]. A major challenge in the development of antibodies, much like small molecule drugs [6], however, is maintaining the effective flow of information and translation of accumulated knowledge throughout the drug development process (Fig. 1). The design of effective translational strategies from early stages of the antibody development process is necessary not only to reduce development time and cost, but also to foster implementation of rational decision-making processes throughout various development phases.

Successful translational strategies for the development of monoclonal antibodies should allow understanding of the relationship between the 'unit dose' and 'unit effect' with respect to both beneficial and deleterious effects from early stages of development. The flow of information from later to earlier stages of development should provide opportunities to facilitate selection of more

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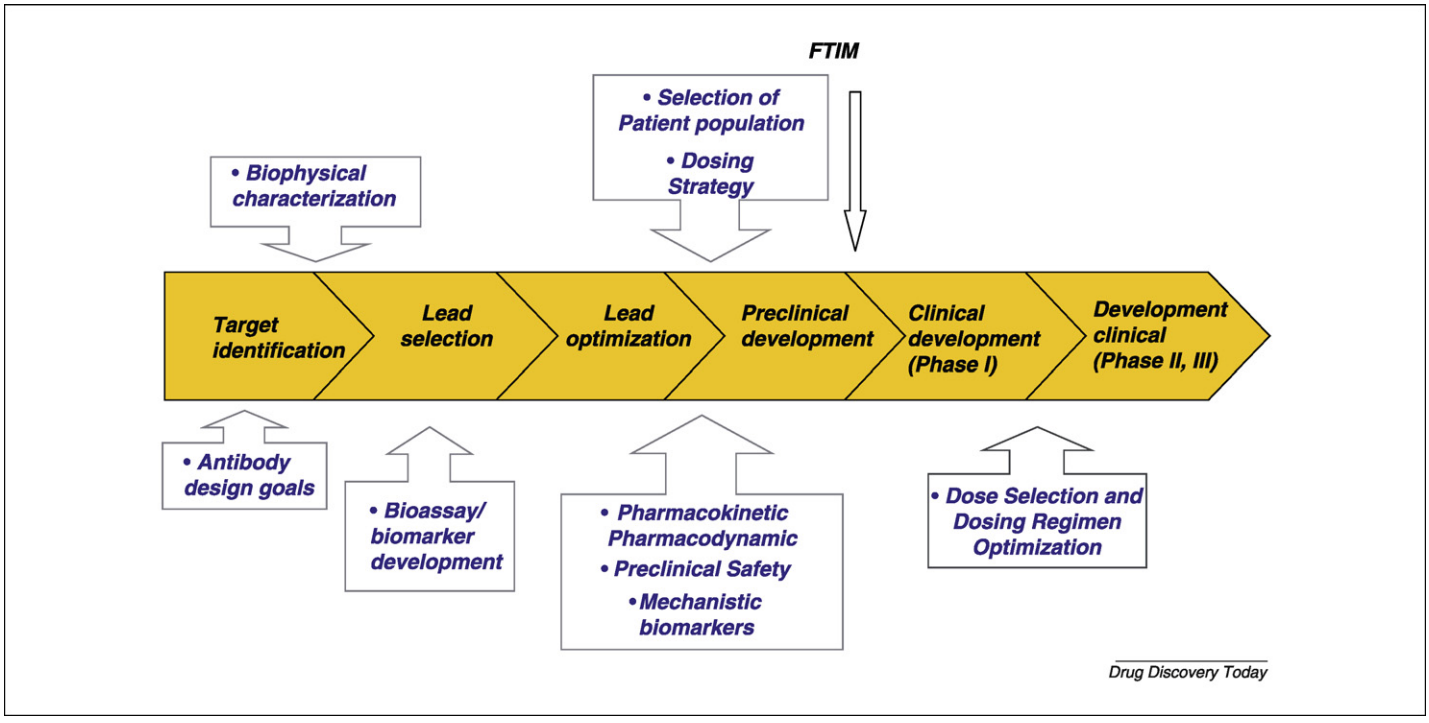

FIGURE 1

Continuum of information flow during the design of effective translational strategies in development of monoclonal antibodies (FTIM: first-time-in-man).

effective drug candidates [7]. To achieve this objective, integration of knowledge with respect to the target antigen properties that is, antigen distribution, expression profile, kinetic properties, target pharmacology, antigen isoforms and pharmacological redundancy in health and disease, as well as antibody design criteria, such as antibody isotype, affinity, pharmacokinetics, pharmacodynamics and safety is necessary (Fig. 1 and [4,7–9]). This review will examine important considerations necessary for the design of effective translational strategies during the development of monoclonal antibodies.

Characterization of antibody–antigen interactions

Antibody–antigen complexes can be described thermodynamically and kinetically by the affinity or the equilibrium dissociation constant (K_D), the association rate constant (k_{on}), or dissociation rate constant (k_{off}). Biophysical measurements are one of the critical components necessary for developing effective translational strategies with respect to lead selection, evaluation of the relevant (appropriate) animal species for the conduct of safety and efficacy studies and the design of effective clinical dosing strategies. Additionally, biophysical techniques prove highly effective in evaluation of cross-reactivity to orthologous antigens from species other than human.

Generally, screening studies using biophysical methodologies provide superior information content compared with standard ELISA screening experiments and thus help in eliminating antibodies with undesirable affinities and in the selection of the best lead drug candidates [10,11]. Implementation of effective biophysical screening strategies early during the development phase allows selection of promising drug candidates with respect to homologous (for addressing potential safety concerns) and orthologous antigen selectivity (for selection of the relevant toxicology species) [4,12]. Three biophysical techniques, Biacore®, kinetic exclusion assay (KinExA®) and fluorescence activated cell-sorting (FACS) are routinely employed for the screening of therapeutic antibody molecules.

The premier technique used for determining antigen/antibody kinetics is Biacore®. With Biacore technology, one reactant is immobilized to the biosensor surface while the other binding partner is flowed across the surface. Binding is followed in real time by surface plasmon resonance (SPR), which is directly related to refractive index changes at the biosensor surface that occur during complex formation and dissociation [13,14]. Biacore experiments do not require labeling of either antigen or antibody before analysis. Figure 2 illustrates a typical sensorgram (data) from a Biacore experiment. The starting baseline is flat in Fig. 2 as it assumes one interactant has already been immobilized and only buffer is flowing across the surface. Following injection of analyte, the refractive index at the surface changes in response to binding. Depending on the concentration of the flowing reactant relative to the K_D, the k_{off} of the interaction and the length of injection, a steady-state or equilibrium condition may be obtained, as indicated by the formation of a plateau of the sensorgram. After the injection ends, buffer is again flowed across the surface and any bound reactant dissociates from the surface. In the regeneration step, a chemical condition, like a short pulse of acid or base, is determined for the dissociation of the binding partner from the biosensor surface in a matter of seconds to minutes, which leaves the immobilized molecule active and ready to bind in another injection. In a typical Biacore experiment, five to eight different concentrations of the analyte are injected in triplicate. Differential equations describing the kinetics of a 1:1 reversible interaction are used to fit, nonlinearly, the sensorgrams in order to determine the k_{on}, k_{off} and $K_D = k_{off}/k_{on}$ [14,15]. When performing Biacore studies

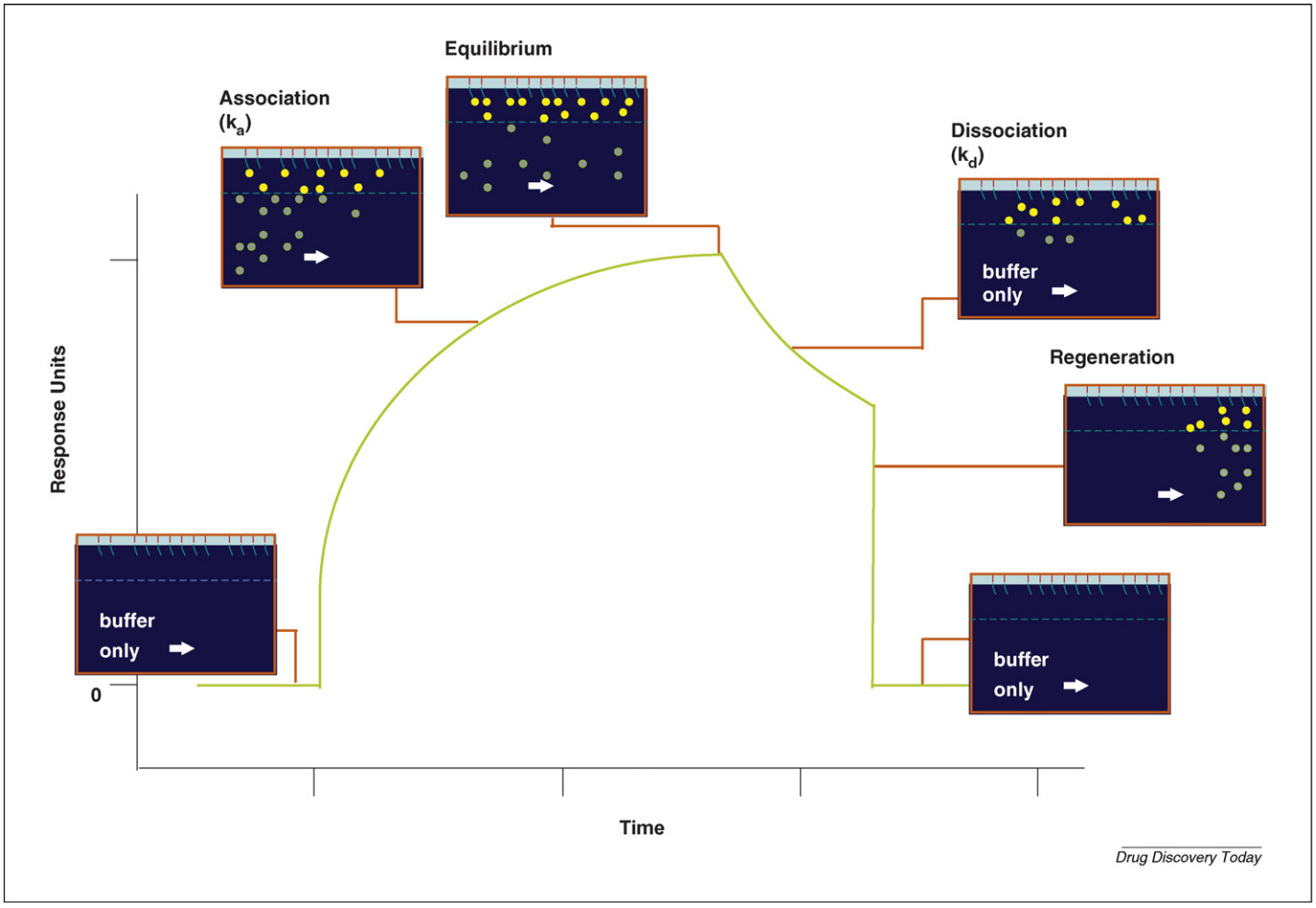

FIGURE 2

Representation of a typical sensorgram from a surface plasmon resonance (SPR) kinetic experiment.

with multivalent molecules, it is extremely important to avoid orientations that lead to artifactual results. It is imperative that antibody be immobilized to the biosensor surface and that univalent antigen is flowed across the sensor in order to obtain the microscopic or site-binding constant which generates the greatest amount of structural information for the interaction [16,17]. Challenges encountered during Biacore analysis and performing high quality Biacore experiments have been discussed in several recent publications [16–19].

The KinExA is a method that incorporates the use of a flow spectrofluorimeter and is solution based, in contrast to Biacore [16,17,20–22]. In a KinExA experiment, equilibrated solutions of antigen and antibody are flowed through a bead bed with immobilized antigen, and free antibody-binding site from the equilibrated solution binds to the bead pack. The captured antibody on the bead pack is detected by a secondary fluorescently labeled species specific polyclonal antibody and is quantitated using epifluorescence. The percentage of the free antibody versus total antigen concentration is plotted, and this titration curve is fitted to a 1:1 equilibrium model to calculate the K_D of the interaction. It is also possible to extract the k_{on} and k_{off} for the interaction by KinExA [16,17,20–22]. Biacore and KinExA are highly complementary techniques and provide excellent confirmatory information using different physical approaches.

In a cell-based FACS method, a titration experiment allows measurements of the K_D of a monoclonal antibody to a cell surface receptor where a constant number of cells in solution are titrated with increasing concentrations of a given antibody and the solutions are allowed to reach equilibrium. A fluorescently labeled polyclonal antibody is then used for detection of cell-bound antibody. FACS flow cytometry instrumentation measures the fluorescence of cells moving individually through an excitation laser, hence, FACS is an ideal methodology for detecting cell-bound antibody. A nonlinear fitting equation, based on the multiple independent binding site equation (MIBS), has been derived that relates the mean fluorescence intensity (MFI) measured by FACS to the amount of cell-bound antibody [23]. The K_D of the antibody to the cell receptor is estimated by a nonlinear fit of the FACS MFI as a function of the antibody concentration. The derived nonlinear equation takes into consideration whether the cell-based titration is under 'K_D-controlled' conditions or 'receptor-controlled' conditions, hence it is a much more rigorous analysis than models that commonly assume only a K_D influence, and it avoids the limitations of traditional (and outdated) Scatchard plots [24,25]. The technique is also relatively high throughput since a 96-well plate format can be used with FACS instrumentation, and multiple antibodies can be analyzed in a single experiment and compared directly.

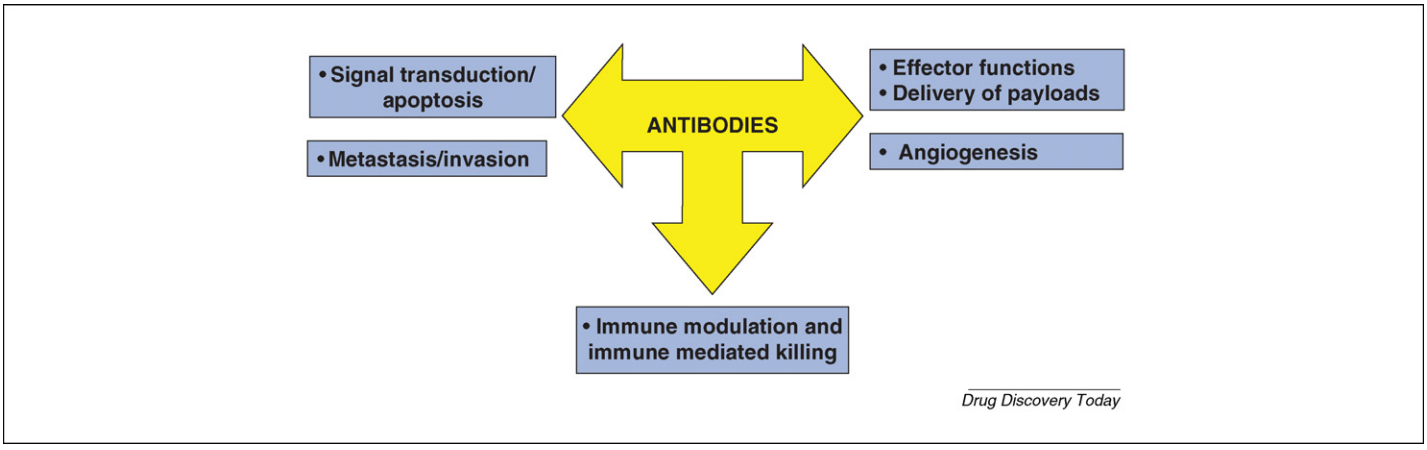

FIGURE 3

Antibodies can mediate their biological activities via multiple mechanisms: binding to receptor, or their cognate ligands; sterically prevent the receptor from assuming the requisite conformation for the homo/heterodimerization step required for signal transduction; modulate (activate or inhibit) the immune system; by mediating effector functions, namely complement-dependent cytotoxicity (CDC) and/or antibody-dependent cell-mediated cytotoxicity (ADCC).

Translational considerations for preclinical development of monoclonal antibodies

Selection of a target antigen is the first step in the generation of an antibody therapeutic. Understanding target antigen biology and its role in the pathogenesis of disease is of primary importance. Surveying appropriate tissues for validation of target expression by immunohistochemistry, or equivalent methodologies, is vital to establishing disease linkage and verifying that the target antigen is not abundantly expressed in normal tissues. Also, functional validation of the target is critical [26]. For example, molecular approaches to modulate target expression via knockdown technologies (e.g. antisense or RNAi, overexpression and transgenic animals), can elucidate target biology [27–29]. Functional redundancy of the target is an additional consideration; if the target antigen belongs to a conserved protein family, downmodulation of the target may not result in the desired phenotypic outcome [30]. Upon antigen target selection, a panel of antibodies can be generated via a number of platform technologies (Box 1) and interrogated for biological activity *in vitro* and *in vivo*. Antibodies are ranked for affinity, potency and safety and a lead candidate may be selected and advanced to clinical development, if warranted [31].

Antibodies can mediate their biological activities via multiple mechanisms (Fig. 3) [32,33]. Growth factor receptors and/or their activating ligands are often overexpressed in a number of malignancies and can promote tumor cell growth and resistance to chemotherapeutic agents. By binding to growth factor receptors, or their cognate ligands, antibodies can interfere with ligand binding and, hence, disrupt signaling pathways [32–34]. Alternatively, an antibody can sterically prevent the receptor from assuming the requisite conformation for the homo/heterodimerization step required for signal transduction [33,34]. Interference of signal transduction pathways can thus mediate apoptosis and/or inhibit cellular proliferation. Antibodies also exhibit the potential to bind and thereby inhibit the biological activity of molecular targets implicated in the invasion or metastasis of tumor cells. They can target antigens uniquely expressed on the neovasculature of tumors, or growth factors that promote angiogenesis, thereby impacting the blood supply to tumors [33,35] and can further modulate the immune system by enhancing tumor-antigen specific immune responses, or alternatively, suppressing immune activity, as in the case for treatment of autoimmune disease [36,37]. Additionally, antibodies can target tumor cells by mediating effector function, namely complement-dependent cytotoxicity (CDC) and/or antibody-dependent cell-mediated cytotoxicity (ADCC). In the case of CDC, upon antibody binding to a tumor-associated antigen, the complement cascade is initiated, triggering the formation of the membrane attack complex and ultimately,

BOX 1

Antibody technology platforms: The first generation of mAbs entered clinical evaluation in the early 1980s and were of murine origin. As murine mAbs progressed into clinical trials, the full potential of these agents had yet to be realized. The human immune system recognizes murine mAbs as foreign, producing human anti-mouse antibodies (HAMAs), which rapidly clears them from the body, thereby limiting any therapeutic benefit. Furthermore, murine mAbs are inefficient at recruiting the human immune system to trigger effector function [41]. To increase efficacy and decrease immunogenicity, chimeric and humanized mAbs derived from both human and mouse DNA were produced. Technological advances also led to generation of fully human mAbs through the development of transgenic animals, whereby endogenous antibody genes were replaced by the equivalent human sequences, and from human antibody phage-display libraries [41–44]. The transgenic mouse is ideally suited for generating antibodies against multispanning membrane proteins or protein–antigen complexes difficult to produce and purify in functional form. Also, this platform may facilitate generation of higher affinity leads not requiring further affinity maturation. Furthermore, the transgenic mouse is useful for generation of antibody panels for target validation prior to identifying lead mAb candidates. Display technology is, however, better suited for isolation of antibodies against infectious disease targets or toxins. Display is preferable where an immunogen is immunosuppressive, or if the antigen target is highly conserved between mouse and human; a high degree of conservation may render the antigen insufficiently immunogenic in the mouse [31].

tumor cell lysis [33]. ADCC involves activation of immune effector cells actively lysing tumor target cells that are bound by a tumor-antigen specific antibody [33]. Human IgG1 is efficient at supporting effector functions, [31,33] while IgG2 and IgG4 are most suitable as blocking agents and generally ineffective at mediating ADCC or CDC. Therefore, selection of the appropriate isotype (IgGs 1, 2 or 4) is a critical consideration in antibody development. The effector functions associated with IgG1 isotype can result in the enhancement of the efficacy profiles when applicable [4,8,9]. A promising means for augmenting the antitumor potency of antibodies is through enhancement of effector function via engineering of IgG1 Fc variants [31,38]. Antibodies can also be effective agents for the delivery of a cytotoxic payload such as a chemotherapy drug, toxin or radioisotope [33,39,40].

Selection of appropriate rodent efficacy models is critical to antibody drug discovery and development, as well as for translational strategies. For cancer models, the human tumor xenograft model is the primary tool to evaluate *in vivo* efficacy. Key endpoints include inhibition of local growth, metastasis or prolongation of survival.

The transgenic knock-in mouse is an informative tool to evaluate antibodies that exhibit poor or no cross-reactivity to the murine ortholog of the antigen target. These animals are genetically engineered to express the human ortholog and enable one to predict better the effect of the antibody on tumor–host interactions [45,46]. Moreover, the transgenic knock-in mouse enables one to circumvent the use of surrogate antibodies. Generation of knock-in models can, however, be costly and time-consuming for breeding and back-crossing to a suitable immune-compromised murine strain appropriate for xenograft models.

Furthermore, xenograft models often do not fully recapitulate all stages of cancer progression [47–49]. Consideration should be given to the route of tumor implantation as well as selection of tumor cell line. Frequently, transplanted tumors adapted to grow in animals exhibit a higher proliferative capacity as compared to the original patient tumor [48,49]. The vascularity of the transplanted tumor may differ, with transplanted tumors exhibiting improved blood supply and reduced necrosis [49,50].

What approaches should be employed to establish more clinically accurate tumor models? For example, orthotopic implantation of primary tumor fragments directly obtained from patients may be more predictive. Orthotopic implantation allows human tumors to mimic clinical-like tumor growth and metastasis, in contrast to subcutaneous implantation, which is not a common site for human tumors, yet is the usual site of implantation in preclinical models [49]. Also, primary tumor fragments, as opposed to cell lines, are histologically intact, maintain tissue architecture and preserve the original genetic lesions of the disease [47]. In summing up, refining rodent models to more closely recapitulate disease will enable scientists to predict drug efficacy better and develop more clinically relevant translational strategies.

Considerations for translation of preclinical safety information

Characterization of safety in relevant species is pivotal to effective translational strategies. The purpose of preclinical safety evaluation for small and large molecules is to identify potential risks to humans. These data are used to recommend a safe starting dose, guide dose escalation schemes and other risk mitigation strategies during early clinical development. The objective is to reveal potential target organs of toxicity with an assessment of dose–response, reversibility and monitorability, as well as establishing no-observed adverse effect levels (NOAELs) or minimally anticipated biological effect levels (MABELs) [51–53]. It is essential, therefore, that these pivotal preclinical studies are conducted in a pharmacologically relevant species and in compliance with good laboratory practice (GLP).

Safety concerns associated with many monoclonal antibodies are often an extension of their intended pharmacological activity [4]. This 'exaggerated' pharmacological response may be the result of a more profound modulation of the target or may occur as a consequence of antigen expression on nontarget cells [54–56]. Nonspecific effects can occur following dosing with monoclonal antibodies. Hypersensitivity reactions are commonly associated with immunogenicity of the antibody [4,57]. Despite advances in humanization and the introduction of fully human antibodies (Box 1), immunogenicity remains a significant clinical problem that is poorly predicted by preclinical studies. In these toxicity studies, measurement of plasma exposure and presence of any host anti-antibody response is essential to data interpretation [4,8,9,57].

Selection of the appropriate species to be used for preclinical safety assessment must be considered. The primary requirements are that the species should express the antigen, and the antibody should bind with sufficient affinity to modulate the target in a manner comparable to that in man. Data supporting species relevance will also include: cross-species comparison of antigen tissue distribution, sequence similarity, epitope binding and functional potency assays. Antibody-binding profiles in human and animal species, assessed by using biophysical techniques and immunohistochemistry across a wide panel of tissues, is another required step in the selection of the appropriate species. Where antibodies possess agonistic properties, further characterization of potency and downstream signaling pathways may be necessary to establish whether the toxicological consequences of target modulation can be fully recapitulated in preclinical models [4].

When an appropriate species cannot be identified, for example if the target is not expressed in animals, or if there is low conservation of the epitope across species, additional approaches will need to be considered. This might include the use of a surrogate antibody(s) that has similar characteristics to that of the intended therapeutic antibody or the generation of transgenic animals that do express the human antigen. Development and validation of the transgenic animals, or the surrogate antibodies is likely to incur significant time and resource demands. The transgenic animal must be characterized for antigen expression profile and functional integrity. In the case of surrogates, an antibody is required that reflects as much as possible, the characteristics of the clinical candidate with respect to affinity, isotype, functional activity, effector function and so on [4,8,9,12,51,52,58].

Immunogenicity of therapeutic antibodies may be a significant problem in the therapeutic use of antibodies containing xenogeneic protein sequences. All currently marketed antibodies have exhibited some level of immunogenicity [4,8,9]. Although evolution in the generation of monoclonal antibodies, that is moving away from murine antibodies to humanized or human antibodies,

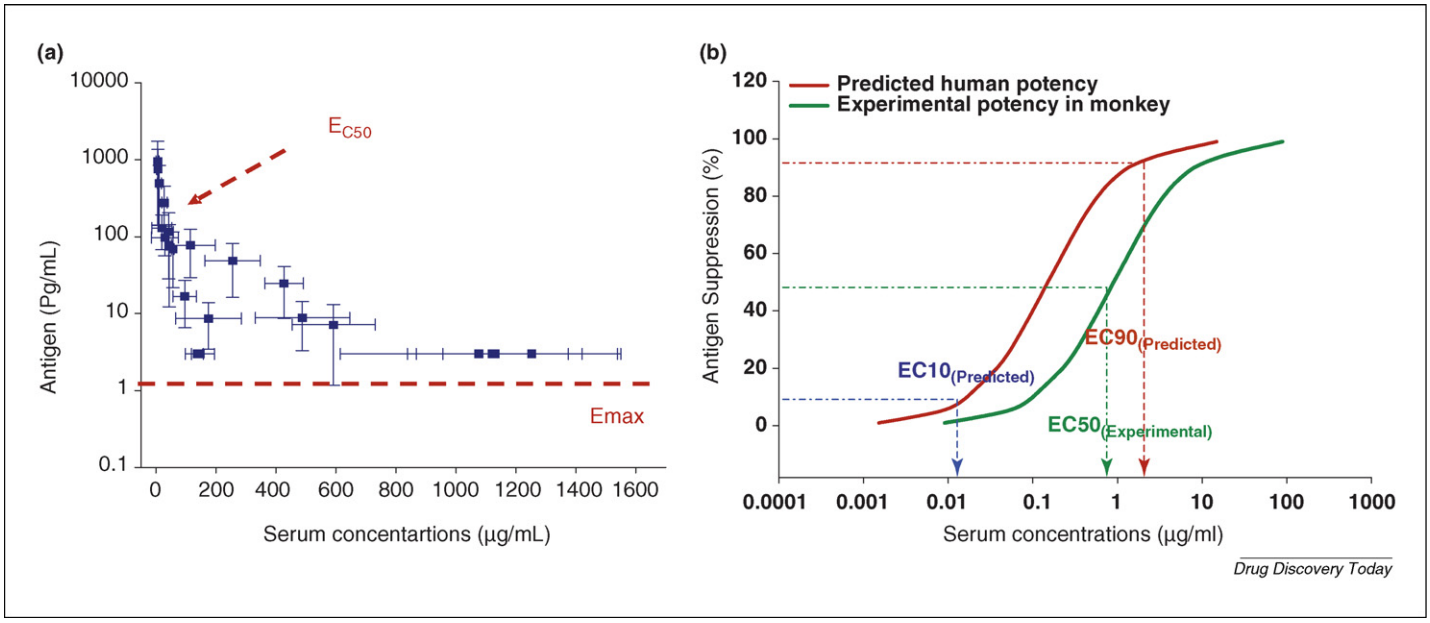

FIGURE 4

Relationships between serum antigen and antibody concentrations in monkeys **(a)**. Predicted pharmacodynamic system efficiency (EC_{50} and E_{max}) in patients from the experimental data obtained in monkeys **(b)**.

has been crucial in reducing the immunogenicity profiles of the marketed antibodies, the anti-drug-antibody (ADA) response to therapeutic antibodies still has clinical relevance. ADA response may not only alter pharmacokinetics by impacting clearance, but can reduce efficacy (anti-idiotypic neutralizing antibodies) and introduce serious safety concerns as well [4]. In general, the predictability of nonclinical studies for evaluation of immunogenicity is low due to inevitable immunogenicity of human proteins in animals [58,59]. Although recent methods in the use of computational tools for identification of T-cell epitopes have improved drastically [60], predictive values of these tools remains to be determined.

Translation of exposure–response data from discovery into the clinic

Conducting PK and PD studies in appropriate animal models can greatly enhance the translation of information across species. When appropriate immunoassay methodologies are available, relationships between antibody exposure and the effect on free antigen reduction or antigen binding to the antibody can be evaluated. Evaluation of the relationships between the free antigen and antibody concentrations *in vivo* can provide invaluable information regarding the antibody potency, EC_{50} (antibody concentrations resulting in 50% suppression of the antigen) and the maximum system efficiency, E_{max} [4,9]. The relevant experimental knowledge obtained from biophysical studies (see previous sections) can allow correction for affinity differences between the orthologous antigens in man and the relevant animal model. Additionally, information on antigen concentrations can be obtained experimentally by direct measurements of the target antigen in patients and be related to the concentrations in preclinical models.

Recently, we implemented this approach for the determination of exposure–response (antigen suppression) relationships for a fully human antibody against a soluble circulating antigen in humans from data obtained in monkeys (Fig. 4). Following administration of the antibody in monkeys, concentration-dependent suppression of the free antigen was observed (Fig. 4a). Biophysical experiments previously revealed an eightfold lower antibody affinity for the monkey orthologous antigen in monkeys compared to that in humans. Correcting for affinity differences and adjusting for antigen concentration differences in the target patient population allowed determination of the pharmacological system efficiency (EC_{10}, EC_{50} and E_{max}) in patients (Fig. 4b). Understanding the relevance of earlier nonclinical studies (both efficacy and safety models, see previous sections) and the antibody pharmacokinetics across species [4,8,9,12], allowed for a rational design of the early clinical studies and justification of clinical dose selection.

This approach has proved highly effective for the design of translational strategies from discovery to the clinic, with many of the currently marketed antibodies against soluble antigens, including adalimumab and omalizumab, or a membrane-associated antigen such as efalizumab [61–63]. Omalizumab is a humanized IgG1 anti-IgE antibody that inhibits binding of IgE to its high affinity receptor in both man and monkey with different affinities [62,64]. The magnitude of reduction in the antigen (IgE) was directly related to the efficacy of omalizumab in asthma, and the concentration–response for omalizumab was corrected for affinity differences across species and demonstrated to be highly predictable from preclinical studies in monkeys [62,64,65]. Similarly, implementation of a science-based translational strategy for efalizumab, a humanized IgG1 antibody against CD11a, proved highly effective during the development of this antibody [63,66–68]. Highly predictive information was obtained from preclinical studies with respect to antibody internalization, antigen binding, and *in vivo* potency in chimpanzee. This information allowed

determination of therapeutic effective doses during early development [63,66–68].

Conclusions

An effective translational strategy should deliver a superior therapeutic candidate into clinical development for the treatment of the appropriate patient population. Understanding of the relationship between the 'unit dose' and 'unit effect' with respect to both beneficial and deleterious effects is essential in accomplishing this objective. A science-based decision-making approach for transition of the exposure–response relationships during the various stages of the antibody development is vital for effective clinical development of antibody-based therapeutics. Incorporation of translational strategies from early stages of antibody development can reduce the development time and cost and increase clinical success.

Conflict of interest

At the time of submission of this article, MT, SK, AD and LR were employed by MedImmune. RK and GB were employed by AstraZeneca. MedImmune is a wholly owned subsidiary of AstraZeneca. SK and AD are currently employed by Takeda San Francisco.

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