Review Article

Theme: Pharmacokinetic/Pharmacodynamic Modeling and Simulation in Drug Discovery and Translational Research

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Application of Quantitative Pharmacology in Development of Therapeutic Monoclonal Antibodies

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Abstract. The advancement of therapeutic monoclonal antibodies during various stages of the drug development process can be effectively streamlined when appropriate translational strategies are applied. Design of successful translational strategies for development of monoclonal antibodies should allow for understanding of the dose- and concentration-response relationships with respect to both beneficial and toxic effects from early phases of drug development. Evaluation of relevant biomarkers during early stages of drug development should facilitate the successful design of safe and effective dosing strategies. Moreover, application of quantitative pharmacology is critical for translation of exposure-response relationships early on.

KEY WORDS: monoclonal antibodies; pharmacodynamics (PD); pharmacokinetics (PK); quantitative pharmacology; translational strategies.

INTRODUCTION

Therapeutic monoclonal antibodies continue to emerge as one of the major contributors to the underlying growth observed in the biologics market (1,2). Since their introduction in the late 1980s, therapeutic antibodies have dramatically impacted the prescription pharmaceutical market and treatment options available to patients across a variety of therapeutic areas. With >20 approved antibodies already in the US marketplace and another ~200 more in various stages of development, sales of antibody-based therapeutics are expected to continue growing beyond the US\$35 billion worldwide sales mark attributed to this segment of the biologics industry (1,2). Therefore, application of effective translational strategies is critical for determining the key development attributes that assist in maximizing the therapeutic potential of new antibody drug candidates in the biologics marketplace. During the preclinical development stage, relative competitiveness of a new antibody candidate can be understood by evaluating the pertinent parameters that provide comparative assessment of the new generation lead *versus* the existing counterpart in a head-to-head fashion (3).

Similar to small-molecule drugs, translation of the exposure-response relationships during development of monoclonal antibodies can be a major challenge hindering effective development of this class of biologics (4-6). The importance of translational challenges encountered during antibody development is highlighted by the severe adverse events experienced in the first-in-human (FIH) clinical trial in healthy subjects receiving the starting dose of TGN1412 (7). As established by the TGN1412 example, effective translation of information across species will require comparative investigations of the target antigen properties, species-dependent pharmacology, and antibody design criteria in the pharmacologically relevant species (4-6,8). Assessment of the factors that regulate antibody exposure-response relationships in the relevant animal models is critical for the design of successful translational strategies from discovery to the clinic (4-6). Additionally, evaluation of the pharmacodynamic (PD) system efficiency and stimulus-response mechanisms that convert receptor occupancy into the pharmacological response(s) along with effective application of quantitative pharmacology (QP) are among the key translational considerations throughout the antibody development process (5,6,9). Depending on the specific clinical indication involved, unmet medical needs within a patient population may require that the efficacy or dosing-related attributes for the existing antibody be improved. An in-depth understanding of the QP-related properties for the original lead can greatly facilitate evaluation of the optimized attributes of the second-generation construct (3,4,9). This review will focus on the application of quantitative pharmacology in the development of monoclonal antibody therapeutics.

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Application of PK–PD Modeling. Implementation of successful translational strategies during development of monoclonal antibodies necessitates integration of knowledge with respect to antigen expression and kinetic properties, target pharmacology, PD system efficiency and redundancies, antibody isotypes as well as evaluation of composite factors that regulate or impact antibody pharmacokinetic (PK) and PD properties (Fig. 1) (3,5,10). Interaction of antibody with soluble or cell-associated targets provides a unique opportunity for selection and evaluation of relevant biomarkers during the early preclinical stage (4,10). Proof-of-mechanism (POM) biomarkers should allow for evaluation of antibody interaction with the molecular target while proof-of-principle (POP) biomarkers further address whether target modulation results in measurable downstream activity and signaling. As safety concerns associated with antibody-based therapeutics are often an extension of their intended pharmacological activity (11), evaluation of desirable or deleterious outcomes may be accomplished by the use of proof-of-concept (POC) biomarkers (4).

Application of QP can greatly facilitate the seamless flow of information across various development stages (5,10,12,13). Similar to small-molecule drugs, the relationship between the antibody dose or concentration(s) and the observed pharmacological response(s) can be characterized by linear and log-linear, sigmoid $E_{\max}$, biophase distribution, or indirect response models (14–25). The relationships between the drug dose or concentrations and the observed pharmacological effect can be characterized by linear or log-linear PD models in the form of $E = E_0 + m \bullet C$ (or $\ln C$), where E and E_0 denote the observed and baseline (no-drug) effects, respectively, and m is the slope of the concentration–effect relationship. For some drugs, however, these simple models do not sufficiently capture the concentration–effect profile. In these instances, the $E_{\max}$ model or the sigmoid $E_{\max}$ model may be a more suitable PD model. Further complexities such as counterclockwise or clockwise hysteresis loops can be observed in the concentration–effect relationships at non-steady-state serum or tissue concentrations. The underlying causes for these additional complexities in the exposure–response relationships can be diverse and related to factors such as active or inhibitory metabolites, indirect

response characteristics, a transient time delay between concentration and effect, or an equilibration delay in the distribution of drug to the biophase compartment (5,12,14,16,17,22–24,26–28). Additionally, target-mediated PK models (21,29) and bi- or tri-molecular interaction PK–PD models (4,30) that describe antibody PK, the interactions between antigen(s) and antibody, and the elimination of free antigen(s) are highly informative tools used for prediction of safe and effective dosing strategies (13,31).

Factors Impacting PK and PD. Many factors can regulate or impact antibody pharmacokinetics and a number of comprehensive reviews have addressed this topic in detail (21,27,32). Briefly, antigen properties, such as antigen distribution (soluble *versus* cell-associated), antigen concentration, as well as Fc receptor (both FcRn and FcγR) expression and distribution can influence antibody pharmacokinetics and biodistribution (10,26). Other factors, such as antibody structure and engineering, host factors, concurrent medications, and immunogenicity can also alter antibody pharmacokinetic profiles (32).

Antibodies can mediate their biological activities via multiple mechanisms such as neutralizing target function, activating receptors by mimicking endogenous receptor ligand, delivering toxins to specific cells, and eliciting effector functions in conjunction with target modulation (5,9,27,33–35). By binding to a target receptor or its associated antigen (s), antibodies can interfere with antigen binding and hence disrupt signaling pathways. In these instances, the data obtained from *in vitro* and *in vivo* studies should facilitate construction of relevant PK–PD models accounting for antibody PK, antibody affinity for the antigen, antigen turnover rates, receptor occupancy, and elimination of antibody–antigen complexes (4,30). When appropriate immunoassay methodologies are available (see below), relationships between antibody PK and the ensuing effects on POM or POP biomarkers can be effectively examined. Application of QP should then guide the design of efficacy and long-term safety studies in the relevant species (5,6,9,36). Adjusting for differences in antibody affinity, antigen concentrations, and the pharmacokinetic differences across species, the POM biomarker profiles may be correlated with the downstream POP or POC biomarker effects (inhibition of downstream

Fig. 1. Integration of relevant information necessary for evaluation of antibody PK and PD properties and clinical dose selection. *POM* proof-of-mechanism, *POP* proof-of-principle, *POC* proof-of-concept biomarkers

receptor signaling events) in the appropriate efficacy models (4,5). Through the use of mechanism-based PK–PD models, contribution of target to antibody clearance as well as the extent of receptor occupancy, the system potency and the maximum response efficiency can be determined (28,37). Predictions of the extent of receptor occupancy in patients using preclinical information should then allow for the design of appropriate dosing strategies (4,5,9,10).

Bioanalytical Considerations. Development of robust bioanalytical methodologies is the first step in implementing effective translational strategies during antibody development (38,39). Universal (UAs) or antigen capture (ACAs) immunoassays can be used for quantification of therapeutic antibody in various biological matrices (Fig. 2).

The configuration for UAs, where two noncompeting antibodies against various constant regions of the human therapeutic antibody are used as capture and detection reagents (Fig. 2a), allows for detection of the total (bound and free) antibody concentrations in biological matrices from various species such as rodents or nonhuman primates (NHPs). Universal assays are effective during early preclinical development with the advantage of maximizing efficiency while minimizing cost and development time. Unlike ACAs, UAs are not suitable for use in human studies as the presence of endogenous human IgGs in the matrix will interfere with determination of the fully human or humanized therapeutic antibodies in this assay format. Therefore, ACAs are frequently used for evaluation of the antibody concentrations in human serum. In contrast to UAs, ACAs use a specific antigen for capturing of the therapeutic antibody (Fig. 2b). Since the antigen bound to the antibody will compete with the capture reagent, this assay format will measure the unbound antibody serum concentrations. In principle, when ACAs are used, nonlinearity in the antibody pharmacokinetic profile can be observed due to this competition, which should not be misinterpreted as an antigen-mediated elimination mecha-

nism. For example, the apparent nonlinearity in the PK profile of a fully human IgG2 antibody in NHPs is shown in Fig. 3 when the ACA was used. Linear PK was evident when a universal assay was employed for determination of the antibody concentrations in the same samples (Fig. 3b). As the ratio of the free-to-total antibody concentrations decreased with increases in the dose, the two assays measured serum concentrations closely at higher doses (*i.e.*, 10 and 60 mg/kg). As shown in Fig. 3, the most pronounced impact on the measured serum antibody concentrations between the two assays was observed at the lowest dose (1.0 mg/kg).

Immunogenicity of therapeutic antibodies can be a major limiting factor in the therapeutic application of constructs that contain xenogeneic protein sequences with potential impact on PK, PD, and safety. Robust immunoassays are critical for evaluation of antibody immunogenicity (IM) during both preclinical and clinical phases (40–44). Various methodologies have been used for IM testing; however, these methodologies differ with respect to sensitivity for measuring both low- and high-affinity antibody responses (45,46). Enzyme-linked immunosorbent assay methodology has been broadly used in IM testing. In its simplest form, a sandwich configuration of the therapeutic antibody used as capture (nontagged) and detection (tagged) reagents, respectively, should permit detection of both anti-isotypic and anti-idiotypic antibody responses (Fig. 2c) (47,48).

Appropriate immunoassay methodologies should also allow for the effective examination of the relationships between antibody PK and the ensuing effects on POM biomarkers. Assays for detection of the free antigen post-antibody-dosing *in vivo* can employ appropriate configurations where the therapeutic antibody is used as the capture reagent. Detection of the free antigen can then be accomplished using a tagged anti-antigen antibody that recognizes the noncompeting epitopes on the antigen (Fig. 2d). Similarly, appropriate assay configurations can be employed for determination of the bound antibody–antigen complex in samples

Fig. 2. Bioanalytical methodologies can facilitate design and implementation of effective translational strategies during antibody development

Fig. 3. Application of ACA and UA for determination of fully human IgG2 antibody concentrations in monkey serum following administration of single intravenous doses. Same samples were subjected to analysis using UA and ACA. **a** Serum concentration-time profiles following administration of single intravenous doses. **b** The relationships between the area under the serum concentrations curve and the administered dose. Approximately a threefold decrease in clearance was observed when ACA was used: the mean clearance for free antibody in this study was 10.5 ± 2.5 , 16.7 ± 5.1 , and 31.4 ± 4.8 mL/day per kilogram following administration of the 60, 10, and 1 mg/kg dose, respectively. When the UA is employed, the mean clearance for total antibody was 16.4 ± 9.8 , 17.1 ± 1.8 , and 12.6 ± 3.2 mL/day/kg following administration of the 1, 10, and 60 mg/kg dose, respectively

collected from *in vivo* studies post-antibody-treatment (Fig. 2e).

Selection of Relevant Animal Models. Safety concerns associated with many therapeutic antibodies are often an extension of their intended pharmacological activity (5,11). This “exaggerated” pharmacological response may be the result of a more profound modulation of the target or occurs as a consequence of antigen expression on normal tissues (6,11). Hence, often the greatest challenge in the development of a novel antibody is finding the most pharmacologically relevant species for evaluation of antibody pharmacology and safety. It is essential to note that “relevant” signifies not only that the antibody binds appropriately in a tissue-specific manner but also that it generates the expected pharmacology (*i.e.*, epitope and functional comparability). This topic has been addressed in a number of recent comprehensive reviews (6,36,49).

Antibodies Against Soluble Antigens. Many of the therapeutic antibodies raised against soluble antigens such as circulating TNF, vascular endothelial growth factor (VEGF), IgE, and various cytokines (*e.g.*, interleukin (IL)-8, IL-5, IL-12/IL-23) have undergone extensive research in both various animal models and clinical studies in patients (13,50–64). For this class of compounds, application of PK–PD modeling approaches can prove highly effective for the design of successful translational strategies (4,5,9,10). Following administration of therapeutic doses of an antibody, it is anticipated that the free concentrations of the antigen are suppressed (4,13,30,65). However, as the elimination rate of small antigens is generally reduced following binding to an antibody, a simultaneous increase in the antibody–antigen complex is observed (30,65). The magnitude of the increase in antibody–antigen complex concentrations *in vivo* will be

dependent on the turnover rate of the antigen (*i.e.*, antigen synthesis and clearance rate) relative to elimination rate observed for the antibody and the clearance rate of the antibody–antigen complex (10,13,30,66).

Following administration of multiple doses of bevacizumab in patients with advanced cancer, total VEGF concentrations in serum were increased two to four times the baseline values 72 days following initiation of therapy while the unbound (free) serum VEGF was not detectable following administration of the lowest dose (0.3 mg/kg) (65,67). The decrease in the clearance of human VEGF following complex formation with bevacizumab was also confirmed in a study conducted in a non-cross-reactive species (rat) where the contribution of host factors such as antigen binding and the impact of endogenous rat antigen on the pharmacokinetics of human VEGF were insignificant (66). Similarly, dose-dependent reductions in unbound IgE levels were reported to be correlated with simultaneous increases in the bound antibody–IgE complex following omalizumab administration (30,58,68). Following intravenous administration of omalizumab in humans, the clearance of total IgE decreased, resulting in increases in omalizumab–IgE complex concentrations in serum (30). Hayashi *et al.* developed a bi-molecular interaction PK–PD model for evaluation of omalizumab binding to IgE. The model accounted for the free IgE clearance and the antigen production rate based on the baseline IgE concentrations, the free antibody PK according to the patient’s body weight, and the *in vivo* free and total IgE concentrations as the relevant POM and POP biomarkers (30). Free IgE levels were shown to decrease dose-dependently following increases in omalizumab doses. Additionally, dose-dependent increases in the total IgE were observed, consistent with the hypothesis that omalizumab–IgE complexes cleared at a slower rate than the free antigen. The inverse relationships between the changes in the free and bound antigen concentrations have also been demonstrated with HAE1, the second-

generation high-affinity anti-IgE monoclonal antibody, following administration of single subcutaneous doses in patients ranging from 7.5 to 360 mg. With increases in dose, the duration of suppression of the free IgE was increased while the bound IgE concentrations in serum increased dose-dependently. Both the free and bound IgE concentrations returned to baseline in parallel with decreases in the free antibody concentrations (13). These results highlight the application of translational POM such as evaluation of the bound antigen concentrations where experimental evaluation of the free antigen is not achievable. In many instances, when determination of free antigen concentrations may not be feasible, application of the bi- or tri-molecular PK-PD modeling approaches may allow extrapolation of the free antigen concentrations when the bound antigen-antibody complex and the antibody concentrations are experimentally determined (69).

Quantitative pharmacology using indirect PD response models (17) has also been successfully employed for evaluation of exposure-response relationships during the development of ustekinumab (63,64). Psoriasis is a chronic immune-mediated skin disorder, and IL-12 and IL-23 have been implicated in the pathogenesis of this disorder. Ustekinumab is a human IgG1 monoclonal antibody that binds the p40 subunit of IL-12/IL-23, blocks the interaction of this subunit with IL-12R β 1 surface receptors, and has shown impressive efficacy in treating moderate to severe plaque psoriasis (70). The pharmacokinetic properties of ustekinumab were described using a one-compartment linear model with a first-order absorption and first-order elimination process (64). The Psoriasis Area and Severity Index (PASI) scores in patients were evaluated as the PD response endpoint, *i.e.*, disease severity index. The indirect response model described the PASI score-time data relatively well. Following subcutaneous administration, population PK analysis demonstrated a trend of lower median exposure to the antibody in nonresponders (CL/F

0.844 L/day) and partial responders (CL/F 0.609 L/day) relative to patients responding to ustekinumab treatment (CL/F 0.462 L/day). Additionally, a 30-fold higher potency (lower IC₅₀) was reported in the patients responding to the treatment relative to the partial responders, suggesting that this population may require higher doses of ustekinumab or more frequent dosing to achieve comparable efficacy (63). The model was proposed to support future alternative dosing strategies during development of ustekinumab.

Membrane-Associated Antigens. Many of the antibodies in development or currently marketed recognize membrane-associated antigens (28,32,37,71–80). Interaction of antibodies with this class of target antigen can greatly impact their PK. When a target antigen alters the clearance of an antibody, the effect is usually manifested as a dose-dependent clearance rate and half-life (32). The dose-dependent saturation of the nonlinear clearance pathway can be used as an effective translational POM or POP biomarker across species as it relays information regarding the degree of saturation of the target antigen *in vivo* (11). In many instances, the target-mediated clearance can be effectively correlated to additional POC or other efficacy markers.

CNT095 is a fully human monoclonal antibody against α_v integrins that inhibits angiogenesis and tumor growth (76). The α_v integrins are expressed on the surface of most epithelial tumors modulating cell proliferation and survival. Following administration of CNT095 in an FIH study in cancer patients, nonlinear PK was observed (Fig. 4a). The dose-dependent decreases in the clearance parameter reflected saturation of the antigen sink at the highest dose, *i.e.*, 10 mg/kg. Interestingly, a partial prolonged response was observed in one patient with angiosarcoma, a tumor of malignant endothelial cells, after multiple-dose administration of the 10 mg/kg dose where the antigen sink was saturated (76). Similarly, nonlinear PK properties have been observed

Fig. 4. **a** The relationship between clearance and dose for the fully human antibody, CNT095, against a membrane-associated, internalizing antigen α_v integrin following single-dose administration in cancer patients (data adapted from (76)). The high antibody clearance at low doses represents the impact of the antigen sink on antibody clearance following administration of single doses. **b** Predicted degree of saturation of the antigen sink *versus* steady-state serum concentrations for panitumumab. More than 98% antigen saturation is predicted at steady-state serum concentrations greater than 30 μ g/ml (predictions were made from the reported K_m estimate from (83))

for volociximab, a chimeric IgG4 antibody that inhibits the $\alpha_v\beta_1$ integrin and is in development for treatment of solid tumors. The clearance of volociximab decreased from 142 to 9.7 ml/h, with increasing doses from 0.5 to 15 mg/kg, respectively. Saturation of the target antigen on the circulating monocytes was observed in patients receiving doses >2.5–5.0 mg/kg (81,82). Following treatment with the antibody, stable disease was reported in six out of 21 patients who had documented progressive malignancies prior to study entry (82). A mechanism-based receptor-binding model was developed to describe the volociximab PK–PD properties (81). The model was further employed to explore the potential application of the proposed POM biomarker (*i.e.*, antigen saturation on circulating monocytes) for selection of dose in future clinical studies.

Similar to these results, a population PK model was developed to describe the nonlinear PK of panitumumab in patients with advanced solid tumors (83). Disposition of panitumumab, a high-affinity fully human antibody targeted to the extracellular domain of the human epidermal growth factor receptor (EGFr), was best described by a two-compartment model with parallel linear and nonlinear (Michaelis–Menten) elimination pathways. The contribution of both the linear and nonlinear clearances to panitumumab elimination was consistent with the reported antibody clearance via the reticuloendothelial system (linear) and the target-mediated clearance due to the binding to EGFr (nonlinear clearance pathway). Among the baseline covariates such as age, gender, body weight, and cancer type, only body weight was found to have the highest impact on antibody exposure. The parameters characterizing the nonlinear clearance, $V_{\max}$ (the maximum elimination rate), K_m (the Michaelis–Menten constant), and $V_{\max}/K_m$ (maximum nonlinear clearance) were 10.6 mg/day, 0.426 $\mu\text{g/ml}$, and 28.4 l/day, respectively (83). As the K_m value represents serum concentrations of panitumumab at which the antibody is eliminated at 50% of the $V_{\max}$, it is possible to estimate the degree of saturation of the antigen sink at steady state based on the estimated K_m following administration of multiple doses of the antibody. Figure 4b represents the degree of saturation of the antigen sink over the range of predicted steady-state serum concentrations. As shown, more than 98% antigen occupancy is predicted at the steady-state serum concentrations >30 $\mu\text{g/ml}$. In line with these predictions following administration of the recommended dose regimen (6 mg/kg once every 2 weeks) for panitumumab, the steady-state peak and trough serum concentrations of 231 ± 59 and 39 ± 14 $\mu\text{g/ml}$ were reported, indicating complete saturation of the antigen sink in cancer patients at a therapeutically effective dose (83,84). Previously, the dose-related skin toxicity, a potential surrogate (POP) marker, observed with panitumumab also was shown to occur at antibody doses that correspond to the maximum saturation of the antigen sink (37).

Evaluation of the stimulus–response mechanisms that convert receptor occupancy into the downstream activity (POP/POC markers) along with application of QP has been effectively employed during development of an anti-receptor activator of NF-kappa B ligand (RANKL) antibody. Denosumab (AMG162) is a fully human Xenomouse®-derived IgG₂ antibody directed against human RANKL (85) and is currently undergoing clinical evaluation in cancer and osteoporosis (86–88). Pharmacokinetic properties of denosumab are highly influenced by target (RANKL) expression and

density (28,86,87). A strong correlation has been observed between the target saturation and other mechanistic bone resorption markers such as N- and C-telopeptides and NTX and CTX (*i.e.*, POP biomarkers) in NHPs and human patients (28,86,87,89). These POP biomarkers additionally have shown a strong correlation with other POC markers such as bone mineral density, a critical endpoint for evaluation of denosumab therapeutic efficacy (89–92). Disposition of denosumab was initially described by a two-compartment model with parallel linear and nonlinear (Michaelis–Menten) elimination pathways (93). The relationships between denosumab PK and its rapid effect on urinary NTX marker were characterized using an indirect response model. A similar profile in the PK and PD of denosumab was observed in monkeys; allometric adjustment of the clearance rates, the antigen properties, and the antigen-mediated elimination across species allowed predictions of human PK–PD relationships in a phase 1 dose escalation study (89). Overall the clinical PK–PD was reasonably predicted from the monkey data, and the simulations appeared to have successfully accomplished their objectives in the design of the phase 1 study (89). Recently, Marathe *et al.* described an integrated cellular bone homeostasis model for evaluation of denosumab PK–PD in multiple myeloma patients (28). Using this mechanistic model, nonlinear PK profiles of denosumab were described by a target-mediated disposition model as described previously (21). Integration of denosumab PK profiles into a mechanistic cellular model of bone homeostasis allowed characterization of the time course of changes in serum NTX profiles in multiple myeloma patients. Additionally, the model provided a mechanistic description of critical processes involved in the regulation of the clinical POP biomarker (NTX) and facilitated evaluation of the role of cellular system variables to drug responses.

Recruitment of Effector Functions. Interaction of human IgG antibodies with Fc γ receptors and the complement pathway (C1q) is of critical importance for antibody function as the PD and safety profiles of therapeutic antibodies *in vivo* can be partly regulated by these interactions (4,5,9,11,32). Engagement of antibody with various immune cells and complement proteins can result in activation of effector functions like antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), respectively (94,95). Optimization of antibody interactions with immune effector cells or complement proteins has been employed to impact favorably the clinical efficacy profile of therapeutic antibodies. Engineering of antibodies with enhanced affinity for Fc γ receptors has resulted in greater effector activation and killing of antibody-coated target cells (94). Additionally, modification of the n-glycans attached to the antibody constant region has been employed for enhancing antibody ADCC-mediated activity (95–97). As nonfucosylated IgGs show stronger affinity for Fc γ RIIIa binding, superior *in vivo* efficacy has been demonstrated with nonfucosylated therapeutic antibodies in both animal studies and human clinical trials (98–100). Therefore, application of QP along with considerations regarding affinity differences and the diversity in the efficiency and potency for the effector systems across species will be of critical significance for the design of effective preclinical safety studies and dose selection across species (5,6,9).

In general, the stimulus–response mechanisms that convert receptor occupancy into pharmacological response are nonlinear and in some instances disproportional (9). Diversity in the relationships between receptor occupancy and response intensity could range from highly efficient to nonefficient responses (101). For highly efficient responses, the binding curves denoting occupancy lie to the right of the concentration–response curves ($KD > EC50$) due to large amplification factors in signaling cascades (101). For antibodies where effector functions (ADCC, CDC, T cell recruitment) contribute significantly to the underlying mechanism of action, highly efficient pharmacological responses are anticipated (3,102–105). However, the magnitude of the pharmacological responses can be impacted by various factors such as antibody affinity for the target and effector cells, the effector cell-to-target ratio within the immediate tissue microenvironment, and the drug transit time through the relevant tissue compartments.

Recently, the role of the cellular microenvironment and its potential impact on rituximab-mediated B cell ablation in various tissues was described (106). In studies conducted in a murine model of human CD20, Gong *et al.* provided evidence that depletion of B cells residing in the lymphoid tissues required access to the vasculature in order to deliver the targeted B lymphocytes to the effector compartment within the reticuloendothelial system (106). Additionally, a longer duration of time was reported for depletion of slower recirculating lymph node B cells compared with the circulating B cells. However, in contrast to the circulating B cells, in the marginal zone compartment, B cells exhibited a greater dependence on complement-dependent mediated mechanisms for depletion (106). In line with these observations, the impact of effector cells-to-target ratio on *in vitro* lysis of targeted tumors by human T cells was previously described for various BITE® constructs (107,108). The differential sensitivity of various tissues such as spleen and bone marrow to *in vivo* administration of a CD19/CD3-specific BITE construct was further demonstrated in a human CD19 transgenic mouse model following subcutaneous administration of single doses of a hybrid surrogate antibody capable of recruiting mouse effector T cells (99). These data underline the significance of species selection where comparable pharmacodynamic/toxicodynamic properties are to be anticipated and further emphasize evaluation of the critical considerations for ascertaining the integrity of preclinical results in identifying potential risks to humans in clinical studies.

CONCLUSIONS

QP is a critical translational tool facilitating information flow throughout the drug development process. Consideration of appropriate mechanistic PK–PD models that account for both the antibody and target antigen properties early on serve as an informative tool in the design of clinical dosing strategies. Utilization of robust bioanalytical techniques allows for development of relevant methodologies to address the impact of therapeutic intervention on relevant biomarkers at the preclinical stage. Evaluation of appropriate translational factors in the intended patient population such as

antigen expression and distribution, antibody affinity, and effector function efficiency can facilitate transition of exposure–response relationships across various species. Additionally, therapeutic properties of second-generation bio-improved therapeutic candidates can be further optimized with an in-depth understanding of QP-related properties of the first-generation counterpart.

Conflict of Interest At the time of submission of this article, MT and HS were employed by AnaptysBio, Inc., and CF was employed by Takeda San Francisco.

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