Research Article

Factors Influencing Magnitude and Duration of Target Inhibition Following Antibody Therapy: Implications in Drug Discovery and Development

Anjaneya P. Chimalakonda,^{1,3} Rajbharan Yadav,² and Punit Marathe¹

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Abstract. Antibodies or antibody-related fusion proteins binding to soluble antigens in plasma form an important subclass of approved therapeutics. Pharmaceutical companies are constantly trying to accelerate the pace of drug discovery and development of these antibodies and identify superior candidates in face of significant attrition rates. Understanding the interplay between drug- and target-related factors on magnitude and duration of target inhibition is imperative for successful advancement of these therapeutics. Simulations using a target-mediated drug disposition model were performed to evaluate the influence of antibody-target binding affinity, baseline target concentration, and target turnover on magnitude and duration of soluble target inhibition. These simulations assumed intravenous dosing of the antibody and evaluated multiple parameters over a wide range. These simulations reveal that improvement in affinity reaches a point of diminishing returns following which further improvement in affinity does not alter the magnitude and more importantly the duration of target inhibition. Evaluation of unbound antibody and target kinetics indicated that point of diminishing returns in duration of inhibition was due to target-mediated binding and subsequent elimination of antibody at later time points. Similarly, influence of baseline target concentration and target turnover on magnitude and duration of target inhibition in plasma is shown. Additionally, the fraction of dose eliminated *via* target mediated elimination (F_{el}^{TM}) can be a useful tool to enable selection of strategies to increase duration of target inhibition. The implications of these simulations in drug discovery and development with regard to target identification, antibody optimization, and backup candidate selection are discussed.

KEY WORDS: antibody; drug discovery; lead optimization; PK-PD; TMDD.

INTRODUCTION

Monoclonal antibodies and related fusion proteins are an important class of therapeutics (1,2). Many pharmaceutical and biotechnology companies have antibody platforms targeting various therapeutic areas. This is, at least in part, due to better safety profile and historically higher approval rates of antibody platforms compared to traditional small molecule platforms (2,3). In spite of these advantages and historical precedent, development of antibodies is challenging and there is significant pressure on pharmaceutical companies to accelerate the drug discovery and development timelines of antibodies and identify superior candidates (2,4).

Antibodies or antibody-related fusion proteins that bind to soluble antigens in plasma form an important subclass of approved therapeutics (2,5), accounting for up to 30–40% of approved monoclonal antibodies (2). For these antibodies,

significant inhibition of soluble target in plasma is generally necessary for demonstration of efficacy (6–9). To enable robust target engagement, optimization of multiple properties like binding affinity, is required to select the best candidate with optimal projected human efficacious dose. Therefore, it is important to understand the factors governing the magnitude and duration of target inhibition to enable selection of appropriate drug candidates. These factors include identification of target binding affinity, targeted human efficacious dose, and dosing regimen.

Target-mediated drug disposition (TMDD) models have been used over the past decade to capture the temporal responses of antibody and/or target kinetics following drug administration (10–13). In addition, these models are increasingly being used to understand the interplay between the antibody and soluble target (12–15). Previous reports have used TMDD models to evaluate the influence of some factors on target inhibition with the magnitude of target inhibition (14,15) and/or area under the target inhibition *vs.* time (8), as the key metrics of target inhibition. Alternatively, influence of certain factors on the efficacious dose was evaluated at a predetermined duration of inhibition (15). However, the absolute duration of target inhibition, one of the key determinants of dose and dose frequency, was not explored in detail. Moreover, in some of these reports (8,15), the range of

¹ Metabolism and Pharmacokinetics, Department of Pharmaceutical Candidate Optimization, Bristol-Myers Squibb Co., Mail Stop: 17-2.04, Pennington, New Jersey 08534, USA.

² Pharmaceutical Candidate Optimization, Biocon Bristol-Myers Squibb R & D Center (BBRC), Syngene International Limited, Biocon Park, Plot 2 and 3, Bommasandra IV Phase, Bangalore 560-099, India.

³ To whom correspondence should be addressed. (e-mail: anjaneya.chimalakonda@bms.com)

parameters explored in the simulation exercise was not extensive. Therefore, in this manuscript, we present simulations that systematically investigate the influence of various factors on the duration and magnitude of target inhibition using the original TMDD model, across a wide parameter (3,000- to 125,000-fold) and dose range (50- to 1,000-fold) using multiple metrics of target inhibition (maximal inhibition of target, minimal level of target, and duration of target inhibition). While complexities in antibody and target disposition may require more integrated models (16,17), the original TMDD model may serve as an initial tool to assess these changes. We show using simulations that optimizing binding affinity may reach a point of diminishing returns following which improvements in binding affinity may lead to no meaningful increase in the magnitude and more importantly the duration of target inhibition. The mechanism underlying this threshold in magnitude and duration of target inhibition is also discussed. Moreover, the influence of baseline target concentration and target turnover half life on the magnitude and duration of target inhibition is demonstrated. These simulations will provide utility in target selection and subsequent lead optimization/identification strategies for monoclonal antibodies and other related platforms.

MATERIALS AND METHODS

A TMDD model (Fig. 1) was used to perform all the simulations reported in this manuscript. The detailed description of this class of models along with the key assumptions has been reported previously by Mager and Jusko (11) and Gibiansky and Gibiansky (18). Brief definitions of the parameters, rate constants, and variables used in the TMDD model (Fig. 1) are presented in Table I. The differential equations describing the model are shown below

$$\begin{aligned} d/dt(\text{Drug}) = & \text{Input} - k_{\text{drug}} \cdot \text{drug} - k_{\text{on}} \cdot \text{drug} \cdot \text{target} \\ & + k_{\text{off}} \cdot \text{complex} \end{aligned} \quad (1)$$

$$\begin{aligned} d/dt(\text{Target}) = & k_{\text{syn}} - k_{\text{target}} \cdot \text{target} - k_{\text{on}} \cdot \text{drug} \cdot \text{target} \\ & + k_{\text{off}} \cdot \text{complex} \end{aligned} \quad (2)$$

$$\begin{aligned} d/dt(\text{Complex}) = & k_{\text{on}} \cdot \text{drug} \cdot \text{target} \\ & - k_{\text{off}} \cdot \text{complex} - k_{\text{complex}} \cdot \text{complex} \end{aligned} \quad (3)$$

$$d/dt(\text{Elim_NTME}) = k_{\text{drug}} \cdot \text{drug} \cdot V_c \quad (4)$$

$$d/dt(\text{Elim_TME}) = k_{\text{complex}} \cdot \text{complex} \cdot V_c \quad (5)$$

The initial conditions for the three compartments are as follows

$$\text{Drug}(t = 0) = \text{dose}/V_c \quad (6)$$

$$\text{Target}(t = 0) = \text{baseline target} \quad (7)$$

$$\text{Complex}(t = 0) = 0 \quad (8)$$

$$\text{Elim_NTME}(t = 0) = 0 \quad (9)$$

$$\text{Elim_TME}(t = 0) = 0 \quad (10)$$

Briefly, elimination of the drug from the central compartment (volume, V_c), via nontarget-mediated mechanisms, was assumed to be first order with a rate constant k_{drug} .

Fig. 1. A target-mediated drug disposition (TMDD) model used to simulate the impact of alterations in key variables on magnitude and duration of target inhibition. Briefly, the antibody is assumed to bind to the target in plasma resulting in an antibody–target complex. The pharmacokinetics of the antibody along with the turnover of the target and the antibody–target complex are represented in the model. The details of the model are provided in the experimental section. Adapted from Mager and Jusko (11)

Table I. Brief Definitions of Parameters, Rate Constants, and Variables Used in the Target Mediated Drug Disposition Model (Fig. 1)

Variables/ parameters/rate constant	Brief definition
Drug	Systemic concentration of the antibody
Target	Systemic concentration of the target protein
Complex	Systemic concentration of the antibody–target complex
<i>Elim_NTME</i>	Hypothetical compartment that aggregates the amount of drug that is eliminated <i>via</i> target independent mechanism
<i>Elim_TME</i>	Hypothetical compartment that aggregates the amount of drug that is eliminated <i>via</i> target dependent mechanism
Maximal inhibition	Maximal inhibition of the free target protein relative to baseline (pre-dose) target concentration (expressed as percentage)
<i>Fel</i> TM	Fraction of total drug dose that is eliminated <i>via</i> target binding and subsequent elimination
Baseline target	Target concentration prior to drug administration (at $t=0$)
V_c (L/kg)	Central compartment volume of distribution of antibody
k_{drug} (day ⁻¹)	First order rate constant for the elimination of the antibody
k_{target} (day ⁻¹)	First order rate constant for the elimination of the target protein
$k_{complex}$ (day ⁻¹)	First order rate constant for the elimination of the antibody-target complex
kon (nM ⁻¹ day ⁻¹)	The forward rate constant for association of antibody to the target
$koff$ (day ⁻¹)	The backward rate constant for dissociation of antibody from the target

The drug in the systemic circulation was assumed to reversibly bind to the target with rate constants kon and $koff$. The production and elimination rates of the target were assumed to be *via* zero order ($ksyn$) and first-order rate constant (k_{target}) processes, respectively. The elimination rate of the complex was assumed to be first order *via* a rate constant, $k_{complex}$. The target concentration prior to drug administration was assumed to be constant and at steady state. To enable calculation of the amount of dose that is eliminated *via* target-independent and target-dependent (*via* complex elimination) mechanisms, two hypothetical compartments, namely *Elim_NTME* and *Elim_TME* were included in the model, that aggregate the amount of drug eliminated *via* target-independent (*via* k_{drug}) and target-dependent (complex elimination *via* $k_{complex}$) mechanisms, respectively. Pharmacokinetic and target-related parameters for a representative antibody; Omalizumab (Xolair®), presented in Table II, were obtained from literature reports and used for simulating the influence of various factors on the magnitude and duration of target inhibition.

Influence on Maximal Inhibition of the Target

The influence of changes in binding affinity (KD), baseline target concentration, and target turnover on maximal

Table II. PK-PD Parameters, Obtained From Published Literature on Xolair® (Omalizumab), Used as Base Parameters for Evaluating the Influence of Drug and Target Related Parameters on Magnitude and Duration of Target Inhibition

Parameter (units)	Estimate	Reference
V_c (L/kg)	0.06	Agoram <i>et al.</i> (14,15)
k_{drug} (day ⁻¹)	0.0315	Kuester and Kloft (29)
k_{target} (day ⁻¹)	0.8	Agoram <i>et al.</i> (14,15)
$k_{complex}$ (day ⁻¹)	0.0797	Meno-tetang <i>et al.</i> (8)
kon (nM ⁻¹ day ⁻¹)	2.82	Meno-tetang <i>et al.</i> (8)
$koff$ (day ⁻¹) ^a	7.49	Meno-tetang <i>et al.</i> (8)
Baseline target concentrations(nM)	2.6	Agoram <i>et al.</i> (14,15)

^a A lower $koff$ (1.41 day⁻¹) was used for simulations evaluating the duration of inhibition

decrease in free target protein (relative to baseline) and minimum level of free target were evaluated following single-dose administration of the antibody as shown in the equations below.

$$\text{Maximal inhibition} = 100 - \left(\frac{\text{min Target}(t)}{\text{baseline target}} \times 100 \right)$$

$$\text{Minimum level of free target} = \left(\frac{\text{min target}(t)}{\text{baseline target}} \times 100 \right)$$

where *Min Target* (t) is the lowest concentration of the free target following drug administration and *Baseline target* is the target concentration prior to drug administration.

As the maximal inhibition of the target following antibody dosing is dependent on multiple factors (19), systematic evaluation of the influence of each factor on maximal inhibition of the free target was performed.

Initially, the influence of changes in KD of the antibody–target interaction on the magnitude of inhibition was evaluated. The KD was altered over a wide range from 2 pM to 250 nM either by altering the association rate constant (kon) or the dissociation rate constant ($koff$) maintaining all other parameters constant (Table I). Next, the influence of baseline target concentration was evaluated over a wide range from 1 pM to 100 nM keeping all other parameters constant (Table I). Lastly, the target turnover was altered by changing the k_{target} over a 3,000-fold range (corresponding to target turnover half lives of 0.5 h to 70 days; calculated as $0.693/k_{target}$). All simulations were performed at four dose levels (0.1, 1, 10, and 100 mg IV for a 70 kg individual) spanning a 1,000-fold dose range.

Influence on Maximal Duration of Target Inhibition

The influence of changes in KD, baseline target concentration, and target turnover on duration of target inhibition was evaluated. Duration of target inhibition was defined as the time over which the free target was inhibited by $\geq 90\%$ compared to the baseline target concentration. The range of KD, baseline target concentration, and target turnover half life evaluated in these simulations were similar to values used

in the previous simulations evaluating the magnitude of inhibition. A lower *k_{off}* value was used in these simulations (1.41 day^{-1}).

All simulations were performed at four dose levels (10, 50, 100, and 500 mg IV per 70 kg individual) spanning a 50-fold dose range. Maximal duration of target inhibition values at lower doses (<10 mg) are not presented as the duration of inhibition was minimal (<1 day).

Evaluation of Unbound Antibody and Unbound Target Kinetics

To provide a mechanistic insight on the influence of evaluated factors on the maximal inhibition of the target, the ratio of maximum unbound antibody concentration (in molar units) to the baseline (predose) target concentration (in molar units) was calculated. As the antibody is administered IV in these simulations, the plasma concentration at time 0 was used as the maximum unbound antibody concentration. Similarly, to elucidate the underlying reason for the observed limit for the duration of inhibition (*vide infra*), simulations investigating the time course of unbound antibody and unbound target concentrations were performed following administration of 100 mg dose over a 135-fold range of binding affinity (KD range, 0.02–2.7 nM). Additionally, the fraction of total drug dose that is eliminated *via* target binding and subsequent elimination (FeI^{TM}) was calculated using the following equation

$$FeI^{TM} = \frac{\text{cumulative molar drug-target complex in Elim-TME compartment}}{\text{molar drug dose}}$$

All simulations presented in this paper were performed using Berkeley MadonnaTM (version 8.3.18, Berkeley, CA, USA).

RESULTS

Influence on Maximal Inhibition of the Target

The plots of maximum inhibition of target as a function of KD at four dose levels are shown in Fig. 2. Panel a presents the plots when KD is changed *via kon* and panel b when KD is changed *via koff*. For clarity, the Y-axis is shown in logarithmic scale on the right and linear scale on the left. Decreasing the KD (*i.e.*, improving the binding affinity) results in increased maximal inhibition of the target ultimately reaching an inflection point following which further improvement in binding affinity does not lead to greater inhibition. This inflection point appears to shift to the right as the dose increases (Fig. 2). Counter intuitively, a different picture emerges when the data are plotted using the minimal concentration of unbound target (expressed as percent of baseline) on the Y-axis as shown in Fig. 3. In this case, decrease in KD (*i.e.*, improvement in binding affinity) achieved *via* decreasing the *k_{off}* (Fig. 3b) results in a lower limit of minimal concentration of target following which further decreases in *k_{off}* (and correspondingly KD) do not result in significant lowering of the target concentration. In contrast, improvement in KD *via* increasing *kon* (Fig. 3a)

results in a progressive lowering of the minimal concentration of the target.

Figure 4 presents the plot of maximal inhibition of target following changes in baseline target concentration (Fig. 4a) and target turnover half life (Fig. 4b). Initially, increases in baseline target concentration do not result in alteration of the maximal target inhibition; however, an inflection point is reached wherein further increase in baseline target concentration results in progressive lowering of the maximal target inhibition (Fig. 4a). An opposite trend is noted for target turnover half life (Fig. 4b) wherein increases in target turnover half-life results in greater maximal inhibition of the target.

Influence on Maximal Duration of Target Inhibition

The plots of duration of target inhibition as a function of KD at four dose levels are shown in Fig. 5. Panel a presents the plots when KD is changed *via kon* and panel b when KD is changed *via koff*. For clarity, the Y-axis is shown in logarithmic scale on the right and linear scale on the left. It can be noted that decreasing the KD (*i.e.*, improving the binding affinity) has a marked effect in increasing the duration of target inhibition. Surprisingly, a further decrease in KD results in point of diminishing returns wherein the duration of effect is essentially similar.

Figure 6 presents the plot of duration of target inhibition following changes in baseline target concentration (Fig. 6a) and target turnover half life (Fig. 6b). Initially, changes in baseline target concentration do not result in alteration of duration of target inhibition; however, an inflection point is reached wherein further increase in baseline target concentration results in progressive lowering of the maximal duration of target inhibition (Fig. 6a). An opposite trend is noticed for target turnover half life (Fig. 6b) wherein increase in target turnover half-life results in greater duration of target inhibition.

Evaluation of Unbound Antibody and Unbound Target Kinetics

The ratio of maximum unbound antibody concentration over the baseline target concentration at varying baseline target concentrations is provided in Table III. At the baseline concentration (2.6 nM) used for simulating the influence of KD, the ratio of maximum unbound antibody concentration over the baseline target concentration is <1 at the 0.1 and 1 mg dose (absolute ratios of 0.061 and 0.61, respectively). At 10 and 100 mg (Table III), the ratio increases to 6.1 and 61, respectively. The ratios are similar when KD is changed *via kon* or *via koff*. As expected, the ratio decreases as a function of baseline target concentration.

The time course of unbound antibody (panel a) and unbound target concentrations (panel b) following administration of a 100 mg dose at a range of KD values is shown in Fig. 7. The time course of unbound antibody concentrations is similar during the initial 24–28 days (Fig. 7a) following which, a rapid drop in concentration is observed. The rate of decline of unbound antibody concentrations is dependent on the affinity of target interaction, with a steeper decline in unbound antibody concentration with higher affinity. The

Fig. 2. Plots of maximum inhibition of target as a function of KD at four dose levels. **a** The plots when KD is changed *via kon* and **b** when KD is changed *via koff*. The Y-axes in the figures are shown in logarithmic scale on the right and linear scale on the left side. Simulations were performed following an IV bolus dose of the antibody

time course of unbound target concentrations also shows a dependence on binding affinity (Fig. 7). As expected, for a low-binding affinity scenario, the inhibition of target concentration during the initial 24–48 days is less prominent

compared to a high-affinity scenario (Fig. 7b). However, at around 33–35 days, a rapid decrease in target inhibition is noticed, resulting in the target concentrations returning to baseline (Fig. 7b).

Fig. 3. Plots of minimum level of the target (as percent baseline value) as a function of KD at four dose levels. **a** The plots when KD is changed *via kon* and **b** when KD is changed *via koff*. The Y-axes in the figures are shown in logarithmic scale on the right and linear scale on the left side. Simulations were performed following an IV bolus dose of the antibody

Fig. 4. A plot of maximal inhibition of target following changes in baseline target concentration (a) and target turnover half life (b). Simulations were performed following an IV bolus dose of the antibody assuming a K_D of 2.7 nM

The plots of fraction of total drug dose eliminated *via* target binding and subsequent elimination (F_{el}^{TM}) as a function of K_D (panel a), baseline target concentration (panel b), and target turnover half-life (panel c) are shown in Fig. 8. In Fig. 8a, F_{el}^{TM} was low (<0.1) at all the doses when the binding affinity is poor. However, as the affinity increases, F_{el}^{TM} change becomes dose dependent, with lower doses (10–50 mg) demonstrating high F_{el}^{TM} values and the highest dose (500 mg) showing only a modest increase in F_{el}^{TM} (~ 0.2). In Fig. 8b, F_{el}^{TM} increases with increase in baseline target concentration reaching its theoretical maximum of 1 at high baseline target concentrations. The increase in F_{el}^{TM} is dose dependent with the lower doses reaching the plateau at relatively lower baseline target concentration compared to higher doses. An opposite trend is noted in Fig. 8c where F_{el}^{TM} decreases as target turnover half-life is increased, and the decrease is dose dependent.

DISCUSSION

Monoclonal antibodies are a major class of therapeutic agents (2,4) with more than 200 in various phases of clinical trials (1). Many pharmaceutical and biotechnology companies have multiple antibody or antibody-related products in their pipeline (2,4). Given this competition, it is recognized that there is significant pressure to rapidly evaluate antibodies or related therapeutic agents in discovery and progress them quickly into development (4). Among the multiple factors that determine clinical and therapeutic success of antibodies, target engagement ranks high (20). Therefore, understanding the key determinants of target engagement can guide discovery and development teams to select optimal antibodies for progression into the clinic.

Fig. 5. Plots of maximum duration of target inhibition as a function of K_D at four dose levels, following IV antibody administration. **a** The plots when K_D is changed *via* k_{on} and **b** when K_D is changed *via* k_{off} . The Y-axes in the figures are shown in logarithmic scale on the right and linear scale on the left side

Fig. 6. A plot of duration of target inhibition following changes in baseline target concentration (a) and target turnover half life (b). Simulations were performed following an IV bolus dose of the antibody assuming a KD of 0.5 nM

As anticipated, improvement in binding affinity has a significant influence on the magnitude and duration of inhibition. However, this influence is dose dependent, *i.e.*, at the lower doses (0.1 and 1 mg) we do not see complete inhibition of the target even when the KD reaches single digit picomolar levels (Fig. 2). At these doses and potency values, the binding is limited by the availability of the antibody, with the ratio of the molar maximum unbound antibody concentration over the molar baseline target concentration is <1 (Table III), hence complete inhibition cannot be achieved. In contrast, at higher doses (10 and 100 mg), while the ratio of maximum antibody concentration to the baseline target concentration is considerably >1 (Table III), the maximal inhibition of target is also dependent on the binding affinity, with higher affinity values leading to near complete inhibition (Fig. 2). The underlying implication of this finding is that, for soluble protein targets that are expressed at a relatively high

level in plasma, a (relatively) high dose of a potent antibody is required, to enable a high degree of target inhibition. The exact dose and degree of inhibition would depend, at least in part, on the ratio of the molar unbound drug concentration over the target concentration.

It appears that optimizing *kon* as opposed to *koff* results in a more robust inhibition of the target (Fig. 3). While at the 10 and 100 mg doses, optimizing either *kon* or *koff* results in near complete inhibition of the target (Fig. 2), a different picture emerges when viewing the data as minimum level of the target relative to baseline (Fig. 3). Improving *kon* (increasing binding affinity) results in progressively lower concentrations of the target as opposed to decreasing the *koff*, for which an apparent plateau is reached. Aston *et al.* in an elegant mathematical analysis (19), have explained the underlying basis for this observation. When *kon*→∞ (*i.e.*, highly potent), the minimum level of the target will be close

Table III. The Ratio of Maximum Unbound Antibody Concentration (at *t*=0) Over the Baseline Target Concentration at Varying Baseline Target Concentrations as a Function of Dose

Baseline target conc. (nM)	Ratio of maximum molar unbound antibody concentration (at <i>t</i> =0) to the baseline target conc.			
	0.1 mg	1 mg	10 mg	100 mg
0.001	160	1,600	16,000	160,000
0.01	16	160	1,600	16,000
0.1	1.6	16	160	1,600
1	0.16	1.6	16	160
2.6 ^a	0.061	0.61	6.1	61
10	0.016	0.16	1.6	16
100	0.0016	0.016	0.16	1.6

Ratios are rounded to two significant digits
^a Baseline target concentration used in simulations investigating the influence of KD and target turnover half-life on magnitude of inhibition

Fig. 7. Time course of unbound antibody (a) and unbound target concentrations (b) following IV administration of a 100 mg dose of the monoclonal antibody for a range of binding affinity values ($K_D = 0.02$ – 2.7 nM)

to zero (*i.e.*, complete inhibition), whereas when $k_{off} \rightarrow 0$ (again highly potent), the minimum level of the target has a non-zero value which is dependent on the level of the target along with the k_{on} and k_{target} (equations 18 and 19 of Aston *et al.* (19)). The implication of this is twofold; first, in lead optimization strategy for monoclonal antibodies, in case where near complete inhibition of the target is desired, targeting improvements in k_{on} may be more impactful. Second, a cutoff value of K_D is reached, when optimizing *via* k_{off} would not significantly alter the magnitude of inhibition. Indeed Agoram *et al.* and subsequently Aston *et al.* have shown through a series of simulations that improvement in binding affinity of Omalizumab (Xolair) may not result in substantially increased inhibition (≤ 2 - to 4-fold) of IgE (14,15,19). This theoretical prediction has subsequently been shown to recapitulate in clinical trials with a high affinity anti-IgE antibody, HAE-1 (19,21).

Baseline target concentration has a significant influence on the maximum inhibition of target (Fig. 4a). As expected, a trend towards decrease in the maximum inhibition of target with increase in baseline target concentration is observed (Fig. 4a) which roughly correlated with a decrease in ratio of maximum unbound antibody concentration to baseline target concentration (Table III). At lower doses (0.1 and 1 mg),

Fig. 8. Plots of fraction of total drug dose eliminated *via* target binding and subsequent elimination (F_{el}^{TM}) as a function of K_D (a), baseline target concentration (b), and target turnover half life (c)

even at a low baseline target concentration (1 pM), the maximum inhibition of target is only in the range of 4–32% (Fig. 4a). Interestingly, in this case, the ratio of maximum unbound antibody concentration to baseline target concentration (Table III) is considerably higher than unity (range, 160–1,600), suggesting that there is significant excess of antibody relative to the target. This apparent contradiction is because while, a higher ratio of maximum unbound antibody concentration to baseline target concentration appears to result in a higher magnitude of inhibition, the absolute value of K_D in relation to baseline target and unbound antibody concentrations is important. In this case, as

the KD used in these simulations ($KD=2.7$ nM) is higher than the baseline target concentration (<0.01 nM), maximal target inhibition is low (4–32%), even with significant excess of the antibody present. This illustrates one of the limitations in using the ratio of maximum unbound antibody concentration to baseline target concentration as a tool to understand maximal inhibition and the value of understanding the interplay between the absolute values of KD with the antibody and target concentration along with their ratios.

Under the conditions used in the simulations, it appears that baseline target concentration has a major impact on target inhibition compared to target turnover half life (Fig. 4). Initially, it was suspected that this may be due to a higher KD (2.6 nM; Table 1) used for this simulation. However, simulations performed using a tenfold lower KD (data not shown) also demonstrated a similar trend. While it was shown by Aston *et al.* (19) that minimum concentration of unbound target is dependent on both the baseline target concentration and target turnover half life, under the conditions used in these simulations baseline target concentration may be a dominant factor. This may appear intuitive under some conditions, as maximal inhibition of the target occurs soon after IV administration of antibody (except when *kon* is slow) and therefore, baseline target concentration plays a major role in determining maximal inhibition. In contrast, the target turnover half-life demonstrates a greater impact on the duration of inhibition (Fig. 6, *vide infra*).

Duration of target inhibition is a major factor, along with magnitude of inhibition, in determining efficacy following antibody administration (12). Therefore, it is important to understand influence of antibody and target-related factors on the duration of target inhibition, something that has been seldom investigated in detail. While an initial improvement in binding affinity has a considerable influence on improving the duration of target inhibition (Fig. 5), an inflection point is reached where further decrease in KD does not dramatically influence the duration of inhibition (Fig. 5). Interestingly, this maximum duration of inhibition is similar regardless of the mode of KD change (*i.e.*, *via kon* or *koff*), which is in contrast to their influence on minimum level of the target relative to baseline (Fig. 3). This finding would be important for drug discovery and development teams, as it implies that, at a certain dose, duration of inhibition is fixed for a particular antibody regardless of the binding affinity. While the duration of inhibition (Fig. 6) is also dependent on the target turnover and baseline target expression (like IgE in this case) this threshold is dose dependent. Consider a therapeutic area that requires a self administered subcutaneous injection. Assuming subcutaneous bioavailability of 50% (22) and aqueous solubility of 100 mg/ml (22,23), the upper limit of bioavailable dose is 50 mg (per 70 kg man). At this dose and under the conditions used in these simulations, the maximum duration of inhibition would be roughly 25 days. The implications of these results in drug discovery and development are twofold (1) for a first-in-class antibody, in the lead optimization stage, improvement in binding affinity beyond a certain value (in this case 0.1–0.3 nM) may not result in longer duration of inhibition and (2) while pursuing a potentially “better” backup antibody, these simulations will enable identification of factors that might reduce the dose or dosing frequency. Therefore, during lead optimization of antibodies, it is

imperative that quantitative mathematical modeling be used to determine the point where further improvement in binding affinity would not be of value in terms of duration of inhibition. Moreover, such modeling can point to the maximum duration of action of an antibody and help identify additional approaches which may result in a lower dose and/or reduced frequency.

Target-mediated binding and subsequent elimination of the antibody, especially at later times, is the underlying mechanism for the observed limit for the duration of target inhibition (Fig. 5). Simulations performed at a fixed (100 mg) dose and a range of KD values revealed that during initial days (~day 25 post-dose; Fig. 7a), the plasma concentration time profile did not show a significant dependence on KD, due to a lower contribution of target mediated binding/elimination to the overall elimination of the antibody. However, as the antibody concentrations approach the baseline target concentrations, a sudden drop in plasma antibody concentration, was observed (Fig. 7a). This correlated with the rise of the unbound target to its baseline concentrations (2.6 nM; Table 1). Due to higher target-mediated elimination as a consequence of higher binding affinity to the target, the steepest decline in plasma concentrations was noted for antibody with the lowest KD. Therefore, target-mediated binding and subsequent elimination of the antibody, especially at later times, was the underlying mechanism for the observed limit for the duration of target inhibition. Similar observation was also reported by Roskos *et al.* (24), in simulations investigating the influence of potency improvements on antibody half life of a cell surface antigen that has a rapid internalization rate (internalization half life of 40 min). Given the rapid internalization rate compared to the simulations in this manuscript (complex turnover half-life, calculated as $0.693/k_{\text{complex}}$, of 8.7 days) and perhaps the dose (not reported in (24)), target-mediated drug elimination and corresponding loss of target inhibition was noted very early (<1.5 days).

To gain additional insight into the underlying mechanism for the observed limit for the duration of target inhibition (Fig. 5) and more importantly to enable selection of mitigation strategies, the contribution of target-mediated elimination to the overall elimination of the antibody was investigated by calculating Fel^{TM} (Fig. 8). Interestingly, in simulations of target inhibition for a high affinity antibody, while the duration of inhibition reaches a fixed value at each dose (Fig. 5), the Fel^{TM} value differs with dose (Fig. 8a). At the lowest dose (10 mg), $>90\%$ of the total dose is eliminated *via* target-mediated elimination, whereas at the highest dose (500 mg), it is considerably lower ($\sim 22\%$). This indicates that the duration of target inhibition could theoretically be increased if non-target mediated elimination is decreased (*via* decreasing k_{drug}) at the highest dose, whereas at the lowest dose, it is challenging to increase the duration of inhibition by decreasing nontarget-mediated elimination. The implication of this in drug discovery and development is that, while maximum duration of target inhibition cannot be increased beyond a certain point by improving binding affinity, depending on the Fel^{TM} value, other strategies to improve the duration of target inhibition can be implemented. In case wherein the Fel^{TM} value is low (indicating

predominantly a nontarget-dependent elimination), strategies like modulating FcRN binding affinity (25,26) (to decrease k_{drug} can potentially increase the duration of target inhibition. Preliminary simulations (data not shown) at the 500 mg dose assuming high affinity (KD 1 pM) indicate increase in the duration of target inhibition with a decrease in k_{drug} . In case where the Fel^{TM} value is high (indicating predominantly a target dependent elimination), alternate strategies like pH-dependent target binding as elucidated by Igawa *et al.* (27) and Chaparro-Riggers *et al.* (28) may need to be adopted to increase the duration of target inhibition. Therefore, while the maximum duration of target inhibition cannot be increased beyond a certain point by improving binding affinity, alternate strategies can be adopted depending upon the Fel^{TM} value.

Duration of target inhibition is also significantly but differentially influenced by baseline target concentration and target turnover half-life. Increases in the baseline target concentration results in a reduction in duration of inhibition (Fig. 6a), in part, due to target-mediated binding and subsequent elimination of the antibody (Fig. 8b). At very low target concentrations (<0.1 nM), the duration of inhibition reaches a plateau and is dose-dependent. At these low target concentrations (<0.1 nM), the elimination of drug at all the dose levels is predominantly by non-target mediated elimination as the Fel^{TM} value is <20%. Therefore, if an increase in duration of target inhibition is desired, strategies like modulating FcRN binding affinity (25,26), to improve the half life and correspondingly decrease k_{drug} , can be employed along with affinity improvement. Alterations in the target turnover half-life result in more dramatic changes in the duration of inhibition for rapidly turned over targets (*i.e.*, low target turnover half life; Fig. 6b). Therefore, targets with relatively high baseline concentration and/or high turnover may result in short duration of target inhibition thus requiring high dose and/or frequent administration of antibody as eluded to by Igawa *et al.* (27). As a result, in the target identification stage of drug discovery, it is necessary to assess the dynamics of a soluble target in the anticipated disease population and incorporate a quantitative modeling plan to evaluate if an antibody-directed approach would even be feasible (17). If a target with relatively high baseline concentration and/or high turnover has already been selected, resulting in a high Fel^{TM} (Fig. 8b and c), strategies like pH-dependent target binding as elucidated by Igawa *et al.* (27) and Chaparro-Riggers *et al.* (28) may need to be adopted.

In conclusion, we have shown *via* simulations, the impact of antibody and target-related factors on the magnitude and duration of target inhibition. Additionally, Fel^{TM} , the fraction of dose eliminated *via* target mediated elimination can be a useful tool to enable design of appropriate strategies to increase the duration of target inhibition. These theoretical analyses provide an improved understanding of the impact of these factors on target inhibition and will enhance decision making in drug discovery and development of antibodies targeting soluble ligands in plasma.

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