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ORIGINAL ARTICLE

AFIR: A Dimensionless Potency Metric for Characterizing the Activity of Monoclonal Antibodies

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For monoclonal antibody (mAb) drugs, soluble targets may accumulate several thousand fold after binding to the drug. Time course data of mAb and total target is often collected and, although free target is more closely related to clinical effect, it is difficult to measure. Therefore, mathematical models of this data are used to predict target engagement. In this article, a ''potency factor'' is introduced as an approximation for the model-predicted target inhibition. This potency factor is defined to be the time-Averaged Free target concentration to Initial target concentration Ratio (AFIR), and it depends on three key quantities: the average drug concentration at steady state; the binding affinity; and the degree of target accumulation. AFIR provides the intuition for how changes in dosing regimen and binding affinity affect target capture and AFIR can be used to predict the druggability of new targets and the expected benefits of more potent, second-generation mAbs.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Mathematical models for target mediated drug disposition of mAbs are widely used to guide drug development by predicting the dosing regimen at which a certain threshold of target inhibition is achieved. Although many mathematical analyses of these models have been published, there has not yet been a demonstration for how the key model parameters (like binding affinity and average drug concentration) link to target engagement in repeated dosing scenarios.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ How do the PK and binding properties of the mAb impact target engagement?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ A simple nondimensional potency factor (AFIR) links target engagement to three key quantities: average drug concentration, binding affinity, and total target accumulation.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ The AFIR metric provides intuition for standard TMDD models and can be used to rapidly predict the druggability of new targets and the expected benefits of second-generation, more potent mAbs.

Monoclonal antibodies (mAbs) are one of the fastest growing classes of therapeutic agents with 47 approved as of November 2014 and an expectation of about 4 new approvals per year.[1] Unlike small molecules, which have a molecular weight of about 500 Da and that are cleared mainly by the liver and kidneys, mAbs are large molecules with a molecular weight of about 150 kDa and are cleared mainly through cellular uptake followed by proteolytic degradation. Whereas small molecules typically have a half-life of hours, fully human mAbs exhibit long half-lives of around 3 weeks due to the FcRn receptor, which binds to the mAb after pinocytosis and rescues it from undergoing lysosomal degradation.[2] There are two classes of targets for mAbs: membrane-bound and soluble. Antibodies with membrane-bound targets (e.g., trastuzumab/HER2, demosumab/RANKL, nivolumab/programmed cell death protein 1) have an additional route of clearance via receptor-mediated internalization, which can lead to a nonlinearity in the drug pharmacokinetics (PKs); this phenomenon is known as target-mediated drug disposition (TMDD). Antibodies with soluble targets (e.g., omalizumab/Immunoglobulin E, bevacizumab/vascular endothelial growth factor, siltuximab/interleukin-6) often demonstrate significant target accumulation after single (**Figure 1a,b**) or repeated dosing (**Figure 1c**) because the mAb-target complex often has a much longer half-life than the free target molecules.[3–5] Although this accumulation plateaus at large doses, this plateau does not necessarily imply a plateau in efficacy; and increasing the dose after the plateau has been associated with further reduction of the free target concentration,[6] greater inhibition of downstream biomarkers,[7,8] and improved efficacy.[9,10]

To understand why the plateau in total target concentration does not imply a plateau in efficacy, and to understand how changes in the dose regimen or drug properties may impact target engagement, it is useful to characterize the free and bound concentrations for the drug and target using the model in **Figure 2**, which describes both TMDD and the accumulation of total target during therapy. This model, often referred to as a TMDD model,[11,12] is mathematically more complex than the usual compartmental models for describing the PK of small molecules, because the kinetics of the free drug, free target, and drug-target complex all need to be considered. Given a dosing regimen and estimates for the

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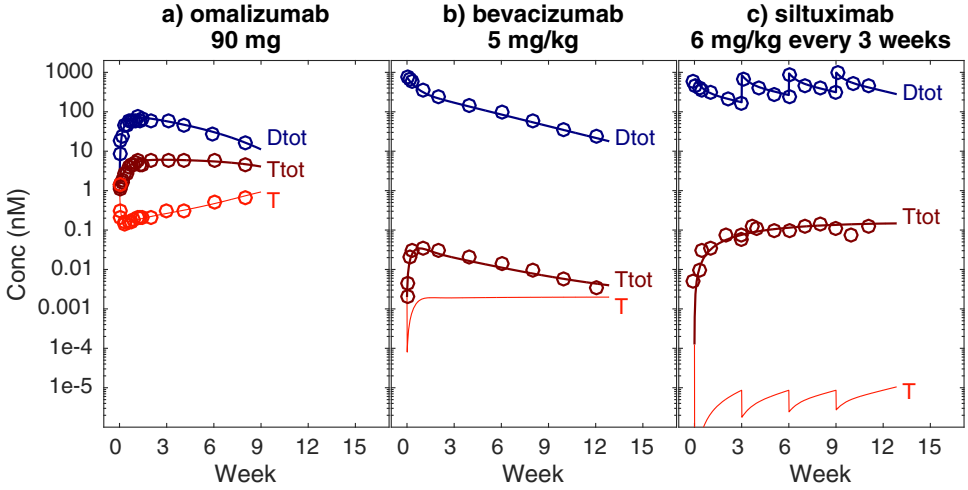

Figure 1 Data: The time course of drug concentration (D_{tot}), total target (T_{tot}), and free target (T) for omalizumab/immunoglobulin E,[3] bevacizumab/vascular endothelial growth factor,[4] and siltuximab/interleukin-6.[5] The circles are data digitized from the literature. Free target data was collected for omalizumab, but not for bevacizumab or siltuximab. Model: The lines denote model simulations using the parameters in **Table 1**. The model provides predictions for the free target concentration in cases in which it was not measured.

parameters governing drug kinetics, target kinetics, and binding affinity, the TMDD model allows one to make predictions for the target inhibition, as shown in **Figure 1** where data and model predictions are shown for three antibodies.

The TMDD model has been used to support many aspects of drug development[13] including:

1. Early evaluation of the druggability of a target,[14] in which if the target level or turnover is too high, an unfeasibly large dose may be required for efficacy.
2. Identification of a minimally active dose in phase I first-in-human studies.[15,16]
3. Comparison of different drugs with the same target to determine whether a second-generation mAb with higher affinity is expected to outperform the first-generation drug.[17]

Modelers have sought to develop rules of thumb for predicting how changing the drug properties or dosing regimen will impact measures of drug activity, such as maximum target inhibition or duration of effect of a single dose.[18–22] Thus far, the existing metrics and analyses apply only to single-dose scenarios, which is of limited value because most mAbs are dosed repeatedly in the clinic.

In this article, a new potency factor based on multiple dosing scenarios is derived. The potency factor is named the Average Free target concentration to Initial target concentration Ratio (AFIR), which depends upon the structural model (**Figure 2**) and the parameters (**Table 1**) that describe the drug, target, and binding kinetics. AFIR provides an intuitive understanding for how dosing regimen and binding affinity affects target inhibition. Examples showing how this intuition can be used to support drug development decisions are also provided.

Theory

In this section, the ratio of steady-state free target (under regular dosing) to baseline free target is derived. This ratio represents the relative degree of suppression of free target after binding to the drug. The TMDD model commonly used to describe biologics binding to their target is given in **Figure 2** and by the equations below. The model parameter descriptions and parameter values for three drugs (omalizumab, bevacizumab, and siltuximab) are provided in **Table 1**. The model parameters were chosen to match the clinical data from,[3–5] noting that varying k_{off} and k_{on} over multiple orders of magnitude while keeping the binding affinity ($K_d = k_{off}/k_{on}$) fixed would yield similar curves. This model describes both subcutaneous and intravenous dosing, distribution of the drug to the peripheral tissue, binding of the drug to target in the serum, synthesis of the target, and elimination of the drug, target, and complex. Distribution,

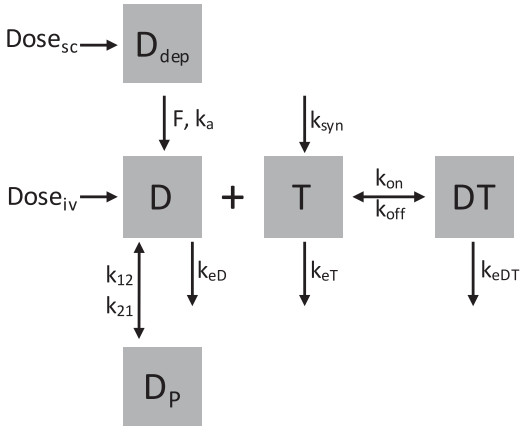

Figure 2 Compartmental model for drug pharmacokinetics and target binding. The model parameters and initial target concentration are defined in **Table 1**. The initial conditions for the drug and complex concentration are zero. D, drug; T, target; DT, complex.

Table 1 Model parameters for omalizumab (one-compartment, subcutaneous dosing), bevacizumab (two-compartment, intravenous dosing), and siltuximab (two-compartment, intravenous dosing)

Type	Parameter	Description	Omalizumab	Bevacizumab	Siltuximab	Units
		Target molecule	IgE	VEGF	IL-6	
Amount	$\text{Dose}_{sc}(t)$	Subcutaneous dosing function				nmol
Amount	$\text{Dose}_{iv}(t)$	Intravenous dosing function				nmol
Conc	D_{dep}	Drug in subcutaneous depot compartment				nM
Conc	D	Free drug concentration				nM
Conc	T	Free target concentration				nM
Conc	(DT)	Complex concentration				nM
Conc	D_{tot}	Total drug concentration $=D+(DT)$				nM
Conc	T_{tot}	Total target concentration $=T+(DT)$				nM
Conc	C_{avg}	Average total drug concentration at steady state				nM
Conc	C_{min}	Trough total drug concentration at steady state				nM
Drug	τ	Dosing interval				d
Drug	k_a	Subcutaneous absorption rate	0.35	–	–	1/d
Drug	F	Subcutaneous bioavailability	0.42	–	–	–
Drug	k_{12}	Drug central → peripheral distribution	–	0.11	0.14	1/d
Drug	k_{21}	Drug peripheral → central distribution	–	0.15	0.19	1/d
Drug	k_{eD}	Drug elimination rate	0.005	0.064	0.058	1/d
Drug	V_c	Central volume	3	3.1	4.1	L
Drug	CL	Drug clearance $=k_{eD}V_c$	0.015	0.2	0.24	L/d
Drug	Q	Intercompartmental clearance $=k_{12}V_c=k_{21}V_p$	–	0.36	0.56	L/d
Drug	V_p	Peripheral volume $=k_{12}V_c/k_{21}$	–	2.4	3	L
Target	k_{syn}	Target synthesis rate	1.4	0.014	0.005	nM/d
Target	k_{eT}	Target elimination rate	0.93	7	40	1/d
Target	T_0	Initial target concentration $=k_{syn}/k_{eT}$	1.5	0.002	1.2e–4	nM
Complex	k_{eDT}	Complex elimination rate	0.2	0.07	0.03	1/d
Complex	$T_{tot,ss}$	Steady state total target $=k_{syn}/k_{eDT}$	7	0.2	0.17	nM
Complex	T_{acc}	Target accumulation ratio $=T_{tot,ss}/T_0$	4.7	100	1.3e3	–
Complex	k_{off}	Off-rate	23	36	0.2	1/d
Complex	k_{on}	On-rate	10	2	10	1/(nM·d)
Complex	K_d	Dissociation constant $=k_{off}/k_{on}$	2.3	18	0.02	nM

IgE, immunoglobulin E; IL-6, interleukin-6; VEGF, vascular endothelial growth factor.
These parameters were chosen to provide good agreement with the data in **Figure 1** and, thus, differ slightly from the population estimates reported in the literature.

binding, and turnover of the target in peripheral tissue generally are not modeled due to limited available data in the peripheral tissue.

Input Absorption Distribution Binding Elimination

$$\begin{aligned} dD_{dep}/dt &= \text{Dose}_{sc}(t)/V_c - k_a D_{dep} \\ dD/dt &= \text{Dose}_{iv}(t)/V_c + k_a F D_{dep} - k_{12}D + (k_{21}V_p/V_c)D_P - k_{on}D\cdot T + k_{off}(DT) - k_{eD}D \end{aligned} \quad (1)$$

$$\begin{aligned} dD_P/dt &= (k_{12}V_c/V_p)D - k_{21}D_P \\ dT/dt &= k_{syn} - k_{on}D\cdot T + k_{off}(DT) - k_{eT}T \end{aligned} \quad (2)$$

$$d(DT)/dt = k_{on}D\cdot T - k_{off}(DT) - k_{eDT}(DT) \quad (3)$$

The key quantity of interest in predicting drug effect over time is the ratio of free target to baseline target (T/T_0). This ratio can be written as the product of two ratios as shown below, where $T_{tot,ss}$ is the steady state total target concentration under a repeated dosing regimen of large doses.

$$\frac{\text{Free Target}}{\text{Baseline Target}} = \frac{T}{T_0} = \left(\frac{T}{T_{tot,ss}}\right)\left(\frac{T_{tot,ss}}{T_0}\right) \quad (4)$$

To compute the first ratio $T/T_{tot,ss}$, the quasi-equilibrium (QE) approximation is used,[23] which assumes that binding and unbinding occurs rapidly compared with other processes, such that the drug, target, and complex are in quasi-equilibrium.

$$k_{on}D\cdot T = k_{off}(DT) \rightarrow D\cdot T/(DT) = k_{off}/k_{on} = K_d \quad (5)$$

Substituting the equation for the total target $[(DT)=T_{tot}-T]$ into Eq. 5 and solving for T/T_{tot}, gives the equation below. The first approximation holds when $D \gg K_d$ and the second approximation holds when $D_{tot} \approx D$, which occurs when the drug is dosed in vast molar excess to the target, as is the case for most mAb drugs in the clinic.

$$\frac{T}{T_{tot,ss}} = \frac{K_d}{D+K_d} \approx \frac{K_d}{D} \approx \frac{K_d}{D_{tot}} \quad (6)$$

The second ratio $T_{acc}=T_{tot,ss}/T_0$, from Eq. 4, is computed by adding Eqs. 2 and 3 for the free target (T) and the complex (DT) to give an equation for the total target (T_{tot}):

$$dT_{tot}/dt=k_{syn}-k_{eT}T-k_{eDT}(DT) \tag{7}$$

Solving Eq. 7 for steady state when no drug [$(DT)=0$] is present gives $T_0=k_{syn}/k_{eT}$. When large amounts of drug are present for a long time during intervals of regular dosing, Eq. 6 shows that very little target is free and, thus $T\approx 0$ and $T_{tot}\approx(DT)$,[24] giving:

$$dT_{tot}/dt\approx k_{syn}-k_{eDT}T_{tot}. \tag{8}$$

At equilibrium, $T_{tot,ss}=k_{syn}/k_{eDT}$, and the target accumulation ratio (T_{acc}) is computed as follows:

$$T_{acc}=\frac{T_{tot,ss}}{T_0}=\frac{k_{syn}/k_{eDT}}{k_{syn}/k_{eT}}=\frac{k_{eT}}{k_{eDT}}. \tag{9}$$

Substituting Eqs. 6 and 9 into Eq. 4 gives the equation below:

$$\frac{T}{T_0}\approx\frac{K_d\cdot T_{acc}}{D_{tot}} \tag{10}$$

For drugs with linear PK that are dosed at regular intervals (τ), the Trough Free target concentration to Initial target concentration Ratio (TFIR) can be computed using the trough drug concentration at steady state $D_{tot,min}$, (referred to here as C_{min} to match the more commonly used nomenclature), which can be written as a sum of exponentials.[25]

$$C_{min}=D_{tot,min}=F\cdot\text{Dose}\cdot\sum_i\frac{C_i\exp(-\lambda_i\tau)}{(1-\exp(-\lambda_i\tau))}$$

Substituting into Eq. 10 gives:

$$\text{TFIR}=\frac{K_d\cdot T_{acc}}{C_{min}} \tag{11}$$

The AFIR is given in the equation below, where t_{ss} denotes a time at which the drug and target have reached steady state.

$$\text{AFIR}=\frac{1}{\tau}\int_{t_{ss}}^{t_{ss}+\tau}\frac{K_d\cdot T_{acc}}{C(t)}dt \tag{12}$$

In the case of linear PK, recall that the average drug concentration is given by $C_{avg}=D_{tot,avg}=(F\cdot\text{Dose})/(CL\cdot\tau)$.[25] When the drug is given as an infusion at rate Dose/τ, then the steady-state drug concentration is a constant ($C(t)=C_{avg}$) giving:

$$\text{AFIR}=K_d\cdot T_{acc}\cdot\left(\frac{CL\cdot\tau}{F\cdot\text{Dose}}\right) \tag{13}$$

$$=\frac{K_d\cdot T_{acc}}{C_{avg}} \tag{14}$$

In practice, mAbs are usually dosed every 2–8 weeks and the above equations are approximations rather than exact solutions. It will be shown in the next section that this approximation is often good.

Thus, the average target inhibition (AFIR) depends upon three quantities: the dissociation constant (K_d), the target accumulation ratio (T_{acc}), and the average drug concentration (C_{avg}).

A number of assumptions were made in developing the AFIR metric. When these assumptions do not hold, three alternative formulas for AFIR have been derived in the **Supplementary Material**.

1. When the dose is not large enough for the total target to reach its steady-state plateau, AFIR_{avg} may be used.
2. When the drug concentration is not in vast excess to the target concentration, AFIR_{QE} may be used.
3. When the irreversible binding approximation is more accurate than the quasi-equilibrium approximation, which may occur when $k_{eT}>k_{off}$, then AFIR_{IB} may be used.

METHODS AND RESULTS

Basic sensitivity analysis

To gain a better understanding of the AFIR and TFIR ratios and to explore the conditions under which these potency metrics accurately describe the system, a basic sensitivity analysis is performed using the parameters for siltuximab (**Figure 3**), as well as omalizumab and bevacizumab (**Supplementary Material**). Equation 13 demonstrates that AFIR depends on 8 different parameters; 7 parameters are explored (excluding F because changing F has the same effect as changing Dose) and k_{syn} is also included to confirm that target synthesis does not affect AFIR. Each row of plots in **Figure 3** explores the sensitivity of a different quantity: $\{D_{tot}, T_{tot}, T/T_0, \text{AFIR}, \text{TFIR}\}$. In each column, is the result of changing one parameter while holding the other parameters fixed. The parameter that is changed and the range over which it is changed is shown at the top of each column. To test the approximations for AFIR and TFIR, a direct calculation of the target inhibition using Eqs. 11 and 14 is compared to a numeric calculation from the model simulation after 2 years of therapy.

For siltuximab, there was generally good agreement between the theory and the numeric estimates for AFIR and TFIR. However, there is divergence from the theory in each of the AFIR and TFIR plots when $\text{AFIR}, \text{TFIR} > 30\%$ and the target does not accumulate to $T_{tot,ss}$, leading to a lower observed target accumulation ratio (T_{acc}) than would be observed for a larger dose. In this case, the AFIR_{avg} calculation described in the **Supplementary Material** should be used. The inaccuracy of the AFIR equation can be especially pronounced in the limit where the dose and drug concentration approach zero and the theoretical approximations for AFIR approach infinity, even though the true behavior is that $\text{AFIR}=1$. Although the numerically calculated AFIR generally approaches its theoretical value as AFIR falls below 30%, divergence from the theory is observed for very small k_{off}, (see k_{off} sensitivity plots of

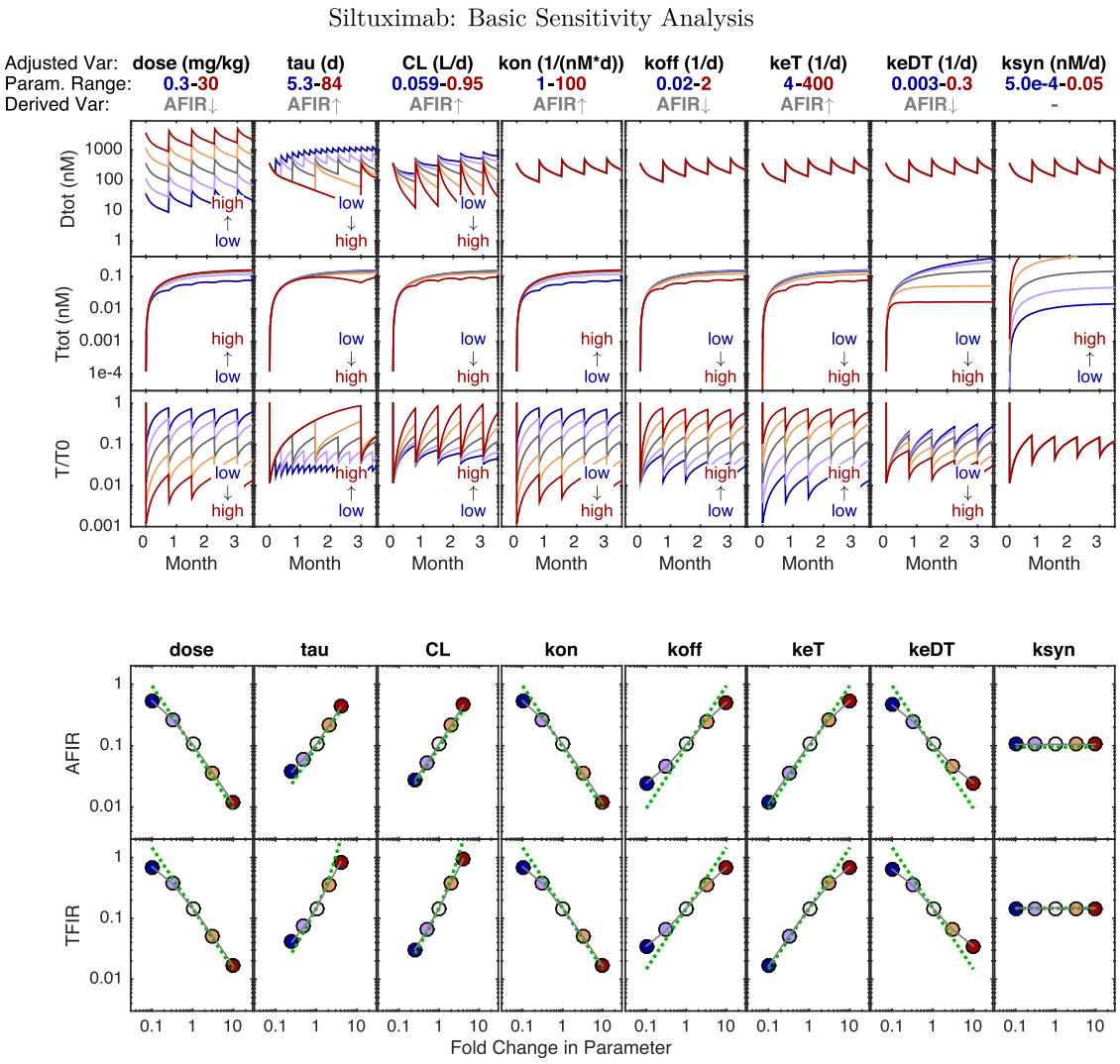

Figure 3 Basic sensitivity analysis for siltuximab centered about 3 mg/kg dosing every 3 weeks. For each column of plots, the parameter in the title is varied relative to the parameters in **Table 1** by either 16-fold (4× lower to 4× higher for *CL* and τ), or 100-fold (10× lower to 10× higher for all other parameters). Each row represents a different variable of the system. The green dashed line in Averaged Free target concentration to Initial target concentration Ratio (AFIR) and Trough Free target concentration to Initial target concentration Ratio (TFIR) plots show the theoretical calculation compared to the estimate from the numerical simulation (circles).

AFIR and TFIR) where the quasi-equilibrium assumption is less accurate because the target elimination rate (k_{eT}) is larger than k_{off}. In this case, drug-target binding approaches the irreversible-binding approximation such that further reduction of k_{off} has no additional benefit. In this scenario, the $AFIR_{IB}$ calculation should be used (see **Supplementary Material** for details).

Lumped sensitivity analysis

A lumped parameter sensitivity analysis was performed in **Figure 4**; the system is reparameterized, replacing rate constants $\{k_{on}, k_{syn}, k_{eT}\}$ with lumped parameters $\{AFIR, T_{tot,ss}, T_0\}$. The rate constants for the model are then calculated from the lumped parameters as shown:

$$k_{syn} = T_{tot,ss} \cdot k_{eDT}$$

$$k_{eT} = \frac{k_{syn}}{T_0}$$

$$k_{on} = \frac{k_{off}}{AFIR} \cdot \frac{T_{tot,ss}}{T_0} \cdot \left(\frac{CL \cdot \tau}{F \cdot Dose}\right)$$

By parameterizing the system in this way, the effect of changing parameters while keeping AFIR fixed can be examined and minimal impact on the free target dynamics is observed. This can be seen by noting that the AFIR and TFIR plots in **Figure 4** are flat. However, there are some exceptions. For large τ (3 months) or large *CL* (1 L/d), the true AFIR is larger than what is theoretically predicted while the TFIR calculation remains accurate. The inaccuracy in the AFIR theoretical prediction is due to large changes in

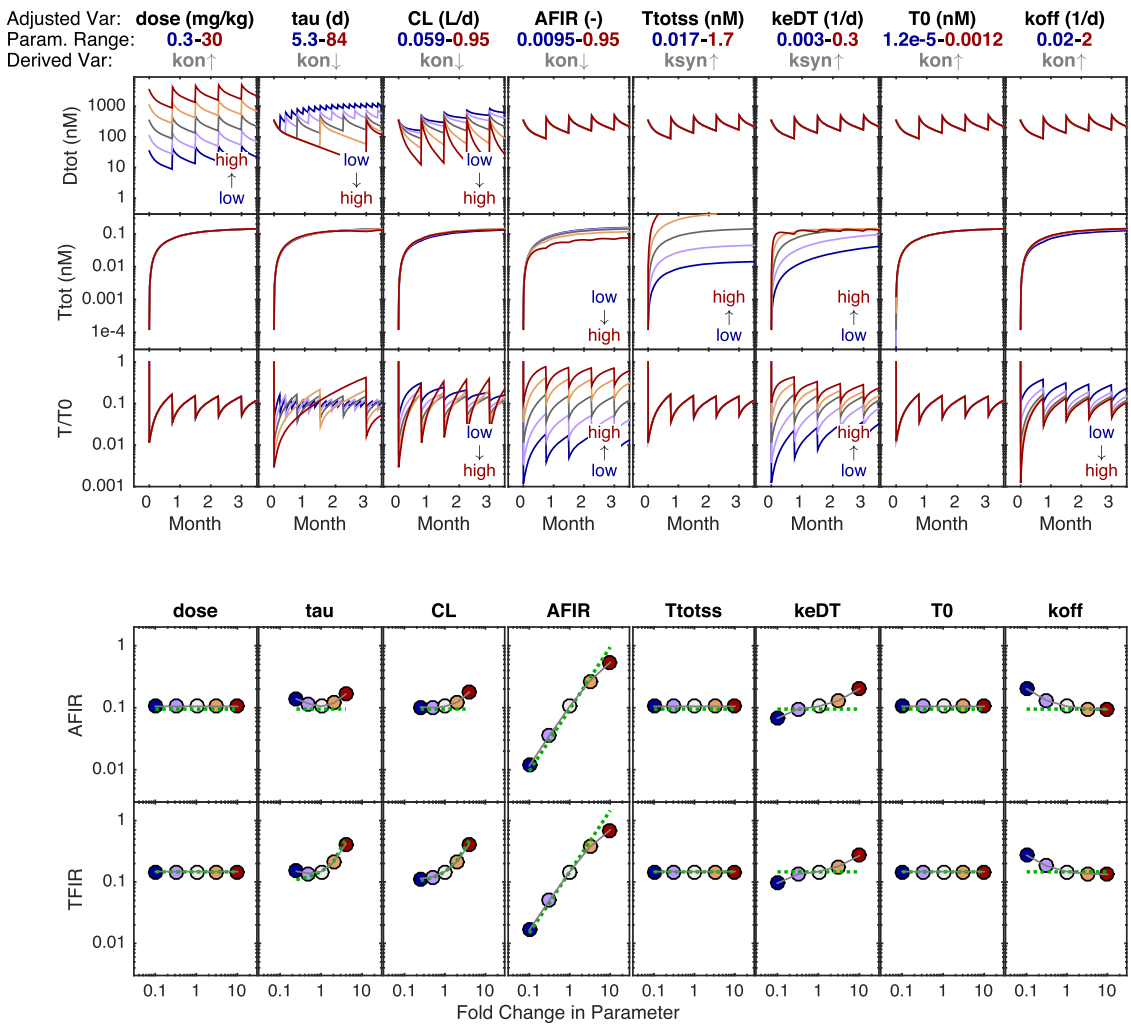

Figure 4 Lumped sensitivity analyses for siltuximab centered about 3 mg/kg dosing every 3 weeks. For each column of plots, the parameter in the title is varied relative to the parameters in **Table 1** by either 16-fold (4× lower to 4× higher for *CL* and τ), or 100-fold (10× lower to 10× higher for all other parameters). Each row represents a different variable of the system. The green dashed line in Averaged Free target concentration to Initial target concentration Ratio (AFIR) and TFIR plots show the theoretical calculation compared to the estimate from the numerical simulation (circles). Ttotss, Total target at steady state; Dtot, Total Drug.

drug concentration over the dosing interval such that the assumption of a constant drug concentration over the dosing interval leads to inaccuracies. Although high $CL = 1$ L/d is typically not observed for mAbs, infrequent dosing (τ=3 months) is sometimes prescribed, as is the case for ustekinumab. As for the basic sensitivity analysis, when $k_{eT} > k_{off}$, both AFIR and TFIR are higher than predicted by the theory and the irreversible binding approximation should be considered because the quasi-equilibrium approximation declines in accuracy.

Effect of increasing dose on total target and free target

It is instructive to focus on the effect of changing the dose on the total target and free target, as shown in **Figure 5a**. Notice that above 1 mg/kg, further increases in dose do not have much impact on the total target accumulation. However, this plateau in total target does not necessarily imply a plateau in free target reduction or efficacy, as demonstrated by the free target curves (T/T_0) and as observed elsewhere.[9,10]

Identifiability of the dissociation constant and baseline target concentration

In considering the identifiability of the four key parameters governing the target dynamics $\{k_{syn}, k_{eT}, k_{eDT}, K_d\}$, it is useful to reparameterize the model as $\{T_0, T_{tot,ss}, k_{eDT}, K_d\}$; T_0 is the target concentration before drug is given; $T_{tot,ss}$ is the total target concentration after the total target reaches its plateau following a large enough dose; k_{eDT} governs the rate at which the total target approaches its plateau (see Eq. 8 and ref. 24); and K_d determines the dose needed for the target to approach its plateau (lower K_d means the total

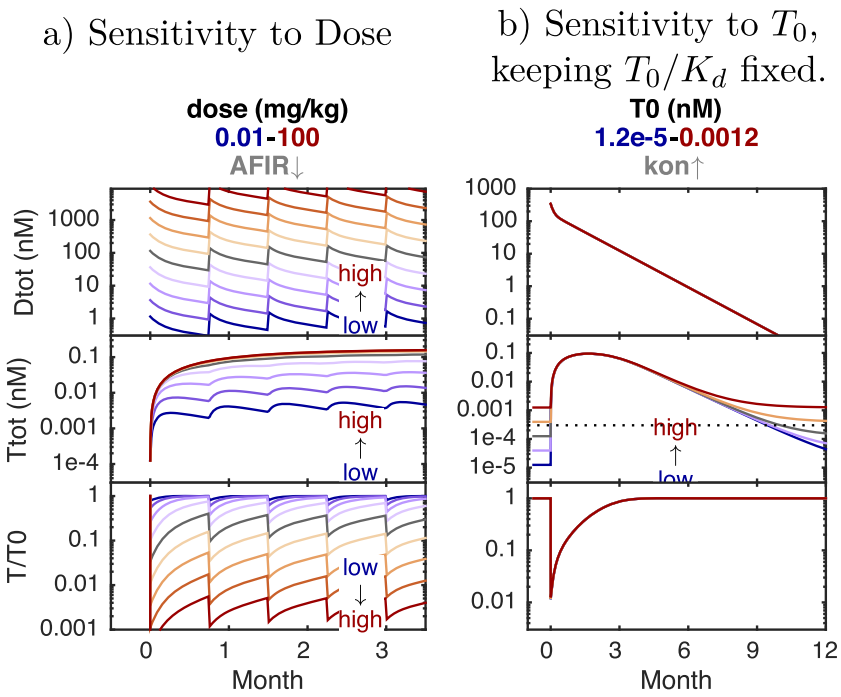

Figure 5 (**a**) Sensitivity analysis for siltuximab where dose is varied over 10,000× (from 0.01 mg/kg to 100 mg/kg). Note that above 1 mg/kg (gray line), there is a plateau in target accumulation, but the free target to initial target ratio continues to decline with dose, as predicted by Averaged Free target concentration to Initial target concentration Ratio (AFIR). (**b**) Sensitivity analysis for siltuximab where T_0 and k_{on} are simultaneously varied over 100× such that AFIR stays fixed. Note that the blue and grey curves are almost overlapping above the dotted line (potential limit of quantification), so in the event that the baseline target is not measurable, the parameters T_0 and $K_d = k_{off}/k_{on}$ are unidentifiable.

target will approach its plateau at lower doses). Thus, all four parameters are identifiable as long as enough measurements are taken and the target assay is sufficiently sensitive.

In practice, the assay for measuring total target is often not sensitive enough to detect the baseline target concentration before the drug is given, as was the case for siltuximab.[5,7] Although the steady-state plateau ($T_{tot,ss}$) and the time scale for reaching it (k_{eDT}) can still be identified, the baseline target level (T_0) is no longer identifiable and this leads to unidentifiability of K_d as well. This can be observed graphically in **Figure 5b**, which shows the kinetics after a single dose of siltuximab, where both T_0 and k_{on} are simultaneously increased while all other parameters are held fixed. The dotted line indicates where a limit of quantification of the total target assay could lie. The sensitivity analysis shows no impact on the profiles for D_{tot} and T/T_0. For T_{tot}, the blue and gray curves ($T_0 < 0.3$ pM and $K_d < 50$ pM) are overlapping indicating that while the quotient $K_d/T_0 = 160$ is identifiable, only an upper bound for T_0 and K_d can be identified. AFIR is also identifiable as can readily be seen because it can be written as $\text{AFIR} = (K_d/T_0) \cdot T_{tot,ss}/C_{avg}$. Thus, if the goal is to predict AFIR, estimation of the ratio (K_d/T_0) may be sufficient and a total target assay that is sensitive enough to measure baseline target levels may not be needed.

Simulation code

All simulations were performed using Matlab R2015a. Code for generating all figures in this article is available in the **Supplementary Material**.

DISCUSSION

The key insight from this work is that under many clinically relevant scenarios, AFIR can be estimated using three parameters: the dissociation constant (K_d), the target accumulation (T_{acc}), and the average drug concentration (C_{avg}).

$$\text{AFIR} = \frac{K_d \cdot T_{acc}}{C_{avg}}$$

This simple formula provides intuition for how changing the dosing regimen or improving the binding affinity of the drug would be expected to alter target inhibition: doubling the dose, halving the dosing interval, or halving the dissociation constant by using a higher affinity drug would all reduce the free target concentration by 50%. Although target accumulation plateaus at large doses, the AFIR formula shows that further increasing the dose continues to reduce the free target levels (as illustrated in **Figure 5a**), which could then

potentially lead to greater biomarker inhibition,[5,8] and efficacy.[9,10]

Although target occupancy (ratio of bound target to total target) provides another metric to assess target engagement, this metric is misleading for soluble targets because it does not account for target accumulation. For example, consider a scenario where there is 99% target occupancy together with a 100-fold accumulation of target. In this scenario, no reduction in the absolute concentration free target has been achieved and, thus, achieving 99% target occupancy would not be expected to provide clinical benefit. Thus, for soluble targets, AFIR is a preferred metric of target inhibition.

Applications

The AFIR potency metric allows for a rapid assessment of new drugs without requiring extensive simulation. Specifically, the formula $\mathrm{AFIR} = K_d \cdot T_{acc} \cdot CL \cdot \tau / (F \cdot \mathrm{Dose})$ allows the drug developer to quantify how a second generation drug with better PK properties or higher binding affinity could lead to improved target inhibition. Alternatively, AFIR could be used to identify dosing regimens that allow for less frequent dosing, ultimately leading to a reduction in the number of injections, the number of visits to the doctor, and the cost of goods.

In designing a phase II dose-finding study, if one has an idea of what level of target inhibition is required, AFIR provides a means to identify the largest dose to be given. When designing preclinical studies, the AFIR metric indicates that it is possible to predict target inhibition without estimating all parameters of the TMDD model. In particular, it is sufficient to have an estimate of the dissociation constant K_d and the expected target accumulation ratio T_{acc}. The target accumulation can be predicted either by first measuring the baseline target concentration and then measuring the total target after a large dose, or by computing the ratio of estimates of the half-life of the target and the complex. Furthermore, if one has rich time-course data for the total target dynamics, even if the assay is not sensitive enough to estimate the baseline target concentration T_0, it may be reasonable to fix the baseline level at a value from the literature; although this will affect the estimate for K_d, it will not impact the AFIR prediction. The overarching principle is that because the AFIR metric is a lumped parameter, it is not necessary to estimate each individual rate constant of the model to make predictions for target engagement.

Caveats

When interpreting the AFIR metric, one must ensure that the following conditions hold:

1. AFIR $< 30\%$: otherwise the total target has not reached its plateau and AFIR_{avg} should be used.
2. Drug is in excess to the target: otherwise, there is not enough drug to bind all target molecules and AFIR_{QE} should be used.
3. $k_{off} > k_{eT}$: otherwise, there is near-irreversible drug binding and AFIR_{IB} should be considered.

Each of these terms is derived in the **Supplementary Material**, although performance of the metrics has not yet been numerically explored. It is also important to recognize that the TMDD model analyzed here is a simplification and leaves out many physiological processes such as:

1. The PK nonlinearity that occurs for many membrane-bound targets.[26,27]
2. Target synthesis and distribution in both peripheral tissue[16] and the target tissue (e.g., the joint or tumor).[28,29]
3. Competition for target binding sites between the drug and the target's endogenous ligand.[30]
4. Feedback mechanisms[31] leading to either an increased synthesis of the target in the presence of drug[32] or a decrease in target synthesis or expression.[16,33]
5. A drug binding multiple targets (which could also include shed receptors); the current model assumes that all of the target is measured, but for infliximab binding tumor necrosis factor-α, target exists in both membrane-bound and soluble forms, and a model that only accounts for soluble tumor necrosis factor-α may considerably underestimate K_d.[3]
6. This analysis was primarily developed for mAbs and may need to be amended when analyzing other biologics, such as bispecifics.

Implicit in this work is the assumption that the model prediction of free target based on total drug and total target data is accurate, because usually free target data is not available. Thus far, the free target prediction has only been validated for omalizumab.[16] Others tried to test this prediction with siltuximab, but because dosing of siltuximab led to an immediate input of IL-6 into the blood, a more complex model was needed to describe the system and many possible free target levels and AFIR ratios were consistent with the data.[34] Publication of other systems in which both total and free target are measured would help to validate the assumption that the model prediction for free target is generally accurate.

Furthermore, target engagement is only the first pharmacodynamic step toward achieving efficacy, and thus AFIR must be interpreted with caution. Even though the AFIR equation demonstrates that the free target concentration will continue to decline with increasing dose, there may be a threshold (e.g., 5%) such that further reduction in AFIR no longer leads to improved clinical efficacy. Understanding this process can be aided with collection of a downstream pharmacodynamic biomarker (e.g., C-reactive protein and neutrophil levels for anti-IL-6 therapy[7,35]). Finally, AFIR should not be applied to agonists like TGN1412[16] or blinatumomab,[36] where only small amounts of target binding (AFIR $> 90\%$) are necessary.

CONCLUSION

In summary, the AFIR potency metric has been derived. This metric predicts the target inhibition at steady state under a repeated dosing regimen using three quantities: the average drug concentration at steady state, the dissociation constant, and the target accumulation ratio. AFIR provides intuition for how various physiological properties of the system impact target engagement. In addition, AFIR has been used for rapid assessment of the druggability of

new targets and exploration of the binding affinity, half-life, bioavailability, and dosing regimen needed for a second-generation drug to achieve comparable or superior efficacy to a marketed compound.

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