Estimating drug potency in the competitive target mediated drug disposition (TMDD) system when the endogenous ligand is included.

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Abstract
Predictions for target engagement are often used to guide drug development. In particular, when selecting the recommended phase 2 dose of a drug that is very safe, and where good biomarkers for response may not exist (e.g. in immunoncology), a receptor occupancy prediction could even be the main determinant in justifying the approved dose, as was the case for atezolizumab. The underlying assumption in these models is that when the drug binds its target, it disrupts the interaction between the target and its endogenous ligand, thereby disrupting downstream signaling. However, the interaction between the target and its endogenous binding partner is almost never included in the model. In this work, we take a deeper look at the in vivo system where a drug binds to its target and disrupts the target’s interaction with an endogenous ligand. We derive two simple steady state inhibition metrics (SSIMs) for the system, which provides intuition for when the competition between drug and endogenous ligand should be taken into account for guiding drug development.

Keywords Monoclonal antibody · Target mediated drug disposition · Pharmacokinetics and pharmacodynamics · Pharmacometrics

Introduction
Characterizing target engagement (i.e. receptor occupancy) for a drug binding to its target is regularly done to support drug development. Applications include assessing the druggability of a target, choosing a drug lead candidate and selecting the dose-regimen. Generally, for monoclonal antibodies, if the drug is safe at high doses, the goal is to identify the dose that can give 90-95% target engagement at steady state in the majority of patients. This differs from antibody-drug conjugates and bispecific target engagers, where toxicity limits how high these drugs can be safely dosed.

In this manuscript, we focus specifically on monoclonal antibodies (mAbs). Unlike small molecules (< 500 Da), mAbs are large molecules (≈ 150 kDa) that often exhibit target mediated drug disposition (TMDD) and have long half-lives (≈ 3 weeks). With the recent increase in number of mAb approvals for cancer treatment, there continues to be a need to identify robust response biomarkers, and PK/PD modeling and simulation plays a role in establishing dosing regimen for early oncology trials (e.g. atezolizumab [1, 2, page 9-10]).
Recently, two metrics \([3, 4]\) have been developed for predicting target engagement in circulation and in the target tissue. However, these (and most other) target engagement metrics do not account for the binding of the target to its endogenous ligand. Many mAbs (e.g. pembrolizumab) are antagonists that confer their activity by binding the target (e.g. PD-1) resulting in disruption of target - endogenous ligand interaction (e.g. PD-1 - PD-L1 interaction) and inhibition of downstream signaling. Most metrics for target engagement use receptor occupancy (RO) to estimate inhibition of target signaling. RO is given by:
\[
RO = \frac{C}{K_{ssDT} + C},
\]
where \(C\) is the drug concentration and \(K_{ssDT}\) is the quasi-steady-state binding constant between the drug and target. This RO metric does not account for the competitive endogenous target-ligand interaction.

However, if the disassociation rate for the target-endogenous-ligand complex is very small, then even if a drug binds \(>95\%\) of the target, it has not necessarily disrupted \(95\%\) of the target-ligand interactions. Additionally, in cases with soluble endogenous ligand, drug-target interaction may result in an increase in concentrations of soluble ligand either due to reduced target-ligand interaction that in turn limits ligand elimination through receptor internalization, or due to an up-regulation in ligand synthesis rate in response to the inhibition \([5–7]\). This increase in ligand levels is observed in Fig. 1 where tocilizumab binds its target IL-6R, which then leads to an accumulation of its ligand, IL-6. Accounting for this ligand accumulation is important for characterizing target inhibition.

In this paper, in order to better understand the drug-target-ligand binding system, we have analyzed the model shown in Fig. 2. This model is an extension of the standard target mediated drug disposition (TMDD) model. In the standard TMDD model, a drug \((D)\) binds its target \((T)\) and forms a complex \((DT)\), all three quantities have an elimination rate \((k_{eD}, k_{eT}, k_{eDT})\), and the target is also synthesized at a constant rate \((k_{synT})\). The model extension presented here, called the competitive TMDD model, assumes that the target also binds competitively to its endogenous ligand; the model extension also accounts for ligand synthesis \((k_{synL})\) and turnover \((k_{eL}, k_{eTL})\). This system has been studied previously where the ligand was considered to be a second drug \([8–10]\) or a plasma binding protein \([11]\). Peletier and Gabrielsson also studied a similar system with one drug and two targets \([12]\). However, none of this previous work focused on a steady state inhibition metric following repeated dosing.

In this work, we have developed a new potency metric for the competitive TMDD system: ASIR (Average Signaling complex to Initial complex Ratio), that accounts for endogenous ligand. ASIR is defined to be \(\frac{TL_{ss}}{TL_0}\), which is the ratio of the target-ligand signaling complex concentration at steady state \((TL_{ss})\) to the signaling complex concentration at baseline in absence of drug \((TL_0)\). We also show how the degree of observed ligand accumulation can be directly related to ASIR. To put this work in context, we also define a steady-state inhibition metric (SSIM), which is given by \(SSIM = \frac{C_{ss}}{C_{ss} + IC_{50}}\), where \(C_{ss}\) is the steady-state drug concentration (trough or average), and \(IC_{50}\) is the drug concentration needed for 50\% inhibition. In Fig. 3, we summarise the results from this work together with other potency metrics to show how the approximate \(IC_{50}\) varies across different ligand binding systems.

![Fig. 1](image)

*Fig. 1* Single dose of tocilizumab at 10 mg/kg with experimental data (symbols)[25] and model simulation (lines). The drug \((D)\) and free endogenous ligand \((L, IL-6)\) were the two quantities that were both measured and modeled. The target \((T)\) in this simulation was membrane-bound IL-6R. It was not measured and neither were its complexes \((DT\) and \(TL)\), but all three components were simulated. Soluble IL-6R was measured, but it was not included in the model. Accumulation of both the soluble target (IL-6R) and endogenous ligand (IL-6) indicates that tocilizumab is engaging its target.
Theoretical

Drug concentration notation

Throughout this manuscript, we use two different variables to denote drug concentration: $C$ and $D$. We use $D$ when describing the differential equations that describe the system of interest, because $D$ denotes drug. But when discussing any target inhibition metrics, we switch to $C$ (for concentration) since this is the standard nomenclature used for receptor occupancy.

Model equations

Figure 2 shows the model diagram used in the calculations and in the derivation of ASIR and SSIM. The drug ($D$) can distribute to a peripheral compartment ($D_2$). The drug can also bind its target ($T$) to form a complex ($DT$) and the target can also bind its endogenous ligand ($L$) to form a complex ($TL$). The target and its ligand are synthesized at rates $k_{synT}$ and $k_{synL}$, and all species are eliminated at rates: $k_{eD}$, $k_{eT}$, $k_{eL}$, $k_{eDT}$ and $k_{eTL}$. The dose is described by $Dose(t)$ into the central compartment and it can either be a constant infusion where $Dose(t)$ is set to a constant, or a series of bolus doses, given at times $t = n\tau$, where $n$ is an integer and $\tau$ is the dosing interval. In these equations, the drug, target, and ligand concentrations are molar.

The differential equations for this system are shown below. Both the target and the ligand can be either membrane-bound or soluble. In the case of a membrane-bound target, $k_eT$ is the internalization rate of the target receptor, and $k_{eDT}$ and $k_{eTL}$ are the internalization rates of the drug-target and target-ligand complexes. In the case of a soluble target, $k_eT$ is the elimination rate of the soluble target, $k_{eDT}$

![Fig. 2 Compartmental model for the competitive target mediated drug disposition system to study drug pharmacokinetics, and target and endogenous ligand dynamics. The model includes drug distribution to a peripheral tissue, drug elimination, and drug binding to its target and forming a complex. Both target and ligand can also bind each other, and are synthesized and eliminated at constant rates. The target-ligand complex $TL$ is the key component of the model that ultimately leads to downstream signaling and the mechanism of action for the drug is to bind the target to reduce the formation of $TL$.]

![Steady State Inhibition Metric (SSIM) = $\frac{C_{SS}}{C_{SS} + IC_{50}}$]

| Name      | Binding | TMDD          | Competitive TMDD | Tissue TMDD |
|-----------|---------|---------------|------------------|-------------|
| Tissue    | in vitro| Circulation   | Circulation      | Tissue      |
| Ligand    | no      | no            | yes              | no          |
| $IC_{50}$ | $K_{ss}$| $K_{ss} \cdot T_{fold,D,L}$ | $K_{ss} \cdot T_{fold,D,L} \cdot I_{fold,D,L}$ | $K_{ss} \cdot T_{fold,D,L}$ |

**Fig. 3** Summary of compartmental models and corresponding $IC_{50}$ derivations explored in this work and in previous studies [3, 4, 18]. All the above ligand binding systems can be described with the same steady state inhibition metric (SSIM) equation. For the in vitro system, $IC_{50} = K_{ss}$. However, for the in vivo system, $IC_{50}$ depends on additional parameters that describe target dynamics, ligand dynamics, and drug biodistribution.
is the elimination rate of the drug-target complex, and \( k_{cTL} \) is the internalization rate of the target-ligand complex.

\[
\frac{dD_2}{dt} = \left( k_{12} \frac{V_1}{V_2} \right) D - k_{21}D_2
\]  
\( \text{(1)} \)

\[
\frac{dD}{dt} = \text{Dose}(t) \frac{V_1}{V_1} - k_{onDT} D \cdot T + k_{offDT}(DT)
\]
\[- k_{cD} D - k_{12} D + \left( k_{21} \frac{V_2}{V_1} \right) D_2
\]  
\( \text{(2)} \)

\[
\frac{dT}{dt} = k_{synT} - k_{onDT} D \cdot T + k_{offDT}(DT)
\]
\[- k_{onTL} T \cdot L + k_{offTL}(TL) - k_{eT} T
\]  
\( \text{(3)} \)

\[
\frac{dL}{dt} = k_{synL} - k_{onTL} T \cdot L + k_{offTL}(TL) - k_{eL} L
\]  
\( \text{(4)} \)

\[
\frac{d(DT)}{dt} = k_{onDT} D \cdot T - k_{offDT}(DT) - k_{eDT}(DT)
\]  
\( \text{(5)} \)

\[
\frac{d(TL)}{dt} = k_{onTL} T \cdot L - k_{offTL}(TL) - k_{eTL}(TL)
\]  
\( \text{(6)} \)

The initial conditions for the above equations are set before the drug is given, so \( D(0) = D_2(0) = DT(0) = 0 \). Then, the other terms are defined to be \( T(0) = T_0, L(0) = L_0, \) and \( TL(0) = TL_0 \). Here, \( T_0 \) and \( L_0 \) are set based on literature data since these numbers are often readily available, and \( TL_0, k_{synT} \) and \( k_{synL} \) are derived, such that the system is at steady state before the drug is given, using Eqs. 13-17 derived further below.

It is useful to define the total drug, total target and total ligand concentration as shown below, where all three equations (including \( D \)) are for only the central compartment

\[
\begin{align*}
D_{tot} &= D + DT \\
T_{tot} &= T + TL + DT \\
L_{tot} &= L + TL
\end{align*}
\]  
\( \text{(7)} \)

\( \text{(8)} \)

\( \text{(9)} \)

Differentiating each side of these equations and then substituting in the right hand side from Eqs. 2-6 above gives

\[
\frac{dD_{tot}}{dt} = \text{Dose}(t) \frac{V_1}{V_1} - k_{cD} D - k_{cDT}(DT) - k_{12} D + \left( k_{21} \frac{V_2}{V_1} \right) D_2
\]  
\( \text{(10)} \)

\[
\frac{dT_{tot}}{dt} = k_{synT} - k_{eT} T - k_{eTL}(TL) - k_{cDT}(DT)
\]  
\( \text{(11)} \)

\[
\frac{dL_{tot}}{dt} = k_{synL} - k_{eL} L - k_{eTL}(TL)
\]  
\( \text{(12)} \)

### Steady state of the system

Before the drug is given, at steady state we set Eq. 6 to zero and solve for \( TL_0 \) giving:

\[
TL_0 = \frac{T_0 \cdot L_0}{K_{synL}}
\]  
\( \text{(13)} \)

where the quasi-steady-state constants \([13, 14]\) for the drug-target and target-ligand complex are

\[
\begin{align*}
K_{synT} &= k_{offDT} + k_{cDT} \\
&= \frac{(D)(T)}{DT}
\end{align*}
\]  
\( \text{(14)} \)

\[
\begin{align*}
K_{synL} &= \frac{k_{offTL} + k_{cTL}}{k_{onTL}} \\
&= \frac{(T)(L)}{TL}
\end{align*}
\]  
\( \text{(15)} \)

The parameters \( k_{synT} \) and \( k_{synL} \) are then computed by setting the right hand side of Eqs. 11-12 to zero, giving:

\[
\begin{align*}
k_{synT} &= k_{cT} T_0 + k_{cTL}(TL_0) \\
k_{synL} &= k_{eL} L_0 + k_{cTL}(TL_0)
\end{align*}
\]  
\( \text{(16)} \)

\( \text{(17)} \)

It is also useful to define two quantities \( T_{tot, largeD} \) and \( L_{largeD} \) where, for very large drug concentrations, almost all the target is bound to drug and so \( (DT) \approx T_{tot} \) and \( T \approx (TL) \approx 0 \). In other words

\[
\frac{\lim}{D \to \infty} T = 0
\]
\[
\frac{\lim}{D \to \infty} (TL) = 0
\]

Substituting these expressions into Eqs. 8-9 gives that \( T_{tot} \approx (DT) \) and \( L_{tot} \approx L \), or in other words

\[
\frac{\lim}{D \to \infty} T_{tot} = (DT)
\]
\[
\frac{\lim}{D \to \infty} L_{tot} = L
\]

Finally, substituting these relationships into Eqs. 11-12 and solving for steady state gives:

\[
T_{tot, largeD} = \lim_{D \to \infty} T_{tot} = \frac{k_{synT}}{k_{cDT}}
\]  
\( \text{(18)} \)

\[
L_{largeD} = \lim_{D \to \infty} L = \frac{k_{synL}}{k_{eL}}
\]  
\( \text{(19)} \)

It will be useful to also define the fold-change in total target \( T_{fold, largeD} \) and the fold-change in free ligand \( (L_{fold, largeD}) \) at steady state and for large doses. By fold-change, we are referring to the multiplicative factor that relates the baseline level to the steady state level of drug or target.

\[
T_{fold, largeD} = \frac{T_{tot, largeD}}{T_0}
\]  
\( \text{(20)} \)

\[
L_{fold, largeD} = \frac{L_{largeD}}{L_0}
\]  
\( \text{(21)} \)
For soluble targets, the elimination of the drug-target complex is often much slower than that of the target itself and so \( T_{\text{fold,largeD}} \) will typically be 20-1000 [3]. To our knowledge, \( L_{\text{fold,largeD}} \) when the target is soluble has not been measured, though we expect that it would be close to one. For membrane-bound targets, \( T_{\text{fold,largeD}} \) can be measured in vitro. The fold change in internalization rates of receptors when bound to their target or drug has been observed to range from 0.25-2 for EGFR/EGF [15], insulin receptor/insulin [16], and CD3ε/otelixizumab [17]. \( L_{\text{fold,largeD}} \), on the other hand, can be much greater than one, as shown in Fig. 1.

**Definition of previous inhibition metrics: AFIR and AFTIR**

In previous work, two inhibition metrics were defined. AFIR is the Average Free target to Initial target Ratio for target inhibition in circulation [3], and AFTIR is the Average Free Tissue target to Initial target Ratio in the tissue of interest (e.g. a tumor) [4]. The formulas for both metrics are provided below:

\[
AFIR = \frac{T}{T_0} = \frac{K_{\text{ssDT}} T_{\text{fold,largeD}}}{C_{\text{avg,ss}}} \tag{22}
\]

\[
AFTIR = \frac{T_{\text{tissue}}}{T_{0,\text{tissue}}} = \frac{K_{\text{ssDT}} T_{\text{fold,largeD}}}{B \cdot C_{\text{avg,ss}}} \tag{23}
\]

All terms above have already been defined except \( B \), which is the biodistribution coefficient; it gives the fraction of drug in circulation that makes it into the interstitial fluid of the tissue of interest at high doses. In the next section, we describe a new target inhibition metric for the system where the competitive endogenous ligand (\( L \)) is taken into account.

**Definition of ASIR and SSIM**

The effect of a drug binding its target is that the amount of target binding its endogenous ligand (\( TL \)) decreases, and therefore, there is a decrease in downstream signaling. A metric for characterizing this decline is the Average Signaling complex to Initial complex Ratio (ASIR). It is defined to be:

\[
ASIR = \frac{TL_{ss}}{TL_0} \tag{24}
\]

For a drug that is dosed at a constant infusion, ASIR will be constant. For repeated dosing regimens, ASIR value can be considered as the averaged values of this ratio, or the ratio at trough.

We also define the concept of Steady-State Inhibition Metric (SSIM), which for the case of the model in Fig. 2, is given by:

\[
SSIM = 1 - ASIR = \frac{TL_0 - TL_{ss}}{TL_0}. \tag{25}
\]

SSIM has the intuitive property that as one increases the dose and achieves greater target inhibition, SSIM approaches 100%. For a number of different systems, SSIM can be approximated by the equation below, where the \( IC_{50} \) depends on the system that is modeled.

\[
SSIM_{\text{system}} \approx \frac{C_{\text{ss}}}{C_{\text{ss}} + IC_{50}} \tag{26}
\]

A summary of \( IC_{50} \) expressions for four different ligand binding systems is shown in Fig. 3. The results are based on the work from this paper as well as on previous studies that explored different compartmental models [3, 4, 18]. Throughout the rest of this manuscript, the term SSIM will refer to the inhibition metric for the competitive TMDD model shown in Fig. 2; we will derive this expression and the \( IC_{50} \) value for this system.

**Relating SSIM to ligand concentration**

We derive the relationship between SSIM and the steady-state ligand concentration (\( L_{ss} \)) by starting with the expression for the total ligand concentration in Eq. 12 at steady state (setting \( dL_{\text{ss}}/dt = 0 \), giving

\[
0 = k_{\text{synL}} - k_{\text{dL}} L_{ss} - k_{\text{eTL}} TL_{ss} \tag{27}
\]

Solving for \( L_{ss} \) gives the equation below, where we emphasize that \( L_{ss} \) and \( TL_{ss} \) will change depending on the dosing regimen.

\[
L_{ss}(\text{Dose}) = \frac{k_{\text{synL}} - k_{\text{eTL}} TL_{ss}(\text{Dose})}{k_{\text{dL}}} \tag{28}
\]

Before the drug is given, we have

\[
L_0 = L_{ss}(\text{Dose} = 0) = \frac{k_{\text{synL}} - k_{\text{eTL}} TL_0}{k_{\text{dL}}} \tag{29}
\]

And for a very large dose, almost all the target will be bound to the drug, which will result in depletion of the target-ligand complex. Thus

\[
L_{\text{targetD}} = \lim_{\text{Dose} \to \infty} L_{ss}(\text{Dose}) = \frac{k_{\text{synL}}}{k_{\text{dL}}} \tag{30}
\]

Now, consider the ligand ratio (\( L_{\text{ratio}} \)) defined below. Then substitute the Eqs. 28-30 in, and cancel terms to show that \( L_{\text{ratio}} = \text{SSIM} \)
When the ligand is measurable in circulation, as is the case for IL-6, then the steady state inhibition metric (SSIM) can be estimated using Eq. 31, and the ligand values observed at baseline, at large doses ($L_{\text{largeD}}$), and at the dose of interest ($L_{\text{ss}}(\text{Dose})$).

It should be noted that the receptor IL-6R can exist in both soluble and membrane-bound forms. In this scenario, there are both membrane-bound ($T_M L$) and soluble ($T_S L$) complexes. Modifying Eq. 32 accordingly gives:

$$T_M L \cdot \frac{L_{\text{ratio}}}{L_{\text{ratio}}} = 1 - \frac{k_{\text{TL}} (T_M L)_{ss}}{k_{\text{TL}} (T_M L)_0 + k_{\text{TL}} (T_S L)_0}$$

(32)

For tocilizumab, membrane-bound receptor internalization is faster than soluble receptor elimination: $k_{\text{TL}} L \approx 8/d [19] \text{ vs } k_{\text{TL}} L \approx 1.2/d [20]$. Thus it may be the case that the $L_{\text{ratio}}$ in Eq. 31 is dominated by the membrane-bound elimination terms and can be approximated by the simpler expression. Checking this assumption would require an estimate of $(T_S L)$ and $(T_M L)$. These quantities may be difficult to measure directly and estimating them would require further assumptions about the system. This more complex system, with both membrane-bound and soluble target is beyond the scope of this manuscript and will not be explored further, here.

In the derivation of $L_{\text{ratio}}$, it was assumed that $k_{\text{synl}}$, does not change in the presence of drug. If $k_{\text{synl}}$ does change, due to up-regulation of ligand synthesis when the target is inhibited, then the expression in Eq. 32 would become more complex and in particular, one cannot cancel the $k_{\text{synl}}$ terms to arrive at Eqn. 32. Understanding the behavior of the system where $k_{\text{synl}}$ varies with drug concentration is left to future research.

**Definition of ASIR potency metric**

Recall that we define $\text{ASIR} = TL_{\text{ss}} / TL_0$. This ratio can also be expanded and calculated from two ratios relating the signaling complex concentrations to the total target concentration at steady state ($T_{\text{tot,ss}}$):

$$\text{ASIR} = \frac{TL_{\text{ss}}}{TL_0} = \left( \frac{TL_{\text{ss}}}{T_{\text{tot,ss}}} \right) \left( \frac{T_{\text{tot,ss}}}{TL_0} \right)$$

(34)

We will first derive an expression for the $TL_{\text{ss}} / T_{\text{tot,ss}}$ term in the equation above.

**Deriving signaling complex ratio ($TL_{\text{ss}} / T_{\text{tot,ss}}$)**

To find $TL_{\text{ss}} / T_{\text{tot,ss}}$, we start by solving equation 8 for $DT$

$$DT = T_{\text{tot}} - T - TL$$

(35)

We then solve Eq. 14 for $DT$:

$$DT = \frac{(D)(T)}{K_{\text{ssDT}}}$$

(36)

and combine these two equations to get

$$\frac{(D)(T)}{K_{\text{ssDT}}} = T_{\text{tot}} - T - TL$$

(37)

Dividing both sides by ($TL$) and rearranging terms gives:

$$\frac{T_{\text{tot}}}{TL} = \frac{D}{K_{\text{ssDT}}} + \frac{T}{(TL)} + 1$$

(38)

Finally, taking the inverse of each side and substituting the expression from Equation 15 gives $[21]$:

$$\frac{TL}{T_{\text{tot}}} = \left( \frac{K_{\text{ssTL}} \cdot D}{K_{\text{ssDT}} \cdot L} + \frac{K_{\text{ssTL}}}{L} + 1 \right)^{-1}$$

(39)

If the drug concentration is large enough, the PK will be approximately linear and $C_{\text{ss}} \approx \text{Dose} / (CL \cdot \tau)$, where $\text{Dose}/\tau$ is the infusion rate. This gives:

$$\frac{TL_{\text{ss}}}{T_{\text{tot,ss}}} \approx \left( \frac{K_{\text{ssTL}} \cdot C_{\text{ss}}}{K_{\text{ssDT}} \cdot L_{\text{ss}}} + \frac{K_{\text{ssTL}}}{L_{\text{ss}}} + 1 \right)^{-1}$$

(40)

Previously, it was shown that the onset of nonlinear PK happens approximately at concentration $C_{\text{crit}} = k_{\text{synl}} / k_{\text{TL}} [22]$.

**Deriving ASIR in the limit of large drug concentrations**

Substituting Eq. 40 into the definition of ASIR in Eq. 34 gives:

$$\text{ASIR} = \frac{TL_{\text{ss}}}{TL_0} = \frac{T_{\text{tot,ss}}}{TL_0} \left( \frac{K_{\text{ssTL}} \cdot C_{\text{ss}}}{K_{\text{ssDT}} \cdot L_{\text{ss}}} + \frac{K_{\text{ssTL}}}{L_{\text{ss}}} + 1 \right)^{-1}$$

(41)

To further simplify this equation, assume that the drug concentration is very large with
modify the ASIR approximation. We define arbitrarily small doses and drug concentrations, ASIR simple formula, large drug concentrations were required; for small doses since holds over small drug concentrations should approach one derivation for AFTIR [4]. An expression for ASIR that approaches infinity. This property was also observed in the In defining Eq. 45, which we call the “simple” ASIR formula, large drug concentrations were required; for arbitrarily small doses and drug concentrations, ASIRsimple approaches infinity. This property was also observed in the derivation for AFTIR [4]. An expression for ASIR that holds, K_{ssTL} = T_0 · L_0/TL_0 and so the last fraction in Eq. 43 is equal to 1.

Multiplying by the ratios \( T_0/T_0 \) and \( L_0/L_0 \) and rearranging the terms gives:

\[
\frac{TL_{ss}}{TL_0} = \frac{T_{tot,ss} K_{ssDT} L_{ss} K_{ssTL} T_0}{C_{ss} T_0 L_0} \frac{T_0 \cdot L_0}{C_{ss} T_0 \cdot K_{ssTL} (43)}
\]

For large doses, we have that \( T_{tot,ss} \rightarrow T_{tot,largeD} \) and \( L_{ss} \rightarrow L_{largeD} \) and so we define:

\[
\text{ASIR}_{simple} = \lim_{\text{Dose} \to \infty} \frac{TL_{ss}}{TL_0} = \frac{K_{ssDT} T_{fold,largeD} L_{fold,largeD}}{C_{ss}} \quad (45)
\]

For the TMDD system without ligand, the previously derived expression for average free target vs initial target ratio (AFIR) in the absence of ligand is similar, missing only the \( L_{fold} \) term [3].

\[
\text{AFIR} = \frac{T_{ss}}{T_0} = \frac{K_{ssDT} T_{fold,largeD}}{C_{ss}} \quad (46)
\]

**Approximating ASIR at low drug concentrations**

In defining Eq. 45, which we call the “simple” ASIR formula, large drug concentrations were required; for arbitrarily small doses and drug concentrations, ASIRsimple approaches infinity. This property was also observed in the derivation for AFTIR [4]. An expression for ASIR that holds over small drug concentrations should approach one for small doses since \( TL_{ss} \) should approach \( TL_0 \). Therefore, as for the AFTIR metric, we were motivated by [18] to modify the ASIR approximation. We define \( IC_{50}^{ASIR} \) to be

\[
IC_{50}^{ASIR} = K_{ssDT} T_{fold,largeD} L_{fold,largeD} \quad (47)
\]

And then we assert that ASIR can be approximated by

\[
\text{ASIR} \approx \frac{IC_{50}^{ASIR}}{C_{ss} + IC_{50}^{ASIR}} \quad (48)
\]

This assertion is an intuitive leap-of-faith and this approximation will be checked by simulations. This equation has the property that as the dose approaches infinity:

\[
\lim_{\text{Dose} \to \infty} \text{ASIR} = \text{ASIR}_{simple}
\]

And for very small doses:

\[
\lim_{\text{Dose} \to 0} \text{ASIR} = 1
\]

Then, the steady state inhibition metric (SSIM) for the competitive TMDD system is similar to the traditional receptor occupancy formula, as shown in Fig. 3.

\[
\text{SSIM} = 1 - \text{ASIR} \approx \frac{C_{ss}}{C_{ss} + IC_{50}^{ASIR}} \quad (49)
\]

**Final comments on derivation**

Many assumptions were made in this derivation for ASIR and SSIM. In the next section, we will describe the methods used to test the scenarios under which this approximation is accurate.

Note that in the derivation of ASIR and SSIM above, although the terms \( T_{fold,largeD} \) and \( L_{fold,largeD} \) appear, the way these terms relate to the model parameters in Eqs. 18-21 is not explicitly used. A more complex system where the drug leads to an up-regulation in the synthesis of target or ligand would have addition parameters that describe this feedback and the formulas for \( T_{fold,largeD} \) and \( L_{fold,largeD} \) would change, but the expression of ASIR and SSIM as a function of \( T_{fold,largeD} \) and \( L_{fold,largeD} \) would remain the same.

**Methods**

To test the accuracy of the SSIM metrics, we performed both local and global sensitivity analyses of the model system and compared the theory to the simulation.

**Parameter ranges for local and global sensitivity analyses**

The parameter ranges used for the analyses for both the local and global sensitivity analyses are provided in Table 1, and then the dose and the other rate constants for the model were computed using the equations below. The PK parameter ranges came from a review of the PK of monoclonal antibodies [23]. We explored a large range of baseline target and ligand levels (0.1 pM - 1 μM) and a large range of target and ligand elimination rates (0.1/day to 1400/day). We simulated both soluble and membrane-bound targets. For the soluble targets, we assumed that
once the target binds the drug, the elimination of the complex \((k_{eDT})\) would be comparable to the rate of elimination of the drug \((k_{eD})\) (0.036-0.14/day) [23]. For the membrane-bound target, we assumed that after binding the drug, the drug-target internalization rate \((k_{eDT})\) would not change that much from the internalization rate of the target \((k_{eT})\), and be a factor of 0.1-10 fold different from \(k_{eT}\). The binding affinities range from \(K_{dDT} = 1\) pM - 100 nM and \(K_{dTL} = 1\) pM - 1000 nM. \(k_{onDT}\) and \(k_{onTL}\) were permitted to range from 1-10,000 nM/day. A typical antibody has a binding rate of 1-100 nM/day [24] and we explored a slightly larger range for a larger upper bound to account for faster binding of smaller drug molecules. Using the parameters from Table 1, the equations below were used to calculate the other model parameters.

\[
k_{eD} = CL/V_1
\]
\[
k_{12} = Q/V_1
\]
\[
k_{21} = Q/V_2
\]
\[
k_{offDT} = K_{IDT} \cdot k_{onDT}
\]
\[
k_{offTL} = K_{ITL} \cdot k_{onTL}
\]
\[
k_{obsTL} = K_{ITL} + k_{onTL}/k_{offTL}
\]
\[
T_{L0} = T_0 \cdot L_0/K_{ITL}
\]
\[
k_{synTL} = T_0 \cdot k_{eT} + k_{eTL} \cdot T_{L0}
\]
\[
k_{synL} = L_0 \cdot k_{eL} + k_{eTL} \cdot T_{L0}
\]

**Local sensitivity analysis**

A local sensitivity analysis where each parameter was changed while holding all others fixed was performed for four drugs: atezolizumab (anti-PD-L1), pembrolizumab (anti-PD-1), tocilizumab (anti-IL-6R) and siltuximab (anti-IL-6). For atezolizumab and pembrolizumab, the target and ligand are both membrane-bound. Whereas for tocilizumab, there is a membrane-bound target and soluble ligand, and for siltuximab, there is a soluble target with a membrane-bound ligand. For PD-1, PD-L1, and IL-6R, the targets are also known to exist in soluble form, but in this work, only the membrane-bound form of the target was considered.

A summary of all parameters is provided in Table 2. Excel tables detailing the references and calculations used for each parameter are provided in the Supplementary Material. For all four drugs, the linear PK parameters \((V_1, k_{eD}, k_{12}, k_{21})\) came from the literature, as did the binding affinities \((K_{dDT} \text{ and } K_{dTL})\). When the on rates \((k_{onDT}, k_{onTL})\) were not available, typical values between 1-100/(nM/day) were assumed and the off rates \((k_{offDT}, k_{offTL})\) were then calculated.

For the drugs targeting IL-6 (siltuximab) and IL-6R (tocilizumab), we fit tocilizumab, nonlinear PK and IL-6 data from an individual patient [25] to the model in Equations 1-6 to estimate \(k_{synT}, L_0, k_{eTL}, k_{eL}\), assuming that \(k_{eT}\) and \(k_{eDT}\) were equal to \(k_{eTL}\). We computed \(T_0 = (k_{synT} - k_{eTL} \cdot T_{L0})/k_{eT}\). For siltuximab (anti-IL-6), we used similar parameters, but with the definition of target and ligand reversed.

For the drugs targeting PD-1 (pembrolizumab) and PD-L1 (atezolizumab), only the membrane-bound target was modeled and all internalization rates \((k_{eT}, k_{eDT}, k_{eL}, k_{eTL})\) were assumed to be 6/day (half-life of 2 hours).
baseline concentration of target ($T_0$) and ligand ($L_0$) were estimated from the literature. The synthesis rates were then calculated using Eqs. 16 and 17.

In the above estimates, there is some uncertainty regarding the true parameter values. The goal of the local sensitivity analysis in this work was not to make definitive predictions about these four drugs, but rather to explore the behavior of this model and the agreement between theory and simulation using a realistic set of parameters based on approved drugs. Thus some degree of uncertainty is acceptable.

Global sensitivity analysis

For the global sensitivity analysis, we performed Latin Hypercube Sampling in the log-transformed parameter space, within the parameter ranges specified in Table 1. We generated two sets of 10,000 parameters for membrane-bound and soluble targets. For each parameter set, the model was simulated for both a constant infusion and for a more realistic dosing regimen that was chosen randomly to be every 2, 3, or 4 weeks (each was selected with 1/3 probability), and so there were a total of 40,000 simulations. We then compared the theoretical calculation of AFIR and ASIR to the simulated calculation of AFIR and ASIR. For constant infusion, $C_{ss} = \frac{Dose}{CL \cdot \tau}$ and for every 2-4 week dosing, $C_{ss} = C_{rough,ss}$ as calculated for the linear, two compartment model PK model with no target or ligand [26, Table 19-1].

In selecting the parameters to simulate, the model was parameterized by AFIR (from 0.0001 to 1) and then the dose was calculated using the equation below from [3].

\[
\text{Dose} = K_{sdT} \cdot \frac{k_{CL}}{k_{sDT}} \cdot \frac{CL \cdot \tau}{AFIR}
\]

Only parameters where the dose was less than 100 mg/kg for a 70 kg patient were selected, to focus our simulations on clinically relevant scenarios. We parameterized the model in this way to ensure that a large range of AFIR and ASIR values were explored. Without this restriction, there were very few parameter sets that gave simulations with significant target inhibition.

From the simulations, a histogram was generated of the ratio between the theoretical calculation and the simulation for AFIR and ASIR, and we examined the scenarios when all assumptions needed for AFIR and ASIR were met and when they were not. The list of assumptions needed for the ASIR formula to hold were:

- The quasi-steady-state approximation is accurate.
- ASIR $< 30\%$ (otherwise the ad-hoc “IC50” formulation will not be accurate)
- $C_{rough,ss} > 2T_{tot,largeD}$ (Drug must be in excess of target in the ASIR derivation)
- $C_{rough,ss} > 4C_{crit}$ (Otherwise PK won’t be linear and constant engagement won’t be maintained. Here, $C_{crit} = k_{synT}/k_{sD}$, as defined previously [22])
- $C_{rough,ss} > 2L_{ss} \cdot K_{sdDT}/K_{ssTL}$ ($C_{ss}$ needed to be larger than this ratio in the ASIR derivation)

We also check under which conditions the AFIR formula (which does not account for ligand turnover) matches ASIR. All simulations were done in R and the code can be found at https://github.com/iamstein/TMDD_EndogenousLigand.

| Parameter | Units | Atezolizumab | Pembrolizumab | Siltuximab | Tocilizumab |
|-----------|-------|--------------|---------------|------------|-------------|
| $V_1$     | L     | 3.3          | 3.5           | 3.5        | 3.7         |
| $k_{12}$  | 1/d   | 0.17         | 0.23          | 0.14       | 0.054       |
| $k_{21}$  | 1/d   | 0.15         | 0.2           | 0.19       | 0.059       |
| $k_{D}$   | 1/d   | 0.061        | 0.063         | 0.058      | 0.078       |
| $L_0$     | nM    | 0.01         | 0.15          | 0.4        | 0.0015      |
| $T_0$     | nM    | 0.15         | 0.0099        | 0.00012    | 1.3         |
| $k_{ET}$  | 1/d   | 6            | 6             | 10         | 5.9         |
| $s_{dT}$  | 1/d   | 6            | 6             | 0.03       | 5.9         |
| $s_{TL}$  | 1/d   | 6            | 6             | 1          | 1           |
| $K_{sdT}$ | nM    | 0.4          | 0.42          | 0.02       | 3.5         |
| $K_{sTL}$ | nM    | 1            | 1             | 1          | 1           |
| $s_{advT}$| 1/(nM*d) | 5           | 8.2          | 10         | 1.4         |
| $s_{advTL}$| 1/(nM*d) | 5           | 5             | 5          | 5           |

The references are available in the Excel Files in the Supplementary Material
Reverse log transform scale

For plotting receptor occupancy and the steady state inhibition metric, we used the reverse log transform scale (revlog) using the `xgx_scale_y_reverselog` function from the `xgx` R package. The reverse log transform of a variable \( x \) is defined to be the function \( f(x) = -\log(1 - x) \). The reverse log transform for SSIM is shown below, where the approximation indicates the behavior for large drug concentration.

\[
\text{revlog}(x) = -\log(1 - x)
\]

\[
\text{revlog}(\text{SSIM}) = -\log\left(1 - \frac{C}{C + IC_{50}}\right)
\]

\[
\approx -\log\left(\frac{IC_{50}}{C}\right)
\]

\[
= -\log(IC_{50}) + \log(C)
\]

When the PK is linear and dose is proportional to concentration, then a plot of the log transformed dose vs the reverse log transformed SSIM appears linear at large doses.

\[
\log(\text{Dose}) \sim \log(C) \sim \text{revlog}(\text{SSIM})
\]

Plotting SSIM on the reverse-log transform scale, rather than the linear scale, makes it easier to see the rate at which SSIM approaches 100% and to confirm that the theory matches the simulation.

Results

To provide intuition for how the model behaves, we first show the simulations of the four mAbs used in this analysis (pembrolizumab, atezolizumab, siltuximab, and tocilizumab) when dosed at 10 mg/kg every three weeks (Fig. 4). For pembrolizumab and atezolizumab, both the target and ligand were membrane-bound, and the elimination rates of PD-1 and PD-L1 did not change when bound. Therefore, no accumulation of \( DT \) (which is approximately equal to the total target) or the free ligand (\( L \)) was observed. For siltuximab, when the drugs binds its soluble target (IL-6), an accumulation of the drug-target complex is observed (\( DT \)), because the complex is eliminated much more slowly than the free target. However, the ligand levels (membrane-bound IL-6R) stay constant. On the other hand, for tocilizumab, no accumulation of the total target was observed, but the ligand levels did increase because tocilizumab blocked one route of ligand elimination (target mediated internalization).

Figure 5 shows the relationship between dose and SSIM for all four drugs on the reverse log-transform scale. At doses at and above 10 mg/kg, the theoretical and simulated calculation for ASIR agree for all four drugs. For the AFIR target engagement metric, which ignores the endogenous ligand, there is also good agreement for pembrolizumab, atezolizumab, and siltuximab. However, for tocilizumab, where the endogenous ligand is soluble, we see there is not good agreement with AFIR. This is because the AFIR metric from Eq. 46 does not account for the change in ligand concentration in the presence of the drug \( (L_{\text{fold,largeD}}) \).

To explore the accuracy of the \( L_{\text{ratio}} \) formula in Eq. 31, the PKPD relationship for tocilizumab was simulated and the drug, ligand (IL-6), and SSIM levels are plotted for both continual infusion and repeated dosing (every 4 weeks) over a range of 0.1-100 mg/kg, as shown in Fig. 6a). Consistent with the theory, Fig. 6b showed strong correlation between \( L_{\text{ratio}} \) and SSIM along the identity line for tocilizumab, and more generally across all simulations from the global sensitivity analysis.

The results of the local sensitivity analysis are shown in Fig. 7. Each row in the plot is a different drug and each column is a model parameter. All model parameters except the PK parameters (which only impact drug concentration) are plotted. Overall, good agreement between the theoretical and simulated calculation for SSIM was observed. For poor binding affinity (large \( k_{dTT} \)) and for large target (\( T_0 \)) or ligand (\( L_0 \)) concentrations, SSIM is low. In addition, for fast target turnover (large \( k_{eT} \)) or slow drug-target complex turnover (small \( k_{dLT} \)) there will be a greater accumulation of target (larger \( T_{\text{fold,largeD}} \)) and SSIM is lower. We also see that for tocilizumab, high affinity between target and ligand (low \( K_{dTL} \)) predicts lower SSIM. And in addition for tocilizumab, larger \( k_{eL} \) and lower \( k_{eTL} \) predict a larger accumulation of the ligand (larger \( L_{\text{fold,largeD}} \)) and therefore a lower SSIM. All these observations are consistent with the insights from Eqs. 47-48 and the assumptions needed for these equations to hold.

The key result from the global sensitivity analysis is shown in Fig. 8. This figure subdivides all simulations into two categories, stratified by whether or not the drug saturates the target such that the PK is approximately linear, with \( C_{\text{sat}} > 4C_{\text{crit}} \). Agreement between theory and simulation is assessed using the ratio of the theoretical calculation for ASIR in Equation 48 to its simulated value \( (\text{ASIR}_{\text{thy}}/\text{ASIR}_{\text{sim}}) \). When this ratio is between 0.75-1.25, we characterized this as a case where the theory matched the simulation and the histogram was colored black. The histogram was colored red when the ratio between theory and simulation was less than 0.75 and blue when greater...
than 1.25. In Fig. 8, over 99% of simulations fell into one of three categories:

1. In 40% of simulations, \( C_{ss} > 4C_{crit} \), and there was good agreement between theory and simulation. (black area in the left panel of Fig. 8).
2. In 38% of simulations, \( C_{ss} < 4C_{crit} \), and the theory predicted too low a value for ASIR, meaning that it predicted greater inhibition than was actually observed (red area in the right panel of Fig. 8).
3. In 21% of simulations, \( C_{ss} < 4C_{crit} \), but the theory still accurately predicted the simulations (black area in the right panel of Fig. 8). In many cases, this was because the inhibition was so low that \( ASIR \approx 1 \) as predicted by both simulation and theory.

Thus although many assumptions were required for the derivation of ASIR, we found that as long as \( C_{ss} > 4C_{crit} \), the ASIR formula in Equation 48 almost always agreed with the simulation. A more detailed summary of how theory compares to simulation for various underlying assumptions being true or false is also shown in Fig. 9. A deeper look into the global sensitivity analysis is provided in the Appendix.
Discussion

**Key SSIM equations and parameters**

The key insight from this work is that for the drug, target, and endogenous ligand binding system in Fig. 2, we have derived a steady-state inhibition metric (SSIM).

\[
SSIM \approx \frac{C_{ss}}{C_{ss} + IC_{50}}
\]

where the \( IC_{50} \) is given by

\[
IC_{50} = K_{ssDT} \cdot T_{fold.largeD} \cdot L_{fold.largeD}
\]

Here, \( K_{ssDT} \) is the quasi-steady-state binding constant, \( T_{fold.largeD} \) is the maximum fold accumulation of target at steady state for large doses, due to changes in target elimination after it binds to the drug, and \( L_{fold.largeD} \) is the maximum fold change in ligand in the presence of the drug at steady state for large doses, which may be due to changes in ligand elimination (from the drug binding a target receptor and blocking a route of target-mediated elimination) or changes in ligand synthesis (due to homeostatic feedback when the target is blocked by drug). Similar \( IC_{50} \) relationships had previously been described.
for other systems that do not account for the endogenous ligand (see Fig. 3).

We can compare the formula for the \( IC_{50} \) above to the formula of the apparent value of the Michaelis-Menten constant \( K_{\text{app}} \) in the presence of a competitor inhibitor, where \( K_m \) is the Michaelis-Menten constant without an inhibitor [27, section 8.5].

\[
K_{\text{app}} = K_m(1 + L_{ss}/K_{ssTL})
\]

Similar to SSIM in Fig. 3, the 50% inhibition term in the absence of competitive ligand (in this case \( K_m \)) is multiplied by an additional factor that depends upon the ligand levels. However, the multiplicative factor is different, where the Michaelis-Menten formula has an additional dependency on \( K_{ssTL} \). While \( K_{ssTL} \) does appear in the model assumptions (see Section 5.2), it does not appear in the final \( IC_{50} \) formulation when the dose of the drug is assumed to be large.
All parameters for the SSIM equation above are estimable. $K_{ssDT}$ can be estimated from in vitro experiments and $C_{ss}$ can be measured in the clinic from traditional pharmacokinetic studies. $T_{fold,largeD}$ and $L_{fold,largeD}$ can also be measured in the clinic by observing the baseline and steady-state target and ligand levels for large doses. If the target or ligand is soluble, enzyme-linked immunosorbent assays (ELISA) can be used to estimate $K_{DET}$ and in this system, $K_{DET} \approx K_{ssDT}$ when $k_{DET}$ is small, as expected since monoclonal antibodies bound to soluble target are also expected to have long half-lives. If the target is membrane-bound, cell-based receptor occupancy assays can be used where the binding, synthesis, and elimination processes are all present [28].

In addition, it has been shown that when homeostatic feedback does not play a role, then SSIM can be directly linked to changes in the soluble ligand levels:

$$SSIM \approx L_{ratio} = \frac{L_{ss}(Dose) - L_0}{L_{largeD} - L_0}$$

where $L_0$ is the baseline free ligand concentration in circulation, $L_{largeD}$ is the maximum, steady-state ligand concentration that is observed in the presence of a large dose of drug, and $L_{ss}(Dose)$ is the steady-state ligand concentration that is observed at the dose of interest. As mentioned above, for cases where the target is a membrane-bound receptor and the competitive ligand is soluble, an ELISA assay can be developed to measure $L_{ratio}$ in the clinic. However, this ratio will only be equal to SSIM when the drug does not induce changes in the ligand synthesis rate. The importance of feedback will depend on the particular target biology and until this process is better understood for a particular target of interest, it may be that the $L_{ratio}$ equation above is mathematically interesting, but harder to directly apply to drug development.

**Assumptions**

Although the derivation of the equation: $SSIM = C_{ss}/(C_{ss} + IC_{50})$ required many assumptions, we found through the global sensitivity analysis that for the wide range of parameters explored for describing monoclonal antibodies, the only assumption that was needed to achieve close agreement between theory and simulation was that of approximately linear PK, i.e. $C_{ss} > 4C_{crit}$.

It should also be noted that this analysis only applies to drugs that act as competitive inhibitors. For allosteric inhibitors that alter the binding affinity of a target to its endogenous ligand but do not directly prevent target-ligand binding, a different derivation would be needed.

The $L_{ratio}$ equation required the assumption that there is no homeostatic feedback that significantly affects the ligand synthesis or elimination rate. At this time, we are not aware of a simple method for checking these assumption.

For membrane-bound targets, it is often the case that there is also a soluble form. In particular, for the drugs modeled here, PD-1, PD-L1, and IL-6R are all known to have both a membrane-bound and soluble form. Analysis of a model where the target has both a membrane-bound and soluble form is left to future work. Thus an additional assumption of this analysis is that it is sufficient to model only one form of the target.

One limitation of this model is that it focuses on target inhibition in circulation rather than in the target tissue of interest. A reasonable guess would be that if one combined the insights from the competitive TMDD model with the tissue TMDD model in Fig. 3, then including both processes would give $IC_{50} = K_{ssDT} \cdot T_{fold,largeD} \cdot L_{fold,largeD}$, where $B$ represents the fraction of drug that distributes from circulation to the interstitial fluid of the tissue of interest at large doses. A global sensitivity analysis of the competitive TMDD + tissue model could confirm this result and this idea is left to future research.

**Applications**

The main application of this work is in further supporting dose selection of monoclonal antibody drugs where the maximum tolerable dose is not be identifiable and there is not a promising enough biomarker to guide dose selection from the efficacy perspective (e.g. atezolizumab [1]). For atezolizumab, the selection of the Phase 2 dose and ultimately the dose specified in the drug label was based mainly on receptor occupancy (RO) calculations and then confirmed to show a good benefit/risk ratio in subsequent trials. Many different methodologies exist for using an RO prediction to guide dose selection and the pharmacometrics community has not yet aligned on the details of a preferred approach. One challenge is that the RO calculation depends on the underlying assumptions of the model. Usually, binding of the target to its endogenous ligand is assumed to be unimportant, but this assumption is almost never explicitly stated or justified. In this work, we have explored simulations of a system with drug, target, and its endogenous ligand and we have shown that the typical RO calculation, which depends only on drug concentration and the binding affinity (Binding model in Fig. 3) is only accurate when neither the target nor the ligand accumulates in the presence of drug. If the target or ligand accumulates, these processes must be accounted for in the target inhibition calculation.

Often, due to the rapid speed and high cost of drug development, assays are not developed for measuring the endogenous ligand concentration. In that scenario, the
SSIM formula gives a means to quickly perform sensitivity analyses (e.g. by varying $L_{\text{fold,largeD}}$ over a range of plausible values) without making assumptions about every parameter in the model in Fig. 2. However, the model presented here is also a simplification. Often, the target may exist in many forms (e.g. both soluble and membrane-bound PD-1) and the target may interact with more than one endogenous ligand (e.g. PD-1 binds both PD-L1 and PD-L2). Thus even the model presented here may be simpler than needed. Moreover, the degree of signaling complex suppression needed for efficacy is often not known and this too requires assumptions. We have found that most approved mAbs in oncology have over 90% target engagement [29], but whether this degree of suppression is needed for efficacy has not been confirmed.

An additional result from this work is that when the target is membrane-bound and the endogenous ligand is soluble, it may be possible to estimate the target inhibition by using changes in the endogenous ligand concentration. However, the $L_{\text{ratio}}$ formula requires the assumption that there is not an up-regulation of ligand synthesis in the presence of the drug. This is an assumption that may be difficult to check and will likely depend on the system of interest. Further work would be needed to understand the degree to which this result is accurate enough to be applicable to drug development. If it were applicable, however, it could simplify PD assay development. For instance, measuring soluble factors in the blood is much easier than measuring receptor occupancy [28]. This is because total target is easier to measure than free target since any dilution of the sample could change the equilibrium of the system and thereby change the receptor occupancy measurement. Thus if it were possible to use soluble ligand instead of membrane-bound receptor occupancy, this could lead to a simpler assay development program.

This work also provides insight and intuition for how the competitive model TMDD parameters impact target inhibition via the IC$_{50}$ parameter in Fig. 3. While the model in Fig. 2 has 14 parameters, it turns out that only 4 lumped parameters are needed to predict inhibition for high doses of the drug: drug concentration, quasi-steady-state binding constant of drug to target, and steady state fold accumulation of both target and ligand in the presence of large doses of the drug; the IC50 for steady state inhibition metric increases with $K_{\text{ssDT}}$, $T_{\text{fold,largeD}}$, and $L_{\text{fold,largeD}}$. If ligand accumulation does not occur in the system of interest, then $L_{\text{fold,largeD}} = 1$ and simpler measures of target inhibition (such as target engagement from the classical TMDD model) can be applied as long as the necessary assumptions hold. One might have expected that other parameters, such as the target-to-ligand ratio ($T_0/L_0$) to also be important for predicting SSIM, but the analysis shown here shows that as long as $C_{\text{ss}} > 4C_{\text{crit}}$, then the SSIM formula will hold.

The SSIM formula also provides a rapid way to estimate the target inhibition at steady state without the need for computer simulations. The ASIR$_{\text{simple}}$ equation is particularly helpful because it illustrates that at high doses, doubling the dose or halving the quasi-steady-state binding constant will halve the number of signaling complexes present. This insight can be useful in discussions with project teams, when any questions about how the model predictions change with a different dose or quasi-steady-state binding constant can quickly be answered without requiring additional simulations.

### Summary/conclusions

In summary, we have extended previous work to develop the SSIM potency metric for in vivo drug-target binding systems that include drug, target, and its endogenous ligand. This metric predicts target inhibition at steady state under a repeated dosing regimen using four quantities: the steady-state drug concentration, the drug-target quasi-steady-state binding constant, and the fold-increase of both the target and the ligand in the presence of drug. All four quantities can be measured by experiment. SSIM provides intuition for how the model parameters impact target inhibition and it can be used to provide a rapid estimate for target inhibition without a need for computer simulations. This work also highlights that when the endogenous ligand concentration changes in the presence of the drug, it is important to take this accumulation into account when predicting target inhibition.

### Appendix

This appendix contains more details of the global sensitivity analysis.

The threshold value of 4 in the requirement $C_{\text{ss}} > 4C_{\text{crit}}$ was based on Fig. 10, where in the yellow shaded area, as long as $C_{\text{ss}} > 4C_{\text{crit}}$, then the simulated value for $C_{\text{ss}}$ is at least 75% of that predicted by the theoretical calculation from the linear PK model. As $C_{\text{ss}}$ is one of the components of ASIR in Equation 48, it is important that this value be estimated accurately. Because the theoretical calculation assumes linear PK in order to estimate the steady state drug concentration, this formula requires a large enough dose for the linear approximation to hold.

When the simulation disagreed from the theoretical calculation, usually the theoretical calculation predicted a lower ASIR (greater inhibition) than observed in the
Fig. 9 Summary of the global sensitivity analyses. The x-axis gives the ratio between the theoretical (ASIR_thy) and simulated (ASIR_sim) calculation for ASIR for all simulations. Good agreement between theory and simulation was defined to occur when this ratio was between 0.75-1.25 (black). Ratios of $<0.75$ and $>1.25$ are colored in red and blue respectively. The numbers above the histogram indicate the percentage of all simulations that fall into these categories. The facet labels on the top and left of the plot indicate whether or not the 4 key assumptions for deriving ASIR were met. Facet labels surrounded by three asterisks (***) indicate that this particular assumption was met. Based on this figure, we realized that $C_{ss} > 4C_{crit}$ was the key assumption for the ASIR formula to apply.

Fig. 10 This figure shows how the ratio of steady state drug concentration to $C_{crit}$ (x-axis) affects the agreement between the theoretical and simulated values of the steady state drug concentration (y-axis). Good agreement between the theoretical and simulated value occurs when the ratio between the two quantities falls between 0.75-1.25 (black dots). Here, we see that when the ratio of $C_{ss,thy}/C_{crit} > 4$ (i.e. $C_{ss} > 4C_{crit}$), there is almost always good agreement between theory and simulation (yellow shaded rectangle).

Fig. 11 This figure assesses the accuracy of the quasi-steady-state approximation (QSS) and how its accuracy impacts the accuracy of the theoretical calculation for ASIR. We plot only the simulations where $C_{ss} > 4C_{crit}$. At QSS, the ratio $(K_{ss,TL}/T_{ss,TL}) = 1$, as observed for most simulations, but for a few simulations, the ratio goes as low as 0.4. The blue dots with the greatest error between theory and simulation predict larger ASIR than expected by the theory, and so in these few cases, when QSS does not apply, ASIR is over-estimated and the amount of inhibition (SSIM) is underestimated.
simulation (i.e. red area is much larger than blue area in Fig. 8). But in a few cases, the theoretical calculation for ASIR was actually higher than the simulation, i.e. more inhibition was observed in the simulation than predicted by the theory. We found that the cause for the underestimate was that the quasi-steady-state approximation did not always hold and that in the simulation, $K_{\text{ssTL}} < T_{\text{ss}}L_{\text{ss}}/T_{\text{ss}}L_{\text{ss}}$. This was further explored in Fig. 11, where the ratio $\left(K_{\text{ssTL}} \cdot T_{\text{ss}}\right)/\left(T_{\text{ss}} \cdot L_{\text{ss}}\right)$ was compared to the ASIR theory to simulation ratio. While usually the approximation was reasonable and the $K_{\text{ssTL}}$ ratio fell between 0.75-1.25, there were a few simulations were $\left(K_{\text{ssTL}} \cdot T_{\text{ss}}\right)/\left(T_{\text{ss}} \cdot L_{\text{ss}}\right) < 0.75$ and this is what lead to the largest over-estimate of the theoretical ASIR value (blue dots in upper left quadrant).

In Figure 12 we examine the relationship between ASIR and AFIR by plotting $L_{\text{fold,largeD}}$ vs the ASIR theory-simulation ratio. As long as the fold accumulation of ligand ($L_{\text{fold,largeD}}$) was less than 2, the ASIR theory and simulation matched to within a factor of 2 (yellow shaded rectangle). However, for large $L_{\text{fold,largeD}}$ values, there can be significant disagreement between AFIR an ASIR, as expected.
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