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Target-Mediated Exposure Enhancement: A Previously Unexplored Limit of TMDD

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Abstract

Target-mediated drug disposition (TMDD) is often observed for targeted therapeutics, and manifests as decreases in clearance and volume of distribution with increasing dose as a result of saturable, high affinity target binding. In the present work, we demonstrate that classically defined TMDD is just one of the characteristic features of the system. In fact, for molecules with rapid non-specific elimination relative to target-mediated elimination, binding to target may actually lead to improved exposure at sub-saturating doses. This feature, which we refer to as target-mediated exposure enhancement (TMEE), produces the opposite trend to classical TMDD, i.e., with increasing dose levels, clearance and volume of distribution will also increase. The general model of TMDD was able to well-characterize the pharmacokinetics of two molecules that display TMEE, ALX-0081 and linagliptin. Additional fittings using the commonly reported TMDD model approximations revealed that both the quasi-equilibrium and quasi-steady-state approximations were able to well-describe TMEE; however, the Michaelis-Menten approximation was unable to describe this behavior. With the development of next-generation therapeutics with high affinity for target and rapid non-specific elimination, such as antibody fragments and peptides, this previously unexplored limit of TMDD is anticipated to become increasingly relevant for describing pharmacokinetics of investigational therapeutics.

Keywords

Pharmacokinetics; Target-mediated drug disposition; Pharmacokinetic modeling; Nonlinear pharmacokinetics

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INTRODUCTION

Nonlinear pharmacokinetics (PK) is a common observation for many targeted therapeutics, in particular monoclonal antibodies (mAbs) and their derivatives [1,2]. This phenomenon, referred to as target-mediated drug disposition (TMDD) was first described by Gerhard Levy in his seminal 1994 publication [3]. By definition, TMDD is a pharmacokinetic (PK) phenomenon wherein specific binding between drug and target has a significant impact on drug behavior *in vivo*. TMDD is observed with increasing regularity in drug discovery & development, due to high affinity interactions between drug and receptors, which are often internalizing in nature. In the majority of reported cases, TMDD manifests as non-linear PK, defined by saturable clearance (CL) and volume of distribution (V). The root cause of these saturable PK processes is capacity limitation of the target, as once the target molecule is fully occupied (i.e. as receptor occupancy approaches 100%), the fraction of drug that is unbound increases, leading to dose-dependent decreases in CL and V . Considering the amount of drug in the body to be a combination of free and receptor bound, each with its own distinct clearance, allows one to envision that at high doses, PK parameters will approach that of the unbound drug, while at low doses, parameters will approximate those of the drug-receptor complex. Ultimately, at very high doses, CL and V will asymptotically approach the values expected for a compound with no target affinity. For example, mAb PK parameters will often approach that of a non-specific immunoglobulin G (IgG) of the same isotype.

A quantitative framework to describe this pharmacokinetic observation was provided by Mager and Jusko who proposed a general model of TMDD in 2001 [4]. Since their original publication, numerous approximations of this model have been proposed, including the quasi-equilibrium (Q_E), often referred to as the rapid binding, [5] quasi-steady state (Q_{ss}) [6], and Michaelis-Menten (M-M) [6] approximations, as well as extensions to situations where target binding is localized in the peripheral compartment, termed peripheral TMDD (pTMDD) [7,8]. Additionally, numerous mathematical evaluations of the system have been performed to understand its limits and to probe how changing properties of the drug, target, or system would be expected to lead to changes in PK [7,9]. These published frameworks for describing the influence of target binding on pharmacokinetics have been widely applied throughout the drug development timeline to characterize non-linear pharmacokinetics of investigational and approved drugs.

Out of the many evaluations of this system, there has been remarkable consistency with respect to changes in pharmacokinetic parameters with dose. As described above, TMDD typically manifests as dose dependent decreases in clearance and/or volume of distribution, down to a limiting value; however, the opposite behavior (e.g. dose-dependent increases in clearance/volume) has been described for small molecule compounds with target in the plasma [10]. With the advent of new molecular constructs, particularly rapidly cleared antibody fragments such as single chain variable fragments (scFv) and single-domain antibodies (sdAb), among others, there is potential for this atypical TMDD-like behavior to be observed. These small affinity ligands are often eliminated extremely quickly, due to rapid renal filtration, with *in vivo* half-lives ($t_{1/2}$) of hours. It could be envisioned for a rapidly cleared molecule with high affinity binding to a target with slow turnover, that

pharmacokinetics could be prolonged in the presence of target binding, similar to that described for some small molecules. We define this phenomenon as target-mediated exposure enhancement (TMEE) as a distinct limit of the overarching TMDD system. We searched for published examples of molecules that had properties that would promote this type of pharmacokinetic behavior. While there were limited published examples of TMEE, it is distinctly possible, even likely, that many molecules in development could display this type of PK behavior.

Herein, we describe an observation where specific interaction with target does not lead to saturable clearance, but rather leads to dose-dependent *increases* in clearance. In particular, this has relevance when targeting circulating drug concentrations *in excess* of accessible receptor concentrations, as is often done for biologics directed against soluble targets. In this unique situation, a pharmacokinetic penalty must be paid at high doses, which runs counterintuitive to traditional logic. In this report, we demonstrate the relevance of this mathematical limit for two drugs of distinct classes, an anti-von Willebrand Factor (vWF) antibody fragment and a small-molecule inhibitor of dipeptidyl peptidase-4 (DPP-4). Additionally, we applied the well-characterized TMDD model approximations to describe the PK at this limit of the system. Through application of a sensitivity analysis, we demonstrate the relative importance of drug- and target-specific parameters that are required to produce TMEE.

METHODS

Data Sources:

A literature search was performed to identify molecules with: (1) rich blood pharmacokinetic data at a range of doses following intravenous administration, (2) high affinity binding to target, and (3) less than dose-proportional increases in area under the concentration vs. time curve (AUC). Data describing the blood PK of ALX-0081 in cynomolgus monkeys [11] and linagliptin in healthy, adult, male subjects [12] were obtained from the literature. Digitization of data was performed using WebPlotDigitizer 4.2 (<https://apps.automeris.io/wpd/>).

General TMDD Model Structure:

All data were fit to the general TMDD model [4] (Figure 1, Equations 1–4). Non-specific disposition of drug was described using a 2-compartment model for both drugs. Briefly, drug in the central compartment (C_1 , V_1) is able to distribute into the peripheral compartment (C_2 , V_2) via a distribution clearance term (CL_D), be eliminated via a linear clearance term (CL), or interact with free target (R). It was assumed that target expression, turnover, and binding were confined to the central compartment. Target binding was described by a second-order association rate constant (k_{on}) and a first-order dissociation rate constant (k_{off}), with the ratio of k_{off}/k_{on} being the equilibrium dissociation constant (K_D). The turnover of free target was assumed to proceed via a zero-order synthesis rate constant (k_{syn}) and a first-order elimination rate constant (k_{deg}). Drug-target complex (DR) was assumed to be eliminated via a first-order process (k_{imd}). Because it was unlikely that linagliptin binding would affect DPP-4 turnover, linagliptin PK was modeled assuming constant concentrations

of DPP-4 in plasma. As these estimates repeatedly converged to values approaching zero despite changes in initial estimates, they were fixed to 0.00 h^{-1} for further model fitting, suggesting that DPP-4 turnover occurs more slowly than the time course of PK.

$$\begin{aligned} \frac{dC_1}{dt} &= -\frac{CL}{V_1} \times C_1 - \frac{CL_D}{V_1} \times (C_1 - C_2) - k_{on} \times C_1 \times R + k_{off} \times DR; \quad C_1(0) \\ &= \frac{Dose}{V_1} \end{aligned} \quad (1)$$

$$\frac{dC_2}{dt} = \frac{CL_D}{V_2} \times (C_1 - C_2); \quad C_2(0) = 0 \quad (2)$$

$$\frac{dR}{dt} = k_{syn} - k_{on} \times C_1 \times R + k_{off} \times DR - k_{deg} \times R; \quad R(0) = \frac{k_{syn}}{k_{deg}} \quad (3)$$

$$\frac{dDR}{dt} = k_{on} \times C_1 \times R - k_{off} \times DR - k_{tmd} \times DR; \quad DR(0) = 0 \quad (4)$$

Parameter Estimation and Simulation:

All model fitting and simulations were performed using ADAPT 5 [13]. Total drug concentrations ($C_1 + DR$) were fit to digitized concentration vs. time data. Parameter estimation was performed using the maximum likelihood estimation option in ADAPT 5 with a linear variance model $((\sigma_{inter} + \sigma_{slope} (y(t))^2)$. ADAPT 5 model code for all models described can be found in the Electronic Supplementary Material.

Sensitivity Analysis:

In order to evaluate the impact of key parameters related to non-specific and target-mediated clearance (target expression, binding, turnover) on the observed phenomenon, simulations were performed in which individual parameters were varied and changes in non-compartmental parameters were calculated. Simulated concentration vs. time profiles were used to perform non-compartmental analysis in order to derive key PK parameters. Relationships between apparent clearance, defined as Dose/AUC, and parameters of interest were derived from these simulations.

TMDD Model Approximations:

The Q_E/Q_{ss} (Equations 5–8), and M-M (Equations 9–11) approximations were fit to the concentration vs. time data for ALX-0081. Because the Q_E and Q_{ss} models are mathematically identical, these were evaluated together as a single model, termed Q_E/Q_{ss} . The base model structure was the same as for the full TMDD model, with changes to target binding included as described in the original references [5,6]. In the M-M model, the assumption was made that $V_{max} = R_{tot} \times k_{tmd}$. Comparisons between the full model and the approximations were made using metrics such as percent prediction error (%PE) and confidence in parameter estimates.

$$\frac{dC_{tot}}{dt} = -\frac{CL}{V_1}C - \frac{CL_D}{V_1} \times (C - C_2) - \frac{R_{tot}k_{tmd}C}{K_{SS} + C}; \quad C_{tot}(0) = \frac{Dose}{V_1} \quad (5)$$

$$\frac{dC_2}{dt} = \frac{CL_D}{V_2} \times (C - C_2); \quad C_2(0) = 0 \quad (6)$$

$$\frac{dR_{tot}}{dt} = k_{syn} - k_{deg} \times R_{tot} - (k_{tmd} - k_{deg}) \frac{R_{tot}C}{K_{SS} + C}; \quad R_{tot}(0) = \frac{k_{syn}}{k_{deg}} \quad (7)$$

$$C = 0.5 \left[(C_{tot} - R_{tot} - K_{SS}) + \sqrt{(C_{tot} - R_{tot} - K_{SS})^2 + 4K_{SS}C_{tot}} \right] \quad (8)$$

$$\frac{dC_1}{dt} = -\frac{CL}{V_1}C_1 - \frac{CL_D}{V_1} \times (C_1 - C_2) - \frac{V_{max}C_1}{K_M + C_1}; \quad C_1(0) = \frac{Dose}{V_1} \quad (9)$$

$$\frac{dC_2}{dt} = \frac{CL_D}{V_2} \times (C_1 - C_2); \quad C_2(0) = 0 \quad (10)$$

$$\frac{dR_{tot}}{dt} = k_{syn} - k_{deg} \times R_{tot} - (k_{tmd} - k_{deg}) \frac{R_{tot}C_1}{K_M + C_1}; \quad R_{tot}(0) = \frac{k_{syn}}{k_{deg}} \quad (11)$$

RESULTS

Linagliptin Pharmacokinetics

Concentration vs. time data for linagliptin following IV infusion in healthy subjects were obtained from the literature [12]. Dose-normalized PK profiles (Fig. 2a) and NCA (Table 1) revealed dose-dependent increases in CL_{NCA} (2.51 – 14.3 mL/h) and V_{ss} (380 – 1540 L). Similar to ALX-0081, the full TMDD model was used to describe the PK of linagliptin. The data were well-described by the TMDD model (Fig. 2b) and final parameter estimates are shown in Table 2. In general, parameters were estimated with good confidence.

ALX-0081 Pharmacokinetics

Data describing the plasma PK of ALX-0081, a bivalent anti-vWF nanobody, in cynomolgus monkeys were digitized [11]. Dose-normalization of PK profiles (Fig. 3a) and NCA (Table 3) revealed less than dose-proportional increases in AUC, along with increasing CL_{NCA} (NCA-derived clearance) (1.32 – 70.7 mL/h/kg) and V_{ss} (56.4 – 1830 mL/kg) with increasing dose, indicative of nonlinear PK. Even though the PK parameters did not follow the expected trends for TMDD, it was hypothesized that interactions with vWF could be driving the observed nonlinearities in ALX-0081 PK. Non-specific nanobodies have been reported to have a half-life of ~2 hours, while vWF circulates with a half-life of ~12 hours, suggesting that binding to vWF could, in theory, prolong the circulation time of ALX-0081.

The digitized data were fit to the TMDD model and model estimated PK profiles were in agreement with the digitized data (Fig. 3b). Final parameter estimates are shown in Table 4. In general, the model was able to well-describe the digitized data across the entire range of administered doses. There was reasonable confidence in all parameter estimates except for the equilibrium dissociation constant, K_D , and the dissociation rate constant, k_{off} . Poor confidence in parameters related to binding affinity and kinetics is suggestive of overparameterization of the model and relative insensitivity to the absolute values of k_{on} and k_{off} . Because the estimated value for k_{off} repeatedly converged to values representing very rapid dissociation kinetics relative to the time frame of sampling (half-life of dissociation ~12 minutes), we decided to apply the Q_E and Q_{ss} approximations to describe this data (discussed in more detail below), as they have the underlying assumption of equilibrium binding.

Impact of Drug-Target Complex Elimination Rate

In order to develop hypotheses regarding properties of drug molecules and targets that would result in TMEE, sensitivity analyses were performed. To assess the impact of the relative efficiencies of non-specific (CL_{ns}) and target-mediated (CL_{tmd}) clearance processes on TMDD, simulations were performed for the 3 reported doses of ALX-0081 (0.02, 0.4, 8 mg/kg). These parameters have been defined as the model-estimated CL parameter (CL_{ns}) and as the product of k_{tmd} and V_I (CL_{tmd}). The drug-target complex elimination rate (k_{tmd}) was varied over a wide range in 10-fold increments and apparent clearance was calculated via NCA (Dose/AUC). The initial model estimates correspond to a CL_{ns}/CL_{tmd} ratio of 6.52. All other parameters were fixed to the model-estimated values for ALX-0081. The results of this analysis are shown in Figure 4 and demonstrate the limits of the system. When $CL_{ns} < CL_{tmd}$, classically defined TMDD is observed (e.g. decreasing clearance with increasing dose). However, when $CL_{ns} > CL_{tmd}$, TMEE is observed, and clearance will increase with increasing dose. As dose increases, the effect of changes in drug-target complex elimination rate are blunted, as shown by simulations for an extremely high dose (8000 mg/kg), where apparent clearance is calculated to be equal to CL_{ns} (103 mL/h/kg) at all simulated parameter sets. At low doses, where target is not saturated, the increased importance of target-mediated clearance leads to an increased sensitivity to this parameter. These results highlight the potential impacts of target binding for molecules with a wide range of pharmacokinetic properties.

Impact of Baseline Target Expression

Simulations were first performed to evaluate the relative pharmacokinetics of ALX-0081 in the presence and absence of vWF expression. It was hypothesized that as target concentration increased relative to dose, clearance would correspondingly decrease. The model predicted that clearance of linagliptin would dramatically increase in the absence of target binding/expression (Fig. 5a), consistent with the notion that target interactions enhance exposure of ALX-0081. Further simulations across a range of doses and baseline target expression values show that with increasing target expression, clearance is expected to decrease (Fig. 5b). While not depicted on the graph due to the logarithmic x-axis, ALX-0081 clearance in the absence of vWF expression was predicted to be equal to the model-estimated CL (103 mL/h/kg). On the converse, as baseline target concentrations begin to

exceed drug concentrations, NCA-calculated clearance approaches zero. This is the polar opposite of classically defined TMDD, where NCA-calculated CL would approach $k_{tmd} \times V_I$ at low drug/target ratios and would approach CL (non-specific clearance) at high drug/target ratios.

Evaluation of TMDD Model Approximations

Model-fitted concentration vs. time curves for ALX-0081 are shown for the Q_E/Q_{SS} (Fig. 6a), and M-M (Fig. 6b) approximations. Final parameter estimates are shown in Table 5. While the Q_E/Q_{SS} approximation is able to well-characterize the pharmacokinetics of ALX-0081, the M-M approximation does not capture the observed data. Mean %PE values were all less than 20% for the full TMDD and Q_E/Q_{SS} models, while it ranges from 19.6 – 4620% for the M-M approximation (Table 6). There was no clear trend towards increasing or decreasing %PE values with dose for the full TMDD or Q_E/Q_{SS} models; however, %PE increased with dose for the M-M approximation, likely reflecting model misspecification. The failure of the M-M model to describe TMDD is largely due to the underlying structure of this model, as this model does not explicitly account for differences in behavior between free and bound drug, due to the assumption that total target concentrations are negligible, and total and free drug concentrations are essentially equivalent. Due to this misspecification, these results were confirmatory of our hypothesis that the M-M approximation would be inappropriate in this situation. Comparison of the M-M model to the linear 2-compartment model revealed identical predictions of concentration vs. time profiles for ALX-0081 (data not shown), demonstrating that the M-M model is unable to account for the nonlinearity observed in ALX-0081 PK.

DISCUSSION

Classically, target-mediated drug disposition manifests as non-linear pharmacokinetics, with dose-dependent decreases in clearance, volume of distribution, or both, due to high affinity interactions between drug and target. As next generation therapeutics are developed, it is likely that TMDD will continue to be a relevant PK complication that must be considered when designing dosing regimens. Many of these new affinity ligands are small protein therapeutics, peptides, or even small molecules, which may have advantages over full-length mAbs, including improved tissue penetration and the ability to bind to otherwise undruggable epitopes. However, with small size often comes the penalty of very rapid elimination, sometimes on the order of minutes to hours, compared to full-length mAbs, which circulate for weeks in the absence of TMDD. In the limit described here, target-mediated elimination occurs at a slower rate than non-specific elimination, manifesting in target-dependent enhancement of pharmacokinetics. While this phenomenon has been previously described for small molecule drugs [10], to our knowledge, this is the first mathematical formalism of this particular limit of the TMDD system. Additionally, target binding may limit the apparent volume of distribution, leading to an increased extent of distribution at target-saturating doses. In other words, it would be anticipated that pharmacokinetics would be non-linear, with less than proportional increases in the area under the blood (or plasma or serum) concentration vs. time curve and dose-dependent increases in both clearance and volume of distribution.

In the present work, the general model of TMDD [4] has been applied to describe the pharmacokinetics of two molecules that display TMEE. We define TMEE as the limit of TMDD where high affinity target binding protects drug from rapid elimination, leading to increased relative exposure at sub-saturating doses. This phenomenon of high affinity binding interactions increasing blood exposure of a molecule is not a new phenomenon; however, it is not typically reported for drug molecules. The general expectation for a mAb against a soluble target (e.g. tumor necrosis factor- α or vascular endothelial growth factor) is that total target concentrations will *increase* in the presence of mAb [14]. This is because the elimination rate of the mAb-target complex is significantly slower than that of the free target. This leads to characteristic profiles of increasing total target concentrations and decreasing free concentrations following mAb administration. In the case highlighted in this manuscript, it is the affinity ligand whose circulation is prolonged by binding to a longer circulating target, resulting in TMEE. Knowing that antibody-antigen binding models can be utilized to predict the dynamics of total soluble target concentrations provided confidence in applying TMDD models to characterize TMEE.

For both compounds used to characterize this limit of the system (ALX-0081 and linagliptin), the model has been used to describe *total* drug concentrations (e.g. free + receptor bound) in plasma. Model estimates suggest that free drug elimination is much more rapid than target-mediated elimination (123 vs 0.405 mL/h/kg for ALX-0081). From these values, it is apparent why target binding leads to reductions in clearance at low doses. When the target is not saturated, the bulk of drug in plasma will be target bound, leading to a low apparent clearance. However, as dose (and plasma concentrations) increase, the fraction of free drug will increase, leading to an increase in the apparent clearance relative to lower doses.

In addition to applying the full TMDD model, we have also evaluated several approximations of the model to describe the PK of molecules exhibiting TMEE. The Q_E/Q_{ss} approximation is able to well-describe the observed data for ALX-0081 PK; however, the M-M approximation is unable to characterize the data. The Q_E/Q_{ss} model has underlying assumptions that processes related to target binding and drug-target complex elimination occur much faster than other pathways controlling PK, which still may hold true for molecules exhibiting TMEE, particularly when binding kinetics are rapid. Because this model contains explicit terms that are used to separate free and receptor-bound drug, it is possible to describe TMEE-like pharmacokinetics, as demonstrated for ALX-0081.

While mechanism-based PK models are useful tools for generation of testable hypotheses, they are not sufficient to define a paradigm. Experimental evidence of TMEE was demonstrated by Ulrichs and co-workers in their study of ALX-0081 pharmacokinetics [11]. Following injection of ALX-0081 into a non-cross-reactive species (mice), they observed rapid elimination from the systemic circulation. However, co-infusion of ALX-0081 and its target (human vWF) led to prolonged circulation of ALX-0081, mirroring the pharmacokinetics of vWF in mice. These results directly demonstrated the role of target binding in the circulation time of ALX-0081 and is in agreement with the model-generated hypothesis that pharmacokinetics of drugs that are cleared more rapidly than drug-receptor complex would be expected to be enhanced at sub-saturating doses. While this was only

tested at one molar ratio of ALX-0081/vWF, it is anticipated that clearance of ALX-0081 would be greater if an excess of ALX-0081 was infused relative to vWF.

An additional area where TMEE would be expected to be relevant is the field of half-life extension. There has been great interest in using long-circulating components of blood, such as albumin, IgG, and erythrocytes [15], as carriers to prolong the circulation time of rapidly cleared therapeutic proteins and peptides. In fact, there are several FDA-approved products that consist of genetic fusions to albumin/Fc fragment or to albumin-binding domains. One preclinical example utilized diabodies (Db), which are 55 kDa bispecific antibody fragments that had been engineered for improved circulation time using the albumin-binding domain of streptococcal Protein G. In the published study, the authors compared the pharmacokinetics of a Db in wild-type and in mice deficient in the neonatal Fc receptor FcRn, which protects albumin from intracellular catabolism, and observed that exposure was greater in wild-type mice [16]. This improvement of PK only in FcRn-expressing mice is another direct demonstration of the TMEE phenomenon, where interaction with a target molecule (albumin) leads to enhanced exposure. By decreasing albumin concentrations through knockout of FcRn, there is an effective reduction in the efficiency of TMEE. While saturation of albumin, IgG, or erythrocytes in half-life extension strategies is unlikely under normal physiological conditions, this could become relevant under pathological conditions.

When considering the broad biological consequences of this observation, it could be argued that target-mediated enhancement of pharmacokinetics is a basic physiological mechanism used by organisms to retain substances in the body for long periods of time. For example, the long circulating time of IgG and albumin in man ($t_{1/2} \sim 3$ weeks) is conferred by interaction with FcRn [17–20]. It has been convincingly demonstrated that: genetic knockout of FcRn [17–19], along with pharmacologic mediation of FcRn function/expression, can lead to large increases in the clearance of traced IgG. This interaction with a ‘secondary’ target confers significant protection to IgG and saturation of FcRn leads to dose-dependent increases in CL. In fact, physiologically-based pharmacokinetic models have been developed to predict differences in IgG PK between wild-type and FcRn knockout mice that utilize equilibrium binding assumptions between IgG and FcRn [21]. These models are able to predict a significant increase in clearance in the absence of binding to FcRn, similar to the results shown here in Figure 5.

In summary, a previously unexplored mathematical limit of target-mediated drug disposition has been evaluated, where high affinity interactions with target lead to extension of pharmacokinetics at sub-saturating doses. Experimental examples of this have been quantitatively evaluated using the gTMDD model and sensitivity analysis has shown that this limit of the system is relevant under conditions where *non-specific elimination of drug is much more rapid than target-mediated clearance*. This limit may be most relevant when using affinity ligands towards either non-internalizing or relatively (compared to drug) long-circulating blood proteins. While the observation of increased clearance at high doses of affinity ligands has been noted in a few cases, this is the first mathematical formalization of this phenomenon. It is anticipated that with further development of next-generation therapeutics, particularly antibody fragments (scFv, nanobody, etc.) and peptides, that TMEE will become a more widely observed phenomenon.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1:

Structure of the general model of target-mediated drug disposition as described in [4]. Drug in the central compartment distributes into the peripheral compartment via a distribution clearance (CL_D), is directly eliminated from the system (CL), or binds to free target (k_{on}) via a second-order process. Drug-target complexes either dissociate (k_{off}) or are eliminated (k_{tmd}) via first-order processes. Free target is produced via a zero-order process (k_{syn}) and eliminated via a first-order process (k_{deg}).

Fig. 2: Pharmacokinetics of linagliptin in healthy subjects. Both (a) dose-normalized and (b) model-fitted concentration vs. time profiles are displayed. Symbols are consistent across panels. Total linagliptin plasma concentrations (free + bound) were measured via high-performance liquid chromatography-tandem mass spectrometry. Data digitized from [12].

Fig. 3: Pharmacokinetics of ALX-0081 in cynomolgus monkeys. Both (a) dose-normalized and (b) model-fitted concentration vs. time profiles are shown. Symbols are consistent across panels. Plasma concentrations of ALX-0081 were measured via enzyme-linked immunosorbent assay. Data digitized from [11].

Fig. 4:

Impact of clearance pathways on TMDD. Simulations were performed at a range of doses and ratios of non-specific and target-mediated clearance to evaluate the limits of the TMDD system. The calculated value of CL represents NCA-derived, or apparent, clearance of the molecule. CL_{ns} represents the CL term in the model and CL_{tmd} was calculated as the product of k_{tmd} and V_I . The region marked TMDD represents the parameter space where 'classical' TMDD behavior would be observed, while the region marked TMEE represents the limit of the system explored here.

Fig. 5:

Impact of target-expression on TMEE. Simulations were performed for ALX-0081 to evaluate the impact of baseline target expression on PK. Simulations in the presence and absence of vWF expression at the lowest tested dose (0.02 mg/kg) are shown in (a) and at a range of baseline target expression values are shown in (b). Solid lines in (a) represent simulations in the presence of target expression and dashed lines are in the absence of target expression. The calculated CL values in panel b represents NCA-derived clearance (Dose/AUC).

Fig. 6:

Performance of TMDD model approximations. Model-fitted concentration vs. time profiles for ALX-0081 using the (a) quasi-equilibrium/quasi-steady-state and (b) Michaelis-Menten approximations of the TMDD model. Symbols are consistent across panels.

Table 1:

Mean NCA-Derived Parameters for Linagliptin

Parameter	0.5 mg	2.5 mg	5 mg	10 mg
AUC _{inf} (nmol×h/L)	422	821	1250	1480
CL (L/h)	2.51	6.42	8.46	14.3
V _{SS} (L)	380	1000	1110	1540

Table 2:

Model-Fitted PK Parameters for Linagliptin (Full TMDD)

Parameter	Final Estimate (%CV)
V_1 (L)	7.15 (13.0%)
V_2 (L)	111 (6.17%)
CL (L/h)	44.3 (3.71%)
CL _D (L/h)	68.5 (5.70%)
K _D (nM)	0.389 (13.6%)
k _{off} (1/h)	0.0130 (8.01%)
k _{on} (1/nM×h) ^a	0.0334 (Derived)
R _{tot} (nM)	7.43 (6.66%)
k _{deg} /k _{tmd} (1/h)	0.00 (Fixed)

^ak_{on} calculated as k_{off}/K_D

Table 3:

Mean NCA-Derived Parameters for ALX-0081

Parameter	0.02 mg/kg	0.4 mg/kg	8 mg/kg
AUC _{inf} (µg×h/mL)	15.2	34.7	113
CL (mL/h/kg)	1.32	11.5	70.7
V _{ss} (mL/kg)	56.4	286	1830

Table 4:

Model-Fitted PK Parameters for ALX-0081 (Full TMDD)

Parameter	Final Estimate (%CV)
V_1 (mL/kg)	39.2 (7.36%)
V_2 (mL/kg)	226 (16.7%)
CL (mL/h/kg)	104 (4.66%)
CL_D (mL/h/kg)	32.3 (12.6%)
K_D (nM)	0.00357 (355%)
k_{off} (1/h)	3.49 (5.89×10^4 %)
k_{on} (1/nM×h) ^a	978 (Derived)
R_{tot} (nM)	32.8 (6.26%)
k_{syn} (nM/h) ^b	0.446 (Derived)
k_{deg} (1/h)	0.136 (26.0%)
k_{tmd} (1/h)	0.407 (13.4%)

^a k_{on} calculated as k_{off}/K_D ^b k_{syn} calculated as $R_{tot} \times k_{deg}$

Table 5:

Model-Fitted PK Parameters for ALX-0081 (TMDD Approximations)

Parameter	Quasi-Equilibrium	Quasi-Steady-State	Michaelis-Menten ^a
V ₁ (mL/kg)	39.2 (3.74%)		45.4
V ₂ (mL/kg)	226 (15.7%)		5.54×10 ³
CL (mL/h/kg)	104 (3.78%)		2.18
CL _D (mL/h/kg)	32.3 (11.3%)		1.24×10 ⁻⁹
K _{SS} or K _m (nM)	0.00347 (307%)		27.7
R _{tot} (nM)	32.8 (6.25%)		2.14×10 ⁻⁷
k _{syn} (nM/h) ^b	0.446 (Calculated)		1.67×10 ⁻⁶
k _{deg} (1/h)	0.0136 (23.2%)		1.45
k _{imd} or V _{max} (1/h)	0.0407 (8.04%)		0.00119

^a Model fit did not generate confidence windows around estimates for Michaelis-Menten model, due to near singular matrix with extremely large standard errors.

^b k_{syn} calculated as R_{tot}×k_{deg}

Table 6:

Comparison of TMDD Model Performance for ALX-0081 (% Prediction Errors)

Dose (mg/kg)	Full TMDD	Q_E/Q_{SS}	M-M
0.02	12.6%	12.6%	19.6%
0.4	12.2%	11.9%	412%
8	15.4%	15.0%	4620%

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