Physiologically-based modeling to predict the clinical behavior of monoclonal antibodies directed against lymphocyte antigens

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ABSTRACT
Many clinically approved and investigational monoclonal antibody (mAb)-based therapeutics are directed against proteins located in the systemic circulation, including cytokines, growth factors, lymphocyte proteins, and shed antigens. Interaction between mAb and target may lead to non-linear pharmacokinetics (PK), characterized by rapid, target-mediated elimination. Several groups have reported that determinants of target-mediated elimination could include mAb-target binding, target expression, and target turnover. Recently, we scaled a physiologically-based pharmacokinetic model for mAb disposition to man and used it to predict the non-linear PK of mAbs directed against tumor epithelial proteins. In this work, we extended the previously described model to account for the influence of lymphocyte proteins on mAb PK in man. To account for the dynamic behavior of lymphocytes in the circulation, lymphocyte cycling between blood and lymphoid organs was described using first-order transfer rate constants. Use of lymphocyte cycling and reported target turnover rates in the model allowed the accurate prediction of the pharmacokinetics and pharmacodynamics (PD) of 4 mAbs (TRX1, MTRX1011a, rituximab, daclizumab) directed against 3 lymphocyte targets (CD4, CD20, CD25). The results described here suggest that the proposed model structure may be useful in the a priori prediction of the PK/PD properties of mAbs directed against antigens in the circulation.

Introduction
Monoclonal antibodies (mAb) are one of the largest and fastest growing classes of drugs globally,1 with over 50 currently approved products, and hundreds more in various stages of clinical development. The first approved mAb product, muromonab-CD3 (Orthoclone OKT3, since withdrawn), was a murine immunoglobulin G (IgG) molecule directed against the T cell marker CD3. Currently, mAbs are used to target several types of antigens, including membrane proteins associated with tissue epithelium and vascular endothelium, membrane proteins associated with circulating cells (e.g., lymphocytes, pathogens), and soluble cytokines and growth factors. It is appreciated that the properties of the target may have a significant effect on the pharmacokinetics (PK), pharmacodynamics (PD), and toxicity of mAbs.2,3 For example, mAbs targeting cell-surface receptors are often subject to target-mediated elimination following receptor binding and internalization.4

Antibody targets present in the circulation are rapidly bound and neutralized by systemically administered mAbs, due to the lack of distributional barriers restricting access to the target. There are several ‘classes’ of targets that may be present in the circulation, including soluble cytokines and growth factors, bacterial/viral proteins, lymphocyte proteins, and tumor shed antigens. Dose-dependent, non-linear PK has been reported for many mAbs targeting lymphocyte antigens, and clearance is often reported to be proportional to receptor expression in the circulation.5 Target-related factors that may influence PK/PD include expression, turnover, and binding affinity. Additionally, for mAbs targeting lymphocyte proteins, the dynamic nature of lymphocyte cycling between the circulation and lymphoid tissues may affect PK/PD. In this work, the interplay of these key factors was considered in order to generate a priori predictions of the in vivo behavior of mAbs that bind to lymphocyte antigens.

Physiologically-based pharmacokinetic (PBPK) models provide a platform for the mechanistic integration of the key determinants of mAb disposition, including binding to the neonatal Fc receptor (FcRn) and target-mediated disposition (TMD). In recent years, several PBPK models have been developed that account for the influence of FcRn-mediated recycling on IgG disposition.6-12 The catenary model of IgG disposition, originally developed for prediction of mAb PK in mice,10 has been extended to account for TMD of mAbs directed against epithelial targets and scaled up to monkey11 and to human.13 This scaled model is able to predict the non-linear plasma PK of mAbs in the presence of target-mediated elimination. In the present work, a PBPK model describing the disposition of IgG in man13 was extended to account for lymphocyte target expression and cycling. The extended model was used to generate a priori predictions of the plasma PK and PD of several mAbs that target proteins present in the systemic circulation.
Results

A schematic of the base PBPK model is shown in Fig. 1 and the lymphocyte cycling sub-model is shown in Fig. 2.

Anti-CD4 mAb (TRX1 and MTRX1011a) pharmacokinetics and pharmacodynamics

Monte Carlo simulations were performed to predict the plasma PK, time course of receptor occupancy, and time course of receptor expression of 2 anti-CD4 mAbs in healthy subjects. Figs. 3 and 4 show the model-predicted PK/PD profiles for TRX1 and MTRX1, respectively. The model was able to generate good predictions of both the PK and PD of TRX1 and MTRX1011a, with simulated results agreeing with digitized data. Some underprediction is observed for the time course of CD4 receptor occupancy; however, this can be readily explained as the digitized values for free CD4 were greater than 100% and the greatest possible free fraction is 100%. In addition, model-predicted values of clearance (\( CL \)) were in agreement with observed values (Fig. 5).

Anti-CD20 mAb (Rituximab) pharmacokinetics and pharmacodynamics

Simulations were performed with the PBPK model to predict the plasma PK and time course of B cell dynamics in recurrent B-cell lymphoma patients, following a single dose of rituximab. The model-predicted PK/PD profiles are shown in Fig. 6. Overall, the model was able to well-predict the in vivo behavior of rituximab across the tested dose range (100–500 mg/m²). Predictions of rituximab clearance were generally in agreement with the observed values of \( CL \) (Fig. 5).

Anti-CD25 mAb (Daclizumab) pharmacokinetics

The PBPK model was used to simulate the plasma PK of daclizumab in adult T-cell leukemia/lymphoma patients across a dose range of 2–8 mg/kg. Model predicted concentration vs. time profiles are shown in Fig. 7. The model was able to accurately predict daclizumab PK at all simulated doses. NCA-derived values of \( CL \) from model-predictions were similar to observed values (Fig. 5).

Discussion

In this work, a PBPK model has been proposed to predict the influence of lymphocyte proteins on the in vivo performance of mAbs in man. To account for the influence of binding to lymphocyte targets on mAb PK, the PBPK model was extended to include the kinetics of lymphocyte cycling between blood and lymphoid tissues, and data describing receptor number and turnover were obtained from the literature. Simulations with this model were able to accurately predict the plasma PK and time course of receptor occupancy and expression of mAbs directed against 3 lymphocyte targets (CD4, CD20, and CD25).

It is appreciated that the lymphocyte pool present in the systemic circulation is not a static pool, but rather it is a small fraction of a constantly cycling pool of cells traveling between the blood and lymphoid tissues (e.g., lymph node, spleen, bone marrow). Because of the large size of lymphocytes relative to antibody molecules, it is likely that mAb associated with lymphocytes would take on the cycling characteristics of the cells. Therefore, in order to adequately describe the in vivo behavior of mAbs targeted against lymphocyte antigens, cycling of cells between the circulation and spleen (as a representative lymphoid tissue) has been described in the current model. In this work, prior data describing the distribution of cycling lymphocytes and the kinetics of lymphocyte cycling in man have been

\[ \text{Figure 1. PBPK model of mAb disposition. Tissues in the model are connected in an anatomical fashion, via plasma (Q) and lymph (L) flows, and drug in the lymph system is returned to the plasma through the lymph node. All organs are divided into tissues representing the vascular, endosomal space of the vascular endothelium, interstitial, and cell spaces. IgG in the vascular space of tissues enters tissue either via convection directly into the interstitial space or by fluid-phase pinocytosis into the endosomal space. The endosomal space is divided into a series of 5 compartments, connected in a catenary fashion. Within the endosomal space, IgG interacts with FcRn via binding rate constants (} k_{\text{on}} \text{ and } k_{\text{off}} \text{) in a pH-dependent manner and any unbound mAb in the last transit compartment is eliminated, while bound mAb is recycled to the vascular or interstitial space. Antibody in the interstitial space is allowed to interact with target in the interstitial space or enter the lymphatics or endosomal space. Target is expressed on the surface of cells and is produced at a zero-order rate (} k_{\text{syn}} \text{) and by a first-order rate process (} k_{\text{int}} \). Antibody-bound target can be eliminated as well by a first-order rate process (} k_{\text{md}} \). \]
integrated to allow for prediction of the PK/PD of mAbs directed against lymphocyte antigens. While it is acknowledged that lymphocytes traffic to tissues other than the spleen (e.g., lymph nodes, Peyer’s patches), the extravascular pool of lymphocytes was lumped into the spleen as a representative lymphoid tissue. As quantitative information becomes available describing tissue-specific homing patterns of lymphocytes, the model could be adjusted to describe cell type-specific

![Figure 2. Model structures for mAbs directed against lymphocyte targets. Lymphocytes in circulation are able to move between the circulation and the interstitial space of the spleen, representing the extravascular lymphocyte pool (kpt and ktp). mAb is able to bind target on lymphocytes (kD) and bound mAb takes on the cycling properties of the lymphocyte. Target molecules are continuously synthesized (ksyn) and degraded (kint) and mAb-target complexes are eliminated by a first-order process (ktmd). All other processes in spleen are as depicted in Fig. 1.](image)

![Figure 3. Model-predicted pharmacokinetics and pharmacodynamics of TRX1 in healthy subjects. Data describing TRX1 plasma pharmacokinetics (upper panel), CD4 receptor occupancy (middle panel), and CD4 receptor expression (lower panel) at doses of 1 (left column), 5 (center column), and 10 mg/kg (right column) were digitized. The digitized data are shown as open symbols and the median model-predicted concentration vs. time profile is depicted as a solid line. The 5th and 95th percentiles of model predictions are shown as dashed lines.](image)
lymphocyte cycling in both healthy individuals and in disease. Simulations that did not consider the substantial extravascular pool of lymphocytes (~98% of cycling lymphocytes) generally underpredicted the observed target-mediated elimination (and overpredicted plasma concentrations) and overpredicted the time course of receptor occupancy. However, by accounting for the kinetics of lymphocyte cycling, the entire accessible pool of receptor was adequately described, allowing for prediction of both the plasma PK and time course of receptor occupancy for lymphocyte antigens.

Simulations for rituximab and MTRX1011a under-predicted the observed clearance at the lowest dose. This disagreement

**Figure 4.** Model-predicted pharmacokinetics and pharmacodynamics of MTRX1011a in healthy subjects. Data describing MTRX1011a plasma pharmacokinetics (upper panel), CD4 receptor occupancy (middle panel), and CD4 receptor expression (lower panel) at doses of 0.3 (1st column), 1 (2nd column), 3.5 (3rd column), and 7 mg/kg (4th column) were digitized. The digitized data are shown as open symbols and the median model-predicted concentration vs. time profile is depicted as a solid line. The 5th and 95th percentiles of model predictions are shown as dashed lines.

**Figure 5.** Comparison of model-predicted and digitized clearance for TRX1, MTRX1011a, rituximab, and daclizumab. Non-compartmental analysis was performed to determine the mean and standard deviation of model-predicted clearance for each mAb (gray bars) and compare with observed clearances for each mAb (black bars).
may be explained, in part, by clinical variability, as the evaluation was performed with very limited data (e.g., data from only 3 patients were available for the dose of 100 mg/kg of rituximab). Nonetheless, it is possible that under-prediction of clearance is due to mechanisms that are not represented within the current model, including the possible rapid elimination of complexes of mAb with "shed" antigen. Significant plasma concentrations of shed CD20 and CD4 have been noted in some patient populations. Investigations are currently underway to characterize, and to incorporate within our predictive physiologically-based model, the influence of shed antigen on mAb PK and PD.

In addition to prediction of plasma PK of mAbs targeted against lymphocyte proteins, the PBPK model was able to

Figure 6. Model-predicted pharmacokinetics and pharmacodynamics of rituximab in recurrent B-cell lymphoma patients. Concentration vs. time profiles (upper panel) and data describing the time course of B cell dynamics (lower panel) following rituximab doses of 100 (left column), 250 (middle column), and 500 mg/m² (right column) were digitized. Monte Carlo simulations were performed and the median predicted concentration vs. time profile is shown as a solid line and the 5th and 95th percentiles of model predictions are shown as dashed lines.

Figure 7. Model-predicted concentration vs. time profiles for daclizumab in adult T-cell leukemia/lymphoma patients. Plasma pharmacokinetic data for daclizumab was digitized and compare with model predictions. The median predicted concentration vs. time profile is depicted as a solid line and the 5th and 95th percentiles of predicted profiles are shown as dashed lines.
generate reasonable predictions of the time course of receptor occupancy for mAbs directed against CD4 (TRX1 and MTRX1011a), a characteristic T cell marker. A priori prediction of receptor occupancy is of utility when making projections of safety margins and anticipated dose-response relationships for first-in-human (FIH) studies. Key determinants of the duration and magnitude of receptor occupancy include baseline expression and turnover in the presence and absence of binding. Predictions of receptor occupancy were made across a 30-fold dose range for anti-CD4 mAbs, and the model was able to well-predict the time course of CD4 occupancy at all doses and for both mAbs (TRX1 and MTRX1011a). Previously, the general model of TMD17 was used to describe the PK/PD of TRX118 and of MTRX1011a;19 however, this approach estimated a 2.3-fold difference in total CD4 concentration between 2 studies of healthy subjects (54.9 nM for TRX1 vs. 24.0 nM for MTRX1011a). From literature reports of CD4 receptor number and lymphocyte counts, the total pool of CD4 on cycling lymphocytes was calculated to be 16.3 nmoles, which would correspond to a concentration of 6.03 nM if the whole pool of receptor existed in the blood (representative of the central compartment), which is 4–9-fold lower than the previously reported model-generated values. Additionally, these publications relied on fitting of binding parameters, and did not utilize the in vitro measured affinity ($K_D = 0.6$ nM). Model-estimated values of $K_D$ were 32-fold18 and 21-fold19 greater than the in vitro measured values. Because these models required the use of estimated parameters for system- (CD4 expression) and antibody-specific ($K_{D_0}$) parameters, the predictive value of this approach is limited. In the present work, prior information was extracted from the literature describing receptor expression and affinity, and the model was able to make good predictions of anti-CD4 mAb PK/PD using values readily available from the literature and from in vitro studies. This suggests that the use of the approach described here may be more useful in prediction of the PK/PD profiles of mAbs targeted against lymphocyte antigens than compartmental models that require the use of model-fitted parameters that do not agree with measured values.

Following binding of mAbs to target cells, Fc-mediated functions (e.g., antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)) may contribute to removal of the target protein (and cell) from the body. While the relative contribution of these factors in vivo is unclear,20 antibody-mediated removal of target cells is a critical factor that can influence both the PK and PD of therapeutic mAbs.21 In the work described here, the proposed PBPK model was able to predict the plasma PK and modulation of circulating B cells in lymphoma patients following a single dose of the anti-CD20 rituximab. Because there was no quantitative data for CD20 turnover available, it was assumed that the receptor would be eliminated at a very rapid rate following binding and at the same rate as basal B cell turnover in the absence of binding. From the mean cellular receptor number and concentration of receptor in circulation, the number of B cells in the blood was calculated for use in the model. Using these assumptions, the PBPK model was able to predict the long duration of B cell suppression observed following a single dose of rituximab. Previous modeling efforts for anti-lymphocyte mAbs have described changes in blood lymphocyte counts following mAb administration as being due to redistribution of cells from blood to tissue.22 While stimulation of cell redistribution may be justified for certain mAbs, removal of target cells by mAbs is often due to Fc-mediated effector functions (e.g., ADCC, CDC).23 In this work, the removal of B cells was described in a manner consistent with the proposed mechanism(s) of action of rituximab, removal of CD20 cells following mAb binding and engagement of effector functions through the Fc region of the antibody.

The model presented in this work is structured for consistency with an assumption of kinetic independence in the elimination of mAb-receptor complexes and the elimination of unbound receptor. However, it is appreciated that for some antibodies, effector mechanisms such as ADCC and CDC may lead to rapid elimination of antibody-opsonized cells, where a relatively low fractional engagement of target receptor stimulates cellular destruction, and thus stimulates the elimination of unbound cell-surface receptors along with the elimination of bound receptors. If such mechanisms are operational, we would expect an underestimation, at low doses or at low mAb concentrations, of mAb effects on the suppression of total or unbound receptor concentrations. Future work will attempt to investigate the relationship between mAb-receptor cell-surface density and the kinetics of cellular destruction via ADCC and CDC, and to incorporate these relationships within the predictive model.

Here, our use of simplifying assumptions describing lymphocyte cycling, target expression, and target turnover allowed for a priori prediction of the clinical PK/PD of mAbs directed against proteins expressed by T cells (CD4, CD25) and B cells (CD20) both in healthy subjects and in cancer patients. When considering all mAbs approved by the Food and Drug Administration or in regulatory review, lymphocyte molecules are targeted by nearly one-third of all therapeutic antibody products, demonstrating the therapeutic relevance of this class of target. The PBPK model structure proposed in this work is a potential framework for prediction of the in vivo behavior of both approved and investigational mAbs against lymphocyte proteins. Additionally, it is anticipated that this framework could be readily adjusted to account for targets expressed on the surface of pathogens (e.g., bacteria, viruses) and the influence of pathogen distribution in the body on mAb disposition.

In summary, the previously published human PBPK model for mAb disposition15 has been extended to account for the influence of binding to lymphocyte targets on mAb PK/PD. A model structure was proposed that described the kinetics of lymphocyte cycling between blood and lymphoid tissues (using spleen as a representative lymphoid organ). Simulations using this model were able to generate good predictions of the plasma PK of mAbs directed against CD4 (TRX1 and MTRX1011a), CD20 (rituximab), and CD25 (daclizumab). Additionally, the model was able to predict the time course of receptor occupancy and expression for anti-CD4 mAbs and the time course of B cell depletion for anti-CD20 mAbs. Overall, the work described here provides a platform for the prediction of the clinical performance of mAbs directed against targets present in the systemic circulation, and it is likely that it would be readily amenable to prediction of the behavior of mAbs directed
against other classes of circulating targets (e.g., cytokines, growth factors, infectious agents).

**Materials and Methods**

**Model structure and parameters**

The base model structure was as described previously. The model includes compartments representing key sites of IgG disposition, including plasma, lung, liver, spleen, GI tract, heart, kidney, skin, muscle, lymph node, and tumor. The model structure is shown in Fig. 1. All tissues were connected in an anatomical manner via plasma (Q) and lymph (L) flow rates. Physiologic parameters related to tissue size and plasma flow were obtained from the literature, and lymph flow rates were fixed to 0.2% of tissue plasma flow, consistent with previous models. Each tissue was further sub-divided into compartments representing the vascular space, endosomal space of the vascular endothelium, interstitial space, and cellular space.

IgG within the vascular space of each tissue was allowed to enter the endosomal space via tissue-specific fluid-phase pinocytosis rates (CL_{up, tissue}) or pass directly into the interstitial space via convective uptake, governed by L and the vascular reflection coefficient (\( \sigma_v \)), which was fixed to 0.95 for all tissues, except tumor, which was set to 0.842. Within the endosomal space, the interaction between IgG and FcRn was modeled using a catenary structure consisting of 5 sub-compartments, representing the time course of endosomal sorting and acidification. Endosomal volumes were calculated as described previously. In the 5th endosomal sub-compartment, any IgG bound to FcRn was allowed to recycle to the plasma or interstitial space, and free IgG was eliminated from the system.

In order to account for the effect of tissue-specific target expression, receptor expression was included in the interstitial space of each tissue. Values for receptor expression in tissue were obtained via conversion of immunohistochemical (IHC) scores to receptor concentrations, as described previously. All tissue IHC scores were obtained from The Human Protein Atlas. Target protein expression on lymphocytes was obtained from literature reports of receptor number/cell for each evaluated target. This value was then converted into receptor concentration in the circulation using reported values for the number of relevant cells in the circulation in the relevant population. Discussion of the values of these parameters can be found in the section on evaluation of the lymphocyte model. Similar to the base model described above, target turnover values were obtained from the literature for each evaluated target in the presence and absence of binding. In the absence of data for the elimination rate of bound target, it was assumed that mAb-target complexes would be rapidly internalized, at a rate similar to the maximum observed clearance for mAbs in the clinic. To obtain an estimate of the maximal rate of clearance of mAbs, an extensive literature search was obtained, and the non-compartmental analysis (NCA)-derived clearance (CL) estimates were extracted at the lowest administered dose for 91 mAbs (Table S1). The estimate of CL_{IC} was obtained using the reported CL for all mAbs with a maximal CL greater than 100 mL/h (n = 16 mAbs), and the mean (and standard deviation) of CL_{IC} was calculated to be 9.75 (15.9) mL/minute.

**Data sets for evaluation**

Data describing the disposition of 4 mAbs, which are directed against 3 targets, following intravenous dosing were used to evaluate the proposed model structure for mAbs targeting proteins expressed on the surface of lymphocytes. All target-specific parameters used in the lymphocyte antigen model are summarized in Table 1. The first 2 data sets relate to mAbs targeting the T cell-associated protein CD4 in healthy subjects, TRX1, at doses of 1–10 mg/kg, and MTRX1011a, at doses of 0.3–7 mg/kg. Both data sets included not only the plasma PK of mAb, but also the time course of receptor occupancy (expressed as % CD4 free), and total receptor expression (expressed as % CD4 baseline). The expression of CD4 on the surface of CD4+ T cells has been reported to be \( \sim 1.06 \times 10^5 \) molecules/cell and it has been estimated that 50% of
lymphocytes in the circulation of healthy individuals are CD4+ T cells (~5 × 10^8 cells). These values were used to calculate the concentration of CD4 in the circulation and in the spleen, as described above. The turnover kinetics of CD4 in the presence and absence of mAb binding were obtained from in vitro studies reported in the literature. As the only difference between TRX1 and MTRX1011a was a single point mutation in the FcRn by 3-fold, the affinity between TRX1 and MTRX1011a was assumed to occur at the maximal rate of elimination observed in the clinic for mAbs.

### Table 1. Mean target-specific parameters used in the lymphocyte model.

| Parameter (Units) | CD4 | CD20 | CD25 |
|-------------------|-----|------|------|
| \( C_{\text{plasma}} \) (nM) | 0.325^a | 0.320^b | 0.918^c |
| \( C_{\text{spleen}} \) (nM) | 444 | 444 | \( 1.27 \times 10^4 \) |
| \( k_{\text{off}} \) (min^-1) | \( 1.79 \times 10^{-4} \) | \( 2.71 \times 10^{-5} \) | \( 2.10 \times 10^{-3} \) |
| \( k_{\text{total}} \) (min^-1) | \( 4.00 \times 10^{-2} \) | \( 3.61 \times 10^{-2} \) | \( 3.61 \times 10^{-3} \) |

^aFrom46-48  
^bFrom67  
^cFrom66  
^dCalculated based on the observation that 2% of lymphocytes are in blood and the remaining 98% are in the spleen  
^eFrom51-55  
^fFrom58  
^gFrom61  
^hFrom49,50  
^iAssumed to occur at the maximal rate of elimination observed in the clinic for mAbs.
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