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Evaluation of a Catenary PBPK Model for Predicting the *In Vivo* Disposition of mAbs Engineered for High-Affinity Binding to FcRn

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Abstract. Efforts have been made to extend the biological half-life of monoclonal antibody drugs (mAbs) by increasing the affinity of mAb–neonatal Fc receptor (FcRn) binding; however, mixed results have been reported. One possible reason for a poor correlation between the equilibrium affinity of mAb–FcRn binding and mAb systemic pharmacokinetics is that the timecourse of endosomal transit is too brief to allow binding to reach equilibrium. In the present work, a new physiologically based pharmacokinetic (PBPK) model has been developed to approximate the pH and time-dependent endosomal trafficking of immunoglobulin G (IgG). In this model, a catenary sub-model was utilized to describe the endosomal transit of IgG and the time dependencies in IgG–FcRn association and dissociation. The model performs as well as a previously published PBPK model, with assumed equilibrium kinetics of mAb–FcRn binding, in capturing the disposition profile of murine mAb from wild-type and FcRn knockout mice (catenary vs. equilibrium model: r^2 , 0.971 vs. 0.978; median prediction error, 3.38% vs. 3.79%). Compared to the PBPK model with equilibrium binding, the present catenary PBPK model predicts much more moderate changes in half-life with altered FcRn binding. For example, for a 10-fold increase in binding affinity, the catenary model predicts <2.5-fold change in half-life compared to an ~8-fold increase as predicted by the equilibrium model; for a 100-fold increase in binding affinity, the catenary model predicts ~7-fold change in half-life compared to >70-fold increase as predicted by the equilibrium model. Predictions of the new catenary PBPK model are more consistent with experimental results in the published literature.

KEY WORDS: endosomal transit; FcRn; mAb; PBPK; pharmacokinetics.

INTRODUCTION

Twenty-eight monoclonal antibodies (mAbs) have been approved for therapeutic use in the USA, and hundreds of mAbs are currently under clinical evaluation (1,2). All of the approved mAbs are of the immunoglobulin G (IgG) isotype (3). The neonatal Fc receptor (FcRn) protects IgG from intracellular catabolism and, thus, contributes to the long biological half-life of mAbs. The receptor is expressed throughout the body (4,5), including within the vascular endothelium, which is regarded as a prime site of IgG catabolism (6–8). IgG–FcRn binding is pH dependent, where FcRn binds to IgG with high affinity at pH 5.5–6, but binding affinity is reduced by at least 2 orders of magnitude as pH increases from 6 to 7 (9,10). The well-accepted theory of FcRn protection of IgG is shown schematically in Fig. 1.

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As FcRn is responsible for the long half-life of IgG in the circulation, there has been considerable effort to engineer mAb for increased binding to FcRn, as a means of increasing biological persistence. Several groups have shown that increasing the affinity of mAb for FcRn at pH 6 can lead to slower rates of mAb clearance and to increases in terminal half-lives (11–19). However, in several other reports, no clear relationship has been shown between mAb half-life and mAb–FcRn binding affinity at pH 6 (15,16,20–26) (Table I).

IgG antibodies appear to be eliminated through a cascade of events that includes endocytosis, endosomal transit and sorting, delivery to lysosomes, and enzymatic catabolism (27). The timecourse of endosomal processing of IgG has not been studied thoroughly, but it is likely that this process is completed quite rapidly, within minutes. Of note, the endocytosis and recycling pathways for FcRn and the transferrin receptor have been reported to overlap (28), and recycled transferrin has been shown to have an intracellular half-life of ~7.5 min (29). Following endocytosis of extracellular fluid, pH drops slowly due to the action of vacuolar ATPase (30). For example, in Chinese hamster ovary (CHO) cells, endosomal pH drops from 7.4 to an average pH of 6.3 in 3 min, and by 10 min, the endosome pH reaches 6 and below (31). The rate of pH change in endosomes of endothelial cells

Fig. 1. Proposed mechanism for IgG protection by FcRn. IgG is taken up into endosomes *via* fluid-phase endocytosis, and IgG binds to FcRn as endosomes are acidified. Bound IgG is sorted to endosomes that fuse with plasma membrane. At physiological pH, FcRn-IgG complexes dissociate, and IgG is returned to extracellular fluid (plasma and interstitial fluid). Unbound IgG is delivered to the lysosomes for catabolism. Values shown for pH are approximate

has not been reported, and the pH change may occur at a different rate than shown for CHO cells; however, it is likely

that the acidification of endosomes from 7.4 to ≤ 6 occurs gradually, rather than abruptly. Considering the rapid rate of endosomal transit and the non-instantaneous process of endosomal acidification, it is likely that IgG and FcRn share only a brief coexistence at $\text{pH} \leq 6$, prior to endosomal sorting for recycling or for delivery of IgG to the lysosome.

At acidic pH, IgG binds to FcRn with high affinity and with slow rates of dissociation. For example, Vaughn and Bjorkman investigated a series of mAb-FcRn complexes, at pH 6, and found dissociation rate constants in the range of 0.002 to 0.0002 s^{-1} (32), which corresponds to dissociation half-lives of 6–58 min. In the study conducted by Datta-Mannan and coworkers (20), the reported dissociation rate constants (k_{off}) of FcRn complexes with engineered IgGs relate to dissociation half-lives up to 7.2 min. Comparison of the estimates of dissociation half-lives of mAb-FcRn complexes with the timecourse of endosomal transit suggests that it is unlikely that mAb-FcRn binding reaches equilibrium prior to endosomal sorting. We speculate that non-equilibrium binding provides a logical explanation for the lack of a clear relationship between equilibrium binding

Table I. Summary of Published Reports of Observed Changes in Terminal Half-Lives for mAb Engineered for Increased FcRn Binding at pH 6

Backbone	Mutation	FcRn binding affinity, relative to wild-type mAb ^a	Half-life, relative to wild-type mAb ^b	Animal model	Reference
hIgG1	G385D/Q386P/N389S	1.4	0.88	balb/c mice	(23)
hIgG1	M252Y/S254T/T256E	10	0.94	balb/c mice	(23)
hIgG1	H433K/N434F/Y436H	20	<0.88	balb/c mice	(23)
hIgG2	M428L	8	1.83	Rhesus monkeys	(12)
hIgG2	T250Q/M428L	27	1.86	Rhesus monkeys	(12)
hIgG1	T250Q/M428L	38	2.5	Rhesus monkeys	(13)
hIgG1	H433K/N434F	21	0.25	Swiss Webster mice	(26)
hIgG1	M252Y/S254T/T256E	10	4	Cynomolgus monkey	(19)
hIgG1	P257I/N434H	197	0.03	CD-1 mouse	(20)
hIgG1	D376V/N434H	17	0.10	CD-1 mouse	(20)
hIgG1	P257I/Q311I	25	0.10	CD-1 mouse	(20)
hIgG1	P257I/N434H	52	0.71	Cynomolgus monkey	(20)
hIgG1	D376V/N434H	52	0.81	Cynomolgus monkey	(20)
hIgG1	T250Q/M428L	513	0.5 ^c	CD-1 mouse	(21)
hIgG1	T250Q/M428L	40	0.78	Cynomolgus monkey	(21)
hIgG1	M428L/N434S	11	4	hFcRn mouse	(18)
hIgG1	M428L/N434S	11	3.2	Cynomolgus monkey	(18)
hIgG1	N434A	4	2.33	Cynomolgus monkey	(25)
hIgG1	N434W	80	1.56	Cynomolgus monkey	(25)
hIgG1	N434A	1.3	1.02	SCID mice	(16)
hIgG1	N434H	1.8	0.57	SCID mice	(16)
hIgG1	N434A	7.7	1.27 ^d	Cynomolgus monkey	(16)
hIgG1	N434H	23	1.38 ^d	Cynomolgus monkey	(16)
hIgG1	N434H	5.3	1.63	Cynomolgus monkey	(15)
hIgG1	T307Q/N434A	10	2.18	Cynomolgus monkey	(15)
hIgG1	T307Q/N434S	12.3	1.96	Cynomolgus monkey	(15)
hIgG1	T307Q/E380A/N434A	15.7	1.9	Cynomolgus monkey	(15)
hIgG1	V308P/N434A	36	1.8	Cynomolgus monkey	(15)
mIgG Fc	T252L/T254S/T256F	3.4	1.65	Balb/c mice	(17)

hIgG human immunoglobulin G, FcRn neonatal Fc receptor, hFcRn human neonatal Fc receptor, mAb monoclonal antibody, SCID severe combined immunodeficiency

^a Relative binding affinity is calculated as the equilibrium association constant for mAb binding to murine FcRn at pH=6.0 for the engineered antibody ($K_{\text{a,engineered}}$) divided by the equilibrium association constant for FcRn binding to the associated wild-type mAb ($K_{\text{a,wild-type}}$)

^b Relative half-life is calculated as the reported mean half-life for the engineered mAb in mice divided by the reported mean half-life for the wild-type mAb in mice

^c CL relative to wild-type mAb

^d AUC relative to wild-type mAb

affinity at pH 6 and the observed *in vivo* half-life of IgG antibodies.

There have been several published physiologically based pharmacokinetic (PBPK) models for characterizing IgG disposition (33–37). A recent PBPK model, developed by Garg and Balthasar, has incorporated FcRn within the endosomal compartments of the vascular endothelium within each tissue (38). The model was able to predict plasma and tissue disposition of mAb in wild-type and FcRn-deficient mice, with or without co-administration of large doses of exogenous IgG (as a competitive inhibitor of FcRn). Urva *et al.* later revised and extended this model to incorporate a tumor compartment, and specific target-mediated mAb elimination (39). The extended model was able to characterize the influence of specific antigen–antibody interactions on the disposition of T84.66, an anti-carcinoembryonic antigen monoclonal antibody. For both of these models, IgG–FcRn interaction in endosomes was assumed to be driven by equilibrium binding. Consequently, the fraction of unbound IgG in the endosomal space is a simple function of equilibrium binding affinity for FcRn at pH 6. Since unbound IgG in the endosomes undergoes degradation, such models are expected to predict a simple, positive correlation between FcRn binding affinity at pH 6 and *in vivo* IgG half-life. However, as discussed above, this type of prediction is inconsistent with the growing literature surrounding the *in vivo* pharmacokinetic behavior of mAb engineered for increased binding to FcRn.

In this work, we have developed a new physiologically based pharmacokinetic model to more closely approximate the pH and time dependence of IgG binding within endosomes. A series of catenary sub-models were utilized to describe the timecourse of IgG transit through endosomes, the gradual acidification of endosomal fluids, and the (non-equilibrium) kinetics of IgG–FcRn association and dissociation. The model also incorporates tissue-specific FcRn expression. Simulations with the model were compared to those of a PBPK model, derived from the Garg and Urva models, where equilibrium binding of IgG and FcRn is assumed. Relative to the equilibrium model, which predicts a near proportional relationship between equilibrium binding affinity and mAb half-life, the new catenary PBPK model predicts changes in half-life that are much more modest, and much more in line with literature observations (summarized in Table I). The catenary model also predicts a complex relationship between binding and *in vivo* half-life, where increased rates of IgG–FcRn association provide a greater benefit than provided by decreases in the rate of IgG–FcRn dissociation.

MATERIALS AND METHODS

Mouse IgG (T84.66) Production and Purification

To examine the relationships between pH and mAb–FcRn binding, the anti-carcinoembryonic mouse monoclonal IgG1 antibody, T84.66, was produced, purified, and evaluated for binding to murine FcRn. Briefly, hybridoma cells secreting T84.66 (ATCC, HB-8747) were grown in 1-L spinner flasks supplemented with serum-free hybridoma media (Invitrogen, NY) and 5 mg/L gentamicin (Invitrogen, NY). The cells were maintained at 37°C and 5% CO₂, and media was changed two to three times weekly to allow collection of secreted antibodies. T84.66 was purified from the media *via* protein G

affinity chromatography (17-0405-01, GE Healthcare NJ) by using Bio-Rad BioLogic DuoFlow chromatography system (Bio-Rad, CA). Na₂HPO₄ (20 mM, pH=7.0) was used as washing buffer and glycine (100 mM, pH=2.8) was used as the eluting buffer. Antibody concentration was determined by the UV reading at 280 nm, assuming 1.35 AU=1 mg/ml IgG (40).

IgG–FcRn Binding Characterization

The binding of T84.66 to murine FcRn was characterized at pH 6.0, 6.5, 7.0, and 7.4 by surface plasmon resonance (SPR) using a BIAcore T100 instrument (GE Healthcare). All binding experiments were performed at 25°C. Briefly, soluble recombinant mFcRn was immobilized onto a CM5 sensor chip using the amine coupling method. Flow cells on the chip were activated with *N*-hydroxysuccinimide (NHS) (GE Healthcare) and 3-(*N,N*-dimethylamino) propyl-*N*-ethylcarbodiimide (DEC) (GE Healthcare) at a flow rate of 10 µl/min. Recombinant mouse FcRn (175nM) in pH 5.5 acetate buffer (GE Healthcare) was injected over the flow cell to reach a low ligand density (~500 resonance units (RU)). The surface was then blocked with ethanolamine–HCL. For each immobilization, a control flow cell was prepared using an identical manner, except with omission of the FcRn injection.

To measure the binding kinetic parameters at each pH condition, PBS with 0.005% (*v/v*) Tween 20 was prepared, with pH adjusted to the desired value, and used as the running buffer for the analysis (flow rate: 30 µl/min). T84.66 was diluted with the running buffer. Dilutions of the antibody ranging from 8 nM to 4 µM were injected for 2 min over the FcRn immobilized chip, followed by perfusion with running buffer for 7 min, for evaluation of complex dissociation. Surfaces were regenerated with a 30-s pulse injection of PBS (0.005% Tween 20) at pH 8.5, with a flow rate of 50 µl/min. The antibody was also injected over a blank control flow cell to allow for correction for non-specific binding. Binding sensorgrams were analyzed with BIAevaluation software 1.1. Binding parameters were estimated by fitting the binding data to a heterogeneous-ligand model.

Mathematical Modeling

Model Structure

The catenary PBPK model was based on the prior PBPK models of Urva *et al.* and Garg and Balthasar (38,39). The model includes ten tissue/organ compartments to represent: plasma, lung, lymph node, liver, spleen, GI tract, heart, kidney, skin, and muscle (Fig. 2a). Intertissue transfer of antibody is mediated by plasma flow and lymph flow. Consistent with the PBPK model of Urva *et al.* (39), a lymph node compartment collects the lymphatic drainage from all organs and returns the lymphatic fluid to the systemic circulation (Fig. 2b). In the previously published model, it was assumed that all IgG collected by lymph nodes was returned back to the circulation. However, in the present model, it is assumed that complete return of IgG to circulation occurs in wild-type animals, whereas only 28% of IgG in lymph node compartment is returned back to the circulation in FcRn-deficient animals. This model feature was based on the experimental observation

Fig. 2. Schematic representation of the physiologically based pharmacokinetic (PBPK) model of IgG disposition. **a** All major organs are connected in an anatomical fashion with plasma flow represented by solid arrows and lymph flow by dashed arrows. **b** The lymph node compartment collects the lymphatic drainage from organs, and lymph fluid is returned to the systemic circulation. L_{organ} represents lymph flow rate, and σ_I is the lymphatic reflection coefficient. Tau_{LY} is the transit time associated with lymphatic transit. f_{LY} , which describes the fractional return of mAb from lymphatic transport to the systemic circulation, has a value of 1 for wild-type animals and 0.28 for FcRn-deficient mice. C_{organ} and X_{LY} represent the IgG concentration in the tissue interstitial space and amount of IgG in the lymph node compartment

of 28% IgG bioavailability following subcutaneous injection to FcRn-deficient mice (41). Similar to the Garg and Urva models, each tissue compartment was further divided into three main sub-compartments to represent the vascular space, the endosomal space of vascular endothelial cells, and the interstitial space (Fig. 3).

In the prior PBPK models, IgG–FcRn interaction in the endosomal space was assumed to be driven by equilibrium binding. In the present model, the vascular endothelial endosomal space has been described with a series of well-mixed transit compartments, representing the step-wise transport of IgG from endocytosis through endosomal sorting. As shown in Fig. 3, the endothelial endosomal sub-compartments were set to represent different pH environments, with pH ranging from 7.4 (just after endocytosis) to 6.0. This catenary structure allows representation of the time-dependent acidification of the endosomal environment during the process of endosomal transit. The transit time assumed for each endosomal sub-compartment is represented by τ .

In each endosomal sub-compartment, IgG interacts with FcRn with an association rate constant (k_{on}) and dissociation

rate constant (k_{off}), which is specific for the pH environment. In the last endosomal sub-compartment, catabolic elimination of unbound IgG occurs and bound antibody is recycled to the plasma or to the interstitial space. The parameters and variables used in the model are defined in [Electronic Supplementary Material I](#) and the equations for the model are provided in [Electronic Supplementary Material II](#).

Model Parameters

The physiological parameters for plasma flow rate, vascular, interstitial, and total tissue volume were obtained from the literature (35). The vascular reflection coefficient and lymph reflection coefficient values for endogenous antibodies were set to 0.95 and 0.2 for all organs (38). Other parameters were determined, or estimated, as described below.

Endosomal Transit Time, τ . The transit time for endosomal sub-compartments was derived based on the half-life of sorting endosomes. Assuming a half-life of sorting endosomes of 7.5 min (28,29), the total transit time for endosomal sorting was estimated as: total transit time = 7.5 min/0.693 = 10.8 min. The residence time associated with each endosomal sub-compartment, τ , was assumed to be equivalent, and τ was then calculated as the total endosomal transit time divided by the number of transit events: $\tau = 10.8 \text{ min}/4 = 2.7 \text{ min}$.

Tissue Uptake Rates (CL_{tissue}^{tissue}) and Endosomal Sub-Compartmental Volumes (V_{tissue}^E). Fluid pinocytosis rates for monolayer endothelial cells have been reported to be ~50 nL/h/10⁶ cells at high confluency (42,43). The total number of endothelial cells in mice was estimated from the reported endothelial volume 0.625 ml/mouse with the assumption that 10⁶ cells occupy a volume of 1 μ L (44). As such, the total pinocytosis rate by endothelial cells in a mouse was calculated as:

$$50 \text{ nL/h}/10^6 \times 0.625 \text{ ml} \times 10^6 \text{ cells}/\mu\text{L} = 5.21 \times 10^{-7} \text{ L/min}$$

Individual tissues uptake rates were obtained by fractionating the total pinocytosis rate by tissue size. Endosomal sub-compartment volumes (V_{tissue}^E) of individual tissues were calculated as: $V_{tissue}^E = CL_{tissue}^{tissue} \times \tau$. This parameterization allows a constant endogenous IgG concentration within all endosomal sub-compartments (at steady state). Individual tissue endosomal uptake rates and endosomal sub-compartment volumes are listed in Table II.

Binding Kinetic Parameters (k_{on} , k_{off}) for Murine IgG and FcRn. Microconstants (k_{on} and k_{off}) for FcRn binding to T84.66, a model murine IgG1 mAb, were measured by SPR at 25°C, as described above. Since the heterogeneous-ligand binding model assumes two different ligands on the sensor chip, two sets of binding parameters were obtained after each model fitting. The values of k_{on} and k_{off} associated with higher binding affinity, at each pH tested, were used in the PBPK model development and simulation.

Tissue-Specific FcRn Concentrations. FcRn protein concentrations in tissues were assumed to be proportional to measured values of FcRn mRNA. Individual tissue concentrations were coded within the model as $k_{tissue} \times R0$, where

Fig. 3. Schematic representation of intratissue compartments. Each tissue in the PBPK model is divided into three major compartments representing the vascular, endosomal, and interstitial spaces. The endosomal space of each organ is further subdivided into five compartments. Q and L represent the plasma and lymph flow rates. σ_V and σ_I are the vascular and lymphatic reflection coefficients. CL_{uptake} is the uptake rate of IgG from the vascular and interstitial compartments into the endosomal space. FR represents the fraction of FcRn-bound antibody that is recycled to the vascular space. kel is the clearance of unbound IgG from the last endosomal sub-compartment. The endosomal sub-compartments are assumed to have pH ranging from 7.4 to 6.0, to represent the acidification of endosomes. τ represents the transit time associated with each endosomal sub-compartment. k_{on} and k_{off} are the association and dissociation rate constants at different pH conditions

k_{tissue} represents the relative tissue FcRn mRNA expression, determined *via* real-time quantitative RT-PCR in prior work (unpublished data), and where $R0$ represents a concentration constant. Previous mathematical analyses of the pharmacokinetics of a monoclonal anti-FcRn antibody allowed the estimation of a whole-body averaged FcRn concentration of 1,660 nM (unpublished data). In the present modeling approach, the estimated whole-body averaged concentration, 1,660 nM, was assigned as the FcRn concentration of muscle tissue, as muscle is the largest organ represented in the model. FcRn concentrations in remaining tissues were calculated based on the relative expression of FcRn mRNA (unpublished data). mRNA expression data and estimated FcRn protein concentrations are shown in Table III.

Lymph Flow. Experimentally determined values for lymph flow in mice are not available. Widely different values for such

parameters have been used in published pharmacokinetic models for IgG disposition (33,34,38,39,45). In the present model, lymph flow was assumed to be proportional to plasma flow, and the lymphatic flow rate values for all organs were set to be 0.2% of the plasma flow rates.

Endogenous IgG Concentrations at Steady State. Endogenous IgG plasma concentration in untreated Swiss Webster mice was set at 7.4 μ M, as measured by ELISA (Life Diagnostics, Inc., 5010-1). The plasma endogenous IgG concentration was fixed within the model, and simulations were performed to obtain steady-state endogenous IgG concentrations in all tissue compartments. These values were assigned as the initial conditions in the model.

Endogenous IgG Production Rate, $K0$. Endogenous IgG production was assumed to be a zero-order rate process, and was not influenced by exogenous IgG administration. The production rate was estimated using the endogenous IgG plasma concentration as model input. The estimated $K0$ was $2.82E-12$ mol/min ($1.00E-03\%$).

Coefficients Accounting for Non-FcRn-Dependent Tissue Uptake of Exogenous IgG ($F1$, $F2$). Even in cases where target-mediated elimination is unlikely, literature data has shown that antibodies with identical constant regions and similar FcRn binding may display different disposition kinetics (46,47). These data demonstrate that additional factors, beyond FcRn binding (*e.g.*, surface charge), serve as determinants of mAb pharmacokinetics. To account for these factors, the coefficients $F1$ and $F2$ have been applied to modulate the rate of endosomal uptake and the vascular reflection coefficient, such that the model has sufficient flexibility to account mAb-to-mAb variability in factors that

Table II. Individual Tissue Endothelial Uptake Rates and Endosomal Volume

Tissue	Tissue volume (mL)	CL_{uptake} (nL/min)	Endosomal sub-compartment volume V^E (nL)
Lungs	0.19	6.22	16.8
GI	3.45	112	304
Liver	0.95	31.0	83.7
Spleen	0.10	3.26	8.80
Heart	0.13	4.33	11.7
Kidneys	0.30	9.71	26.2
Skin	2.94	95.8	259
Muscle	7.92	258	697

Table III. Tissue FcRn mRNA Expression and FcRn Concentrations

Tissue	Relative FcRn expression	FcRn concentration (nM)
Lungs	4.78	5.83E-06
GI	1	1.22E-06
Liver	10.67	1.30E-05
Spleen	3.12	3.81E-06
Heart	3.57	4.36E-06
Kidneys	6.36	7.76E-06
Skin	5.98	7.30E-06
Muscle	1.36	1.66E-06

FcRn neonatal Fc receptor

influence antibody uptake by endothelial cells and transport through endothelial cell layer. The values of $F1$ and $F2$ for a murine IgG1 mAb (7E3) were estimated by simultaneous fitting to plasma 7E3 pharmacokinetic data from both wild-type and FcRn-deficient mice (38).

Transit Time in Lymph Node Compartment, τ_{LY} . The lymph node compartment collects the lymphatic drainage from all organs and returns the lymphatic fluid to the systemic circulation. The value for τ_{LY} was estimated by fitting (simultaneous with the estimation of $F1$ and $F2$).

Comparison with Equilibrium Binding Model

PBPK Model with Equilibrium FcRn Binding. The new catenary PBPK model was developed under the assumption of non-equilibrium IgG-FcRn interaction within endosomes. It is of interest to compare predictions mAb pharmacokinetics provided by the catenary model with predictions of a PBPK model that assumes equilibrium binding of IgG and FcRn. However, the prior PBPK models developed and utilized by Garg and Balthasar (38) and Urva *et al.* (39) have made the assumption that endogenous and exogenous IgG bind FcRn with the same affinity and, consequently, these models were not structured to make predictions of the disposition of mAb with FcRn affinity that is different from that of endogenous IgG (38,39). To allow a meaningful comparison of the catenary and equilibrium models, we have modified the equilibrium model to allow multiple interactions with FcRn, with differing affinity. Derivations for the equilibrium binding function with two FcRn binding affinities are shown in [Electronic Supplementary Material III](#).

Within the modified equilibrium model, most parameters are consistent with those in the model published by Urva *et al.*; however, the coefficients $F1$ and $F2$ were added, as described for the catenary model above, to allow for mAb-to-mAb differences in antibody uptake and convective transport into the interstitial compartment. $F1$, $F2$, and τ_{LY} were simultaneously estimated by fitting the model to the murine mAb 7E3 plasma data in both wild-type and FcRn knockout animals. The endogenous IgG production rate, $K0$, was then fixed to allow the appropriate steady-state concentration of endogenous IgG. To evaluate the performance of the two models, observed 7E3 plasma concentration data were compared with model predicted data. For each PBPK model, correlation between the model predicted concentration and

observed concentration was calculated. Prediction error at each data point was calculated as:

$$C_{\text{error}} = \frac{(C_{\text{pred}} - C_{\text{obs}})}{C_{\text{obs}}}$$

Comparison of Model Predictions of the Relationship Between FcRn Binding Affinity and mAb Disposition. Simulations were conducted with the catenary and equilibrium PBPK models to examine the relationship between mAb-FcRn binding affinity at pH 6 and predicted disposition profiles. FcRn-mAb binding affinity was modulated 10,000-fold, with equilibrium binding affinity ranging from 0.01- to 100-fold of the FcRn affinity for endogenous IgG. In simulations with the catenary model, microconstants are used to define FcRn-mAb binding, and the equilibrium affinity at pH 6 is the ratio: $k_{\text{on}}/k_{\text{off}}$ within the pH 6 sub-compartment in the endosomal transit cascade. Changes in affinity were achieved by either adjusting k_{on} relative to k_{off} , or by changing k_{off} relative to k_{on} . As such, for the catenary PBPK model, two sets of predictions were made. To facilitate comparison of model predictions, terminal plasma half-lives were calculated from the log-linear decline profile of the simulated data.

Software

All parameter estimations and simulations were performed in ADAPT V (48), using maximum likelihood estimation.

RESULTS

T84.66 Binding to FcRn: Effect of pH

SPR sensorgrams demonstrated decreased binding of T84.66 to murine FcRn with increasing pH. At pH 7.4, there was no detectable binding of the mAb to FcRn. At pH 7.0, binding was only detectable at antibody concentration of 184 nM and greater. However, under more acidic conditions (pH 6.0 and 6.5), T84.66 binding responses were detectable at a wide range of concentrations. Estimated values of k_{on} and k_{off} , as associated with the high-affinity site of the heterogeneous-ligand model, were used as the binding parameter values for the catenary PBPK model (Table IV). Binding capacity (R_{max}) decreased with increasing pH, which indicates a loss of mAb binding capacity, in addition to reductions in binding affinity, with changes in ionization of FcRn (and or IgG).

Table IV. Kinetic Binding Parameters for T84.66-FcRn Interaction at Different pH Conditions Represented in the Catenary PBPK Model

	k_{on} (1/M min)	k_{off} (1/min)	R_{max} (RU)
pH 6.0	7.08E+05	6.96E-02	92.1
pH 6.5	7.14E+04	5.19E-04	34.7
pH 7.0	3.34E+04	2.94E-02	23.6

Both endogenous and exogenous antibodies

Fitting of 7E3 Data

The catenary and equilibrium models each provided very good fitting of previously published plasma concentration vs. time data for a murine monoclonal antibody, 7E3, following i.v. dosing of 8 mg/kg to wild-type mice and to FcRn-deficient (knockout) mice (38). Simulated data is shown with observed data in Fig. 4, and the fitted parameters from each model ($F1$, $F2$, and Tau_{LY}) are listed in Tables V and VI. The correlation coefficient (r^2) for model predicted data and observed data were 0.978 and 0.971 for the equilibrium model and catenary model, respectively. The median concentration prediction errors were 3.38% and 3.79% for the catenary model and equilibrium model, respectively.

Comparison of Model Predictions of the Relationship Between FcRn Binding Affinity and mAb Disposition

Each model predicts that an alteration of FcRn binding affinity at pH 6 will impact mAb disposition and terminal half-life (Fig. 5). As shown in the figure, the equilibrium

Fig. 4. Characterization of 7E3 data by the catenary PBPK model and the modified equilibrium PBPK model. 7E3 data (symbols), following i.v. injection of 8 mg/kg to wild-type and FcRn-deficient mice were collected from prior work (41). Parameters of the equilibrium and catenary PBPK models were determined by fitting to the plasma data. Solid lines represent the best fitted profiles for **a** the catenary PBPK model, and **b** the equilibrium PBPK model

Table V. Estimated Pharmacokinetic Parameters for the Catenary PBPK Model

Parameters	Estimated value	CV%
$F1$	0.26	20.2
$F2$	0.94	0.469
Tau_{LY} (min)	544	13.3
$K0$ (mole/min)	$2.82\text{E}-12$	0.001

model predicts a near proportional increase in terminal half-life with increased FcRn binding affinity at pH 6, while the catenary model predicts much more moderate changes in half-life. Compared to experimental data published in the literature, the predictions of the equilibrium model greatly over-predict the effects of increased FcRn binding on mAb half-life, whereas predictions of the catenary model are more consistent with the published data (Table I). Interestingly, the catenary model also predicts that there is greater benefit found from an increase in k_{on} vs. from a decrease in k_{off} . For example, with a 100-fold increase in affinity (*i.e.*, k_{on}/k_{off}) achieved *via* a 100-fold decrease in k_{off} , the catenary model predicts a 1.5-fold increase in half-life. For the same increase in affinity, achieved with a 100-fold increase in k_{on} , the model predicts a 7.3-fold increase in half-life (Fig. 5).

DISCUSSION

As it is well accepted that FcRn is responsible for the extended systemic persistence of IgG, there has been great interest in modulating mAb–FcRn interaction to improve the pharmacokinetic properties of therapeutic monoclonal antibodies. Although several PBPK models for IgG disposition have been published, none of the prior models have been structured to consider the relationships between the time-course of FcRn binding and the timecourse of endosomal transit. The unique feature of the new catenary PBPK model presented in this work is that it attempts to approximate the pH and time dependence of FcRn binding and endosomal processing of IgG. The catenary PBPK model retains several features that were incorporated in prior PBPK models, and the new model is equally capable of characterizing the disposition of wild-type mAb, including mAb disposition in FcRn-deficient mice. However, the new PBPK model differs dramatically from prior models with respect to the prediction of the relationships between mAb–FcRn binding and mAb disposition *in vivo*. The catenary model predicts much more modest effects relative to equilibrium models, and the catenary model predictions are much more in line with data observed in the published literature.

Table VI. Estimated Pharmacokinetic Parameters for the Equilibrium PBPK Model

Parameters	Estimated value	CV%
$F1$	1.68	24.6
$F2$	1.05	0.097
Tau_{LY} (min)	776	16.24
$K0$ (mol/min)	$7.76\text{E}-12$	0.003

Fig. 5. Model predicted fold-change in half-life with changes in FcRn binding affinity at pH 6. Panel **a** model predicted changes in the terminal half-life of mAb as a function of up to 10-fold changes in mAb-FcRn binding affinity. Panel **b** model predicted changes in half-life with changes in FcRn binding, shown in log scale. Predictions by equilibrium PBPK model are shown in *dash-dot lines*. Predictions of the catenary PBPK model, where k_{on} is altered to adjust binding affinity, are shown with *solid lines*. Predictions of the catenary PBPK model, where k_{off} is altered to adjust binding affinity, are shown with *dashed lines*.

The catenary PBPK model differs from prior PBPK models of mAb disposition in several ways. First, tissue-specific FcRn expression has been incorporated. In the PBPK models of Garg and Balthasar (38) and Urva *et al.* (39), FcRn concentration was assumed to be the same in all tissues. Analysis of FcRn expression indicates that FcRn expression may vary substantially among different organs (7,49–51). To the best of our knowledge, experimentally determined data for FcRn protein concentration in tissues in any species are not available. In recent work, we have investigated FcRn mRNA expression by quantitative RT-PCR (manuscript in review), and we have attempted to approximate tissue-specific FcRn protein expression with the simple assumption that endosomal FcRn concentration is proportional to FcRn mRNA expression. Tissue FcRn concentrations were calculated from the experimentally determined values of relative mRNA expression and a whole-body averaged FcRn concentration. The FcRn concentration estimate was obtained through a model-based analysis of the disposition of an anti-FcRn mAb (1G3) in mice (manuscript in review). The estimated FcRn concentrations are based on several untested assumptions; however, the FcRn concentration estimates have been used simply as a starting point to allow model building. In future work, we plan to employ specific FcRn

probes to determine tissue-specific FcRn protein concentrations, which may allow improvement and further refinement of the model.

The new PBPK model also differs from prior models with respect to the return of IgG from the lymph nodes to the systemic circulation. Based on our prior investigations of the bioavailability of mAb following subcutaneous dosing to wild-type and FcRn-deficient mice, where mAb bioavailability was 82% in wild-type animals vs. 28% in knockout mice (41), the efficiency of IgG return was set as a function of IgG binding to FcRn (detailed in [Electronic Supplementary Material II](#)). The Garg and Urva models assume that all IgG drained from the interstitial compartment through the lymphatics is collected by lymph nodes and transited to the systemic circulation (39).

The catenary PBPK model also differs from the Urva and Garg models with respect to the uptake of IgG into the endosomal space of the vascular endothelium. In the new model, uptake rates for individual tissues have been derived from fluid pinocytosis rates for monolayer endothelial cells, while in previous models (38,39,45), tissue uptake rates were estimated by model fitting, and differed substantially from model-to-model. For example, in the models developed by Urva and Garg (38,39), the estimated endosomal uptake rate constants were 1.96 and 0.715 day^{-1} , and the value used in the model by Ferl *et al.* was $9.6 \times 10^{-3} \text{ min}^{-1}$, corresponding to 13.8 day^{-1} . Due to the use of a tissue-specific endosomal uptake clearance, and as a consequence of setting the endosomal volume to allow a fixed transit time for the endosomal cascade, the endogenous IgG concentrations in (all) endosomes closely approximates the steady-state endogenous IgG concentration in the vascular space. This model feature is desirable, as it is consistent with the present conceptual model of IgG uptake *via* fluid-phase endocytosis.

The catenary PBPK model and the equilibrium PBPK model each provided very good characterization of 7E3 disposition in FcRn-deficient mice and in wild-type mice (Fig. 4). Fitted model parameters ($F1$, $F2$, Tau_{LY} , and $K0$) were estimated with high precision and with similar values for the two models (*i.e.*, within 1–3-fold). However, the parameter estimates were not identical, and the confidence intervals did not overlap for the estimates of Tau_{LY} or for the estimates of $K0$. The finding of different, but precise, parameter estimates illustrates that the “identifiability” of fitted PBPK model parameters does not necessarily reflect the accuracy of the parameters (or, in other words, the accuracy of the quantitative depiction of biological processes). Parameter estimates may be dependent on all aspects of the model structure, and confidence in the model structure is perhaps best built through the evaluation of model predictions (*vs. via* assessment of the precision of fitted parameters).

The catenary model predicts that reductions in the rate constant of FcRn-IgG dissociation (k_{off}) at pH 6 only allow a ~1.5-fold increase in mAb half-life, relative to the half-life of endogenous IgG. This prediction is logical if one considers that the rate of dissociation for wild-type antibody is relatively slow compared to the timecourse of endosomal transit. Reductions of k_{off} are fruitless if the rate of movement of IgG through the endosomal transit process occurs more rapidly than the rate of complex dissociation. Additional simulations of the influence of binding parameters at other pH conditions suggest a strong relationship between the

predicted mAb half-life and rates of mAb–FcRn association (k_{on}) in early endosomes, where pH is 6.5–7. It is plausible that point mutations that allow an increase in mAb–FcRn equilibrium binding at pH 6 may also lead to changes in the rates of antibody association or dissociation at other pH conditions. This type of possibility, which may be easily considered with the catenary model, may explain why some investigators have found that mAb engineered for increased affinity at pH 6 often demonstrate no improvement in half-life (14,16,20,21,23,26), and occasionally demonstrate a decrease in half-life (16,20,23), relative to the wild-type mAb variant.

The catenary model, while more complex than prior PBPK models, is still quite simplistic. Major simplifying assumptions of the new model include the use of fixed pH conditions within the vascular endothelial endosomes, the use of a fixed transit time for all sub-compartments, the use of FcRn binding parameters from a model antibody (T84.66), and the assumption that binding parameters obtained *in vitro* at 25°C will be identical to those found *in vivo*. It is unlikely that the model will serve as an accurate predictor of IgG disposition under all conditions; however, the model does appear to be capable of capturing the major features of mAb disposition, including rapid, monoexponential mAb disposition in FcRn-deficient mice. Additionally, in comparison with equilibrium binding models, the catenary model clearly provides predictions of the effects of altered mAb–FcRn binding that are more consistent with experimental data. Many of the parameters associated with the endosomal transit sub-compartments will be difficult to verify experimentally; however, it is clear that the incorporation of the sub-compartment structure allows a more biologically realistic representation of the time dependence of transit of IgG, with movement through pH environments ranging pH 7.4–6.0, as IgG progresses from the moment of cellular endocytosis through sorting for catabolism or for extracellular release. Further refinement of the model may be possible by measuring binding parameters for each mAb of interest, by incorporating measured concentrations of FcRn protein in tissues, and by investigating tissue concentrations of engineered mAbs. Future work will include prospective evaluation of model predictions of the disposition of engineered mAbs in mice.

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REFERENCES

- Reichert JM. Marketed therapeutic antibodies compendium. *MAbs*. 2012;4(3):413–5.
- Reichert JM. Antibody-based therapeutics to watch in 2011. *MAbs*. 2011;3(1):76–99.
- Nelson AL, Reichert JM. Development trends for therapeutic antibody fragments. *Nat Biotechnol*. 2009;27(4):331–7. doi:10.1038/nbt0409-331.
- Simister NE, Mostov KE. Cloning and expression of the neonatal rat intestinal Fc receptor, a major histocompatibility complex class I antigen homolog. *Cold Spring Harb Symp Quant Biol*. 1989;54 (Pt 1):571–80.
- Story CM, Mikulska JE, Simister NE. A major histocompatibility complex class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus. *J Exp Med*. 1994;180(6):2377–81.
- Ward ES, Zhou J, Ghetie V, Ober RJ. Evidence to support the cellular mechanism involved in serum IgG homeostasis in humans. *Int Immunol*. 2003;15(2):187–95.
- Borvak J, Richardson J, Medesan C, Antohe F, Radu C, Simionescu M, *et al*. Functional expression of the MHC class I-related receptor, FcRn, in endothelial cells of mice. *Int Immunol*. 1998;10(9):1289–98.
- Montoyo HP, Vaccaro C, Hafner M, Ober RJ, Mueller W, Ward ES. Conditional deletion of the MHC class I-related receptor FcRn reveals the sites of IgG homeostasis in mice. *Proc Natl Acad Sci USA*. 2009;106(8):2788–93. doi:10.1073/pnas.0810796106.
- Junghans RP. Finally! The Brambell receptor (FcRB). Mediator of transmission of immunity and protection from catabolism for IgG. *Immunol Res*. 1997;16(1):29–57. doi:10.1007/BF02786322.
- Raghavan M, Bonagura VR, Morrison SL, Bjorkman PJ. Analysis of the pH dependence of the neonatal Fc receptor/immunoglobulin G interaction using antibody and receptor variants. *Biochemistry*. 1995;34(45):14649–57.
- Kacs Kovics I, Kis Z, Mayer B, West Jr AP, Tiangco NE, Tilahun M, *et al*. FcRn mediates elongated serum half-life of human IgG in cattle. *Int Immunol*. 2006;18(4):525–36. doi:10.1093/intimm/dxh393.
- Hinton PR, Johlfs MG, Xiong JM, Hanestad K, Ong KC, Bullock C, *et al*. Engineered human IgG antibodies with longer serum half-lives in primates. *J Biol Chem*. 2004;279(8):6213–6.
- Hinton PR, Xiong JM, Johlfs MG, Tang MT, Keller S, Tsurushita N. An engineered human IgG1 antibody with longer serum half-life. *J Immunol*. 2006;176(1):346–56.
- Petkova SB, Akilesh S, Sproule TJ, Christianson GJ, Al Khabbaz H, Brown AC, *et al*. Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humoral mediated autoimmune disease. *Int Immunol*. 2006;18(12):1759–69. doi:10.1093/intimm/dxh110.
- Yeung YA, Wu X, Reyes 2nd AE, Vernes JM, Lien S, Lowe J, *et al*. A therapeutic anti-VEGF antibody with increased potency independent of pharmacokinetic half-life. *Cancer Res*. 2010;70 (8):3269–77. doi:10.1158/0008-5472.CAN-09-4580.
- Deng R, Loyet KM, Lien S, Iyer S, DeForge LE, Theil FP, *et al*. Pharmacokinetics of humanized monoclonal anti-tumor necrosis factor- α antibody and its neonatal Fc receptor variants in mice and cynomolgus monkeys. *Drug Metab Dispos*. 2010;38 (4):600–5. doi:10.1124/dmd.109.031310.
- Ghetie V, Popov S, Borvak J, Radu C, Matesoi D, Medesan C, *et al*. Increasing the serum persistence of an IgG fragment by random mutagenesis. *Nat Biotechnol*. 1997;15(7):637–40. doi:10.1038/nbt0797-637.
- Zalevsky J, Chamberlain AK, Horton HM, Karki S, Leung IW, Sproule TJ, *et al*. Enhanced antibody half-life improves *in vivo* activity. *Nat Biotechnol*. 2010;28(2):157–9. doi:10.1038/nbt.1601.
- Dall'Acqua WF, Kiener PA, Wu H. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). *J Biol Chem*. 2006;281(33):23514–24. doi:10.1074/jbc.M604292200.
- Datta-Mannan A, Witcher DR, Tang Y, Watkins J, Jiang W, Wroblewski VJ. Humanized IgG1 variants with differential binding properties to the neonatal Fc receptor: relationship to pharmacokinetics in mice and primates. *Drug Metab Dispos*. 2007;35(1):86–94.
- Datta-Mannan A, Witcher DR, Tang Y, Watkins J, Wroblewski VJ. Monoclonal antibody clearance. Impact of modulating the interaction of IgG with the neonatal Fc receptor. *J Biol Chem*. 2007;282(3):1709–17. doi:10.1074/jbc.M607161200.
- Gurbaxani B, Dela Cruz LL, Chintalacharuvu K, Morrison SL. Analysis of a family of antibodies with different half-lives in mice fails to find a correlation between affinity for FcRn and serum half-life. *Mol Immunol*. 2006;43(9):1462–73. doi:10.1016/j.molimm.2005.07.032.
- Dall'Acqua WF, Woods RM, Ward ES, Palaszynski SR, Patel NK, Brewah YA, *et al*. Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol*. 2002;169(9):5171–80.

24. Suzuki T, Ishii-Watabe A, Tada M, Kobayashi T, Kanayasu-Toyoda T, Kawanishi T, *et al.* Importance of neonatal FcR in regulating the serum half-life of therapeutic proteins containing the Fc domain of human IgG1: a comparative study of the affinity of monoclonal antibodies and Fc-fusion proteins to human neonatal FcR. *J Immunol.* 2010;184(4):1968–76. doi:10.4049/jimmunol.0903296.
25. Yeung YA, Leabman MK, Marvin JS, Qiu J, Adams CW, Lien S, *et al.* Engineering human IgG1 affinity to human neonatal Fc receptor: impact of affinity improvement on pharmacokinetics in primates. *J Immunol.* 2009;182(12):7663–71.
26. Vaccaro C, Bawdon R, Wanjie S, Ober RJ, Ward ES. Divergent activities of an engineered antibody in murine and human systems have implications for therapeutic antibodies. *Proc Natl Acad Sci U S A.* 2006;103(49):18709–14. doi:10.1073/pnas.0606304103.
27. Maxfield FR, McGraw TE. Endocytic recycling. *Nat Rev Mol Cell Biol.* 2004;5(2):121–32.
28. Ober RJ, Martinez C, Vaccaro C, Zhou J, Ward ES. Visualizing the site and dynamics of IgG salvage by the MHC class I-related receptor, FcRn. *J Immunol.* 2004;172(4):2021–9.
29. Hopkins CR, Trowbridge IS. Internalization and processing of transferrin and the transferrin receptor in human carcinoma A431 cells. *J Cell Biol.* 1983;97(2):508–21.
30. Lafourcade C, Sobo K, Kieffer-Jaquinod S, Garin J, van der Goot FG. Regulation of the V-ATPase along the endocytic pathway occurs through reversible subunit association and membrane localization. *PLoS One.* 2008;3(7). doi:10.1371/Journal.Pone.0002758.
31. Yamashiro DJ, Maxfield FR. Kinetics of endosome acidification in mutant and wild-type Chinese hamster ovary cells. *J Cell Biol.* 1987;105(6 Pt 1):2713–21.
32. Vaughn DE, Bjorkman PJ. High-affinity binding of the neonatal Fc receptor to its IgG ligand requires receptor immobilization. *Biochemistry.* 1997;36(31):9374–80. doi:10.1021/bi970841r.
33. Baxter LT, Zhu H, Mackensen DG, Jain RK. Physiologically based pharmacokinetic model for specific and nonspecific monoclonal antibodies and fragments in normal tissues and human tumor xenografts in nude mice. *Cancer Res.* 1994;54(6):1517–28.
34. Covell DG, Barbet J, Holton OD, Black CD, Parker RJ, Weinstein JN. Pharmacokinetics of monoclonal immunoglobulin G1, F(ab')₂, and Fab' in mice. *Cancer Res.* 1986;46(8):3969–78.
35. Baxter LT, Zhu H, Mackensen DG, Butler WF, Jain RK. Biodistribution of monoclonal antibodies: scale-up from mouse to human using a physiologically based pharmacokinetic model. *Cancer Res.* 1995;55(20):4611–22.
36. Friedrich SW, Lin SC, Stoll BR, Baxter LT, Munn LL, Jain RK. Antibody-directed effector cell therapy of tumors: analysis and optimization using a physiologically based pharmacokinetic model. *Neoplasia.* 2002;4(5):449–63. doi:10.1038/sj.neo.7900260.
37. Zhu H, Melder RJ, Baxter LT, Jain RK. Physiologically based kinetic model of effector cell biodistribution in mammals: implications for adoptive immunotherapy. *Cancer Res.* 1996;56(16):3771–81.
38. Garg A, Balthasar JP. Physiologically-based pharmacokinetic (PBPK) model to predict IgG tissue kinetics in wild-type and FcRn-knockout mice. *J Pharmacokinetic Pharmacodyn.* 2007;34(5):687–709. doi:10.1007/s10928-007-9065-1.
39. Urva SR, Yang VC, Balthasar JP. Physiologically based pharmacokinetic model for T84.66: a monoclonal anti-CEA antibody. *J Pharm Sci.* 2010;99(3):1582–600. doi:10.1002/jps.21918.
40. Ed Harlow DL. *Antibodies: a laboratory manual.* New York: CSHL Press; 1988.
41. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther.* 2008;84(5):548–58. doi:10.1038/clpt.2008.170.
42. Davies PF, Ross R. Mediation of pinocytosis in cultured arterial smooth muscle and endothelial cells by platelet-derived growth factor. *J Cell Biol.* 1978;79(3):663–71.
43. Davies PF, Selden 3rd SC, Schwartz SM. Enhanced rates of fluid pinocytosis during exponential growth and monolayer regeneration by cultured arterial endothelial cells. *J Cell Physiol.* 1980;102(2):119–27. doi:10.1002/jcp.1041020204.
44. Hahnfeldt P, Panigrahy D, Folkman J, Hlatky L. Tumor development under angiogenic signaling: a dynamical theory of tumor growth, treatment response, and postvascular dormancy. *Cancer Res.* 1999;59(19):4770–5.
45. Ferl GZ, Wu AM, DiStefano 3rd JJ. A predictive model of therapeutic monoclonal antibody dynamics and regulation by the neonatal Fc receptor (FcRn). *Ann Biomed Eng.* 2005;33(11):1640–52. doi:10.1007/s10439-005-7410-3.
46. Boswell CA, Tesar DB, Mukhyala K, Theil FP, Fielder PJ, Khawli LA. Effects of charge on antibody tissue distribution and pharmacokinetics. *Bioconjug Chem.* 2010;21(12):2153–63. doi:10.1021/bc100261d.
47. Igawa T, Tsunoda H, Tachibana T, Maeda A, Mimoto F, Moriyama C, *et al.* Reduced elimination of IgG antibodies by engineering the variable region. *Protein Eng Des Sel.* 2010;23(5):385–92. doi:10.1093/protein/gzq009.
48. D'Argenio DS. ADAPT V User's Guide: Pharmacokinetic/Pharmacodynamic system analysis software. In: *Biomedical Simulation Resource*; 1997.
49. Dzidic A, Mohr A, Meyer K, Bauer J, Meyer HH, Pfaffl MW. Effects of mycophenolic acid (MPA) treatment on expression of Fc receptor (FcRn) and polymeric immunoglobulin receptor (pIgR) mRNA in adult sheep tissues. *Croat Med J.* 2004;45(2):130–5.
50. Akilesh S, Christianson GJ, Roopenian DC, Shaw AS. Neonatal FcR expression in bone marrow-derived cells functions to protect serum IgG from catabolism. *J Immunol.* 2007;179(7):4580–8.
51. Ghetie V, Hubbard JG, Kim JK, Tsen MF, Lee Y, Ward ES. Abnormally short serum half-lives of IgG in beta 2-microglobulin-deficient mice. *Eur J Immunol.* 1996;26(3):690–6. doi:10.1002/eji.1830260327.