

Utility of Physiologically-based Pharmacokinetic Model to Predict Inter-Antibody Variability in Monoclonal Antibody Pharmacokinetics

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Objectives

In this investigation, we tested the hypothesis that a physiologically based pharmacokinetic (PBPK) model incorporating measured *in vitro* metrics of off-target binding can largely explain the inter-antibody variability in monoclonal antibody (mAb) pharmacokinetics (PK). We measured *in vivo* mouse PK and evaluated 10 assays selected to exploit a broad range of mAb physiochemical attributes across a diverse panel of 83 mAbs. We modified an existing PBPK platform to establish meaningful relationships between mAb-specific *in vitro* metrics of off-target binding and tissue uptake clearance. The objective was to evaluate the predictive utility of this PBPK model to triage and de-risk antibodies in early discovery preclinical screening allowing a priori characterization of mAb concentration-time profiles.

Process d

Methods

mAb selection: 83 human IgG1z stable effector functionless mAbs were selected, 19 were eliminated due to mouse cross-reactivity to avoid TMDD and 8 mAbs were removed due to immunogenicity. Remaining 56 mAbs were pursued for this investigation

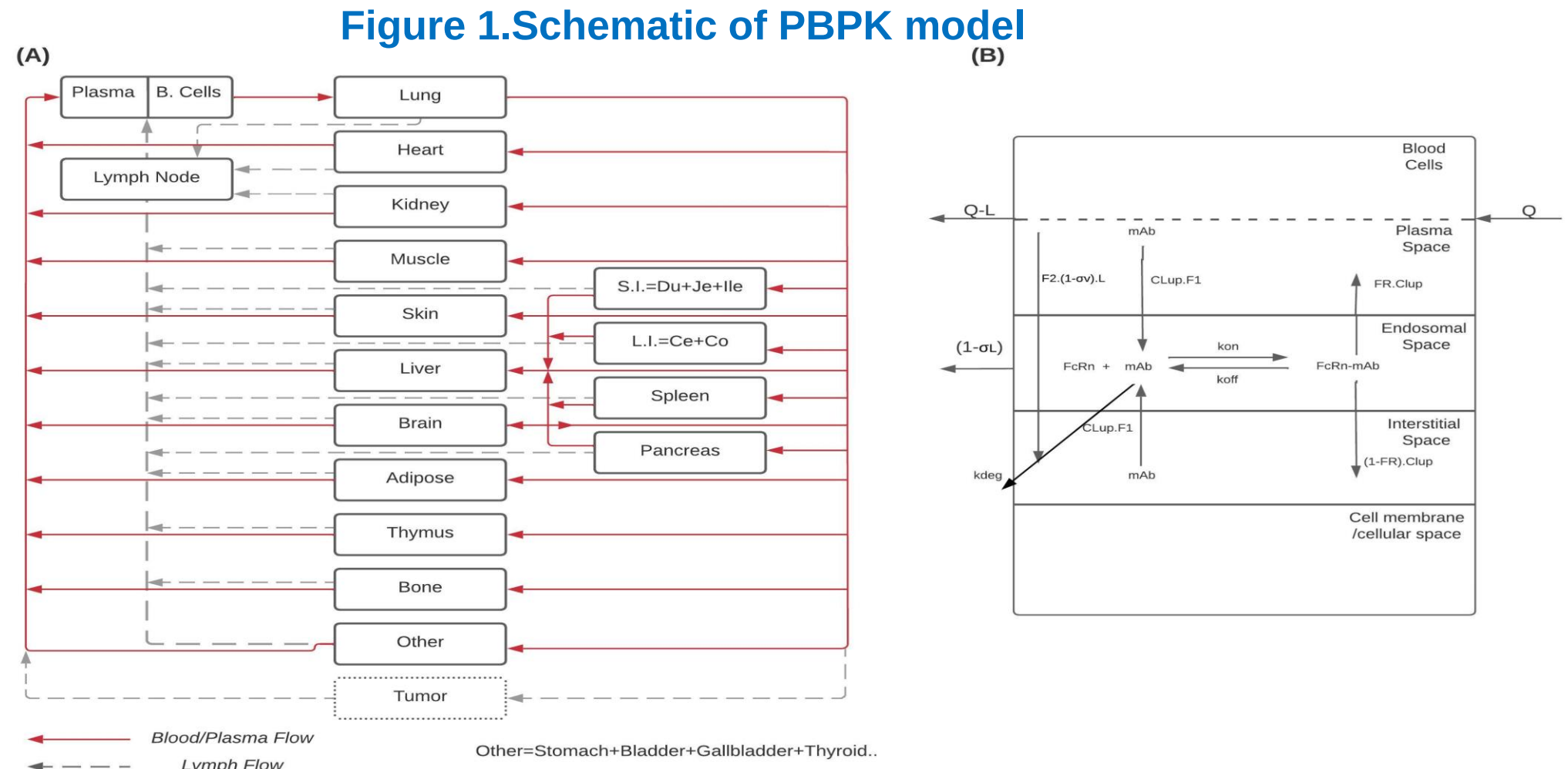
PK Study: All research protocols were reviewed and approved by the Amgen Institutional Animal Care and Use Committee (IACUC).

Female BALB/c mice were ordered at 6–8 weeks of age from Charles River Laboratories (Hollister, CA). For each dosing group, the test articles were combined into a single vial to generate the cassettes (a total of 5 mAbs/cassette). The dose level for each mAb per cassette was 2 mg/kg. mAb cross-reactive to mouse antigens were excluded from the study. Quantitation of mAbs in mouse serum was performed using multi-plex electro-chemiluminescent immunoassays

In vitro Assays: Ten *in vitro* assays as listed in Table 1 were pursued for all 56 mAbs

PBPK model: The base PBPK model is a combination of the platform PBPK model developed by Shah and Betts¹ and variability terms described by Chen and Balthasar²

Briefly, *F1* was multiplied by the pinocytosis rate (*CL_{up}*) to reflect variability in cellular uptake, whereas *F2* was multiplied by organ lymphatic flow (*L*(1 – σ)) to represent variability in the convection (Figure 1 A & B)



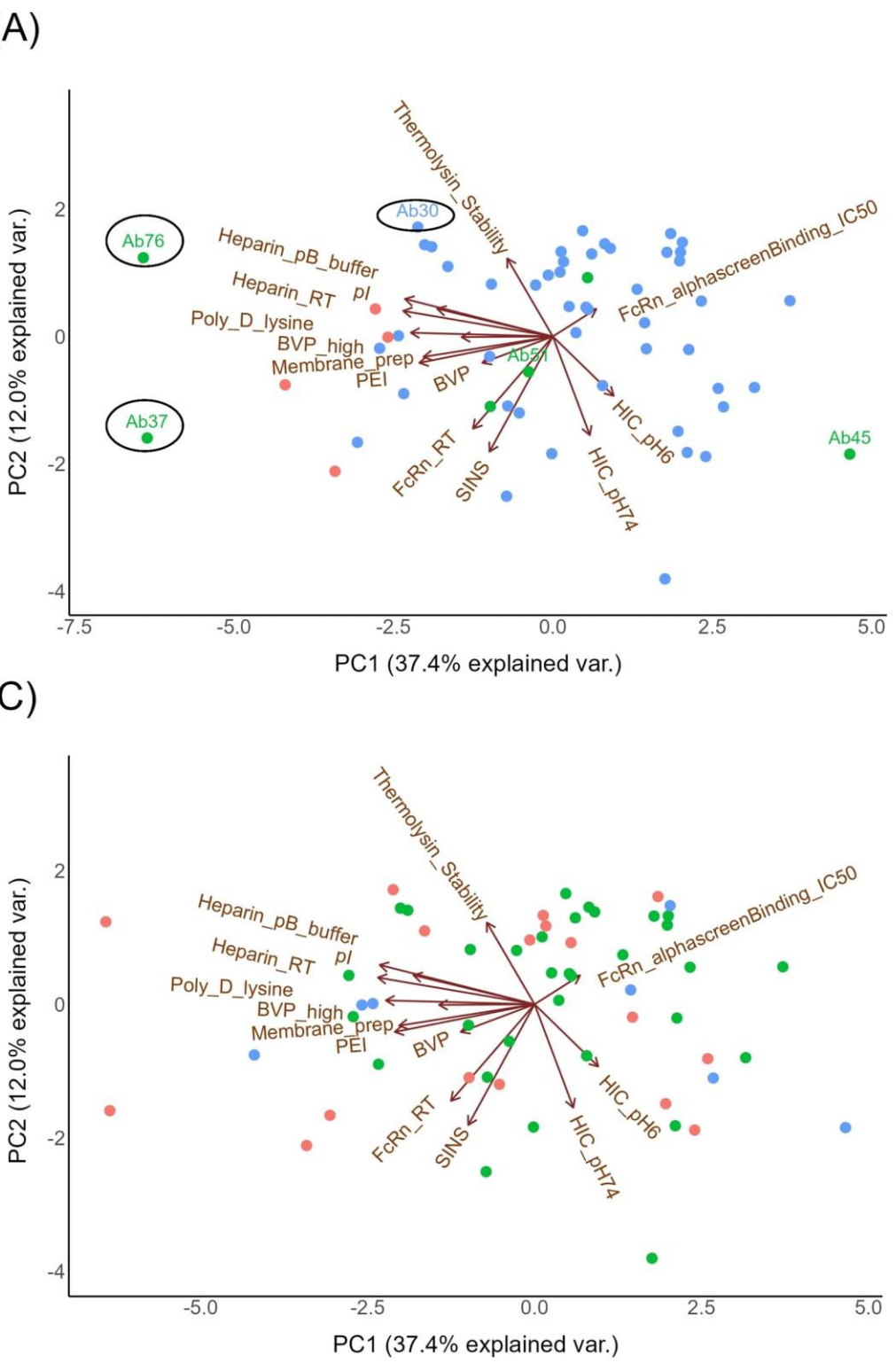
Results

PK data: An approximately 8-fold difference in the area under the curve (AUC) was observed, with the minimal and maximal *AUC*_(0–672h) being 1.74×10⁶ and 1.38×10⁷ ng·h/mL, respectively.

Accordingly, a roughly 10-fold variability in clearance (Figure 2A & 2B) was obtained (2.55 – 26.4 mL/day/kg).

Identification of covariate(s) in the PBPK model: *F1* values were estimated with very good precision (CV%<15%), and the estimates range from 0.419 to 3.61, with the median, mean, and standard deviation of 0.799, 0.961, and 0.593, respectively. The range, median, mean, and standard deviation of *F2* are [1.20e-7, 17.6], 1.44, 2.13, and 2.62, respectively.

Principal component analysis (PCA) plot indicates that many assay outputs are highly correlated, such as BVP, BVP_high, Heparin_RT (Heparin chromatography retention time), Heparin_pB_buffer (Heparin



percent B buffer), poly_D_lysin, PEI, membrane_prep, and calculated pi (Figure 3B). The *F1* PCA plot shows no separation between the low- and medium- *F1* classes, but the mAbs with high *F1* estimates all have high scores in nonspecific binding assays (Figure 3A). In contrast, there is no clear pattern on the *F2* PCA result (Figure 3C). Accordingly, we did not further pursue establishing a covariate relationship for *F2*. A more detailed examination of correlations between estimated *F1* values and individual assays reveals that Heparin_RT is the most significant variable against which *F1* displays a trend (Figure 3D). Similar results were observed in Pairwise correlation analysis (Figure 4)

Figure 4: Correlation matrix of different *in vitro* assay results from the 56 mAbs in the training set. Pearson r linear correlations range from -1 (red) to 1 (blue).

Table 1: Data collected on the antibody panel

Attribute	Method	Conditions
<i>In vitro assay</i>		
Self-interaction	AC-SINS	[mAb] = 0.1 mg/mL 10 mM NaOAc, 150 mM NaCl, pH 5.2
Cross-interaction	BVP ELISA	[mAb] = 33 nM
	Membrane prep ELISA	[mAb] = 33 nM
Hydrophobic interaction	mAb-Pac HIC chromatography	[mAb] = 1 mg/mL
Charged interaction	Heparin chromatography	[mAb] = 0.4 mg/mL, 5 – 400 mM NaCl gradient
	PEI ELISA	[mAb] = 33 nM
	Poly-D-Lys ELISA	[mAb] = 33 nM
FcRn binding	FcRn chromatography	[mAb] = 0.4 mg/mL, pH 5.5 - pH 8.0 gradient
	FcRn AlphaScreen	[mAb] = 2 mg/mL - 0.009mg/mL, pH 6.0
Protease sensitivity	Thermolysin challenge	[mAb] = 2.89 nM, Thermolysin = 28.9 nM
<i>In vivo PK</i>		
Clearance	WT mouse cassette dosing	Female BALB/c mice 2 mg/kg per mAb 5 mAbs per cassette

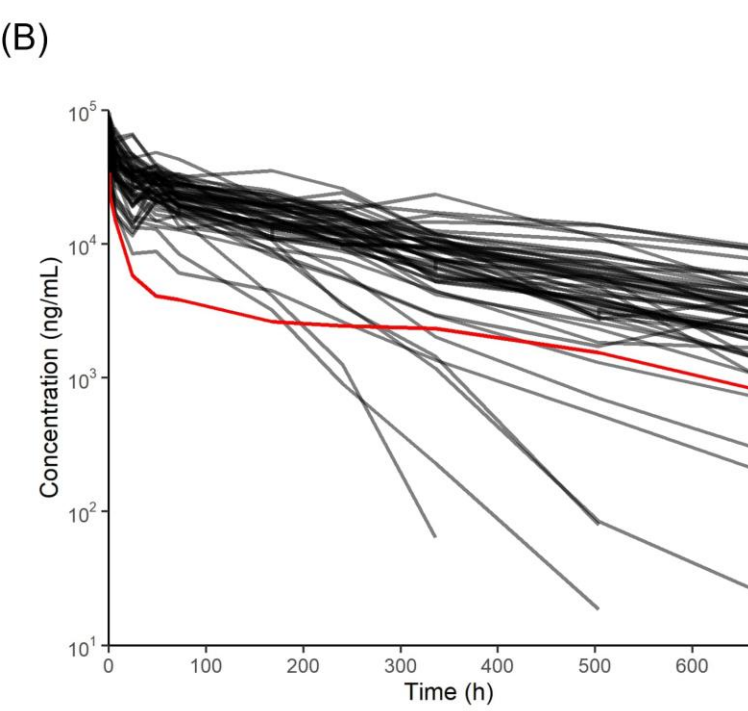


Figure 2B: A spaghetti plot showing the concentration versus time variability of the 56 mAbs. Most mAbs exhibit similar PK profiles with some exceptions.

Fitting of the PBPK model incorporating the Heparin_RT covariate:

The correlation plot (Figure 3D) suggests a sigmoidal relationship between mAb-specific *F1* and Heparin_RT, we expressed *F1* as follows

$$F1 = a + \frac{b - a}{1.0 + \left(\frac{c}{\text{Heparin_RT}} \right)^d}$$

Where *a*, *b*, and *c* are coefficients, and *d* is the exponent. This equation was incorporated into the PBPK model, and *a*, *b*, *c*, and *d* were estimated by simultaneously fitting PK data of all the selected 53 mAbs in the training set.

Estimation and simulation

The PBPK model incorporating the covariate relationship of *F1* was fitted simultaneously using

PK profiles of all the 53 mAbs. The estimates and associated

CV% for the coefficients/constants are

shown in Table 2, and the fitted profiles superimposed on actual data are shown in Figure 5A and 5B. This updated PBPK model captures PK

profiles of the majority of the mAbs with either high or low clearance. Sensitivity analysis of *F1* parameter suggests that plasma

concentrations are only sensitive to *F1* when *F1*>1 (Figure 6). This also indicates that there is marginal benefit to further account for the variability in PK when *F1*<1.

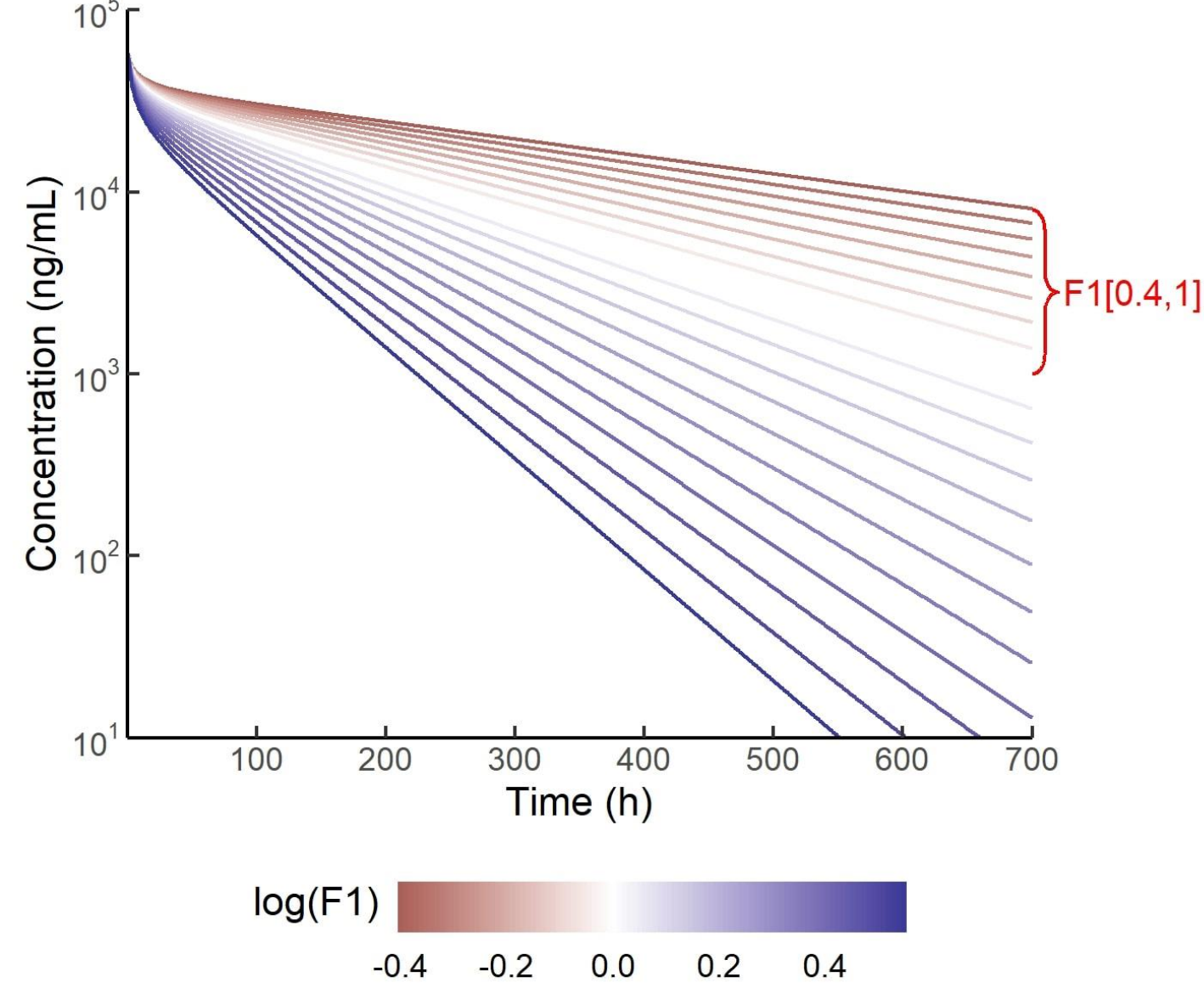


Figure 6: Sensitivity analysis of *F1*. PK profiles following a 2 mg/kg IV dose were shown. The color bar from red to blue indicates an *F1* range of 0.4 to 4.

Validation of the full PBPK model using a post-hoc test dataset

To test whether Heparin_RT is a useful predictor and if the full PBPK model could a priori predict PK data, we constructed monospecific SEFL2 antibody formats of these antibodies and conducted heparin chromatography only and *in vivo* PK studies in a manner similar to the training set²⁹. It was anticipated that this test panel of monospecific mAbs (n=14) would be biased towards exhibiting poor PK. Figure 7 shows model-predicted concentration-time profiles superimposed with raw data

Summary

Here, we used a PBPK model to predict the clearance and PK profiles of a panel of 53 mAbs in mice. To account for differences between the mAbs in the model, we evaluated the panel in 10 *in vitro* assays designed to interrogate a range of mAb physiochemical attributes. Using PCA and nonlinear regression, we found that Heparin_RT was

a key covariate of clearance variability in mice, more specifically, in the pinocytotic uptake coefficient *F1* in our PBPK model. Utilization of this covariate improved our ability to predict mAb PK, and our model was validated using an additional test set of 14 mAbs. Furthermore, heparin chromatography has now been implemented as a routine assay in-house to screen mAbs for potential PK issues prior to running *in vivo* studies

Publication

The data discussed here was recently published in mAb Journal. Citation: Liu, Humphreys, Thomas et. al. Utility of physiologically based pharmacokinetic modeling to predict inter-antibody variability in monoclonal antibody pharmacokinetics in mice mabs Journal 2023. Taylor and Francis, Vol 15

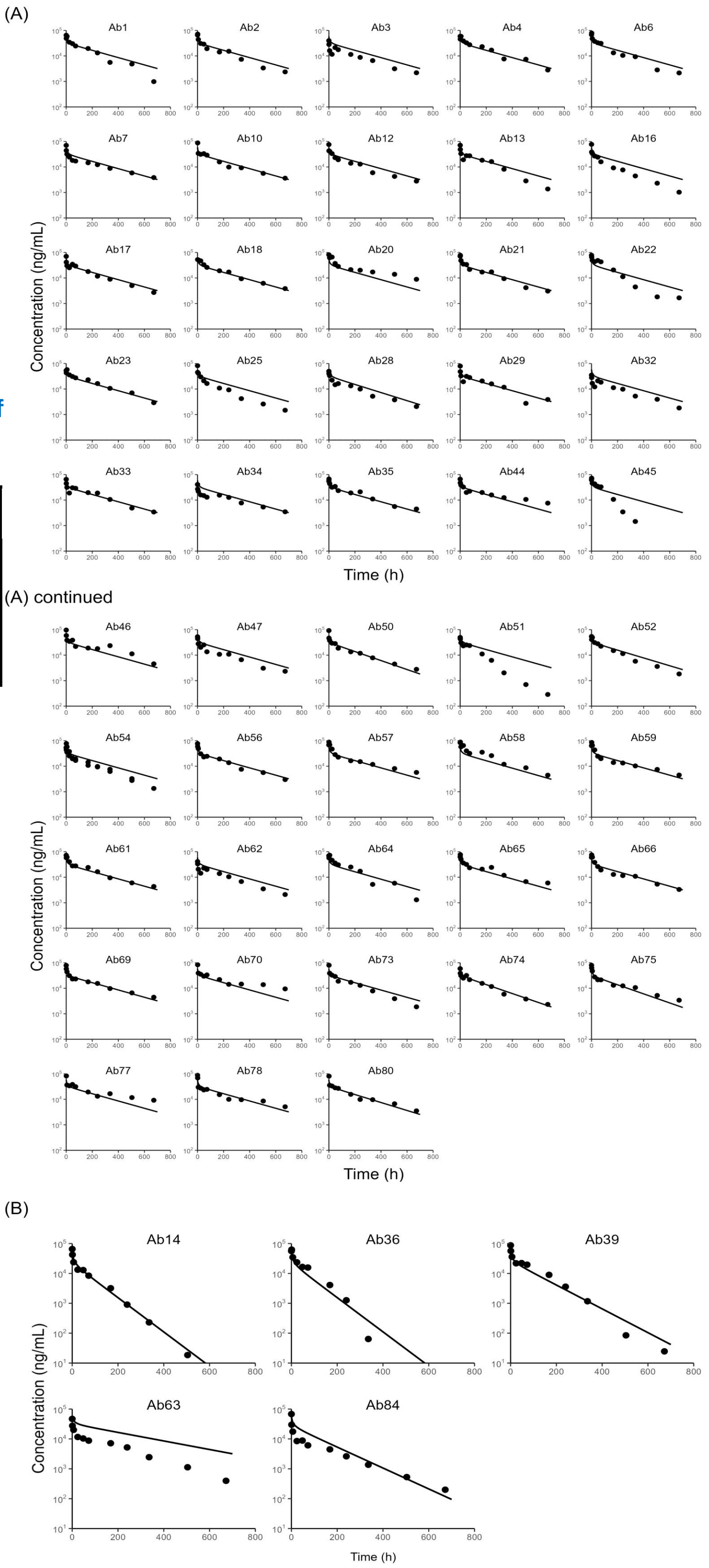


Figure 5: PK of mAbs in the training set that have (A) low clearance and (B) high clearance. Different mAbs were dosed IV into mice at a 2 mg/kg dose. Observed data are shown as solid dots, whereas model-fitted profiles are shown in solid lines.

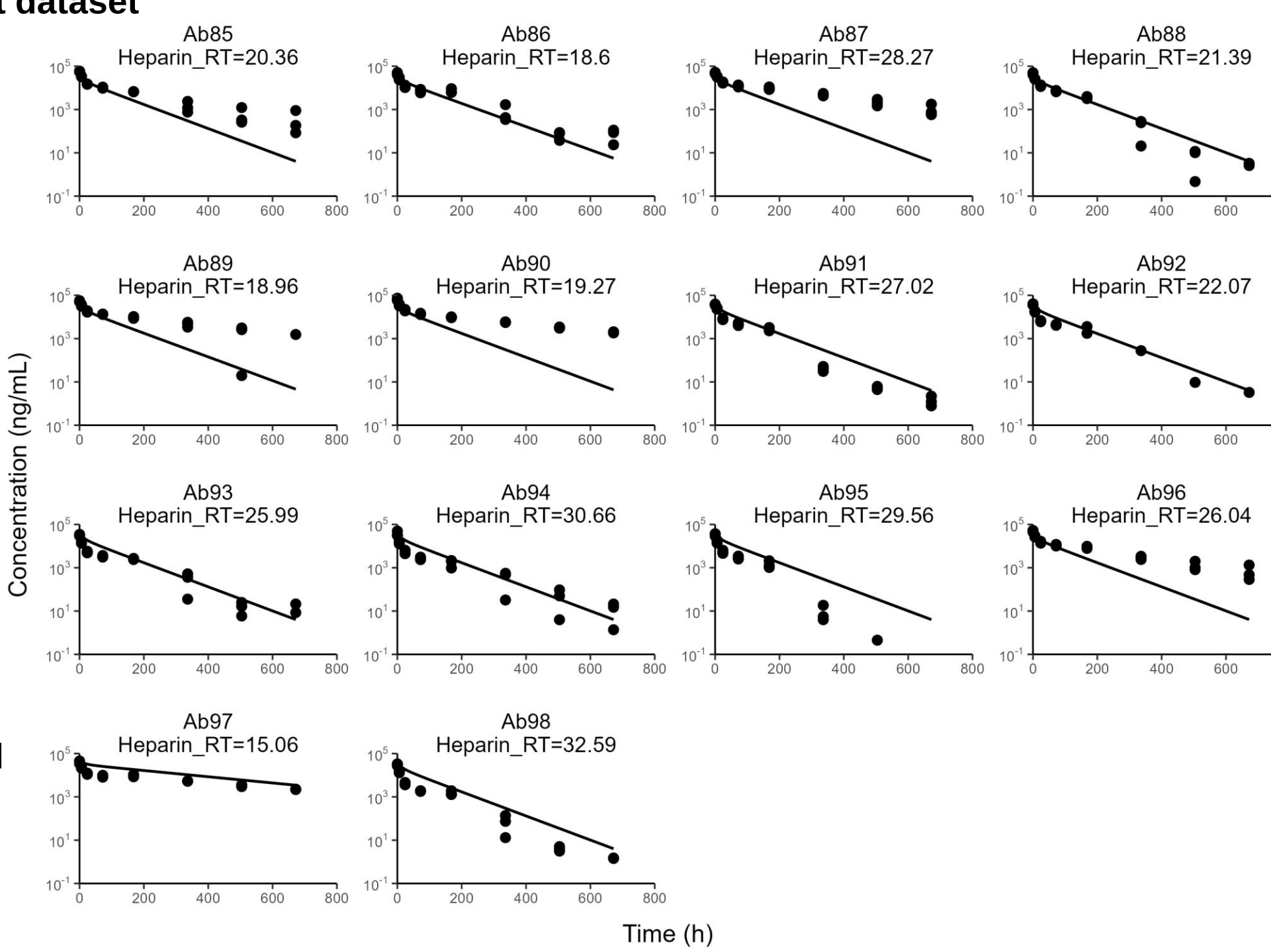


Figure 6: PK of mAbs in the test set. Different mAbs in the test set were dosed IV into mice at a 2 mg/kg dose. Observed data are shown as solid dots, whereas model-predicted profiles using obtained Heparin_RT as inputs are shown in solid lines

References

- [1] Shah, D. K.. and Betts. A. M. *Journal of pharmacokinetics and pharmacodynamics* 39 (2012): 67-86.
- [2] Chen Y. and Balthasar J.P. *AAPS Journal*, Vol. 14, No. 4, December 2012
- [3] Hu S. et.al. *mAbs Journal*. 2022, VOL. 14, NO. 1, e2056944

Conclusions and Perspectives

With the inclusion of heparin chromatography to inform model-estimated pinocytosis, our validated application of PBPK modelling to predict mAb clearance preclinically in wild-type mice provides further validation of a similar approach in humans that was recently published by Hu et al³. Taken together, these studies further substantiate mounting evidence that non-specific binding, largely modulated by interactions between positively charged patches on the mAb surface and negatively charged surfaces in the systemic circulation, the interstitium, the cell surface, and within endosomes, is a significant driver of mAb PK and an attribute that should routinely be evaluated *in vitro* using heparin chromatography prior to testing *in vivo* in animals and humans.