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REPORT

Prediction of human pharmacokinetics of Fc-engineered therapeutic monoclonal antibodies using human FcRn transgenic mice

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ABSTRACT

Human FcRn transgenic mice (Tg32) have been widely used to evaluate the pharmacokinetics of mAbs and predict human pharmacokinetics. This study aims to establish an approach for predicting the human pharmacokinetics of Fc-engineered mAbs with enhanced FcRn binding mutations using Tg32 mice. MAbs were intravenously administered at 10 mg/kg in the absence or presence of IVIG (1000 mg/kg) in Tg32 mice. Pharmacokinetic parameters (CL, Q, V_c, and V_p) estimated in Tg32 mice were compared with clinical data. Optimal allometric scaling exponents were determined to improve the accuracy of human pharmacokinetic predictions for Fc-engineered mAbs. Moreover, we predicted the plasma concentration-time profile after IV injection in humans using parameters estimated based on an optimized exponent. While normal mAbs exhibited a higher CL in the presence of IVIG compared to its absence, Fc-engineered mAbs showed comparable CL in both conditions. The larger difference in CL between normal and Fc-engineered mAbs observed in the presence of IVIG closely matched clinical study results. A significant positive correlation between Tg32 mice and humans was observed in the CL of Fc-engineered mAbs in both the absence and presence of IVIG. The estimated optimal exponents for CL, Q, V_c, and V_p were 0.73, 0.60, 0.95, and 0.87, respectively. Using these exponents, the plasma mAb concentration-time profile after IV injection in humans was accurately predicted. This study establishes a robust methodology for accurately predicting the human pharmacokinetics of Fc-engineered mAbs using Tg32 mice, achieving prediction accuracy comparable to that of cynomolgus monkeys. This approach, as a viable alternative to cynomolgus monkeys, can accelerate the preclinical development of promising Fc-engineered mAbs with enhanced FcRn binding.

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Introduction

Therapeutic monoclonal antibodies (mAbs) have become a promising therapeutic modality for the treatment of various diseases. One of their key advantages is their long half-life compared with other therapeutic modalities. Generally, mAbs show a 10–30 day half-life in humans.[1] This enables dosing regimens as infrequent as weekly to monthly injections.

It has been reported that the binding to the neonatal Fc receptor (FcRn) is a critical determinant of mAbs pharmacokinetics.[2] MAbs have been shown to exhibit significantly shortened half-lives in FcRn knock-out mice.[3] Also, Fc amino acid mutations that delete FcRn binding result in a shorter half-life compared to the parent mAb.[4] Conversely, several reports have shown that enhancing FcRn binding mutations at acidic pH significantly prolongs the half-life of mAbs in animals and humans.[5] Notably, the M252Y/S254T/T256E (YTE) and M428L/N434S (LS) mutations have been extensively investigated in various therapeutic programs. For example, in a clinical trial, motavizumab-YTE exhibited a half-life of 85 days, compared to 26.5 days for the parent mAb motavizumab.[6] Similarly, VRC01-LS showed a half-life of 71 days, significantly longer than the 15 days half-life of the parent mAb VRC01.[7] These YTE or LS mutations can extend the effective duration of mAbs, offering patients longer-lasting therapeutic benefits compared to unmodified parent mAbs. In recent years, the development of Fc-engineered mAbs with enhanced FcRn binding mutations, such as YTE and LS, has increased substantially.[5,8] These Fc-engineered mAbs are primarily being developed for infectious diseases such as HIV and SARS-CoV-2. The effective duration of these Fc-engineered mAbs is strongly influenced by their pharmacokinetic properties.

Generally, mAbs exhibit similar binding affinities to FcRn in cynomolgus monkeys and humans.[9] Thus, cynomolgus monkeys have often been used to predict the pharmacokinetics of mAbs in humans.[10] Previous research has shown that the plasma concentration-time profile of mAbs in humans can be accurately predicted from cynomolgus monkey data using the allometric scaling of linear two compartment model parameters (CL, Q, V_c, and V_p).[11] However, recent research has revealed that conventional allometric scaling clearly underpredicts the human pharmacokinetics of Fc-engineered mAbs with YTE or LS mutations when extrapolated from cynomolgus monkeys.[12] To address this, optimized allometric exponents were needed to accurately predict the plasma concentration-time profiles of engineered mAbs with YTE or LS mutations from cynomolgus monkeys to humans.

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While different scaling approaches are required for normal mAbs and Fc-engineered mAbs, cynomolgus monkeys remain a critically important model for the preclinical development of mAbs. However, the availability of cynomolgus monkeys has been greatly impacted by the SARS-CoV-2 pandemic, leading to a significant reduction in supply. Furthermore, the principles of the 3Rs – Reduction, Replacement, and Refinement – are increasingly emphasized in the context of animal welfare, highlighting the importance of developing alternative methods to reduce reliance on cynomolgus monkeys.

Transgenic mice expressing human FcRn were previously developed by the Jackson laboratory and have been reported to show a good correlation in CL and half-life of mAbs in humans. Among these, homozygous line 32 (Tg32) mice have been widely used to evaluate the pharmacokinetic properties of mAbs at various research facilities.[13,14] Previous reports have established optimal allometric scaling exponents for CL. Avery et al. reported that Tg32 mice showed good prediction accuracy for the human CL of normal mAbs using an exponent of 0.93.[15] Betts et al. further determined optimal exponents for predicting linear two compartment model parameters (CL, Q, V_c, and V_p) of normal mAbs from Tg32 mice to humans.[16] Reported optimal exponents for allometric scaling were 0.90, 0.67, 0.97, and 0.93 for CL, Q, V_c, and V_p, respectively. However, as observed with cynomolgus monkeys, the optimal allometric exponents for Fc-engineered mAbs with enhanced FcRn binding in Tg32 mice might differ from those of normal mAbs. To date, no studies have been published on predicting the human pharmacokinetics of Fc-engineered mAbs from Tg32 mice. Thus, the main purpose of this study is to establish optimized allometric scaling exponents for predicting the plasma concentration-time profiles of Fc-engineered mAbs with YTE or LS mutations from Tg32 mice to humans.

Although Tg32 mice express human FcRn in place of mouse FcRn, a notable difference between Tg32 mice and humans is the level of endogenous IgG. Tg32 mice carry mouse endogenous IgG, which has been reported to bind to human FcRn with very weak affinity.[9] Therefore, the plasma concentration of endogenous mouse IgG in Tg32 mice is significantly lower than the plasma concentration of endogenous human IgG in humans, as there is almost no FcRn-mediated recycling of mouse IgG in Tg32.[17] Due to very weak binding of mouse IgG to human FcRn, competition on FcRn with endogenous IgG in humans is not mimicked in Tg32 mice. Therefore, another goal of this study is to evaluate the impact of human IgG co-injections on the pharmacokinetics of Fc-engineered mAbs in Tg32 mice.

In this study, two parent mAbs and their Fc-engineered counterparts were evaluated in the absence and presence of intravenous immunoglobulin (IVIG) to investigate the impact of IVIG on the pharmacokinetics of normal and Fc-engineered mAbs in Tg32 mice. Additionally, exponents for the allometric scaling of Fc-engineered mAbs were optimized using 11 Fc-engineered mAbs with YTE or LS mutations to accurately predict human pharmacokinetics. The results in this study demonstrate that Tg32 mice can serve as an effective alternative to cynomolgus monkeys for predicting the pharmacokinetics of Fc-engineered mAbs in humans.

Results

Effect of endogenous human IgG on the pharmacokinetics of Fc-engineered mAbs with enhanced FcRn binding mutations

First, the effect of endogenous human IgG on the pharmacokinetics of normal mAbs and Fc-engineered mAbs was investigated in Tg32 mice. The pharmacokinetics of motavizumab, motavizumab-YTE, VRC01, and VRC01-LS in Tg32 mice were evaluated in the presence and absence of IVIG. As shown in Figures 1a,c and 2 and Table 1, motavizumab and VRC01 showed faster CL in the presence of IVIG than in its absence. This is likely due to competition for FcRn binding within the endosome between IVIG and mAbs, which partially inhibits FcRn-mediated recycling of the mAbs. In contrast, as shown in Figures 1b,d and 2 and Table 1, motavizumab-YTE and VRC01-LS showed comparable pharmacokinetics in both the absence and presence of IVIG. In humans, motavizumab-YTE and VRC01-LS showed 4.9-fold and 8.7-fold improvements in CL compared to motavizumab and VRC01, respectively.[6,7] In the absence of IVIG, although motavizumab-YTE and VRC01-LS showed lower CL than their parent mAbs in Tg32 mice, the CL ratios between motavizumab and motavizumab-YTE (1.9-fold) and between VRC01 and VRC01-LS (1.1-fold) in Tg32 mice were clearly smaller than those observed in humans (Figure 2 and Table 1). On the other hand, the co-injection of IVIG greatly increased the CL ratios between parent and Fc-engineered mAbs (7.0-fold in motavizumab-YTE and 5.0-fold in VRC01-LS), and the observed CL ratios in the presence of IVIG were closer to those in humans. This finding suggests that co-injection of IVIG in Tg32 mice mimics the optimal conditions for FcRn-mediated recycling in humans, enabling Tg32 mice to clearly detect the impact of enhanced FcRn binding on the pharmacokinetics of mAbs.

Pharmacokinetics of Fc-engineered mAbs in Tg32 mice

A total of 11 Fc-engineered mAbs (five with YTE and six with LS) were evaluated in Tg32 mice in the presence and absence of IVIG. As shown in Figure 3 and Table 2, all mAbs showed comparable pharmacokinetics in both the presence and absence of IVIG. As shown in Figure 4a, e, CL in Tg32 mice significantly correlated with human CL under both conditions ($r = 0.897$ without IVIG and $r = 0.922$ with IVIG). This result supports the utility of Tg32 mice for selecting Fc-engineered mAb candidates with potentially favorable PK properties in humans. While significant correlations were observed in both conditions, the presence of

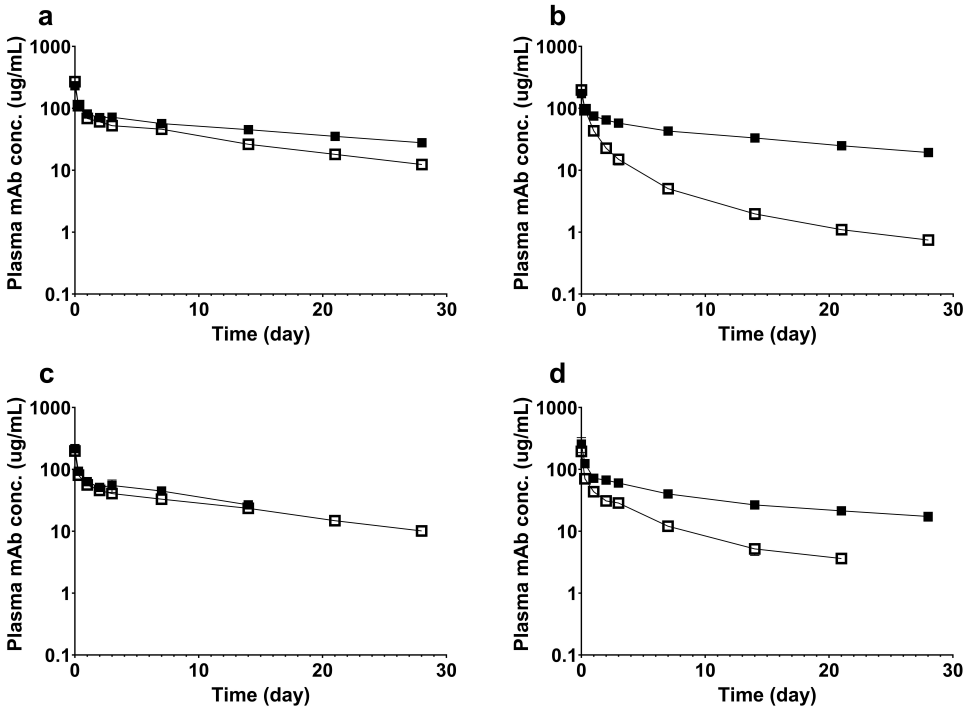

Figure 1. Plasma mAb concentration-time profiles of motavizumab, motavizumab-YTE, VRC01, VRC01-LS after IV injections in Tg32 in the absence and presence of IVIG. A: motavizumab (open square) and motavizumab-YTE (closed square) in the absence of IVIG. B: motavizumab (open square) and motavizumab-YTE (closed square) in the presence of IVIG. C: VRC01 (open square) and VRC01LS (closed square) in the absence of IVIG. D: VRC01 (open square) and VRC01LS (closed square) in the presence of IVIG.

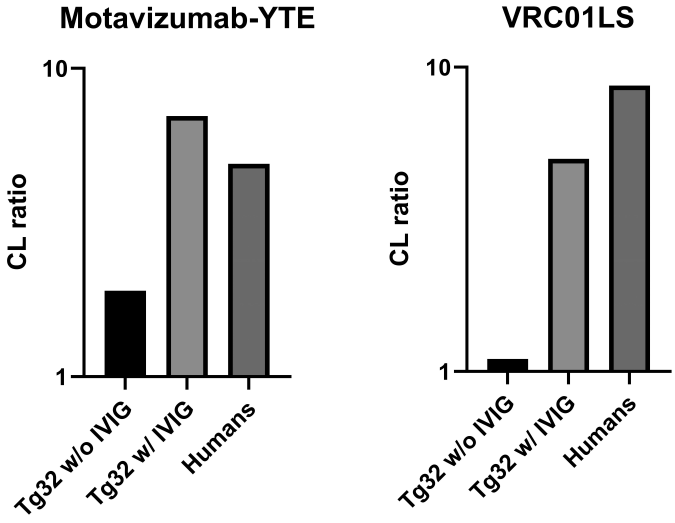

Figure 2. Ratios of CL between parent mAbs and Fc-engineered mAbs in Tg32 mice in the absence and presence of IVIG and humans.

IVIG slightly improved the correlation. Figure 4b,c,f,g demonstrate that Q and V_c showed no significant correlation between Tg32 mice and humans. In contrast, as shown in Figure 4d, h, V_p exhibited a significant and positive correlation between Tg32 mice and humans. This correlation could be due to the high V_p of CAP256V2LS observed in both Tg32 mice and humans. The pharmacokinetics of motavizumab-YTE and VRC01-LS was evaluated twice in two different studies (Tables 1 and 2). Although a slight difference in CL (within 1.5-fold) was observed between the studies, this variation does not affect the overall result and conclusion.

Prediction of two-compartment model parameters of mAbs in humans

Using the two-compartment model parameters of 11 Fc-engineered mAbs obtained from Tg32 mice, the optimal exponents for predicting human parameters were investigated. As a result of this investigation of the prediction accuracy of each parameter, the optimized exponents for CL, Q, V_c, and V_p were 0.73, 0.60, 0.95, and 0.87 in both the absence and presence of IVIG. Prediction accuracy within 1.5-fold of observed values for CL, Q, V_c, and V_p using the optimal exponents was 82%, 73%, 91%, and 73% without IVIG and 82%, 64%, 91%, and 100% with IVIG, respectively. Prediction accuracy within 2-fold of observed values for CL, Q, V_c, and V_p was 100%, 100%, 91%, and 100% without IVIG and 100%, 91%, 91%, and 100% with IVIG. The comparable prediction accuracy under both conditions suggests that IVIG is not necessary for evaluating Fc-engineered mAbs with enhanced FcRn binding mutations in Tg32 mice. In a previous study, the prediction accuracy within 1.5-fold of observed values for CL, Q, V_c, and V_p of Fc-engineered mAbs in humans, based on optimized allometric scaling from cynomolgus monkeys, was 78%, 100%, 89%, and 89%, respectively. These results indicate that Tg32 mice achieve pharmacokinetic prediction accuracy for humans that is nearly equivalent to that obtained using cynomolgus monkeys.

Prediction of plasma mAb concentration-time profiles after IV injection in humans

Using the optimized exponents in both the presence and absence of human IVIG, the plasma mAbs concentration-

Table 1. Clearance of motavizumab, motavizumab-YTE, VRC01, VRC01LS in Tg32 in the absence (w/o) or presence (w/) IVIG.

Antibody		w/o IVIG CL mL/day/kg	w/ IVIG CL mL/day/kg
Motavizumab	Mean	8.51	45.0
	SD	0.39	4.94
Motavizumab-YTE	Mean	4.59	6.46
	SD	0.21	0.81
VRC01	Mean	11.0	30.7
	SD	0.79	4.70
VRC01LS	Mean	10.3	6.16
	SD	1.17	2.01

Table 2. Two-compartment model parameters of Fc-engineered mAbs in Tg32 mice in the absence (w/o) or presence (w/) IVIG.

	Tg32 (w/o IVIG)				Tg32 (w/IVIG)			
Antibody	CL mL/day/kg	Q mL/day/kg	V_c mL/kg	V_p mL/kg	CL mL/day/kg	Q mL/day/kg	V_c mL/kg	V_p mL/kg
Motavizumab-YTE/MEDI-524-YTE	6.60	133	33.0	115	9.04	158	43.3	113
Nirsevimab/MEDI8897	4.66	183	40.4	86.1	4.27	251	52.8	98.3
Tixagevimab/AZD8895	5.42	183	47.1	124	6.05	190	52.1	120
Cilgavimab/AZD1061	7.27	209	48.5	126	6.87	158	57.0	104
Suvratoxumab/MEDI4893	6.28	179	61.1	122	5.69	261	52.3	113
Sotrovimab/VIR-7831	6.94	115	48.6	92.0	6.68	178	54.9	117
Elipovimab/GS-9722	24.5	188	45.8	110	25.2	207	48.5	145
VRC01-LS	7.71	162	34.3	110	7.36	137	38.7	104
VRC07-523LS	10.3	139	32.2	111	10.9	113	41.0	132
N6LS	14.6	130	31.3	110	15.5	169	35.6	144
CAP256V2LS	22.1	211	35.8	179	33.2	174	39.3	230
Geometric mean	9.05	164	40.7	115	9.59	177	46.3	125

time profiles of 11 Fc-engineered mAbs following IV injections in humans were predicted. The observed and predicted plasma concentration-time profiles for each mAb are shown in Figure 5 Overall, plasma concentration-time profiles were accurately predicted based on the optimized exponents estimated from Tg32 data under both conditions. Prediction accuracy within a 2-fold difference of observed values was 93.9% for Tg32 data without IVIG and 93.7% with IVIG (Figure 6). These results demonstrate that both approaches can accurately predict the plasma concentration-time profiles of Fc-engineered mAbs with enhanced FcRn binding.

Discussion

Being able to accurately predict the human pharmacokinetics of mAbs during the preclinical stage is critical for delivering innovative mAb therapeutics to patients. Currently, several Fc-engineered mAbs designed to enhance FcRn binding at acidic pH have been approved and are under clinical and preclinical development. These innovative mAbs offer patients the benefits of long half-lives and reduced dosing frequencies. Recent reports have shown that optimized allometric scaling from cynomolgus monkey data can accurately predict the human pharmacokinetics of Fc-engineered mAbs with YTE or LS mutations. This approach can further accelerate the development of Fc-engineered mAbs. However, due to the limited availability of cynomolgus monkeys, there is a need for alternative, more accessible predictive models. This study is the first to investigate the applicability of Tg32 mice for predicting the human pharmacokinetics of Fc-engineered mAbs.

The first objective of this study is to evaluate the impact of IVIG on the pharmacokinetics of Fc-engineered mAbs in Tg32 mice. As reported previously, Tg32 mice exhibit low plasma concentrations of endogenous mouse IgG due to the very weak binding affinity of mouse IgG to human FcRn.[17] As a result, competition for FcRn binding with endogenous IgG in Tg32 mice is less than that in humans, leading to more efficient FcRn-mediated recycling of mAbs. To mimic endogenous IgG concentrations observed in humans, 1000 mg/kg of IVIG was co-injected with the mAbs in this study. Endogenous IgG concentrations in humans are reported to be approximately 5–20 mg/mL.[18] Given that plasma volume in mice is 50–100 mL/kg, the expected initial IVIG concentration in plasma after a 1000 mg/kg dose is 10–20 mg/mL, which is comparable to endogenous IgG concentrations in humans. Motavizumab and VRC01, which lack YTE or LS mutations, showed significantly higher CL in the presence of IVIG compared to its absence. This finding suggests that IVIG competed with mAbs for FcRn binding within the endosome, partially inhibiting FcRn-mediated recycling. In contrast, the Fc engineered mAbs motavizumab-YTE and VRC01LS showed comparable CL in both the presence and absence of IVIG. YTE and LS mutations have been reported to have about 10-fold stronger FcRn binding affinity at acidic pH compared to their parent mAbs.[19,20] Thus, Fc-engineered mAbs do not appear to be affected by IVIG competition for FcRn binding in the endosome. In humans, motavizumab-YTE showed 4.9-fold improvement in CL over motavizumab, and VRC01-LS showed 8.7-fold improvement in CL over VRC01.[6,7] However, in the absence of IVIG, motavizumab-YTE and VRC01LS showed only 1.9-fold and 1.1-fold improvement in CL compared with motavizumab

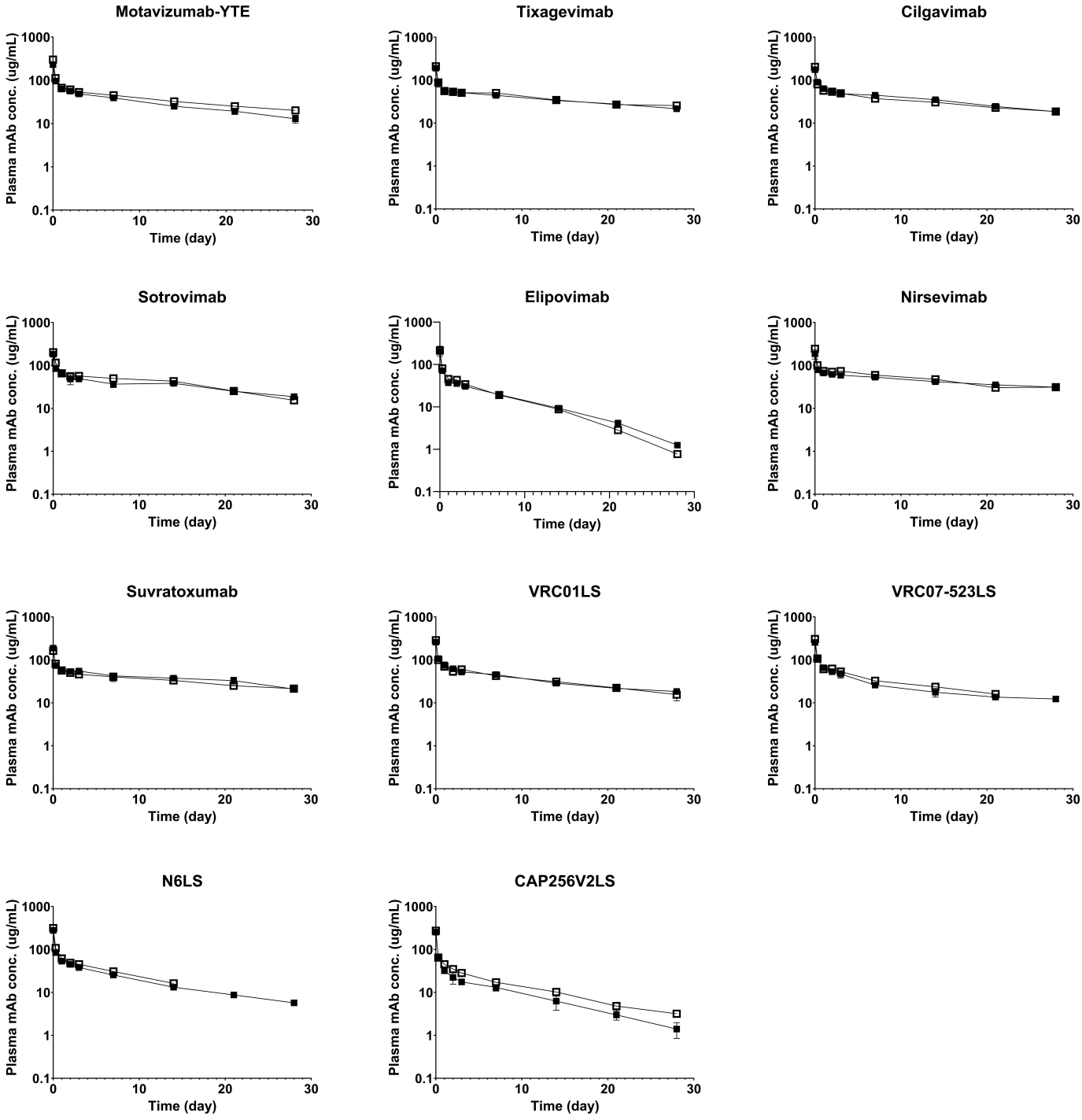

Figure 3. Plasma mAb concentration-time profiles of Fc-engineered mAbs after IV injections in Tg32 in the absence and presence of IVIG. Open squares indicate plasma mAb concentration-time profiles in the absence of IVIG and closed squares indicate plasma mAb concentration-time profiles in the presence of IVIG.

and VRC01. This study found that the difference in CL between parent mAbs and Fc-engineered mAbs was significantly smaller in Tg32 mice than in humans. This may result from the more efficient FcRn-mediated recycling of mAbs in Tg32 mice in the absence of IVIG. Due to the lack of endogenous IgG competition in Tg32 mice in the absence of IVIG, even normal mAbs would show sufficient FcRn recycling, contributing less to the improvement of FcRn recycling by YTE or LS mutations. Conversely, in the presence of IVIG, the difference in CL between parent mAbs and Fc-engineered mAbs in Tg32 mice was larger than that in the absence of IVIG and more closely aligned with observations in humans. These findings suggest that IVIG co-injection in Tg32 mice effectively mimics human FcRn competition and can be valuable for detecting the impact of enhanced FcRn binding on pharmacokinetics. A previous report partially demonstrated the effect of a high dose of human IgG on the pharmacokinetic differences between Fc-engineered mAbs and parent mAbs in Tg32 mice.[21] In that study, dose of 100 mg/kg of human IgG was used, which was expected to result in plasma concentrations far lower than the endogenous levels in humans. Consequently, the impact of Fc-engineering on the half-life

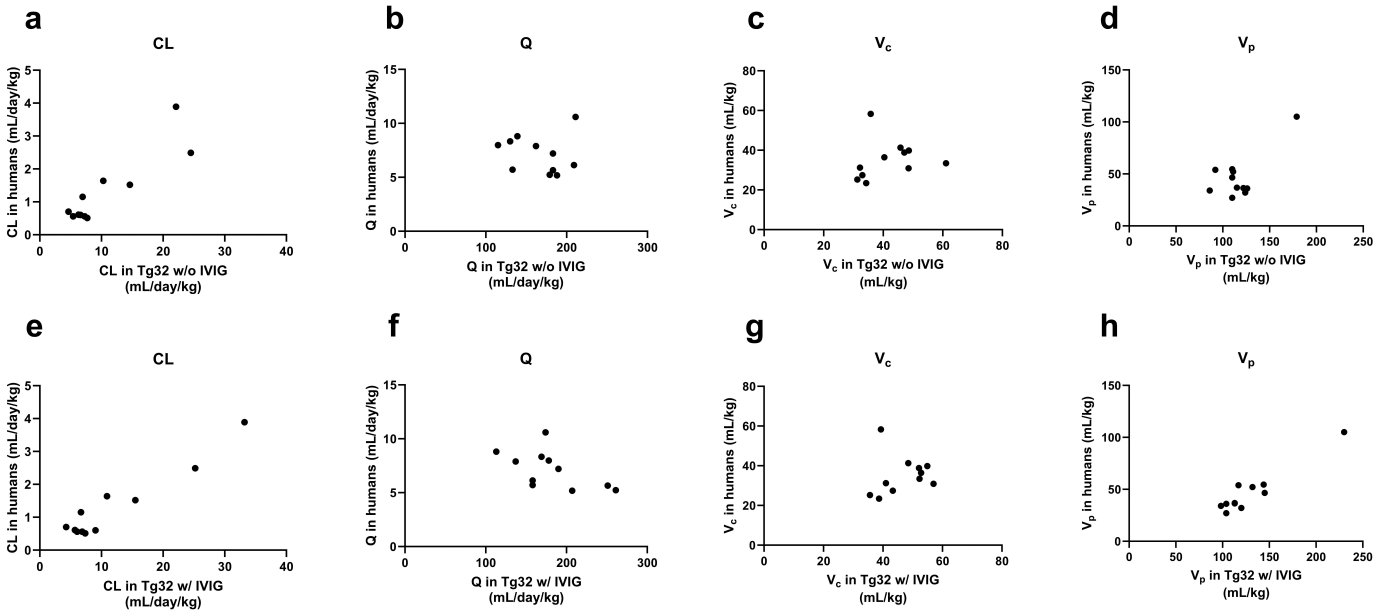

Figure 4. Correlation of two-compartment model parameters of Fc-engineered mAbs in Tg32 in the absence and presence of IVIG and humans. a-d: Correlation of CL (a), Q (b), V_c (c), V_p (d) between Tg32 in the absence of IVIG and humans. E-F: Correlation of CL (e), Q (f), V_c (g), V_p (h) between Tg32 in the presence of IVIG and humans.

of mAbs was small (only a 1.5-fold improvement) suggesting that the IgG concentration was insufficient to adequately mimic the human endosomal FcRn competition.

The second objective of this study was to establish an approach for accurately predicting the pharmacokinetics of Fc-engineered mAbs in humans using Tg32 mice. As previously reported with cynomolgus monkeys, Fc-engineered mAbs require different optimal allometric scaling exponents compared to normal mAbs in Tg32 mice. For normal mAbs, optimal exponents for CL, Q, V_c, and V_p from Tg32 to humans were reported as 0.90, 0.67, 0.97, and 0.93, respectively. However, in this study, the optimal exponents for Fc-engineered mAbs from Tg32 to humans were 0.73, 0.60, 0.95, and 0.87 for CL, Q, V_c, and V_p. The large difference in optimal exponents between normal mAbs and Fc-engineered mAbs was observed only for CL. While the mechanistic basis is still unknown, one potential explanation is the difference in endogenous IgG concentrations. As shown in Figures 1 and 2, normal mAbs showed large differences in CL between absence and presence of IVIG in Tg32 mice. This means that the optimal CL exponent for normal mAbs when extrapolating from Tg32 mice to humans would differ depending on the absence and presence of IVIG. Exact exponents of CL for motavizumab and VRC01 in the absence of IVIG were 0.90 and 0.92, respectively, consistent with previous reports for normal mAbs.[16] However, in the presence of IVIG, the CL exponents for motavizumab and VRC01 were 0.67 and 0.73, respectively. This result suggests that the optimal CL exponent for normal mAbs and Fc-engineered mAbs in presence of IVIG would be similar, whereas it would be different in its absence. A comprehensive pharmacokinetic evaluation of normal mAbs in the presence of IVIG is needed to validate this hypothesis. Overall, in this study, Fc-engineered mAbs showed comparable pharmacokinetics in both the presence and absence of IVIG. However, only CAP256V2LS showed approximately 1.5-fold difference of CL in the presence and absence of IVIG. Although the exact reason for this remains unknown, one hypothesis is that CAP256V2LS has a weaker FcRn-binding affinity compared to the other Fc-engineered mAbs.

Previous research demonstrated that optimized allometric scaling from cynomolgus monkey data accurately predicted the plasma concentration time profiles of nine Fc-engineered mAbs with YTE or LS mutations.[12] This approach predicted 98.6% of plasma mAb concentrations within a 2-fold difference of observed values. In this study, Tg32 pharmacokinetic data achieved 93.9% and 93.7% accuracy in the absence and presence of IVIG in humans within a 2-fold difference of observed values. Thus, while the prediction accuracy of Tg32 mice was slightly lower than that of cynomolgus monkeys, it is highly acceptable for drug discovery and development, suggesting Tg32 mice as a possible alternative to cynomolgus monkeys for predicting the human pharmacokinetics of Fc-engineered mAbs.

This study concludes that the pharmacokinetics of Fc-engineered mAbs in humans can be accurately predicted using Tg32 mice. While IVIG co-injection can be valuable for detecting the impact of enhanced FcRn binding mutations on pharmacokinetics in Tg32 mice, it is not essential for predicting the pharmacokinetics of Fc-engineered mAbs in humans. In addition to YTE and LS mutations, other mutations to enhance FcRn binding, such as H433K/N434F,[22] M428L/N434A,[23] and T250Q/M428L,[24] have been investigated in humans. Since these mutations share the same mechanism of prolonging half-life as YTE and LS, Tg32 mice are expected to be valuable for predicting the human pharmacokinetics of Fc-engineered mAbs with these mutations. This approach has the potential to accelerate the preclinical development of Fc-engineered mAbs with enhanced FcRn binding.

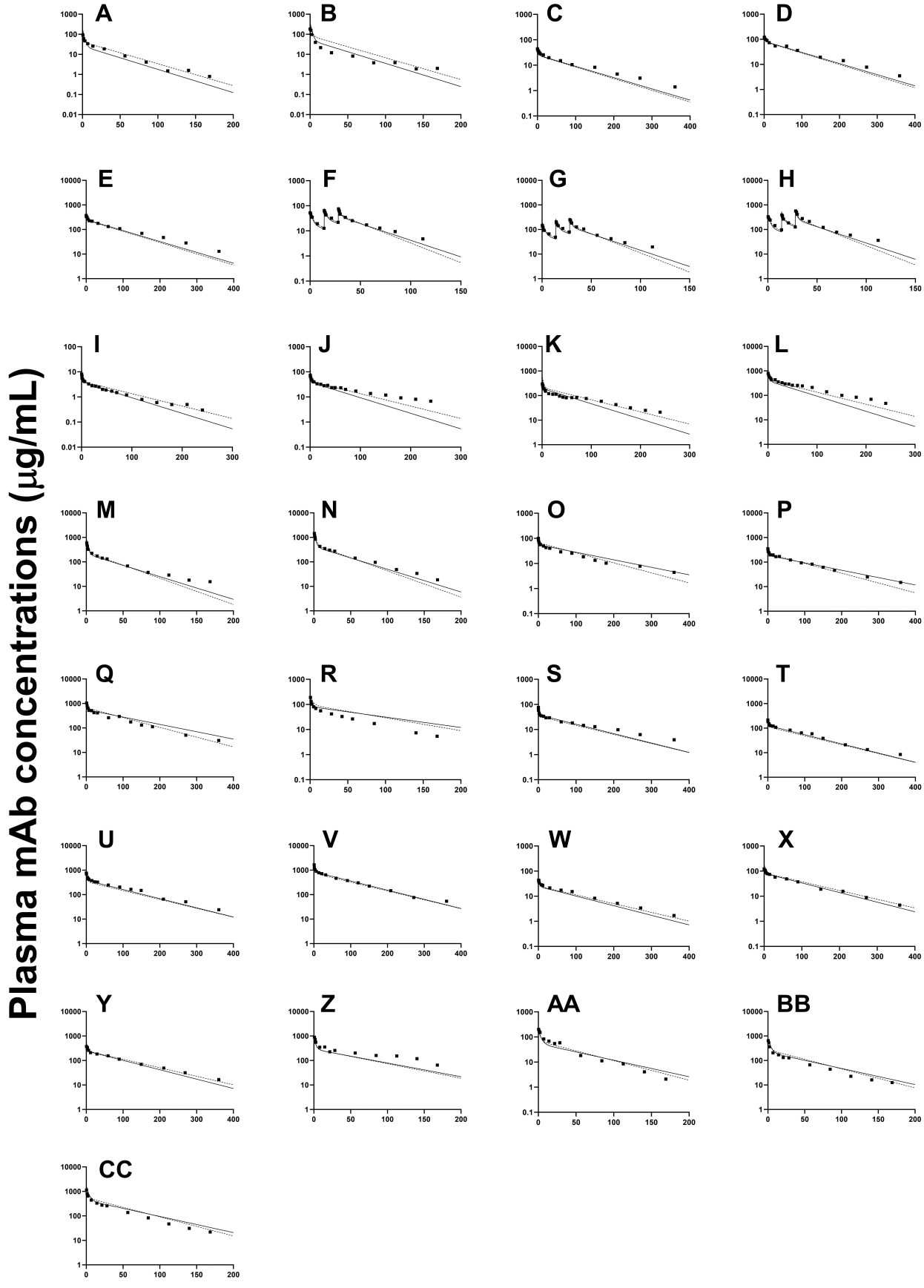

Figure 5. Predicted and observed plasma concentration-time profiles after IV injection in humans using allometric scaling from Tg32. Closed squares indicate observed plasma concentration. Dotted lines indicate predicted plasma concentration-time profiles using Tg32 in the absence of IVIG. Solid lines indicate predicted plasma

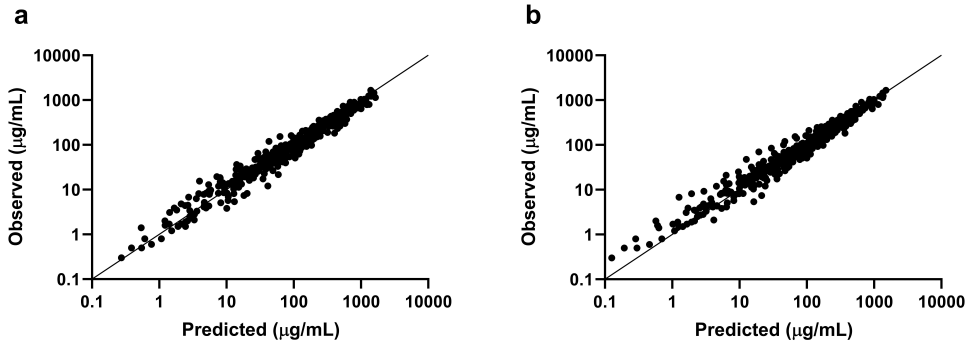

Figure 6. Relationship between predicted and observed plasma mAb concentrations. Solid lines indicate unity. (a) Plasma concentrations in humans predicted from Tg32 in the absence of IVIG. (b) Plasma concentrations in humans predicted from Tg32 in the presence of IVIG.

Materials and methods

Antibody expression and purification

Sequences of nirsevimab, tixagevimab, cilgavimab, suvratoxumab, sotrovimab, elipovimab, and motavizumab were obtained from the IMGT database. The sequence of motavizumab-YTE was designed by introducing M252Y/S254T/T256E mutations into motavizumab. Sequences of VRC01-LS, VRC07-523LS, and N6LS were obtained from the literature.[25] VRC01 was designed by introducing L428M/S434N back mutations into VRC01-LS. CAP256V2LS was designed by introducing the K100mA mutation into the VH domain of CAP256-VRC26.25[26] and M428L/N434S mutations into the wild type human IgG1 CH domain. All the antibodies were transiently expressed using the Expi293 expression system (Thermo Fisher Scientific, MA, USA). Antibodies were purified from the medium with protein A affinity chromatography followed by gel filtration chromatography as needed.

Pharmacokinetic study in Tg32

The pharmacokinetic study was conducted using human FcRn homozygous transgenic mice, line #32 (B6.Cg-Fcgrt < tm1Dcr > Tg(FCGRT)32Dcr/DcrJ, Jackson Laboratories). Mabs were intravenously injected at 10 mg/kg in the absence or presence of human IVIG (Hizentra, CSL Behring) at 1000 mg/kg. Blood samples were collected at 5 min, 7 hr, 1, 2, 3, 7, 14, 21, and 28 days after injection of mAbs. The blood samples were centrifuged to collect plasma samples. Collected plasma samples were stored at −80°C until bioanalysis. Motavizumab-YTE and VRC01-LS were evaluated twice in two different studies (1st study: comparison with parent mAbs, 2nd study: human prediction). All animal experiments in this study were performed according to the Guidelines for the Care and Use of Laboratory Animals at Chugai Pharmaceutical Co., Ltd.

Bioanalysis of plasma mAbs concentrations

Plasma samples for motavizumab, motavizumab-YTE, tixagevimab, cilgavimab, sotrovimab, elipovimab, nirsevimab, and suvratoxumab were pretreated by a modified method from a previous report.[27] After denaturation through sodium dodecyl sulfate (SDS), reduction, alkylation and methanol precipitation, the plasma pellet was digested by protease. Plasma samples for VRC01, VRC01LS, VRC07-523LS, N6LS and CAP256V2LS were subjected to denaturation with urea, reduction, alkylation, and protease digestion without protein precipitation. Calibration standards and quality control samples were prepared by diluting the antibody solution with blank plasma within the concentration range as shown in supplementary data. Concentrations were measured by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) using a ACQUITY UPLC I-Class system with 2D technology and Xevo TQ-S or TQ-XS triple quadrupole instruments (Waters Corporation, Milford, MA.). Chromatographic separation was performed using a ACQUITY UPLC BEH C18 (1.7 μm, 50 mm × 2.1 mm i.d.) (Waters Corporation, Milford, MA.) as a trapping column and ACQUITY UPLC Peptide BEH C18 (1.7 μm, 100 mm × 2.1 mm i.d.) (Waters Corporation, Milford, MA.) as an analytical column. Gradient elution was performed at a flow rate of 250 μL/min with mobile phase A consisting of 0.1% formic acid and 3% dimethyl sulfoxide in water, and mobile phase B consisting of 0.1% formic acid and 3% dimethyl sulfoxide in acetonitrile. Mass spectrometric detection was performed using multiple reaction monitoring (MRM) in positive ion mode. The amino acid sequences of unique surrogate peptides and MRM transitions are shown in the supplementary data. Masslynx software Version 4.1 and 4.2 (Waters Corporation, Milford, MA.) was used for data acquisition and data analysis.

concentration-time profiles using optimized allometric scaling approach. a: CAP256V2LS, 5 mg/kg. b: CAP256V2LS, 10 mg/kg. c: Cilgavimab, 150 mg. d: Cilgavimab, 500 mg. e: Cilgavimab, 1500 mg. f: Elipovimab, 150 mg. g: Elipovimab, 500 mg. h: Elipovimab, 1000 mg. i: Motavizumab-yte, 0.3 mg/kg. j: Motavizumab-yte, 3 mg/kg. k: Motavizumab-yte, 15 mg/kg. l: Motavizumab-yte, 30 mg/kg. m: N6LS, 20 mg/kg. n: N6LS, 40 mg/kg. o: Nirsevimab, 300 mg. p: Nirsevimab, 1000 mg. q: Nirsevimab, 3000 mg. r: Sotrovimab, 500 mg. s: Suvratoxumab, 225 mg. t: Suvratoxumab, 750 mg. u: Suvratoxumab, 2250 mg. v: Suvratoxumab, 5000 mg. w: Tixagevimab, 150 mg. x: Tixagevimab, 500 mg. y: Tixagevimab, 1500 mg. z: VRC01LS, 20 mg/kg. aa: VRC07-523LS, 5 mg/kg. bb: VRC07-523LS, 20 mg/kg. cc: VRC07-523LS, 40 mg/kg.

Table 3. List of 11 Fc-engineered mAbs and two-compartment model parameters in humans.

				Human				
Antibody	Target	subclass	mutation	CL mL/day/kg	Q mL/day/kg	V_c mL/kg	V_p mL/kg	Reference
Motavizumab-YTE/MEDI-524-YTE	RSV	IgG1	YTE	0.60	5.70	27.4	36.8	[12]
Nirsevimab/MEDI8897	RSV	IgG1	YTE	0.70	5.65	36.4	34.1	[11]
Tixagevimab/AZD8895	SARS-CoV-2	IgG1	YTE	0.56	7.21	38.8	32.0	[12]
Cilgavimab/AZD1061	SARS-CoV-2	IgG1	YTE	0.56	6.13	30.9	36.0	[12]
Suvratoxumab/MEDI4893	Alpha-toxin	IgG1	YTE	0.61	5.23	33.4	36.4	[11]
Sotrovimab/VIR-7831	SARS-CoV-2	IgG1	LS	1.15	7.98	39.8	53.9	[28]
Elipovimab/GS-9722	HIV	IgG1	LS	2.49	5.18	41.3	46.5	[12]
VRC01-LS	HIV	IgG1	LS	0.51	7.89	23.4	27.0	[12]
VRC07-523LS	HIV	IgG1	LS	1.64	8.81	31.2	52.2	[12]
N6LS	HIV	IgG1	LS	1.52	8.33	25.2	54.5	[29]
CAP256V2LS	HIV	IgG1	LS	3.89	10.6	58.3	105	[30]
Geometric mean				1.01	6.97	34.0	43.5	

Pharmacokinetic analysis

Measured plasma concentration-time profiles of mAbs in Tg32 mice were analyzed by non-compartment model analysis and two compartment model analysis using Phoenix WinNonlin 8.4 (Certara). In compartment model analysis, the two-compartment model with first order elimination was used to estimate CL, Q, V_c, and V_p.

Clinical data collection

Linear two-compartment model parameters (CL, Q, V_c, and V_p) and plasma mAb concentration-time profiles after IV injection in humans were obtained from published data. Additionally, information on IgG subclass and target antigen was also collected. To exclude the effect of target-mediated drug disposition (TMDD) on analysis, only mAbs showing linear pharmacokinetics without TMDD in humans were selected for analysis. When body weight information was unavailable in published data, a standard body weight of 75 kg for humans was applied for analysis. When CL, Q, V_c, and V_p were available in published data, these values were used for analysis. If CL, Q, V_c, and V_p were unavailable, they were estimated using a two-compartment model based on plasma mAb concentration – time profiles. These profiles were obtained by scanning figures from published data using UnGraph 5 (Biosoft). Linear two-compartment model parameters for a total of 11 Fc-engineered mAbs were collected (Table 3).

Allometric scaling approach

Linear two-compartment model parameters of 11 Fc-engineered mAbs in humans were predicted using the allometric scaling approach as shown in the following equations.

$$CL_{human} = CL_{Tg32} \times \left(\frac{BW_{human}}{BW_{Tg32}}\right)^{e_{CL}}$$

$$Q_{human} = Q_{Tg32} \times \left(\frac{BW_{human}}{BW_{Tg32}}\right)^{e_{Q}}$$

$$V_{c,human} = V_{c,Tg32} \times \left(\frac{BW_{human}}{BW_{Tg32}}\right)^{e_{V_c}}$$

$$V_{p,human} = V_{p,Tg32} \times \left(\frac{BW_{human}}{BW_{Tg32}}\right)^{e_{V_p}}$$

BW and e represent body weight (kg) and exponent. Units in the above equations were mL/day for CL and Q and mL for V_c, and V_p. To determine the optimal exponents for CL, Q, V_c, and V_p, each exponent was examined in 0.01 increments. Then, prediction accuracy for each exponent was evaluated by comparing predicted values with observed values.

Prediction of plasma mAb concentration-time profiles after IV injection in humans

Next, to evaluate the utility of optimized exponents, the plasma mAb concentration – time profiles of Fc-engineered mAbs after IV injection in humans were simulated using predicted two compartment model parameters derived from Tg32 mice in the absence and presence of IVIG. The predicted plasma mAb concentration – time profiles in humans were compared with observed values.

Analysis

All fittings and simulations for plasma mAb concentration-time profiles were performed using Phoenix WinNonlin 8.4 (Certara). Relative weight (1/y^2) was used in all fittings. All figures and statistical analyses were prepared using GraphPad Prism 9 (GraphPad Software, San Diego, CA). The correlation of linear two-compartment model parameters between cynomolgus monkey and humans was statistically analyzed. The Pearson correlation coefficient *r* value was significant when $p < 0.05$.

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