Retrospective analysis of model-based predictivity of human pharmacokinetics for anti-IL-36R monoclonal antibody MAB92 using a rat anti-mouse IL-36R monoclonal antibody and RNA expression data (FANTOM5)

Jennifer Ahlberg, Craig Giragossian, Hua Li, Maria Myzithras, Ernie Raymond, Gary Caveness, Christine Grimaldi, Su-Ellen Brown, Rocio Perez, Danlin Yang, Rachel Kroe-Barrett, David Joseph, Chandrasena Pamulapati, Kelly Coble, Peter Ruus, Joseph R. Woska, Rajkumar Ganesan, Steven Hansel, and M. Lamine Mbow

ABSTRACT
Accurate prediction of the human pharmacokinetics (PK) of a candidate monoclonal antibody from nonclinical data is critical to maximize the success of clinical trials. However, for monoclonal antibodies exhibiting nonlinear clearance due to target-mediated drug disposition, PK predictions are particularly challenging. That challenge is further compounded for molecules lacking cross-reactivity in a nonhuman primate, in which case a surrogate antibody selective for the target in rodent may be required. For these cases, prediction of human PK must account for any interspecies differences in binding kinetics, target expression, target turnover, and potentially epitope. We present here a model-based method for predicting the human PK of MAB92 (also known as BI 655130), a humanized IgG1 monoclonal antibody directed against human IL-36R. Preclinical PK was generated in the mouse with a chimeric rat anti-mouse IgG2a surrogate antibody cross-reactive against mouse IL-36R. Target-specific parameters such as antibody binding affinity (Ka), internalization rate of the drug target complex (kimp), target degradation rate (kdeg), and target abundance (R0) were integrated into the model. Two different methods of assigning human R0 were evaluated: the first assumed comparable expression between human and mouse and the second used high-resolution mRNA transcriptome data (FANTOM5) as a surrogate for expression. Utilizing the mouse R0 to predict human PK, AUC0–∞ was substantially underpredicted for nonsaturating doses; however, after correcting for differences in RNA transcriptome between species, AUC0–∞ was predicted largely within 1.5-fold of observations in first-in-human studies, demonstrating the validity of the modeling approach. Our results suggest that semi-mechanistic models incorporating RNA transcriptome data and target-specific parameters may improve the predictivity of first-in-human PK.

Introduction
MAB92, also known as BI 655130, is a humanized IgG1κ monoclonal antibody engineered for reduced effector function and directed against the human cell-surface receptor, IL1RL2 (IL-36R). Signaling of IL-36R is induced by heterotrimeric binding with its co-receptor, IL-1 receptor accessory protein (IL-1RACP), and one of the three IL-36R cognate agonistic ligands, such as, IL36a, IL36β, or IL36γ, resulting in downstream activation of NF-κB and MAPKs and expression of proinflammatory and profibrotic mediators. An additional ligand, IL-36Ra, competes with the aforementioned ligands, thereby acting as a natural antagonist of IL-36R signaling. A strong link has been established between IL-36R signaling and skin inflammation as demonstrated by the occurrence of generalised pustular psoriasis in patients with a loss-of-function mutation in IL-36Ra. IL-36R agonist ligands are upregulated in psoriatic tissue, and accumulating evidence suggests that the IL-36R signaling pathway plays a role in the pathogenesis of psoriatic and rheumatoid arthritis, asthma, chronic obstructive pulmonary disease, and inflammatory bowel disease, making IL-36R signaling an attractive target for therapeutic intervention in the aforementioned and other epithelial-mediated inflammatory diseases. IL-36R is reported to be expressed on dendritic cells, CD4+ T cells, intestinal lymphocytes, and synovial fibroblasts. In-house immunohistochemistry (IHC) data for MAB92 in human tissue showed mostly mild-to-moderate staining in a variety of epithelial tissues (bladder, breast, eye, esophagus, lung, pituitary, prostate, salivary gland, skin, thymus, tonsil, ureter, and cervix).
MAB92 shows species-specific binding with high affinity against human IL-36R and > 2000-fold reduced affinity towards IL-36R in mouse, rat, hamster, mini pig, and nonhuman primates (cynomolgus, rhesus, and marmoset). As MAB92 targets a cell-surface receptor, target-mediated drug disposition (TMDD) resulting from internalization and subsequent degradation of the molecule was expected to contribute to overall clearance of the antibody. In order to enable in vivo preclinical studies, we identified a chimeric rat anti-mouse mAb, MAB04 (also known as BI 674304), targeted against mouse IL-36R. MAB04 shares key characteristics with MAB92, including affinity, in vitro functional activity (both within ten-fold), and IL-36R domain-2 epitope binding. Intrapерitoneal administration of the mouse surrogate antibody, MAB04, in both the imiquimod- and IL36-induced mouse models of skin inflammation resulted in blockade of the swelling response as well as substantial reduction of inflammatory cytokines. IHC data were not available for the mouse surrogate antibody against murine IL-36R; therefore, it is unknown if staining patterns and/or intensity were comparable between human and mouse.

Although allometric scaling or Dedrick transform of pharmacokinetics (PK) from preclinical species to human is often successful for therapeutic antibodies targeting soluble antigens, prediction of human PK for those targeting cell-associated antigens or otherwise affected by TMDD is significantly more challenging due to potential interspecies differences in target expression or turnover, as well as in binding kinetics. In these cases, a model-based approach incorporating target-specific parameters may improve the predictivity of human PK. However, additional challenges exist in predicting human PK for molecules lacking cross-reactivity in preclinical species. In these cases, as for MAB92, a surrogate molecule cross-reactive to the target in the preclinical species may be required. As a result, in addition to the aforementioned TMDD challenges, discrepancies in linear PK characteristics, such as neonatal receptor (FcRn) binding and recycling as well as in catabolic susceptibility, may exist between human candidate and surrogate molecule.

The purpose of the experiments outlined herein is to characterize the PK of the anti-mouse IL-36R antibody, MAB04, in mice in support of the first-in-human (FIH) clinical trial. In this retrospective analysis, we incorporated molecule- and species-specific parameters, such as volume of distribution (Vc), inter-compartmental transfer rates (k12 and k21), linear elimination (k0), binding affinity (Kd), internalization rate of the drug–target complex (kint), target degradation rate (kdeg), and target abundance (R0), into a semi-mechanistic model. Two different methods of assigning target abundance were evaluated: the first assumed comparable expression between human and mouse, and the second utilized FANTOM5 RNA transcriptome data in a subset of matched tissues as a surrogate for expression in each respective species. FANTOM5 is a comprehensive expression dataset that includes ~1000 human and ~400 mouse tissues, primary cells, and cancer cell lines. This dataset is based on cap analysis of gene expression (CAGE), a method developed at RIKEN in Japan that characterizes transcription start sites across the entire genome at single-base resolution level. Since eukaryotic transcription factors are typically activating, the number of transcription factors on a promotor is predictive of breadth of expression. Human PK profiles were then simulated based on a semi-mechanistic TMDD model incorporating critical target-specific parameters for both the human candidate and mouse surrogate antibodies with R0, either assumed to be the same as that of mouse or corrected for the differences in RNA transcriptome data between species. For the human model utilizing the model-estimated mouse target abundance, Cmax was well predicted; however, AUC0–∞ was substantially underestimated. After correcting for relative differences in RNA transcriptome data between species, the model-predicted human AUC0–∞ and Cmax were largely within 1.5-fold that observed for both nonsaturating and saturating doses.

**Results**

**Monkey PK**

Concentrations versus time curves for MAB92 in cynomolgus monkey following intravenous administration are shown in Figure 1. Noncompartmental PK analysis showed that clearance was dose linear following the intravenous administration of 0.3, 1.5, and 10 mg/kg of MAB92, as expected due to lack of cross-reactivity of MAB92 against cynomolgus monkey IL-36R (Table 1). The clearance, steady-state volume of distribution, and terminal half-life for the three dose groups were similar and were in the range of 0.17–0.22 mL/h/kg, 65.2–83.0 mL/kg, and 284–349 h, respectively. Anti-MAB92 antibodies (ADA) were observed in two of three animals in the 10 mg/kg intravenous dose groups; however, there was no impact of ADA on the PK profile. Monkeys administered 0.3 and 1.5 mg/kg intravenous doses tested negative for ADA.

**Mouse PK**

Noncompartmental analysis for MAB04 PK in female mouse showed dose-dependent clearance with values for clearance (CL/F) of 1.6, 0.47, and 0.13 mL/h/kg for the 0.3, 1.5, and 10 mg/kg doses, respectively, suggestive of TMDD (Table 2). At the highest dose evaluated, 10 mg/kg, MAB04 clearance in the mouse was 0.13 mL/h/kg, which is consistent with published values for therapeutic monoclonal antibodies in that

![Figure 1. Mean monkey concentration versus time data (non-transformed) of MAB92 at 0.3, 1.5 and 10 mg/kg i.v. demonstrating dose linearity and absence of TMDD impact on profiles.](image-url)
species.\textsuperscript{28} In addition, the mouse clearance was comparable to that observed for a 1.5 mg/kg dose of the non-cross-reactive antihuman monoclonal antibody, MAB92, in the monkey (0.17 ± 0.03 mL/h/kg), suggesting that FcRn recycling and catabolic stability were comparable between molecules and species. No ADA titers were detected in any animals.

**Human pharmacokinetic predictions**

**Modeling approach**

Mouse concentrations versus time data for MAB04 were simultaneously fitted to a 2-compartment (CMT) model (Figure 2) with parallel nonlinear and linear elimination from the central compartment. $V_c$, $k_{10}$, $V_{\text{max}}$, and $K_m$ were derived from the model using mean fit (Table 3). Typical linear PK parameter values for mouse (in-house data from a panel of human IgG monoclonal antibodies) were used for absorption rate ($k_a$) and distribution microconstants ($k_{12}$ and $k_{21}$). Monoclonal antibody absorption following intraperitoneal dosing in mice has been demonstrated to be rapid and near complete; therefore, bioavailability was assumed to be 100%.\textsuperscript{29}

The mouse concentration versus time data were subsequently fitted to a 2-CMT semi-mechanistic model (Figure 3) using central volume ($V_c$) and linear clearance ($k_a$) derived from the TMDD model fit. Consistent with the parallel elimination model, typical values for human monoclonal antibodies in mouse were utilized for $k_a$, $k_{12}$, and $k_{21}$. Average plasma membrane turnover rate of 0.5 h was applied for both $k_{\text{deg}}$ and $k_{\text{int}}$.\textsuperscript{30} *In vitro* surface plasmon resonance (SPR) binding affinity data for MAB04 was used for $K_D$, and mouse $R_0$ was estimated by model fitting. The fit and corresponding weighted residual plot for the fit of the mouse data are shown in Figures 4 and 5, and the model parameters are shown in Table 4.

Estimated mouse $R_0$ (0.794 nM) was comparable to the mouse $K_m$ (1.18 nM) derived using simultaneous fitting of the mouse data in the 2-CMT TMDD model, suggesting that estimation of baseline of expression in mouse was reasonable. Dedrick transform was then used to scale monkey concentration–time profiles to human using an allometric exponent of 1.0 for volume of distribution (b) and 0.85 for CL (a) according to the following equations.\textsuperscript{17,31}

$$t_{\text{human}} = t_{\text{monkey}} \times \left( \frac{BW_{\text{human}}}{BW_{\text{monkey}}} \right)^{b-a}$$

$$C_{\text{human}} = C_{\text{monkey}} \times \left( \left( \frac{BW_{\text{monkey}} \times D_{\text{human}}}{BW_{\text{human}} \times D_{\text{monkey}}} \right)^b \right)$$

---

**Table 2.** Non-compartmental analysis parameters for MAB04 in C57BL/6 mouse and MAB92 in cynomolgus monkey following intraperitoneal (clearance only) and intravenous administration, respectively.

| Species        | mAb        | Parameter | Units | 0.3 | 1.5 | 10 |
|----------------|------------|-----------|-------|-----|-----|----|
| Monkey         | MAB92 CL (SD) | mL/kg/h  | 0.22 (0.01) | 0.19 (0.05) | 0.17 (0.03) |       |
| Monkey         | MAB92 V (SD)  | mL/kg    | 83.0 (6.3)  | 65.2 (3.2)  | 75.5 (13)   |       |
| Monkey         | MAB92 t1/2 (SD) | h       | 284 (19)     | 288 (40.5)  | 349 (94)    |       |
| Mouse          | MAB04 CL/F (SD) | mL/kg/h | 1.62 (0.02)  | 0.47 (0.13) | 0.13 (0.02) |       |

---

**Table 3.** Two-compartment semi-mechanistic model parameters for MAB04 (mouse-specific mAb).

| Parameter | Units | Mouse mean | SE |
|-----------|-------|------------|----|
| $V_{\text{max}}$ | nmol/h | 0.000533 | 0.0000626 |
| $k_m$ | nM | 1.18 | 0.729 |
| $k_{12}$ | day$^{-1}$ | 0.086 | Fixed |
| $k_{21}$ | day$^{-1}$ | 0.063 | Fixed |
| $V_c$ | L | 0.000403 | 0.0000308 |
| $k_{10}$ | day$^{-1}$ | 0.00506 | 0.00147 |
| $k_{a}$ | day$^{-1}$ | 0.152 | Fixed |
Dedrick-scaled concentration versus time data were then fitted to the parallel elimination 2-CMT TMDD model previously described in Figure 2 (fit shown in Figure 6) to derive the human linear PK parameters ($V_c$, $k_{10}$, $k_{12}$, and $k_{21}$) shown in Table 4. The differential equations for the mouse and human semi-mechanistic TMDD model are shown below and were fitted using rapid-binding approximation such that the free drug, free target, and complex are in rapid equilibrium determined by the equilibrium constant, $K_D$.

$$A_1 = ka * A_0 - (A_1 * k_{el}) - (A_1 * K_{12} - A_2 * k_{21}) - ((Rtot * kint)/(KD + A_1/V) * A_1)$$

$$A_2 = (A_1 * K_{12} - A_2 * K_{21})$$

$$R_{tot} = kinetics * R_{tot} - (k_{deg} * R_{tot} - (k_{deg} * R_{tot}) * (KD + A_1/V) * A_1/V$$

$$C = A_1/V$$

$$A_0 = -ka * A_0$$

**Scaling of baseline expression**

Two different methods of estimating baseline receptor expression in human were evaluated. The first assumed equivalence of IL-36R expression between species and gender such that $R_0$ was expected to be comparable between the preclinical female mice and the FIH male subjects. Therefore, the mouse model-derived $R_0$ was used in the semi-mechanistic model described previously, and human PK profiles corresponding to the doses used in the FIH clinical trial were then simulated. Utilizing this approach, although $C_{max}$ was predicted within 1.5-fold that observed in human, the $AUC_{0-\infty}$ was substantially under-predicted (Figures 7 and 8, respectively), indicating that IL-36R expression in male human was likely substantially lower than that in female mice.

For the second method, integrating mRNA transcriptome (CAGE) data, the model-derived mouse $R_0$ was multiplied by 0.013, the ratio of human to mouse IL-36R transcripts per million (TPM), for a panel of 14 matched, gender-specific, non-privileged tissues (Figure 9). By integrating the RNA transcriptome data, both $C_{max}$ and $AUC_{0-\infty}$ were predicted within 1.5-fold (Table 5). Within the dose range of 0.030–0.300 mg/kg, exposure of MAB92 increased with increasing dose in a greater than dose-proportional way. However, exposure increased in an approximately dose-proportional manner for the doses of

---

**Figure 4.** Mean mouse 0.3, 1.5 and 10 mpk i.p. concentration versus time data fitted to a 2-CMT semi-mechanistic TMDD model and corresponding weighted residuals.

**Figure 5.** Dedrick transformed mean cynomolgus monkey 10 mg/kg i.v. concentration versus time data fitted to a 2-CMT model. Estimates for the fitted parameters are shown in Table 4.

**Table 4.** Two-compartment semi-mechanistic model parameters for MAB04 (mouse-specific antibody) and MAB92 (clinical candidate).

| Parameter | Units | Mouse mean | Monkey mean* | Translated human parameter |
|-----------|-------|------------|--------------|---------------------------|
| $R_0$     | nM    | 0.794      | -            | 0.010                     |
| $k_{deg}$ | day\(^{-1}\) | 1.39       | -            | 1.39                      |
| $K_D$     | nM    | 0.24       | -            | 0.020                     |
| $k_{int}$ | day\(^{-1}\) | 1.39       | -            | 1.39                      |
| $K_{12}$  | day\(^{-1}\) | 0.086      | 0.0056       | 0.0089                    |
| $k_{21}$  | day\(^{-1}\) | 0.063      | 0.0074       | 0.0110                    |
| $V_c$     | L     | 0.0000403  | 3.0          | 3.0                       |
| $k_{el}$  | day\(^{-1}\) | 0.00506   | 0.0021       | 0.0021                    |
| $k_{a}$   | day\(^{-1}\) | 0.152      | -            | -                         |

Dedrick-scaled concentration versus time data were then fitted to the parallel elimination 2-CMT TMDD model previously described in Figure 2 (fit shown in Figure 6) to derive the human linear PK parameters ($V_c$, $k_{10}$, $k_{12}$, and $k_{21}$) shown in Table 4. The differential equations for the mouse and
0.300 mg/kg and higher, suggesting saturation of TMDD within this dose range.

Discussion

Accurate prediction of human PK is critical for effective and efficient design of FIH clinical trials. Although human PK for protein therapeutics targeting soluble or low-expression antigens has been successfully translated from preclinical data using single-species allometric scaling or Dedrick-transformed monkey PK data, prediction of PK for TMDD-impacted proteins remains challenging. Some success in overcoming these challenges has been achieved by incorporation of in vitro-derived mechanistic parameters into model-based predictions, though in these cases assumptions are often made regarding comparable target expression between preclinical species and human. Since the greatest driver of clearance nonlinearity for highly expressed and/or cell-associated targets...
is typically target density ($R_0$), accurate scaling of this parameter is critical for human predictions. One approach is to experimentally determine target abundance, but converting arbitrary readouts (e.g., light intensity units for flow cytometry) into target concentration does not necessarily translate correctly.\textsuperscript{35} Similarly, the total number of cells expressing target is typically unknown, so estimation of the number of cells carrying the receptor and the number of receptors per cell may not be valid.\textsuperscript{35} Liquid chromatography-mass spectrometry (MS)/MS determination of total receptor in preclinical studies is technically feasible, given the identification of a selective, high-affinity, and tissue-penetrant tracer molecule, but translatability to human is still lacking, and assumptions of comparable expression between preclinical species and human may be invalid.

For the prediction of IL-36R TMDD impact and initial exposure in healthy human volunteers, we evaluated two approaches to translating $R_0$ from mouse to human. As both methods required accurate characterization of the TMDD impact in mouse, whole blood microsampling was used to enable a rich sampling scheme from each individual animal. $R_0$ in mouse was first estimated by fitting mouse data to a semi-mechanistic model incorporating mouse linear PK parameters derived from a 2-CMT model fit and binding data derived from MAB04 in vitro studies. For the human semi-mechanistic model, linear PK parameters in human were estimated by a 2-CMT model fit of Dedrick-transformed monkey PK data and binding data from MAB92 in vitro studies were incorporated. In the absence of comparative species expression data, the first approach to translating $R_0$ from mouse to human was to assume equivalent $R_0$ between the two species. This approach is frequently used for translation of TMDD impact from preclinical species to human despite limitations of low predictivity. Although this method resulted in a good prediction of human $C_{\text{max}}$, $AUC_{\text{0-\infty}}$ was substantially underpredicted at all doses (Figures 7 and 8), demonstrating the limited utility of this approach. The second translation approach was to apply the human to mouse RNA transcriptome ratio for gender- and species-matched organs as a correction factor to the mouse $R_0$. Organs with expected low antibody exposure due to restricted access of large polar molecules (e.g., eye, brain, central nervous system (CNS), and testes) were excluded as exposure to both MAB04 and MAB92 was expected to be limited in those tissues. This method of $R_0$ estimation successfully predicted both $C_{\text{max}}$ and $AUC_{\text{0-\infty}}$ largely within 1.5-fold (Table 4).

The plots for the human PK data against simulation results from the semi-mechanistic model corrected for cross-species differences in RNA expression between mouse and human.

### Table 5. Predicted versus observed $C_{\text{max}}$ and $AUC_{\text{0-\infty}}$ for MAB92 in human. Using a semi-mechanistic model incorporating target-specific parameters including a correction for cross-species differences in RNA expression, $C_{\text{max}}$ and $AUC_{\text{0-\infty}}$ for MAB92 in human were predicted largely within 1.5-fold and 1-fold, respectively.

| Dose (mg/kg) | Pred. $C_{\text{max}}$ (nM) | Obs. $C_{\text{max}}$ (nM) | Pred./Obs. $C_{\text{max}}$ (nM) | Pred. $AUC_{\text{0-\infty}}$ nM*h | Obs. $AUC_{\text{0-\infty}}$ nM*h | Pred./Obs. $AUC_{\text{0-\infty}}$ |
|------------|------------------|------------------|-------------------|------------------|------------------|-------------------|
| 0.03       | 5                | 3                | 2.0               | 374              | 381              | 1.0               |
| 0.05       | 9                | 7                | 1.4               | 1510             | 2108             | 0.7               |
| 0.1        | 18               | 12               | 1.5               | 4259             | 4492             | 0.9               |
| 0.3        | 54               | 43               | 1.3               | 20,376           | 20,595           | 1.0               |
| 1          | 179              | 131              | 1.4               | 84,217           | 91,297           | 0.9               |
| 3          | 538              | 409              | 1.4               | 199,440          | 204,324          | 1.2               |
| 6          | 1077             | 1033             | 1.1               | 549,062          | 548,108          | 0.9               |
| 10         | 1795             | 1587             | 1.2               | 822,862          | 823,784          | 1.1               |
In cases where TMDD is driven primarily by factors other than $R_0$ (e.g., $k_{un}$), the FANTOM5-corrected R0 translation approach might not improve preclinical to clinical prediction of PK.

One obvious limitation to utilizing tissue mRNA tissue transcriptome data as a surrogate for expression is the inability to assess inter-species differences in the extent of the shed and/or soluble target. For those cases, quantitation of circulating target in both species and integration of those differences into $R_0$ corrections would likely be required. Similarly, if the target is differentially expressed across organs and between species, an organ-to-body weight ratio correction might be required to improve the predictivity of TMDD impact and therefore PK in human. An additional limitation is the fact that, for some targets, the panel of tissues assessed is not always consistent for both mouse and adult human. For example, in the case of IL-36R, although the target is expected to be expressed in skin, only mouse had TPM values available for that organ. Although TPM was reported for human fetal skin ($<0.5$), assumptions cannot be made that the same low expression applies in adult human. Recognition of expression level differences between normal and healthy target tissues is another contributor to translational uncertainty.

In spite of the aforementioned limitations, our results demonstrate the potential utility of a rational, semi-mechanistic approach to predicting human PK using preclinical data generated with a surrogate antibody in combination with CAGE-derived mRNA expression transcriptome data (FANTOM5) and in human in vitro parameters. For molecules like MAB92, which lacks cross-reactivity in nonhuman primate, and requires a rodent or other species cross-reactive surrogate molecule for characterization of TMDD impact and prediction of FH PK, integration of RNA transcriptome data may enable improved preclinical to clinical translation of PK. An additional benefit is the potential reduction in the use of higher species for preclinical PK characterization since mouse may provide sufficient data to enable human prediction. However, additional studies are required to validate the general applicability of this approach.

**Materials and methods**

**Reagents**

MAB04 and MAB92 were produced by the Boehringer Ingelheim Research team (Ridgefield, CT). MAB92, also known as BI 655130, is a humanized monoclonal IgG1 antibody produced in Chinese Hamster Ovary (CHO) cells and targeted against human IL-36R. Both L234 and L235 of the heavy chain were mutated to Chinese Hamster Ovary (CHO) cells and targeted against human IL-36R. Both L234 and L235 of the heavy chain were mutated to chimeric monoclonal antibody of the IgG2a isotype that is directed against mouse IL-36R. It utilizes a backbone that has two rhesus, marmoset, or rat IL-36R-Fc.

**Nonclinical pharmacokinetic studies**

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee and conducted in full compliance with the ethical and regulatory principles and local and national licensing regulations.

**Mouse studies**

In-life studies were conducted in house. MAB04 was administered by intra-peritoneal injection to female, C57BL/6 mice at 0.3, 1.5, and 10 mg/kg ($N = 3$ per dose group) to assess potential TMDD impact and saturability as well as clearance (CL/F) across a dose range intended to cover the human therapeutic dose. Blood samples collected via whole blood microsampling (10 µL) over one and two weeks for the 0.3 mg/kg and higher dose groups, respectively, were diluted in phosphate-buffered saline (PBS) and analyzed using an enzyme-linked immunosorbent assay (ELISA) to determine free concentrations of MAB04.

**Nonhuman primate studies**

The in-life studies were conducted at Charles River Laboratory (Reno, NV). Nine male drug-naïve cynomolgus monkeys (Mauritius origin) weighing 2.3–2.7 kg were each administered a single intravenous dose of MAB04 at 0.3, 1.5, or 10 mg/kg as a 10-min constant rate infusion. Serial blood samples were processed to serum and collected for up to 1008 h (42 d) after dosing. Because MAB92 does not bind to cynomolgus monkey, PK characteristics of that molecule address only the catabolic stability and FcRn recycling properties of the molecule, rather than full PK evaluation including possible TMDD.

**Clinical pharmacokinetic study**

Human PK data from a Phase 1, single rising dose study of MAB92 (NCT02525679) were used to validate the PK predictions based on the models. The FIH dose selection was based on a MABEL approach using an in vitro IC$_{10}$ as the target with predicted exposure based on TMDD saturation (linear) PK. The PK data were obtained from healthy male volunteers, and the available data from the 0.03, 0.05, 0.1, 0.3, 1, 3, 6, and 10 mg/kg intravenous doses were used to verify human PK predictions. Noncompartmental analyses were utilized to compare the observed versus predicted $C_{\text{max}}$ and AUC$_{0-\infty}$. All studies were conducted in accordance with the ethical standards of the Declaration of Helsinki.

**Bioanalytical assay**

**MAB04 quantitation in mouse**

MAB04 concentration in the mouse whole blood samples was determined using a protein-capture ELISA. Briefly, recombinant mouse IL-1 Rrp2/IL-1 R6 (R&D Systems, Minneapolis, MN) was immobilized onto Nunc MaxiSorp 96-well plates (ThermoFisher, Waltham, MA). The plates were washed and then blocked with 5% bovine serum albumin in PBS (w/v). Matrix reference standards, quality control, and test samples (10 µL whole blood diluted into 90 µL PBS) were transferred to the blocked plates. The plates were washed again, and horseradish peroxidase (HRP)-conjugated secondary antibody (Southern Biotech, cat#1080–05) was added. The plates were washed, and the BioFx (SurModics) substrate TMBW was added. The plates were allowed to develop at room temperature, and then, the BioFx liquid stop solution (0.2 M H$_2$SO$_4$) was added before the plates were analyzed on a SpectraMax (Molecular Devices) M5 Plate Reader at OD 450 nM. Drug concentrations were derived from the four-parameter fitting model from Softmax Pro software (Molecular Devices). Equivalent MAB04
The ratio of human to mouse TPM for IL-36R was 0.013. TPM in remaining tissues was below the quantitation limit in both species.

**Abbreviations**

IL-36R  interleukin-1 receptor-like 2  
IL-1RAcP  IL-1 receptor accessory protein  
mAb  monoclonal antibody  
PK  pharmacokinetics  
C_{\text{max}}  maximum concentration  
AUC_{0-\infty}  area under the curve from time 0 to infinity  
t_{1/2}  terminal half-life  
CL  clearance  
V  volume of distribution  
FIH  First-in-Human  
TMDD  target-mediated drug disposition  
ITE  Indirect target engagement  
FANTOM  Functional Annotation of the Human Genome  
CAGE  Cap Analysis of Gene Expression.

**Acknowledgments**

The authors acknowledge the following individuals for their contributions in reviewing the manuscript or providing technical expertise: Chia-Hung Tsai, Frank Li, Steven Cañero, Sally Ye, and Jon Hill.

**ORCID**

Jennifer Ahlberg  http://orcid.org/0000-0002-2892-4163  
Danlin Yang  http://orcid.org/0000-0002-5085-5950  
Rachel Kroe-Barrett  http://orcid.org/0000-0003-1413-1223  
Rajkumar Ganesan  http://orcid.org/0000-0002-3431-9664

**References**

1. Towne JE, Renshaw BR, Douangpanya J, Lipsky BP, Shen M, Gabel CA, Sims JE. Interleukin-36 (IL-36) ligands require processing for full agonist (IL-36alpha, IL-36beta, and IL-36gamma) or antagonist (IL-36ra) activity. J Biol Chem. 2011;286:42594–42602.

2. Towne JE, Sims JE. IL-36 in psoriasis. Curr Opin Pharmacol. 2012;12:84–89.

3. Cowen EW, Goldbach-Mansky R, Dirra, ditra, and new insights into pathways of skin inflammation: what’s in a name? Arch Dermatol. 2012;148:381–84.

4. Marrakchi S, Guigue P, Renshaw BR, Puel A, Pei XY, Fraigt S, Zribi J, Bal E, Cluzeau C, Chrabieh M, et al. Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. N Engl J Med. 2011;365(7):620–28.

5. Blumberg H, Dinh H, Dean C Jr., Trueblood ES, Bailey K, Shows D, Bhagavathula N, Aslam MN, Varani J, Towne JE, et al. IL-1R1 and its ligands contribute to the cytokine network in psoriasis. J Immunol. 2010;185(7):4354–62.

6. Sims JE, Nicklin MJ, Bazan JF, Barton JL, Busfield SJ, Ford JE, Kastelein RA, Kumar S, Lin H, Mulero JJ, et al. A new nomenclature for il-1-family genes. Trends Immunol. 2001;22 (10):536–37.

7. Saha SS, Singh D, Raymond EL, Ganesan R, Caviness G, Grimaldi C, Woska JR Jr., Mennerich D, Brown SE, Mbow ML, et al. Signal transduction and intracellular trafficking by the interleukin 36 receptor. J Biol Chem. 2015;290(39):23997–4006.

8. Afonina IS, Muller C, Martin SJ, Beyaert R. Proteolytic processing of interleukin-1 family cytokines: variations on a common theme. Immunity. 2015;42:991–1004.

9. Onoufriadis A, Simpson MA, Pink AE, Di Meglio P, Smith CH, Pullabhatla V, Knight J, Spain SL, Nestle FO, Burden AD, et al.
Mutations in il36rn/Il1l5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. Am J Hum Genet. 2011;89(3):432–37.

10. Frey S, Derer A, Messbacher ME, Baeten DL, Bugatti S, Montecucco C, Schett G, Hueber AJ. The novel cytokine interleukin-36alpha is expressed in psoriatic and rheumatoid arthritis synovium. Ann Rheum Dis. 2013;72:1569–74.

11. Chen H, Wang Y, Bai C, Wang X. Alterations of plasma inflammatory biomarkers in the healthy and chronic obstructive pulmonary disease patients with or without acute exacerbation. J Proteomics. 2012;75:2835–43.

12. Medina-Contreras O, Harusato A, Nishio H, Flannigan KL, Ngo V, Leon G, Neumann PA, Geem D, Lili LN, Ramadas RA, et al. Cutting edge: Il-36 receptor promotes resolution of intestinal damage. J Immunol. 2016;196(1):34–38.

13. Scheibe K, Backert I, Wirtz S, Hueber A, Schett G, Vieth M, Probst HC, Bopp T, Neurath MF, Neufert C. Il-36r signalling activates intestinal epithelial cells and fibroblasts and promotes mucosal healing in vivo. Gut. 2017;66:823–38.

14. Russell SE, Horan RM, Stefanska AM, Carey A, Leon G, Aguilar M, Statovci D, Moran T, Fallon PG, Shanahan F, et al. Il-36alpha expression is elevated in ulcerative colitis and promotes colonic inflammation. Mucosal Immunol. 2016;9(5):1193–204.

15. Wang M, Kussrow AK, Ocana MF, Chabot JR, Lepsy CS, Bornhop DJ, O’Hara DM. Physiologically relevant binding affinity quantification of monoclonal antibody pf-00547659 to mucosal addressin cell adhesion molecule for in vitro in vivo correlation. Br J Pharmacol. 2017;174:70–81.

16. Ganesan R, Raymond EL, Mennerich D, Woska JR Jr., Wirtz S, Neufert C, van Steeg TJ, van der Graaf PH, Avery LB, Jones H, Berkhou J. Linear pharmacokinetic parameters for monoclonal antibodies are similar within a species and across different pharmacological targets: a comparison between human, cynomolgus monkey and hfcrn tg32 transgenic mouse using a population-modeling approach. Mabs. 2018;10:751–64.

17. Hurst LD, Sachenkova O, Daub C, Forrest AR, Huminiecki L, Consortium F. A simple metric of promoter architecture robustly predicts expression breadth of human genes suggesting that most transcription factors are positive regulators. Genome Biol. 2014;15:413.

18. Shah DK, Veith J, Bernacki RJ, Balthasar JP. Evaluation of combined bevacizumab and intraperitoneal carboplatin or paclitaxel therapy in a mouse model of ovarian cancer. Cancer Chemother Pharmacol. 2011;68:951–58.

19. Mollman I, Fuchs R, Hellenius A. Acidification of the endocytic and exocytic pathways. Annu Rev Biochem. 1986;55:663–700.

20. Furth PK, Olichney JM, Fett N, Zhang J, DeGuzman S, Gajewski TJ, et al. Use of the Tm-a model for therapeutic monoclonal antibodies: initial look. J Clin Pharmacol. 2009;49:1382–402.

21. Oitate M, Nakayama S, Ito T, Kurihara A, Okudaira N, Izumi T. Prediction of human plasma concentration-time profiles of monoclonal antibodies from monkey data by a species-invariant time method. Drug Metab Pharmacokinet. 2012;27:354–59.

22. Mayer DE, Jusko WJ. General pharmacokinetic model for drugs exhibiting target-mediated drug disposition. J Pharmacokin Pharmacodyn. 2001;28:507–32.

23. Mayer DE, Neubeboom B, Efthymiopoulos C, Munao A, Jusko WJ. Receptor-mediated pharmacokinetics and pharmacodynamics of interferon-beta1a in monkeys. J Pharmacol Exp Ther. 2003;306:262–70.

24. Consortium F, Forrest AR, Kawai H, Rehli M, Baillie JK, de Hoon MJ, Haberle V, Lassmann T, Kulakovskiy IV, Lizio M, et al. A promoter-level mammalian expression atlas. Nature. 2014;507 (7493):462–70.

25. Kanamori-Katayama M, Itoh M, Kawai H, Lassmann T, Katayama S, Kojima M, Bertin N, Kaiho A, Ninomiya N, Daub CO, et al. Unamplified cap analysis of gene expression on a single-molecule sequencer. Genome Res. 2011;21(7):1150–59.

26. Nemoto H, Kojima M, Ishikawa-Kato S, Kaida K, Kaiho A, Kamor et al. Production and functional characterization of anti-human IL-36R antagonistic monoclonal antibodies. Mabs. 2015;7(7):1143–54.

27. Deng R, Iyer S, Theil FP, Mortensen DL, Fielder PJ, Prabhu S. Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data: what have we learned? Mabs. 2011:3;61–66.

28. Liang J, Zhou H, Jiao Q, Davis HM. Interspecies scaling of therapeutic monoclonal antibodies: initial look. J Clin Pharmacol. 2009;49:1382–402.

29. Oitate M, Nakayama S, Ito T, Kurihara A, Okudaira N, Izumi T. Prediction of human plasma concentration-time profiles of monoclonal antibodies from monkey data by a species-invariant time method. Drug Metab Pharmacokinet. 2012;27:354–59.

30. Mayer DE, Jusko WJ. General pharmacokinetic model for drugs exhibiting target-mediated drug disposition. J Pharmacokin Pharmacodyn. 2001;28:507–32.

31. Mayer DE, Neubeboom B, Efthymiopoulos C, Munao A, Jusko WJ. Receptor-mediated pharmacokinetics and pharmacodynamics of interferon-beta1a in monkeys. J Pharmacol Exp Ther. 2003;306:262–70.

32. Consortium F, Forrest AR, Kawai H, Rehli M, Baillie JK, de Hoon MJ, Haberle V, Lassmann T, Kulakovskiy IV, Lizio M, et al. A promoter-level mammalian expression atlas. Nature. 2014;507 (7493):462–70.

33. Kanamori-Katayama M, Itoh M, Kawai H, Lassmann T, Katayama S, Kojima M, Bertin N, Kaiho A, Ninomiya N, Daub CO, et al. Unamplified cap analysis of gene expression on a single-molecule sequencer. Genome Res. 2011;21(7):1150–59.

34. Nemoto H, Kojima M, Ishikawa-Kato S, Kaida K, Kaiho A, Kamor et al. Production and functional characterization of anti-human IL-36R antagonistic monoclonal antibodies. Mabs. 2015;7(7):1143–54.

35. Deng R, Iyer S, Theil FP, Mortensen DL, Fielder PJ, Prabhu S. Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data: what have we learned? Mabs. 2011:3;61–66.

36. Singh AP, Krzyzanski W, Martin SW, Weber G, Betts A, Ahmad A, Abraham A, Zutshi A, Lin J, Singh P. Quantitative prediction of human pharmacokinetics for mabs exhibiting target-mediated drug disposition. J Pharmacol Exp Ther. 2012;341:702–08.

37. Singh AP, Krzyzanski W, Martin SW, Weber G, Betts A, Ahmad A, Abraham A, Zutshi A, Lin J, Singh P. Quantitative prediction of human pharmacokinetics for mabs exhibiting target-mediated drug disposition. J Pharmacol Exp Ther. 2012;341:702–08.

38. Parm G, Singh P, Pittman DD, Wright K, Leary B, Patel-Hett S, Rakhe S, Steijak J, Peraza M, Dufield D, et al. Translational pharmacokinetic/pharmacodynamic characterization and target-mediated drug disposition modeling of an anti-tissue factor pathway inhibitor antibody, pf-06741086. J Pharm Sci. 2018;109:751–64.

39. Ng CM, Joshi A, Dedrick RL, Garovoy MR, Bauer RJ. Pharmacokinetic-pharmacodynamic- efficacy analysis of efalizumab in patients with moderate to severe psoriasis. Pharm Res. 2005;22:1088–100.

40. Michaelis TE, Sandlie I, Bratlie DB, Sandin RH, Ihle O. Structural difference in the complement activation site of human iggl and igg3. Scand J Immunol. 2009;70:553–64.

Park YM, Squizzato S, Buso N, Gur T, Lopez R. The ebi search engine: ebi search as a service-making biological data accessible for all. Nucleic Acids Res. 2017;45:W545–W549.