Utility of immunodeficient mouse models for characterizing the preclinical pharmacokinetics of immunogenic antibody therapeutics

Maria Myzithras, Tammy Bigwarfe, Hua Li, Erica Waltz, Jennifer Ahlberg, Craig Giragossian, and Simon Roberts

Immune Modulation and Biotherapeutics Discovery, Research, Boehringer Ingelheim, Ridgefield, CT, USA

ABSTRACT
Prior to clinical studies, the pharmacokinetics (PK) of antibody-based therapeutics are characterized in preclinical species; however, those species can elicit immunogenic responses that can lead to an inaccurate estimation of PK parameters. Immunodeficient (SCID) transgenic hFcRn and C57BL/6 mice were used to characterize the PK of three antibodies that were previously shown to be immunogenic in mice and cynomolgus monkeys. Four mouse strains, Tg32 hFcRn SCID, Tg32 hFcRn, SCID and C57BL/6, were administered adalimumab (Humira®), mAbX and mAbX-YTE at 1 mg/kg, and in SCID strains there was no incidence of immunogenicity. In non-SCID strains, drug-clearing ADAs appeared after 4–7 days, which affected the ability to accurately calculate PK parameters. Single species allometric scaling of PK data for Humira® in SCID and hFcRn SCID mice resulted in improved human PK predictions compared to C57BL/6 mice. Thus, the SCID mouse model was demonstrated to be a useful tool for assessing the preclinical PK of immunogenic therapeutics.

Abbreviations: IgG, immunoglobulin G; mAb, monoclonal antibody; PK, pharmacokinetics; B6, C57BL/6; Tg, transgenic; SCID, severe combined immunodeficiency; hFcRn, human neonatal Fc receptor; AUCinf, area under the curve to infinity; MSD, meso scale discovery; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; CL, clearance; T1/2, terminal half-life; IV, intravenous; ADA, anti-drug-antibody; TNF, tumor necrosis factor

Introduction
The preclinical pharmacokinetics (PK) of humanized therapeutic monoclonal antibodies (mAbs) is often confounded by the generation of anti-drug antibodies. Emergent anti-drug antibodies (ADAs) after a single dose can lead to an apparent faster than expected clearance (CL) due to ADA-drug complexes or an inaccurate assessment of the terminal elimination half-life. When there is a sufficient number of animals in a study, it may be possible to exclude ADA-positive samples or animals in order to improve PK parameter estimates. However, in other cases the number of samples per timepoint is insufficient to produce reliable PK parameter estimates, or in the worst case scenario for highly immunogenic proteins, all animals exhibit early onset of anti-therapeutic antibodies, precluding a meaningful assessment of the intrinsic PK properties of the molecule prior to first-in-human studies.

Human neonatal Fc receptor transgenic (hFcRn Tg) mice have been shown to have utility for predicting human CL of mAbs. These mice also offer the advantage, compared to wild-type mice, of being able to evaluate the PK properties of engineered mAbs with enhanced affinity for hFcRn. As such, hFcRn Tg mice can be used as a cost and resource-effective tool for screening and characterizing antibody therapeutics; thereby, leading to the reduced use of non-human primates. Many transgenic mouse strains expressing hFcRn are available; including strains in an immunodeficient (SCID) background. These mice do not possess a functional immune system and are expected to substantially reduce the impact of anti-drug antibodies on antibody clearance compared to immune-competent mice.

We were interested in evaluating SCID mice in both hFcRn Tg and C57BL/6 varieties with three different antibodies, Humira®, mAbX and mAbX-YTE. All three antibodies were previously shown to be immunogenic in mouse and cynomolgus monkey (see supplemental material) and cleared rapidly after roughly 7 days, resulting in an inability to accurately characterize PK parameters. mAbX and mAbX-YTE are the same humanized antibody targeting tumor necrosis factor (TNF); however, mAbX-YTE contains a triple engineered ‘YTE’ (M252Y/S254T/T256E) mutation for increased affinity to the human FcRn receptor, allowing for testing of possible half-life extension. Additionally, ADA response after previous administration of mAbX and mAbX-YTE molecules in cynomolgus monkey masked any potential YTE-related differences in elimination half-life due to limited concentration-time profiles. The aim was to utilize SCID strains to obtain a full concentration timecourse to better characterize and assess the PK of each molecule compared to non-SCID strains.
Results

Humira®, mAbX and mAbX-YTE molecules were administered using a single intravenous dose at 1 mg/kg in four different mouse strains, Tg32 (homozygous) hFcRn, Tg32 (homozygous) hFcRn SCID, B6 (C57BL/6) SCID and B6 (C57BL/6), and concentration-time profiles were determined for 3 weeks. The Tg32 hFcRn homozygous was chosen based on studies where this mouse strain most closely predicted human half-life and clearance of antibodies tested, and was more efficient at FcRn mediated recycling than other available hFcRn transgenic strains.4

After intravenous (IV) administration of Humira®, mAbX and mAbX-YTE at 1 mg/kg in all four mouse strains, the concentration-time profiles of both SCID strains (Tg32 hFcRn and B6) were similar, with no indication of ADA for the entire duration of the study (21 days) for all molecules, while both non-SCID strains (Tg32 hFcRn and B6) had profiles suggestive of ADA after 4 d (Figs. 1–3) for all molecules.

ADAs were determined using an enzyme-linked immunosorbent assay (ELISA) endpoint titer method using timepoints from all animals after day 4 in each dose group. Positive ADA titers were confirmed by calculating fold-change over predose, with any fold-changes above 1 considered positive for ADA. All timepoints tested from non-SCID strains were positive for ADA after 4–7 days, which corresponds to the precipitous drop in drug concentration, while all timepoints for SCID strains were negative for ADA (Figs. 4–6).

Clearance and half-life were calculated for all Tg32 hFcRn SCID and B6 SCID strains because there were sufficient timepoints to characterize the terminal phase with minimal extrapolation of the area under the curve to infinity (Tables 1–3). Clearance and half-life were also determined for the non-SCID strains; however, clearance is predicted to be faster due to ADA-drug complexes, and half-life less reliable due to the lack of timepoints in the terminal phase.

Mean clearance of Humira® in Tg32 hFcRn SCID was 16.5 mL/d/kg with a half-life of 10.3 days, and mean clearance in B6 SCID was comparable at 13.9 mL/d/kg with a half-life of 12.4 d. Conversely, for both non-SCID strains elimination half-life and clearance values could not be accurately characterized due to contributions of ADA-induced drug clearance to total clearance. Using a simple mouse to human species allometric scaling based on body weight and an exponent of 0.85,9 mean human CL for Tg32 FcRn SCID and SCID B6 were predicted to be 4.9 and 4.0 mL/d/kg, respectively. For non-SCID strains, mean human CL was predicted to be faster at 6.7 and 5.2 mL/d/kg for Tg32 hFcRn and B6, respectively. Healthy human subjects dosed intravenously with Humira® cleared the drug at a mean rate of 2.9 to 3.8 mL/d/kg, which is within the variability range of the scaled clearance value from both SCID mouse strains.10

Non-SCID strains were not as predictive as their SCID counterparts, with a higher mean human CL, which is expected given the less accurate scaled mouse CL due to immunogenicity.
Mean clearance and half-life of mAbX-YTE in Tg32 SCID mice was 16.9 mL/d/kg and 5.8 days, respectively, and mean clearance for B6 SCID was 24.2 mL/d/kg with a mean half-life of 4.8 days. Mean clearance of mAbX in Tg32 SCID was 18.5 mL/d/kg with a half-life of 5.9 days, and mean clearance for B6 SCID was comparable to that seen in Tg32 SCID at 18.6 mL/d/kg with a mean half-life of 6.6 days. As was observed for Humira™, half-life and clearance values for non-SCID strains could not be accurately characterized due to contributions of ADA-induced drug clearance to total clearance and lack of timepoints in the terminal phase.

### Discussion

When discovering and developing new monoclonal antibody-based therapeutic drug candidates, it is important to screen for molecules with optimal PK properties to enable desired pharmacological effects while minimizing required dose and dose frequency. Candidates are selected using preclinical animal PK studies, most commonly rodent and non-human primates; however, for immunogenic molecules, clearing and neutralizing anti-drug antibodies generated in both species can preclude accurate characterization of terminal half-life subsequent to ADA-drug complex formation. Human FcRn Tg and B6 mice in SCID background were evaluated for their ability to enable PK characterization of molecules previously demonstrating immunogenicity. Additionally, the PK evaluation of molecules can more easily be done in mouse than in monkey due to minimal material and animal costs, as well as for ethical reasons in terms of reduction in the use of higher species.

After evaluation of three molecules (Humira™, mAbX and mAbX-YTE) in both hFcRn and B6, SCID or non-SCID mice, we found no significant differences in clearance and half-life in Tg32 hFcRn SCID vs. B6 SCID mice for respective groups; all were within a 1.5-fold difference, which is within biological variability. Both SCID strains enabled full timecourses in the absence of immunogenicity, so PK calculations were more accurate than those for their non-SCID counterparts for all three molecules. After using a simple allometric scaling of mouse to human clearance for Humira™, there appeared to be no predictive advantage with the Tg hFcRn SCID strain compared to B6 SCID. However, other studies have shown that hFcRn transgenic mice provided numerically superior human clearance predictions compared to wild-type mice. A recent publication using a larger cohort of antibodies showed the Tg32 hFcRn strain predicted human clearance within 2-fold for 100% of the molecules evaluated, whereas wild-type mice predicted human clearance within 2-fold for 73% of the molecules evaluated.

A YTE engineered mutation in the Fc region of mAbX-YTE was also evaluated in the SCID mouse model and PK properties were compared to mAbX, which is the same molecule without any mutations. This mutation increases affinity to the human FcRn receptor and can promote recycling of the antibody instead of subsequent degradation, therefore prolonging exposure and half-life. A comparison of the two molecules was not possible in previous animal studies due to the presence of ADA and resulting inability to compare terminal phases of each molecule. In other animal studies where the PK of YTE-containing

### Table 1. Pharmacokinetic parameters calculated using non-compartmental analysis for animals dosed with Humira™.

| Mouse Strain | CL (mL/d/kg) | T₁/₂ (d) |
|--------------|--------------|----------|
| Tg32 SCID    | 16.5 (6.0)   | 10.3 (2.0) |
| Tg32         | 24.5 (4.0)   | 5.4 (1.2)  |
| B6 SCID      | 13.9 (4.7)   | 12.4 (4.7) |
| B6           | **18.4 (9.7)** | **6.2 (2.3)** |

### Table 2. Pharmacokinetic parameters calculated using non-compartmental analysis for animals dosed with mAbX-YTE.

| Mouse Strain | CL (mL/d/kg) | T₁/₂ (d) |
|--------------|--------------|----------|
| Tg32 SCID    | 16.9 (3.4)   | 5.8 (0.6)  |
| Tg32         | **31.2 (6.8)** | **4.7 (1.1)** |
| B6 SCID      | 24.2 (1.8)   | 4.8 (0.3)  |
| B6           | **19.7 (5.9)** | **4.2 (1.5)** |

### Table 3. Pharmacokinetic parameters calculated using non-compartmental analysis for animals dosed with mAbX.

| Mouse Strain | CL (mL/d/kg) | T₁/₂ (d) |
|--------------|--------------|----------|
| Tg32 SCID (n = 2) | 18.5 (0.5)   | 5.9 (0.2)  |
| Tg32         | **19.2 (10.4)** | **10.2 (10.4)** |
| B6 SCID      | 18.6 (3.9)   | 6.6 (1.1)  |
| B6           | **17.4 (3.3)** | **4.6 (1.1)** |

*Parameters may not be accurate due to lack of timepoints in the terminal phase and drug-clearing ADAs.*
mutations was assessed, ADA was not detectable (data not shown); therefore, SCID mice are not a necessary prerequisite for assessing the PK of molecules with Fc mutations. In the SCID strains, where full timecourses are available to more accurately calculate PK parameters, there were no significant differences in clearance or half-life in the YTE mutant relative to that of the corresponding non-YTE molecule. The Tg32 SCID strain, having humanized FcRn and no detectable ADA, should have enabled differentiation between YTE and non-YTE mutants if there were differences in binding and recycling; however, mean clearance was comparable between these two groups at 16.9 and 18.5 mL/d/kg. Mean half-life was also comparable at 5.8 and 5.9 d for the YTE and non-YTE mutants, respectively. The lack of differentiation in this study could also be masked by other intrinsic factors in the molecules unrelated to FcRn binding that could affect PK (e.g., aggregation, differences in the isoelectric point or post-translational modifications) since Fc mutations have previously shown differentiation in half-life. Also, in rodents, Fc mutations that increase affinity to the FcRn have been shown to exhibit the same or shorter half-life, and may not correlate to the improvement seen in humans.

In conclusion, the Tg32 hFcRn SCID and B6 SCID mouse strains have proven utility when characterizing the PK profile of three antibodies tested over the non-SCID strains. The absence of immunogenicity in Tg32 SCID and B6 SCID enabled more accurate PK calculations, and, in the case of Humira®, closer human CL predictions using allometric scaling over Tg32 hFcRn and B6. Drug-clearing ADAs formed after 4–7 d in all non-SCID strains. As a consequence, PK parameters were not as accurate due to lack of terminal phase timepoints. The SCID mouse model can therefore be used preclinically to characterize, screen and rank molecules and determine their intrinsic PK properties without the possibility of immunogenicity to confound results. There are other points to consider when using hFcRn mice to screen antibody candidates; for example, if the target is cell-associated or highly expressed, target-mediated drug disposition contributions to clearance may not translate well across species as a result of potential differences in target expression and turnover. Another consideration is that SCID mice lack functional immune systems, including a substantially reduced population of B and T cells, so evaluating the pharmacodynamics effects of therapeutics targeting immune pathways in parallel with PK may not be relevant.

**Materials and methods**

**Molecules**

A single-dose syringe of the human monoclonal antibody Humira® (adalimumab, Abbvie Inc.) was purchased and used for in-house research only. Humanized antibodies mAbX and mAbX-YTE were produced in-house and passed internal quality control (QC) verification for use in animal studies. mAbX-YTE contains an engineered YTE mutation for increased binding to FcRn receptor and mAbX is the same construct as mAbX-YTE without the mutations. All three molecules target soluble TNF; therefore, any possible target-mediated disposition will be avoided due to the low circulating soluble levels.

**In vivo study details**

The in vivo study portion was conducted at the Jackson Laboratory (Bar Harbor, ME) using their transgenic mouse strains following Institutional Animal Care and Use Committee approval and with the study outline shown in Table 4. Nine mice per strain (n = 3 per group) were transferred to the animal room at 6–10 weeks old; the mouse strains were human FcRn transgenic SCID “Tg32 hFcRn SCID” (B6.Cg-Fcgritm1Dcr Prkdcscid Tg(DCCRT)32Dcr/Dcr JAX # 18441) mice, transgenic FcRn mice “Tg32 hFcRn” (B6.Cg-Fcgritm1Dcr Tg (DCCRT)32Dcr/Dcr JAX # 14565), “B6 SCID” (B6.CB17-Prkdcscid/SzJ, JAX # 1913), and “B6” (C57BL/6J, JAX # 664). Samples were collected following Jackson’s microsampling protocol. Briefly, 25 μL of whole blood was collected into tubes containing tri-potassium ethylenediaminetetraacetic acid (K3-EDTA) for each timepoint and processed for plasma, immediately diluted 1:10 (50% glycerol in 1X PBS with 0.05% azide), and then subsequently frozen in 96-well plates at −80°C. A pre-dose sample was collected, and 24 hours later the study was initiated using a single IV bolus dose of 1 mg/kg of each test article. Additional timepoints were collected at 1, 6, 24, 72, 96, 168, 336, and 504 hours post-dose.

| Compound | Tg32 hFcRn SCID | Tg32 hFcRn | B6 SCID |
|----------|-----------------|------------|--------|
| Humira®  | 1a 1b 1c 1d     | 2a 2b 2c 2d| 3a 3b 3c 3d |
| mAbX-YTE |                 | 24 1 6 72 | 96 168 336 504 |

**Reagent labeling**

Biotinylation of an unlabeled polyclonal goat anti-human IgG (Southern Biotech, cat#2045-01) for use as capture reagent was performed at a concentration of 1 mg/mL using a no-weigh EZ-Link Sulfo-NHS-LC-Biotin kit (Pierce Thermo Scientific) using a 1:10 antibody to biotin challenge ratio and one hour incubation with shaking (600 rpm) at room temperature. The resulting biotinylated antibody was desalted using a 0.5 mL Zeba 7K MWCO spin column (Thermo Scientific), pre-washed 3 times with 300 μL of 1X PBS.

For detection, a separate unlabeled polyclonal goat anti-human IgG (Jackson ImmunoResearch, cat#109-005-098) was labeled at 1 mg/mL with an MSD Sulfo-Tag NHS-Ester (Meso Scale Discovery) using a 1:10 antibody to Sulfo ratio, according to manufacturer’s instructions. The labeled antibody was desalted using 0.5 mL Zeba 7K MWCO spin columns (Thermo Scientific), pre-washed 3 times with 300 μL of 1X PBS.
Both labeled reagents are to be diluted in binding buffer; 5% bovine serum albumin (BSA; Seracare) with 0.05% Tween 20 (Sigma).

**Antibody quantitation by MSD**

An immunoassay using the Meso Scale Discovery platform (MSD) was developed to detect total antibody, using a homogenous assay format. A master mix was prepared, combining 0.5 μg/ml biotinylated goat anti-human IgG (Southern Biotech) and 0.5 μg/mL sulfo-labeled goat anti-human IgG (Jackson ImmunoResearch) in binding buffer (5% BSA in 1X PBS with 0.05% Tween 20). This master mix was added to a 96-well, non-binding, light-blocking plate (Fisher Scientific) at 50 μL per well. Twenty-five μL of standards, QC's and study samples (diluted in binding buffer) were added per well in duplicate to the non-binding plate containing the master mix. The non-binding plate was incubated at room temperature on a plate shaker (500 rpm, 1.5 hr). In parallel, an MSD streptavidin (SA) gold 96-well plate (Meso Scale Discovery) was blocked using 150 μL blocking buffer (5% BSA in 1X PBS with 0.05% Tween 20) and incubated at room temperature on a plate shaker (500 rpm, 1.5 hr). After incubation, the MSD plate was washed 3 times with 300 μL per well wash buffer (0.05% Tween 20 in 1X PBS). Fifty microliter aliquots of each sample mix from non-binding plates were added to MSD plates and incubated at room temperature on a plate shaker (500 rpm, 1.5 hr). After incubation, the plate was washed 3 times and 150 μL of 2X Read Buffer T (MSD, stock diluted 2X in dH2O) was added to each well and read immediately on the MSD Sector Imager 2400 using an electrochemiluminescent (ECL) signal. QC and unknown sample concentrations were back-calculated using standard curves fitted to a 4-parameter logistics equation using MSD Discovery Workbench software. PK parameters were calculated with Phoenix 64 (Centara) using non-compartmental analysis.

**ADA confirmation by endpoint titer ELISA**

An enzyme-linked immunoassay (ELISA) was developed to detect signal from mouse anti-drug antibodies from any possible immune response using the endpoint titer method. Nunc Maxisorp 96-well microplates (Fisher Scientific) were coated overnight at 4°C with 1 μg/mL corresponding molecule as capture reagent, Humira®, mAbX or mAbX-YTE. The plates were washed 3 times with 300 μL per well wash buffer (0.05% Tween 20 in 1X PBS) and blocked with 150 μL per well blocking buffer (5% BSA in 1X PBS with 0.05% Tween 20), and incubated at room temperature on a plate shaker (300 rpm, 1 hr). While the plates were blocking, 5-fold serial sample dilutions were prepared in binding buffer (5% BSA in 1X PBS with 0.05% Tween 20) starting with a 1:50 dilution, vertically down each column 1-11 in a non-binding plate for the following timepoints from each individual animal: predose, 96 hr, 168 hr, 336 hr and 504 hr. After incubation, the plates were washed 3 times with 300 μL per well wash buffer and 50 μL of each serial dilution were transferred to the Nunc plate from the non-binding plate, adding only binding buffer to at least one column as background. The plates were incubated on a plate shaker at room temperature for 2 hrs at 300 rpm. After incubation, the plates were washed 3 times with 300 μL per well wash buffer and 100 μL per well of an anti-mouse IgG+IgM antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch, cat#115-035-068) was added at a 1:4000 dilution. The plates were incubated on a plate shaker at room temperature for 1 hr at 300 rpm. After incubation, the plates were washed 3 times with 300 μL per well wash buffer and 100 μL per well of tetramethylbenzidine substrate was added and incubated for 5 minutes. The reaction was stopped with 100 μL per well of H2SO4 and immediately read on a Molecular Devices M3 plate reader at 450 nm and 600 nm dual wavelengths to calculate optical density. Endpoint titer was calculated as the reciprocal of the highest dilution that gives signal above baseline (baseline determined as 5-fold plate background absorbance). ADA titer fold-change was calculated by dividing the sample titer by corresponding predose titer for each animal, and positive results are represented as any fold change over predose.

**Disclosure of potential conflicts of interest**

The authors have no potential conflicts of interest or financial disclosures to report. All in vivo procedures conducted at The Jackson Laboratory were approved by their animal ethics committee.

**ORCID**

Maria Myzithras [http://orcid.org/0000-0002-2295-437X](http://orcid.org/0000-0002-2295-437X)
Jennifer Ahlberg [http://orcid.org/0000-0002-2892-4163](http://orcid.org/0000-0002-2892-4163)
Simon Roberts [http://orcid.org/0000-0002-5743-3769](http://orcid.org/0000-0002-5743-3769)

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