HuPK: A Humanized Platform for therapeutic antibody PK evaluation

FIELD GUIDE

The Jackson Laboratory

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Introduction

Because of evolutionary changes in the Neonatal Fc Receptor, the protein responsible for IgG and albumin recycling, WT mice cannot be used to predict the human PK of candidate therapeutic antibodies. The Jackson Laboratory has developed a series of transgenic mouse lines that exclusively express human FcRn, the HuPK models. These models allow the prediction of the clinical PK of therapeutic antibodies other Fc-based biologics and albumin-conjugated molecules. The different HuPK models carry additional mutations and transgenes to address specific aspects of antibody- and albumin-based drug pharmacokinetics (PK).

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This guide provides an overview of the available models, study design templates, expected results, and troubleshooting tips in the context of the most common applications of JAX's HuPK Platform. It is based on years of experience performing thousands of antibody evaluations for our customers at The Jackson Laboratory.

Scan here if you have any questions regarding our models or services, do not hesitate to contact us by visiting: https://qrco.de/fcrn-cs

Main Applications

A. Compare leads to identify the candidate with the longest half-life

Drug developers need to compare the pharmacokinetic properties of multiple molecules following an antibody discovery campaign or after the engineering of a lead candidate to optimize its therapeutic features. These two situations require slightly different approaches depending on the nature of the molecules and modifications.

I. Comparison of different antibodies against a specific target

Drug developers can use different approaches to identify functional human or humanized antibodies against therapeutic targets. Irrespective of the technology used, antibody discovery teams usually identify several molecules with appropriate antigen affinity and effector functionality. While in vitro assays are an excellent means to identify antibodies with adequate affinity for human FcRn, they fail to predict PK performance in human patients.

Model selection

For an initial evaluation of different antibodies, mice expressing the Tg32 transgene are highly recommended. Antibody-based formats usually have half-lives between 8 and 15 days, depending on their FcRn affinity and other factors. Constructs containing the Fc region (i.e., Fc fusion proteins, bispecifics, and other modular constructs) and albumin-based molecules generally show half-lives between 2 and 8 days.

Occasionally human antibodies with wild-type Fc, and, more frequently, highly engineered molecules elicit anti-drug antibody (ADA) responses (Greg Christianson and Dan Villareal, unpublished data). In these situations, the Tg32 SCID model is the choice for predicting human PK.

STUDY DESIGN

Experimental group size

Since the study's goal is to compare the stability of different molecules rather than predict their half-life in humans with high accuracy, the size of the experimental groups can be limited to four mice.

Suggested controls

A PK study designed to compare antibody candidates directly does not require extensive controls since the molecules are compared one against the other. However, adding a clinical antibody of the same class with known PK in patients can provide a reference to judge the therapeutic candidates' performance. Another situation when a control antibody should be considered is when all molecules in a study are analyzed for the first time. In this case, the control antibody validates that the PK study was performed correctly, assuming the control antibody yields PK data consistent with historical data, should the test antibodies yield unexpected PK outcomes.

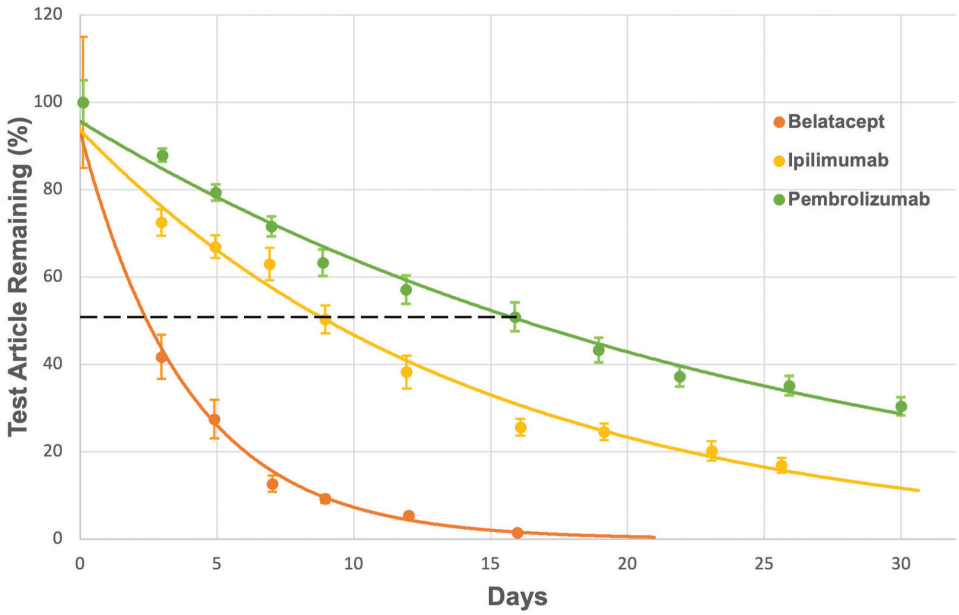
Experimental timelines

For IgG molecules, a typical experiment runs for 28 days. The length of the study is estimated to span about three half-lives for a "typical" human IgG molecule in the Tg32 model. Up to eleven blood draws are scheduled during this period with a higher frequency in the first week to capture the Cmax at the earliest time after IV administration. Potentially, a few additional time points prior to 24 hours can be measured to represent the distribution phase, then less frequent and evenly spaced time points to define the elimination phase of the molecules.

STUDY RESULTS

Expected results

Since the sequence of the antigen-binding domain can influence the PK of antibodies carrying the same Fc region, different antibodies often show measurable differences when compared head-to-head in Tg32 mice.



Serum concentration of three clinical antibodies in Tg32 mice over time expressed as a percentage over the initial concentration (5 minutes post-injection). The significant PK difference between the molecules reflects the clinical situation, with Pembrolizumab being the most stable antibody and Belatacept the most unstable. Calculated half-lives: Belatacept 2,8 days; Ipilimumab 9 days; Pembrolizumab 17 days. SE is shown. Belatacept and Ipilimumab are IgG1 class proteins, while Pembrolizumab is an IgG4.

Pitfalls and troubleshooting

- *All the test articles have long half-lives and show no apparent difference in their PK parameters.* The ideal length of a PK study spans at least three half-lives of the test articles to allow the clear detection of plasma concentration differences to exceed the statistical variation of the ELISAs used. If the antibodies tested in Tg32 mice have a half-life longer than 15 days, the PK curves may appear too flat to estimate their half-life precisely. Consequently, if their kinetics are superimposed, we might be unable to differentiate between the lead candidates. If molecules are expected to yield half-lives exceeding 15 days, Tg276 mice are highly recommended as a means to measure differences more precisely.

- *The test articles show a strong ADA response.* Although the Tg32 model has been extensively used and published to characterize the PK parameters of human IgG antibodies and other FcRn-binding therapeutics, this mouse model is immunocompetent and capable of mounting anti-drug antibody (ADA) responses. ADA production occurs due to the foreign nature of the therapeutics being administered. Human IgG antibodies with no Fc sequence changes are less likely to cause ADA responses. More engineered constructs, such as Fc-fusion proteins, tend to yield more frequent ADA responses. Still, it is not possible to predict which test articles will cause ADA.

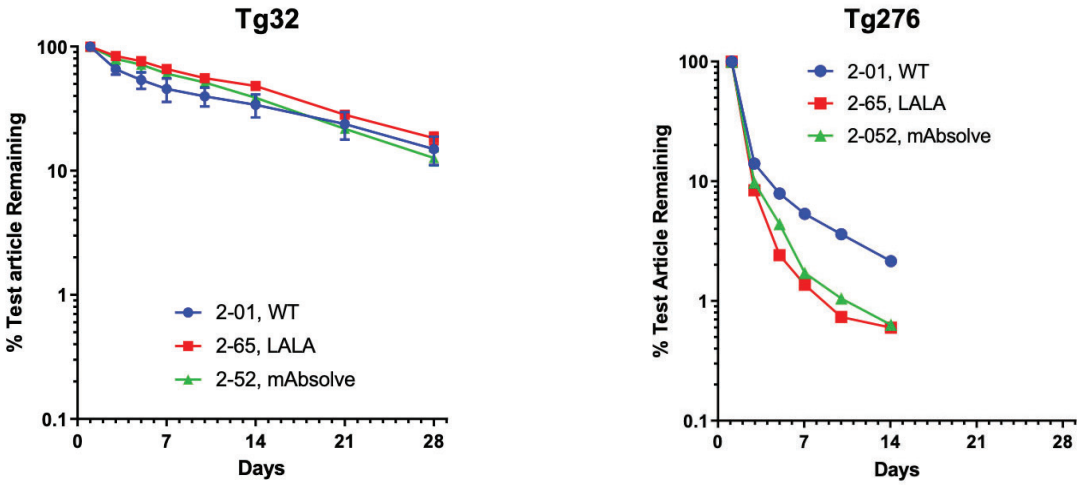
- *The simple solution to avoid ADA responses when performing PK studies is to utilize Tg32 scid mice (stock 018441).* Since these mice carry the scid mutation in the *Prkdc* gene, they lack T and B cells and are incapable of making ADA responses. Our validation data has shown that human antibody PK resulting from Tg32 and Tg32 scid mice are not significantly different.

II. Comparison of variants of the same construct

Most antibody-based therapeutics carry mutations in the Fc region aimed at silencing their immunological function, extending their serum half-life, or both. The preclinical evaluation of the half-life of the variants of an antibody is crucial to select the best clinical candidate in a pool of molecules with similar characteristics. For this reason, it is essential to evaluate PK parameters with high accuracy and select the most appropriate human FcRn transgenic model based on the molecular profile of the lead candidates.

Model selection

In general, mutations that inhibit the interaction with the Fcγ receptors have detrimental effects on the stability of the mutated antibodies. Depending on the half-life of the WT molecule, it might be appropriate to test the candidates in the Tg32 (<15 day half-life) or in Tg276 mice (>15 day half-life). As a rule of thumb, IgG-based drugs should be tested in the Tg276 model, while artificial constructs such as modular antibodies most likely work best in the immunocompromised Tg32 scid mouse model. In the figure below, we show the analysis of Fc variants of rituximab that yield indistinguishable elimination rates from Tg32 mice yet are readily differentiated by their half-life in Tg276 mice.



STUDY DESIGN

Experimental group size

When the PK study aims to screen larger panels of Fc variants to narrow down the lead candidate list for further characterization, as few as 4 mice per treatment group can be sufficient to provide statistical differences between the resulting spread of half-life values. Reducing this number to three per treatment group is possible but runs a risk: Should an animal be removed from the study for any reasons, no statistical differences will be provided for the affected treatment group. For the comparison of small numbers of lead candidates (four or less), five to eight mice should be considered per treatment group to provide the best possible preclinical estimates of PK parameters, particularly if the PK results are intended for publication or IND application.

Suggested controls

If the PK study intends to compare different Fc variants, there may be no need to include a reference antibody. However, when comparing Fc variants having mutations expected to reduce the effector function or extend half-life (LALA or YTE, for example) it is good practice to include the WT version of the antibody or FDA approved therapeutic that is being mimicked. The inclusion of a reference antibody is also essential when testing Fc variants in FcRn humanized mice for the first time, in case the mutations result in unexpectedly short half-lives.

Experimental timelines

Typical PK studies in Tg32 mice have test article quantification through 28 days, with four blood samples collected the first week and the other six collected throughout the remaining three weeks. For PK studies utilizing Tg276 mice which yield higher elimination rates, the study period can be shortened to 14 or 21 days, with up to six blood samples collected in the first week to increase the resolution of the plasma concentration-time curves early before the test articles drop below the lower limit of quantification (LLOQ) of the ELISA.

STUDY RESULTS

Expected results

Choosing which human FcRn platform model to use is critical to obtaining PK data that identify lead candidates. Acquiring test article plasma concentrations that span three half-lives is the optimum for providing statistically reliable preclinical PK estimates.

Pitfalls and troubleshooting

- *Test articles assessed in Tg276 mice might be eliminated too quickly, preventing precise quantification*. For molecules administered to Tg276 mice yielding half-lives shorter than two days, the standard study design might not provide sufficient time point plasma concentrations to generate robust half-life estimates. In such cases, the outcome can be improved by using Tg32 mice that will slow the elimination rate, increase the number of time points yielding test article concentrations above the LLOQ, and yield more robust half-life data.

- *Test articles that yield a strong ADA response. When using Tg276 mice, ADA responses can also occur*. Tg276 scid mice (stock 021146) are available to eliminate the problems associated with ADA. If the test articles are albumin-based therapeutics and ADA responses occur, it is possible to switch to using Tg32 Alb KO scid mice (Strain #:031644).

III. Testing variants of the same construct in the presence of competition from endogenous proteins

Mutations in the Fc binding domain of antibody therapeutics and albumin fusion proteins can affect the equilibrium dissociation constant with FcRn by changing the kon, the koff rate constants, or both. If a mutation decreases both rate constants, the half-life of a therapeutic lead might appear unchanged in the absence of competitor molecules. In the presence of competition, however, such a molecule would be unable to bind efficiently to FcRn, resulting in a shorter half-life.

Model selection

Depending on the therapeutics being characterized, either Tg32 or Tg32 Alb KO mice might be the appropriate model. Preloading the mice by IV injection beforehand with either human IgG (IVIG) or human albumin provides competition for FcRn binding and protection from lysosomal degradation. The advantage of administering competitor molecules makes it possible to change specific parameters (e.g., the concentration of the competitors or timing of administration), providing researchers with flexibility in the study design. On the other hand, the competitor molecules will be eliminated over time rather than keeping a steady-state concentration. An improvement of this design is to administer competitor molecules multiple times, but this results in competitor plasma concentration fluctuations. To avoid ADA responses caused by the repeated injections, use of Tg32 scid, or Tg32 Alb KO scid is highly recommended. Other options include Tg32-hFc mice (stock 029686) which express human IgG1 in place of mouse IgG1 at about 300 ug/mL, and Tg32 hAlb mice (stock TBD) which express human albumin in place of mouse albumin at about 30 mg/mL. Tg32-hFc mice are readily available, and Tg32 hAlb mice will become available by the third quarter of 2023.

STUDY DESIGN

Experimental group size

For models requiring the administration of the competitor molecules, we suggest using larger cohorts, namely five to eight mice. Using more mice per treatment group improves outcomes due to all of the parameters being manipulated in these studies.

Suggested controls

The no-competition control should be included in all of the experiments where the mice are administered vehicle only. Depending on the number of test articles being assessed, injecting the competitor at different concentrations is good practice to confirm the dose-dependent competition.

Experimental timelines

The presence of competition decreases the half-life of antibodies and albumin fusion proteins by a factor dependent upon the level of competition provided, due to FcRn function being saturable. These experiments are, therefore, usually shorter than the typical PK studies using Tg32 mice. The increased elimination of test article resulting from the added competition requires that blood sample collection be crowded early as 6 times in the first week and 4 times in the last 2 weeks of the study.

STUDY RESULTS

Expected results

Competition at the site of FcRn binding typically results in a shortened test article half-, unless the test molecules has an Fc variant with improved FcRn affinity (YTE for example).

Pitfalls and troubleshooting

- *Quantification of test articles being assessed in the presence of competition*. When competition is added to a PK study, an ELISA assay is required that can specifically quantify the test article without crossreacting with the competitor molecule. The cleanest method is to capture the test antibody, then use an anti-human IgG Fc, or anti-human albumin antibody labeled with HRP to quantify plasma concentrations.

- *The test articles or competitor molecules show a strong ADA response.* If test articles or competitor molecules are immunogenic, it is possible to use Tg32 scid mice (Myzitras et al.) for Fc-based therapeutics, or Tg32 Alb KO scid mice (Strain #:031644) for albumin-based therapeutics.

B. Predicting the clinical half-life of antibody-based therapeutics

A major challenge facing clinical drug developers is the selection of a starting dose for first-in-human clinical trials. The dose selection is based on several preclinical parameters, including *in vivo* efficacy, toxicity, and PK data (Muller et al.). The prediction of the half-life of a therapeutic antibody is crucial not only to calculating the serum concentration of a single dose but also defining the correct dosing schedule. Once a lead compound is selected as a candidate for clinical development, it is imperative to predict its stability in humans with high accuracy.

Multiple groups from different pharmaceutical companies have extensively assessed their antibodies and bispecifics in Tg32 mice, demonstrating that this model can reliably predict half-life in patients and pave the way for the use of this model for IND applications (i.e., Avery et al.; Sakshi et al.). Using this transgenic mouse model to explore the PK parameters of clinical leads allows unparalleled flexibility in the experimental design with reasonable costs and short timelines.

STUDY DESIGN

Experimental group size

Since it is crucial to have an exact estimate of the half-life for IND applications, a relatively large group size (8 animals) is recommended. Enrolling this number of animals allows for test article administration and quantification variation to cause the most negligible negative impact on the PK parameters.

Model selection

The most characterized transgenic line for predicting the clinical half-life of antibody-based therapeutics is, by far, the Tg32 mouse model. Scientists from Pfizer, Johnson and Johnson, Sanofi, and Eli Lilly, amongst others, have published multiple articles showing strong correlations for the half-life of human antibodies in Tg32 mice and humans.

Suggested controls

For the assessment of molecules with a short half-life (<7 days), it is highly recommended to add a group consisting of FcRn KO mice to fully understand the extent of protection being provided by human FcRn expressed in Tg32 mice.
This evidence might be significant for IND filings of engineered molecules with short half-lives to prove the beneficial effects of adding the Fc region to the construct. Another important control is the parallel measurement of the half-life of a clinically relevant molecule of the same class as the lead candidate (e.g., IgG, bispecific, etc.). Ideally, the compound should be synthesized using the same procedure as the test article to avoid differences due to the synthesis process.

Experimental timelines

The overall timelines of the experiment depend on the nature of the test article. IgG molecules and their derivatives usually have half-lives longer than 14 days, and we suggest running the experiment for four to six weeks, or longer. A three-week-long experiment is appropriate for shorter-lived molecules with half-lives ≤10 days.

STUDY RESULTS

Expected results

The use of large cohorts of animals will provide a high density of data and, consequently, a precise estimate of the half-life of the test article. Since all of the mice are genetically identical, the variability in the measurements should be minimal.

Pitfalls and troubleshooting

- *The lead candidate has a long half-life, leading to a "flat" elimination curve*. Some life-extended IgGs can have half-lives longer than 50 days. Molecules with such a long half-life should be evaluated in the Tg276 model. Although we are currently validating this transgenic line for allometric scaling, it is not currently possible to use PK data obtained from Tg276 mice to predict half-life in humans. The solution is, therefore, to extend the timeline of the experiment in Tg32 mice to at least 60 days.

- *The test articles show a strong ADA response*. If lead compounds are immunogenic, it is possible to use Tg32 scid mice (Myzitras et al.).

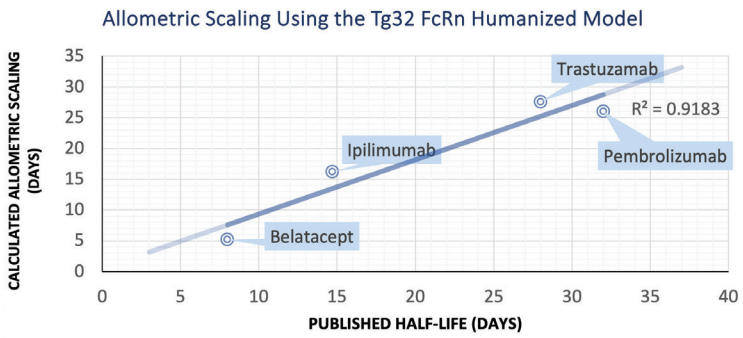
C. Allometric scaling

The linear correlation between the elimination of antibody-based therapeutics in the Tg32 model and humans allows allometric scaling using a simple formula:

$$Y_{HUM} = Y_{Tg32} * (\text{body weight}_{HUM}/\text{body weight}_{Tg32})^{\alpha}$$

Y is the PK parameter of interest (e.g., CL-clearance or VSS-volume of distribution at steady state), and α is the correlation coefficient. It is important to note that the correlation coefficient might vary depending on the model used. For the Tg32 homozygous model, the published literature reports allometric scaling exponents for CL comprised between 0.90 (Betts et al.) and 0.93 (Avery LB et al.), while for VSS is 1.

Interestingly, Valente et al., date report that the allometric scaling exponents can change between WT of Fc-mutated antibodies. Specifically, CL allometric exponents ranged from 0.97 for WT Fcs to 0.91 for mutated Fcs and Vss exponents from 0.96 to 0.93, respectively (only the exponents from Tg32 to NHPs were reported in this article).



Closing

To use the HuPK models to optimize your lead molecules and predict the clinical half-life of your drug candidates, you can take advantages of JAX Preclinical Services. Our experienced scientists can guide you through the choice of the best model and study design, perform the in vivo procedures, and generate the data for you. Alternatively, you can acquire the models directly from JAX to use in your facility.

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