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REVIEW

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Non-human primates in the PKPD evaluation of biologics: Needs and options to reduce, refine, and replace. A BioSafe White Paper

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ABSTRACT

Monoclonal antibodies (mAbs) deliver great benefits to patients with chronic and/or severe diseases thanks to their strong specificity to the therapeutic target. As a result of this specificity, non-human primates (NHP) are often the only preclinical species in which therapeutic antibodies cross-react with the target. Here, we highlight the value and limitations that NHP studies bring to the design of safe and efficient early clinical trials. Indeed, data generated in NHPs are integrated with in vitro information to predict the concentration/effect relationship in human, and therefore the doses to be tested in first-in-human trials. The similarities and differences in the systems defining the pharmacokinetics and pharmacodynamics (PKPD) of mAbs in NHP and human define the nature and the potential of the preclinical investigations performed in NHPs. Examples have been collated where the use of NHP was either pivotal to the design of the first-in-human trial or, inversely, led to the termination of a project prior to clinical development. The potential impact of immunogenicity on the results generated in NHPs is discussed. Strategies to optimize the use of NHPs for PKPD purposes include the addition of PD endpoints in safety assessment studies and the potential re-use of NHPs after non-terminal studies or cassette dosing several therapeutic agents of interest. Efforts are also made to reduce the use of NHPs in the industry through the use of in vitro systems, alternative in vivo models, and in silico approaches. In the case of prediction of ocular PK, the body of evidence gathered over the last two decades renders the use of NHPs obsolete. Expert perspectives, advantages, and pitfalls with these alternative approaches are shared in this review.

Introduction

There has been particular interest in non-human primate (NHP) use in biologics development since it was recognized that they may be the only cross reactive and pharmacologically relevant species for nonclinical assessment of many of these protein constructs. Therefore, an increase in the development of biologics has led to an increase in the use of NHPs, mainly the cynomolgus macaque in both safety and pharmacokinetic/pharmacodynamic (PKPD) assessment. On the other hand, there is an industry-wide push to reduce NHP use for ethical, logistical, and availability reasons. There are several initiatives and guidances that demonstrate this drive to reduce NHP use, including but not limited to the NC3Rs (National Center for the Replacement, Refinement, and Reduction of Animals in Research), which, in collaboration with up to 30 organizations from the pharmaceutical/biotechnology companies, contract research organizations (CROs) and regulatory agencies, have already facilitated cross-company data-sharing initiatives to minimize the increased use in NHPs. These evidence-based approaches have fed into regulatory addendums, e.g., ICH S6 (R1) and ICH M3 (R2) and continue to support the field in using appropriate study designs to answer the scientific questions at hand.[1, 2]

Here, we examine the value of NHP for PKPD assessment of biologics and evaluate the applicability of alternative methods. The value of NHP for the preclinical safety evaluation of biotherapeutics has been previously described by Brennan et al. and is outside of the scope of this review.[3] We focus primarily on monoclonal antibodies (mAb), as they are the most common biologics in development and on the market, but it is worth noting that the principles we describe are also applicable to engineered formats such as multi-specifics or Fc-fusion proteins.[4]

While PKPD is applied along the entirety of the drug discovery and development process, it plays a particularly valuable role in biotherapeutic drug development at the stage where strong evidence has been obtained that a candidate molecule, or a small number of candidates molecules, may be considered sufficiently safe and efficacious in humans based on preclinical data to merit advancement to investigation in human trials. A number of key decisions face the drug development team at this point, including: 1) selection of the best candidate

molecule, 2) prediction of the safe and efficacious exposure range in humans, and 3) determination of the optimal dose, route, and regimen required to achieve that therapeutic window. Important and relevant data essential to informing these decisions can come from in vitro and in vivo pharmacology, efficacy, and safety studies, and are often integrated using PKPD modeling to provide a quantitative and integrated analysis from which one can make comparisons between molecules, determine the safe and effective concentration range for the drug of interest, and guide optimal dosing in humans.

Data from NHPs can provide an invaluable contribution to the decisions described above. While it is rare in biotherapeutic drug development that all the information guiding these decisions comes only from NHPs, it is common that the best source of some of the essential information can be obtained only in studies with NHPs and, frequently the NHP is the only relevant nonclinical species from which in vivo data can be obtained. However, use of NHPs should not be the default, but rather justified by proper rational and science-based decision-making that should both support their ethical use and better inform the safety of clinical studies.[3] There are multiple approaches to reduce the NHP use in PKPD development of biologics by using alternative in vivo as well as in vitro models, optimizing study designs and use of physiologically based pharmacokinetic (PBPK) modeling to improve human prediction.

In the next sections, the utility of NHPs for PKPD purposes and their translational relevance are explained. Case examples are given for successful use of NHP for PKPD assessment, as well as its limitations. Case examples of alternative models and approaches to reduce the number of NHP are discussed to demonstrate the further path forward. A summary of the case examples presented below can be found in Table 1.

Relevance of NHP in PKPD understanding of mAbs

Utility of NHP PKPD studies in drug discovery and development

The relationship between drug concentration and response is the foundation of pharmacology and a critical element of the development of therapeutic molecules, regardless of their composition. During the drug development process, building and refining the exposure-response relationship in various assay and model systems, with tool and candidate drug molecules, provides support in choosing suitable drug targets, discovering and screening candidate molecules, and building the evidence needed to inform the optimal dose and dose regimen for studies in animals and humans. Failure to understand concentration-response relationships in relevant test systems, including NHPs, can have catastrophic consequences as evidenced by the TGN1412 program where gaps in this knowledge were a contributing factor to the tragic events that occurred during a clinical trial.[5] In this case, quantitative and biological differences between NHP and human in the exposure-response relationships were not taken into consideration when interpreting the results of toxicology studies, and PKPD-driven predictions of pharmacologic effects in human were not used, both of which may have led to a maximum recommended starting dose (MRSD) that would have allowed for safe dosing in humans. The evolution of exposure-response, built on literature data and the iterative generation of relevant data derived from multiple species, and test systems, incorporating multiple and diverse endpoints, presents challenges and opportunities to drug development strategy. The inherent complexity of this data provides a unique opportunity for application of PKPD principles and methods to enable holistic and quantitative integration of the available data, enabling a deeper understanding of pharmacologic systems, facilitating hypothesis generation and testing, and allowing rational predictions of novel outcomes.

It is essential that trials in human are conducted in a safe and efficient manner. To achieve this goal requires careful selection of biotherapeutic doses and dose regimens that avoid untoward effects while minimizing the exposure of patients to ineffective levels of a new biotherapeutic. Therefore, it is incumbent on drug developers to design trials that allow the most efficient generation of clinical data to facilitate the development of the drug candidate, and the use of optimized doses and dosing regimens is a critical component of this strategy. These principles apply during all stages of clinical development but may be most challenging when initially taking a new candidate drug into humans due to the lack of

Table 1. Summary of the examples.

Value of NHP in PKPD understanding of mAbs
Determination of FIH dose with pivotal PKPD data from cynomolgus monkeys (Lulizumab)
Successful translation of PKPD and MRSD determination (Rozanolixizumab)
Value of NHP for predicting PKPD in mAbs with half-life extension
Discontinuation of the development of a compound or preventing the discontinuation of a compound using NHPs (QBP359)
Challenges to overcome when using NHP for PKPD purposes
Successful use of allometric scaling to predict the human PK of a TNFR-Fc fusion protein despite immunogenicity (Lenercept)
PKPD assessment in cynomolgus monkey is feasible despite marked anti-drug antibody response in this species (Obinutuzumab)
Pharmacokinetics and immunogenicity investigation in non-naïve cynomolgus monkeys: a human anti-interleukin-17 monoclonal antibody example
An example where NHP did not successfully predict nonspecific clearance in humans (MNRP1685A)
Efforts to optimize the use of NHPs
Leveraging toxicology studies to reduce NHP use for PKPD evaluation (Rozanolixizumab)
Strategic re-use of NHPs
Use of ADA-positive monkeys from PKPD studies to generate reagents for immunogenicity assays
Cassette dosing as a way of reducing primate usage
Efforts to replace and minimize the use of NHP
Development of in silico, in vitro and in vivo methods to replace NHPs
Physiologically based pharmacokinetic modeling as an approach to minimize NHP use
Leveraging existing data to reduce NHP use in ocular drug development

human data to guide dosing. Regulators, investigators, and drug developers pay keen attention to the MRSD in humans, the dose escalation scheme and maximum dose in Phase 1 trials. A number of relevant regulatory guidance documents outline some general principles for drug developers to consider when determining the optimal dosing in early human trials.[1,6–14] To emphasize the importance, the 2017 EMA guidance explicitly states that "Careful dosing selection of an investigational medicinal product is a vital element to safeguard subjects participating in first in human (FIH) and early clinical trials."

NHPs can play an important role in the translational strategy for determining the MRSD, dose escalation scheme and maximum dose in early clinical trials. The determination of the MRSD can be approached in a number of ways, for example by selecting an MRSD that is expected to provide an exposure in humans that is a fraction of the exposure deemed tolerable (e.g., no observed adverse effect level (NOAEL), or highest non-severely toxic dose (HNSTD)) in nonclinical safety studies.[1,6–9] The human equivalent dose (HED) can be determined from toxicology studies run in NHPs. Calculation of the HED will vary depending on the nature of the biologics, its target, and its intended use. Alternatively, drug developers can apply a more pharmacologically based approach to the MRSD by integrating information from in vitro, ex vivo, and in vivo studies in animal and human test systems that allows the estimation of a drug exposure (e.g., minimal anticipated biological effect level (MABEL); pharmacologically active dose (PAD)) that is expected to provide an acceptable pharmacologic effect in humans. These concepts have been discussed in various other articles.[10,11,12,13,14] As described below, the physiologic similarities between humans and NHPs can make drug effect and other PD data from the NHP a critical part of an integrated approach to determining the safe and effective exposure of a candidate drug in humans.

Converting the drug exposures derived from the approaches above into an accompanying dose in humans that can be used as the MRSD is dependent on a robust estimate of the PK of the candidate drug in humans. These human PK estimates underpin not only the determination of the MRSD but also allow the determination of the optimal incremental doses during escalation and an estimate of the maximum acceptable dose to be tested in the FIH trial.

Physiological relevance of NHP for the translation of PK and PD to human

Selection of an appropriate animal species to evaluate the PKPD of mAbs for the prediction of human PKPD should be based on the similarity of relevant characteristics between the animal model and humans.[15] Hence, understanding the main PK determinants for mAbs is important in determining the relevance of the animal model. Several reviews have described the PK of mAbs, and it is generally characterized by slow clearance, long half-life ($t_{1/2}$), and limited tissue distribution.[15–17] Clearance pathways for mAbs can be categorized as specific and nonspecific. The specific pathway is target-dependent and involves binding of the mAb to its target antigen. In these circumstances, the PK of mAbs is dependent on the binding interactions between the mAb and its target

antigen, as well as on the target antigen characteristics such as whether it is soluble or membrane bound, its expression levels, and turnover rate. The nonspecific pathway involves cellular uptake of the mAb by pinocytosis and is mainly influenced by interaction of the Fc region on the mAb with the neonatal Fc receptor (FcRn, Brambell receptor), which recycles it back to the cell surface and systemic circulation.[18–20] Other nonspecific pathways that may affect clearance of mAbs to varying extents depending on glycosylation characteristics of mAbs such as via mannose receptors.

Cynomolgus monkey is typically the preferred species to evaluate the PK of mAbs due to its similarity to humans in both the specific and nonspecific pathways involved in mAb PK. For the specific pathway, which is highly dependent on the binding of the mAb to its target and the physiologic behavior of the target such as target turnover, cynomolgus monkeys have a high genetic similarity with humans, generally leading to greater target antigen sequence homology and similar tissue cross-reactivity profiles. However, it should be noted that differences between healthy monkeys used in PK studies and human patients, such as varying target expression levels and general health status, that could affect mAb PK may still be quite large. Cynomolgus monkey is also a favorable species to evaluate nonspecific clearance mainly due to the similarity in binding affinity of human IgG to cynomolgus monkey and human FcRn. Murine FcRn appears to have a much higher affinity to human IgG than human FcRn, making rodents not as relevant as monkeys in determining the nonspecific PK of mAbs.[21, 22] To circumvent this issue of differential FcRn binding in rodents, many groups are exploring the use of human FcRn transgenic mice to evaluate the nonspecific PK of mAbs, as discussed below.[23–25] An additional route of clearance of IgGs is through their interaction with receptors recognizing the level of glycosylation of proteins.[26, 27] Limited data are available on the interspecies differences in expression and activity of these receptors.[28]

Several approaches are being used to predict nonspecific clearance in humans. Scaling from cynomolgus monkeys to humans using a single species simplified allometric approach with fixed allometric exponents for clearance and volume of distribution appears to provide the best predictions and is recommended by multiple groups.[29–32] While prediction of nonspecific or linear clearance is very accurate using cynomolgus monkeys, the prediction of target-mediated clearance is still challenging due to differences in target antigen characteristics across species, such as target density, expression profiles, target turnover kinetics, and affinity to the target. However, even in this situation, cynomolgus monkeys have been used with varying levels of success in predicting human PK.[33–35] The interaction of IgGs with Fcγ receptors can also lead to target-mediated drug disposition (TMDD). In that context, it is worth bearing in mind the differences in expression and activity of this system between human and NHPs.[36, 37] For prediction of other PK parameters after subcutaneous (SC) dosing, such as rate of absorption and bioavailability, cynomolgus monkey does not appear to be a good model, possibly due to factors such as differences in physiology of hypodermis and the lymphatic system. Other animal models such as minipigs have been evaluated for evaluation of bioavailability after SC dosing with varying success.[38, 39] Although

Table 2. Considerations for the use of NHPs for PKPD purposes.

Antibody-specific characteristics
> Cross-reactivity between species
> Differences in affinity
> Differences in epitope
> Interaction with clearance pathways such as FcRn
> Risk of interference of immunogenicity in results interpretation

System-specific characteristics
> Expression profile (location, soluble/membrane-bound)
> Expression level
> Turnover rate
> Differences in downstream biology
> Relevance of healthy NHPs to the targeted patient population

the need for NHP studies for PKPD purposes needs to be made on a case-by-case basis, key aspects to consider are summarized in Table 2.

Value of NHP in understanding PKPD of mAbs

In this section, we present case studies of lulizumab and rozanolixizumab which illustrate the value of NHP to increase PKPD understanding to support the design of FIH studies. The value of NHPs to assess half-life extension strategies is also discussed. Finally, this section shows how knowledge gathered in NHPs led to the discontinuation of a research program prior to starting clinical development, thus saving volunteers and patients being exposed to an ineffective therapeutic.

Lulizumab a pegylated monovalent anti-human CD28 domain antibody antagonist: determination of FIH dose with pivotal PKPD data from cynomolgus monkeys

Targeting the CD28 pathway has been considered high risk since 2006, when TeGenero's CD28 superagonist mAb (TGN1412) caused severe cytokine release syndrome resulting in long-term damage in 6 healthy volunteers during a Phase 1 clinical trial.[40, 41] Using a pharmacologically based method to establish the FIH starting dose, such as the MABEL approach, is recommended for targets that likely lead to a biological cascade or cytokine release with an amplification.[7] As an antagonist, lack of agonism or costimulatory activity and inhibiting CD28-mediated T-cell proliferation and cytokine production, lulizumab pegol was not anticipated to cause any amplified cytokine release.[42] However, given the inherent risk of targeting CD28, a MABEL approach was conservatively adopted to select the FIH starting dose.[43] The challenges associated with the MABEL approach included: 1) assessing potential differences of sensitivity for the mode of action (preferably under physiological relevant conditions) between human and animals; 2) identifying a relevant animal model to establish in *vitro* to in *vivo* correlation in target engagement (e.g., dissociation constant (Kd), receptor occupancy (RO), and concentration leading to 50% of maximum effect (EC50)); and 3) identifying a translational PD marker for functional activities. This example illustrates how the NHP PKPD data, including systemic exposure, extent of RO, PD activities, and duration of effect, played a critical role in the FIH dose selection.

To assess differences in binding affinity and occupancy between human and monkey model, a comprehensive panel of in vitro or ex vivo assays, including Kd from Biacore assays, EC50 for RO in whole blood cells, and EC50 of a functional effect (inhibiting T cell proliferation in *in vitro* dendritic cell-driven mixed lymphocyte reaction (MLR) assays) were performed with lulizumab or a similar anti-hCD28 domain antibody that only differed from lulizumab by two additional amino acids at the N terminus.[43] The comparable binding affinity and RO along with the inhibitory effect were observed between human and monkey. Interestingly, this finding is consistent with the 100% identical extracellular domain of the CD28 receptor found in both species.[42]

Moreover, using an integrated PKPD modeling approach with the data from a PKPD study conducted in monkeys, Yang et al. demonstrated: 1) a strong correlation between *in vivo* RO EC50 and immunosuppressive activities assessed through the T cell-dependent antibody response (TDAR) to keyhole limpet hemocyanin (KLH) in monkeys, and 2) the relevance (comparable values) between *in vivo* RO EC50 and *in vitro* MLR EC50. This PKPD relationship established in NHPs laid the groundwork in defining MABEL and selecting a subsequent FIH dose. Since no significant immunosuppression was observed in monkeys at an average in vivo RO of ≤30% over approximately 28 days, the MABEL was defined as a target in vivo RO <10% at predicted maximum concentration (Cmax) in humans. In vivo RO EC10 is not yet available until clinical trials are conducted. Therefore, based on the established relevance between *in vivo* RO and *in vitro* MLR EC50, the latter at 10% (MLR EC10) was used to calculate a MABEL dose of 0.01 mg.[43]

PKPD results and the safety profile of lulizumab were reported in healthy volunteers following single- or multiple-dose administration.[44] The strong RO/PD correlation between in vivo RO and TDAR demonstrated in monkeys was able to be recapitulated in humans. Moreover, the extent and duration of the RO as well as the corresponding immunosuppression were comparable in both species. Overall, these results in humans further confirmed the close correlation between target engagement and the proof-of-mechanism marker (i.e., the inhibition of KLH-induced TDAR); hitherto the suitability of using the monkey model to inform study design and dose selection for future drug candidates with similar mode of action has been soundly established.

Looking back, could lulizumab have been developed solely based on a rodent model or an alternative non-rodent species other than NHPs? The answer is probably not due to the lack of cross-reactivity between lulizumab and CD28 from other species. Although Yang et al. were able to establish a similar correlation between RO and the suppression of KLH-induced TDAR in mice as auxiliary evidence to support their final decision, a mouse surrogate had to be used in such study.[43] Given the high risk associated with targeting the CD28 pathway, the technical challenges, and limitations associated with using surrogates made this option unsuitable. Although unavailable at the time of lulizumab development, the recently emerged humanized CD28 immune checkpoint knock-in or conditional knock-out mouse model may serve as an alternative to study the correlation between target engagement and the proof-of-mechanism marker. The utility of humanized

immune checkpoint models can potentially reduce NHP use in future PKPD development of biologics with similar mode of action.

In lieu of the in vitro MLR EC10 with its relevance to immunosuppression established in a PKPD study in cynomolgus monkeys, could the MABEL dose be defined merely with the Kd from the in vitro Biacore assay? The possibility of using the Kd from a Biacore assay to calculate in vivo RO was discussed by Yang et al.[43] The MABEL doses estimated using Biacore Kd and MLR EC10 were 3.1 and 10 μg, respectively. The latter was selected as the FIH starting dose because the MLR assay was considered as a more reasonable and sensitive way to evaluate the extent of RO and it represents a reasonable approximation of the in vivo situation compared to artificial environment in Biacore assay. On the other hand, a too conservative starting dose of 3.1 μg rendered from Biacore Kd may negatively impact drug development timeline and unnecessarily delay patient access to medicines.[11] As evidence to further verify the appropriate selection of the FIH dose based on MLR EC10, Shi et al. reported that the dose of 10 μg led to <5% RO in healthy volunteers in a single-ascending-dose study.[44]

Taken together, data from NHPs were essential to derisk the in vitro approach and build confidence during the development of lulizumab, given the inherent high risk of targeting CD28 pathway and the lack of cross-reactivity in other nonclinical species.

Rozanolixizumab an anti-FcRn mAb: successful translation of PKPD and MRSD determination

Rozanolixizumab is a human FcRn-targeted mAb developed to decrease the levels of circulating pathological IgGs in auto- and allo-immnune diseases.[45, 46] It is a high affinity antibody that competes with endogenous IgGs for FcRn.[47] It blocks the recycling of endogenous IgGs, increasing their clearance and therefore reducing their circulating concentrations. As of late 2022, it is being tested in Phase 2 in patients with chronic inflammatory demyelinating polyradiculoneuropathy (NCT03861481) and in Phase 3 in patients with generalized myasthenia gravis (NCT04124965) and primary immune thrombocytopenia (NCT04224688).

Whilst human FcRn-transgenic mice provided a tool for proof of mechanism, PKPD relationship, dosing regimen, and safety were assessed in cynomolgus monkeys due to the conserved FcRn system between human and primates. Indeed, whilst the cross-reactivity between human FcRn and rodent IgG is poor, human, and cynomolgus monkey IgG4 bind comparably to FcRn.[48, 49] In addition, rozanolixizumab exhibits similar affinity toward cynomolgus monkey and human FcRn whilst it does not bind the rodent receptor.[47] Cynomolgus monkeys initially received a single intravenous (IV) administration of rozanolixizumab at 5, 10, and 30 mg/kg. Free PK was assessed. Circulating concentrations of endogenous IgG were used as a marker of PD effect, while albumin concentrations were monitored as a sign of exaggerated pharmacology since FcRn also recycles albumin. As expected, a strong TMDD effect was observed on the PK of rozanolixizumab due to the impact of FcRn binding. Levels of endogenous IgG decreased by 49, 63, and 69% from baseline following

single IV administration at 5, 10, and 30 mg/kg, respectively. These data were integrated with the affinity and transcytosis in vitro measurements in a semi-mechanistic PKPD model to predict the decrease in circulating IgG in cynomolgus monkey.[50–52] PK and IgG measurements following frequent (30 mg/kg IV loading dose, followed by daily 5 mg/kg IV doses) and infrequent dosing (30 mg/kg IV every 60 days) were used to validate the initial PKPD model.[53] Both dosing regimens led to a decrease of 75% of IgG from baseline. Safety assessment in cynomolgus monkeys showed that rozanolixizumab was safe when dosed at 150 mg/kg IV every 3 days.[54] Such doses led to a decrease in IgG of 85% from baseline, with only small changes in albumin concentrations. As a result, doses for the FIH trial in healthy volunteers (NCT03859219) were defined to cover decreases in IgG ranging from 10 to 50% from baseline. The PK of rozanolixizumab in human was predicted based on allometric scaling of the cynomolgus monkey data. The PD component of the semi-mechanistic PKPD model was translated between cynomolgus monkey and human to include the differences in in vitro binding and FcRn expression between the two species. Doses of 1, 4, and 7 mg/kg administered to healthy volunteers led to decreases in circulating IgG of 14.5, 33.4 and 47.6% following IV dosing and 16.8, 25.9, 43.4% following SC dosing, respectively.[54] Although the level of IgG suppression observed at 1 mg/kg was higher than expected, this example highlights the benefit of using cynomolgus monkeys to predict results in human for biological systems that are well conserved between the two species. In this case, although the human FcRn-transgenic mice were a helpful tool to establish a proof of mechanism, quantitative translation was complex due to the incomplete understanding of the dynamics and abundance of FcRn in this model. As a result, cynomolgus monkey played a critical role in designing a concise Phase 1 trial, limiting exposition of healthy volunteers and expediting clinical development for the benefit of patients.

Value of NHP for predicting PKPD in mAbs with half-life extension

Several strategies have been explored to increase the half-life of antibodies and Fc-fusion proteins using the FcRn-mediated recycling pathway.[55] While antibodies already have a long half-life due to their binding to FcRn, antibody engineering approaches have been devised to further extend their half-life with the aim of decreasing dosing frequency in the clinic. One promising approach to increase half-life of antibodies is engineering of the Fc region to increase affinity to FcRn. Both cynomolgus monkey and human FcRn have similar binding affinity to human IgG, making cynomolgus monkey an ideal species to evaluate the effects of changing binding to FcRn on antibody PK and predict human PK.[15] Several studies have shown that increasing FcRn binding of mAbs by engineering Fc variants with increased binding to human FcRn at pH 6.0 can increase their half-life in cynomolgus monkeys.[56, 57] In one notable example, Dall'Acqua et al. introduced a triple mutation M252Y/S254T/T256E (YTE) into the Fc portion on MEDI-524, a humanized anti-respiratory syncytial virus mAb.[48] This mutation resulted in a 10-fold increase in binding of MEDI-

524 to both human and cynomolgus monkey FcRn at pH 6.0, while efficiently releasing it at pH 7.4. This increase in FcRn binding at pH 6.0 resulted in a 4-fold increase in systemic half-life of the altered MEDI-524-YTE antibody in cynomolgus monkeys compared to the parent MEDI-524 antibody. A subsequent study in humans using the same YTE mutation for motavizumab-YTE showed lower clearance (71 to 86%) and 2-to 4-fold longer systemic half-life than the parent motavizumab.[58] This was the first study of an Fc-modified mAb in humans, showing that motavizumab-YTE exhibited an extended half-life of up to 100 days. This PK extension was similar to that seen in monkeys for the YTE mutation and showed the value of using monkeys to evaluate half-life extension strategies when modulating binding to FcRn. Recently, the use of human FcRn transgenic mice has been investigated to rank half-life extension strategies, suggesting that this rodent model could be used to predict PK in NHP in preparation for safety assessment studies.[59]

Discontinuation of the development of a compound or preventing the discontinuation of a compound using NHPs

During drug development, it is often necessary to determine whether a target is "druggable". The druggability of a target can be related to a number of characteristics, including tissue distribution or location, and expression and turnover. Drug targets with high levels of expression and/or turnover, particularly in non-target tissues can pose challenges due to the high amounts of drug required to sustain sufficient levels of occupancy to drive the desired pharmacologic effects. An excellent example of this is CCL21, a soluble chemokine believed to play a role in modulating inflammation. QBP359 is a human IgG1 mAb that binds specifically to human CCL21 and cross-reacts with cynomolgus monkey, but not with mouse CCL21.[60, 61] The similarity in binding of QBP359 between NHP and humans, and the physiologic similarities between these species, made the NHP a suitable system to explore the PKPD of this novel biotherapeutic for the purposes of estimating human efficacious dose. In a dose-range finding NHP toxicology study, the elimination rate of QBP359 was found to be rapid compared to a typical IgG and decreased as the dose increased, suggesting that CCL21 occupancy was not achieved at the low dose used in that study (10 mg/kg weekly). This raised questions about the ability of QBP359 to sufficiently suppress CCL21 concentrations at manageable doses. Subsequent PK and biodistribution studies in the NHP were conducted to enable a more complete assessment of the PK of QBP359 and the dynamics of CCL21 turnover, and information on the tissue localization of CCL21.

Integration of the information using a semi-mechanistic PKPD model confirmed the high turnover rate of CCL21 and indicated that binding of QBP359 with CCL21 accelerated the elimination of the QBP359. The PKPD model was used, with the incorporation of some human-specific parameters (e.g., allometrically scaled free QBP359 PK, CCL21 concentrations and turnover in humans) to predict the dose and dose regimen that would be required in humans to maintain >90% neutralization of CCL21. These simulations determined that doses of >50 mg/kg/week would be required to achieve this goal, thus making this target undruggable with QBP359.

The data derived from NHPs in this program allowed the developers to make a clear decision to discontinue the project, thus minimizing further use of animals and resources, and avoiding the exposure of humans to an ineffective treatment.

Challenges to overcome when using NHP for PKPD purposes

Immunogenicity can complicate use of NHP data, but can often be managed to still derive valuable information from such studies

General considerations on immunogenicity

It is generally accepted that immunogenicity of therapeutic proteins in animals (NHPs and other species) is not predictive of the immunogenicity in human.[62] In a nonclinical PKPD or safety study, important concerns are that anti-drug antibodies (ADA) (e.g., clearing and neutralizing antibodies) in NHP may affect PK and PD of the dosed therapeutic proteins and interfere with the PKPD assessment.[63] Since human therapeutic proteins are foreign antigens to NHPs, non-rodents, and rodents, administration of human therapeutic proteins will challenge the animal immune system and may elicit formation of anti-drug antibodies.[64, 65] For example, in a study by Thway et al., Sprague Dawley rats were administered a humanized mAb at 50 mg/kg weekly for 4 doses.[66] Rats developed ADA as expected. The concentration of the drug was measured by three bioanalytical methods that were either prone or not prone to interference by ADA. The presence of ADA in samples led to discrepant concentrations depending on the methods used. In another example, when albiglutide was administered to monkeys or mice, anti-albiglutide antibodies were detected in both species.[67] In the monkey study, ADA was detected in in 2/10 and 6/10 monkeys given 15 or 50 mg/kg/week. In the mouse SC postnatal development study, ADA were detected in 6/8, 6/8, and 5/8 in the low-dose, middle-dose, and high-dose lactating dams, respectively, assessed Day 21 postpartum. These examples indicate that the development of ADA response against human therapeutic proteins by animals is not limited to NHPs but also can occur in other animal species. It is worth mentioning that, although ADA can occur in any species, in NHP one can measure, PK, PD, and ADA serially in the same animal, which may not be feasible in rodents due to the blood volume limitation.

Development of ADA responses in NHPs and other animal species can affect PK and safety studies in multiple aspects, including altering the exposure of the drug (decreasing exposure by clearing ADA or increasing exposure by sustaining ADA) and neutralizing the pharmacological actions of drugs by preventing it from binding to its target(s).[68–71] Additionally, the presence of ADAs may interfere with the PK assay used for concentration measurements of therapeutic proteins, thus making it difficult to accurately quantify drug exposure and PK.[66] When the bioanalytical assay measures total drug concentrations using polyclonal antibodies or anti-Fc reagent as the capture reagent, the impact by ADA on the total concentrations is likely to be minimal. However, part of the measured

total drug may be neutralized and rendered pharmacologically inactive. To assess the impact of ADA on drug exposure, a bioanalytical assay that measures the active (or free) drug concentration may be more appropriate. In the free assay, a drug target or blocking anti-idiotypical antibody is used as the capture reagent. Therefore, only drug that is not neutralized by ADA will be measured.[72]

When ADA response develops in NHPs and other animal species, the PK scientist should review and interpret the PK, ADA, and PD data together to assess the impact of the ADA on PK and PD. When the PD biomarker reflects the pharmacological action of the drug and is robust and sensitive to changes in the exposure of the biologically active drug, the first step is to correlate ADA status with PD activities (see example related to the testing of anti-IL17 mAbs in non-naïve NHPs below). If ADA does not affect PD significantly, the exposure from these ADA-positive animals should be included in the mean PK parameter calculation. On the other hand, if PD activities are significantly altered in some ADA-positive animals along with altered drug exposure, these animals likely need to be excluded from the mean PK parameter calculation. In the absence of PD data, the impact of ADA can be assessed in terms of altering active drug concentrations or lack thereof.[73] The active drug exposure in both ADA-positive and ADA-negative animals is compared side-by-side. If ADA does not significantly alter active drug exposure (as measured by the "free" bioanalytical assay) in any animals, the impact of ADA on the study is considered minimal and all animals can be included in the mean PK parameter calculation.[74] However, if ADA alter the drug exposure substantially in some animals in a dose group, it may be appropriate to exclude these ADA-positive animals from the calculation of mean PK parameters. In some cases, the majority or all the treated animals may be ADA positive and have significantly altered drug exposure, which will create challenges in defining the PKPD relationship. These situations will need to be handled on a case-by-case basis. The mitigation strategies include evaluation of drug exposure and PK parameters based on data from the early part of the study where there are sufficient animals whose drug exposure has not been altered significantly (see lenercept and obinutuzumab examples below) and evaluation of the drug exposure from high-dose group where the impact of ADA on drug exposure may be smaller than from the low dose and/or middle-dose groups.

Lenercept: Successful use of allometric scaling to predict the human PK of a TNFR-Fc fusion protein despite immunogenicity

Lenercept is an Fc-fusion protein consisting of the extracellular domain of two human p55 tumor necrosis factor receptors fused to the Fc portion of human IgG1.[75] The PK of lenercept was characterized in several animal species, including cynomolgus monkeys, to support planning of toxicity and pharmacology studies, as well as for predicting the PK in humans by allometric scaling. Lenercept was found to be immunogenic in all animal species tested. In monkeys, the immune response was evident from an accelerated clearance of lenercept starting at 10 days post-dose in all four animals tested and was confirmed by appearance of ADA in plasma samples collected 19 days after dosing. In the PK non-compartmental analysis (NCA), the period of accelerated clearance was disregarded, as the ADA-

mediated accelerated clearance does not reflect the disposition kinetics of the drug itself. This meant that the PK could be followed only over 10 days, which results in an uncertainty in the PK parameters assessed by NCA. This uncertainty is considered acceptable, as the PK could be characterized over about two half-lives. Despite these shortcomings, the obtained PK parameters in cynomolgus monkey are predictive for humans. Using state-of-the-art scaling procedures from monkeys to humans with an allometric exponent of 0.85 for clearance, the lenercept clearance of 12 mL/day/kg in monkey results in a projected human clearance of 7.6 mL/day/kg, calculated for a 70 kg subject.[29] This value is in excellent agreement with the observed value of 6.8 mL/day/kg.[76]

PKPD assessment of the anti-CD20 antibody obinutuzumab in cynomolgus monkey is feasible despite marked anti-drug antibody response in this species

Following single IV administration of the anti-CD20 antibody obinutuzumab to cynomolgus monkeys at two different dose levels (1 and 10 mg/kg, n = 2/dose level), a marked immunogenicity against obinutuzumab was observed.[77] Three of 4 monkeys showed accelerated clearance of obinutuzumab approximately 10 days after dosing. It is of note that TMDD and accelerated clearance due to an immune response may lead to a similar shape of serum concentration-time profiles with an accelerated clearance following a log-linear elimination phase. In the present case, the immune response could be identified as root cause, since: 1) one animal without ADA formation showed a continued log-linear elimination phase, and 2) the onset of rapid clearance occurred at very different serum levels in the different dose groups. Similar to the lenercept case, the PK of obinutuzumab was analyzed using NCA by neglecting the phase of accelerated clearance. The obtained PK data translate well to humans. For the PKPD assessment of obinutuzumab with B-cell depletion as PD endpoint, the accelerated clearance was included in a PK model as an additional time-dependent, linear clearance process, which was up to ~30-fold more rapid than the regular clearance of obinutuzumab. The PK model was included in an indirect-response PKPD model to describe loss and re-population of B cells. The PKPD model predicted a re-population of B cells when obinutuzumab serum levels drop below 0.02 μg/mL. The accelerated clearance shortened the time to loss of PD response, i.e., start of B cell re-population, and thus the duration of pharmacologic effect. Overall, these data show that with appropriate inclusion of the additional immune-mediated clearance pathway in the PKPD model, the PKPD of obinutuzumab in cynomolgus monkeys was well characterized despite a marked anti-drug antibody response. The marked immunogenicity of obinutuzumab in monkeys is not predictive for humans. In the clinic, obinutuzumab showed very low immunogenicity.

PK and immunogenicity investigation in non-naïve cynomolgus monkeys: a human anti-interleukin-17 mAb example

A strategy for screening NHPs for preexisting antibodies to a to-be-tested biotherapeutic was applied in a study of the PK of a human anti-IL17 antibody.[78] A group of 32 male cynomolgus monkeys that had been used once previously for a single-dose PKPD study with a human IgG1 mAb were

screened for ADA against a human anti-IL17 IgG1 mAb. Screening was conducted approx. 2 months prior to use in the anti-IL17 PK study and approximately 3 months after the last mAb dosing. Animals were separated into two groups (n = 17, negative; n = 14, positive) depending on their ADA status at screening and were further divided into treatment groups receiving a single dose of 1, 3, or 10 mg/kg of anti-IL17 mAb IV, or 3 mg/kg SC. Serum sampling was implemented for 50 days following dosing and analyzed for concentrations of free anti-IL17 mAb. Screening and confirmatory ADA were measured, as were ADA titers, predose and at the end of study. PK analysis was conducted using anti-IL17 mAb concentrations.

In the assessment of preexisting ADA approximately 44% of animals had preexisting cross-reactive antibodies to anti-IL17 mAb and there appeared to be no correlation between the presence of ADA and the type of mAb the animal had previously received. The authors tested the specificity of the preexisting ADA against anti-IL17 mAb and found that this varied widely between animals.

There were some instances of a change in ADA status between screening and pre-dose testing (approximately 20–30%) with a nearly equal number switching from negative to positive as from positive to negative. Following anti-IL17 mAb dosing none of the animals that tested negative at both screening and pre-dose were found to test positive. In the animals that tested positive at screening, 57% remained positive at predose and after anti-IL17A dosing. Approximately 20% became negative at screening (and remained negative) and 20% were positive pre-dose but negative after dosing. In the animals classified as positive at pre-dose, titers increased in approximately 70% of those animals.

The mean PK of anti-IL17 mAb in the ADA-negative group (among animals that were ADA-negative throughout) was consistent with previous data in naïve animals. Animals that tested negative before the dosing remained negative at the end, with no individual animals showing PK evidence of ADA. In the animals with preexisting ADA, accelerated clearance was observed in approximately 57% of those animals and was concordant with them also testing positive at the end of the study. The remaining animals tested negative at the end of the study, despite testing positive at screening and, in some cases, also pre-dose.

This study provides supportive evidence that screening for cross-reactive antibodies can be a useful strategy for selecting animals that may be suitable for dosing with biotherapeutics for the purposes of evaluating PKPD. The observation that animals can change ADA status over time in the absence of dosing, and that this can affect PK, suggests that testing more than once may be needed to select animals for further use.

While this study provides a great deal of valuable knowledge, much remains to be done before we can fully understand the risks of re-dosing animals and to also develop strategies to minimize these risks. This study did not evaluate animals that had been dosed with an IgG1 more than once (either multiple IgG1s, or multiple doses of the same IgG1) prior to testing with the anti-IL17 mAb, nor did it assess whether animals could be dosed more than once with a new test article after having been previously dosed with a biotherapeutic. Additionally, similar studies with biotherapeutics other than human IgG1s will be required, including studies looking at the implications of sequential dosing with dissimilar biotherapeutics.

An example where NHP did not successfully predict nonspecific clearance in humans

As discussed above, cynomolgus monkey is typically the preferred species to evaluate the PK of protein therapeutics due to the similarity to humans in terms of both target sequence homology which affects target-mediated clearance pathways, as well as FcRn-binding affinity which affects nonspecific clearance pathways of mAbs. These similarities have allowed the use of only monkeys as a single species to successfully predict human PK.[15, 29] Despite these similarities, there is an interesting case study showing divergence in PK behaviors between the monkey and human. Xin et al. described the development of MNRP1685A, a human mAb against neuropilin-1 (NRP1), where the PK in monkeys underestimated the nonspecific clearance in humans.[79] The PK of MNRP1685A was evaluated in mouse, rat, monkey, and humans following IV administration across a wide dose range. MNP1685A bound to its target, NRP1, in all species tested and showed non-linear PK in all species consistent with widespread expression of this target. Due to the observed PK non-linearity, a two-compartment model with parallel linear (to describe the nonspecific) and non-linear clearance (to describe the target-mediated specific clearance) could adequately describe the data. The model derived nonspecific clearance of MNP1685A in monkey (3.22 mL/day/kg) was within the expected range for a typical antibody, but it was much higher than expected in mouse (60.3 mL/day/kg), rat (19.4 mL/day/kg) and human (8.53 mL/day/kg).[29] The nonspecific clearance in humans was 4-fold higher than that predicted from monkey based on the usual allometric measures. The authors postulated that this unexpected species difference in nonspecific clearance to possible off-target binding in mouse, rat and human, but not in monkey. This is a rare example where using monkey data to predict human nonspecific clearance was misleading, likely due to differences in off-target binding. Some learning from this example suggests that when off-target clearance is suspected in preclinical species, the preclinical PK data may not be as informative to predict human PK. Interestingly, the specific or target-mediated clearance parameter values (Vmax and Km) in humans were close to the predicted values from the monkey PK. This was surprising as the scaling of target-mediated clearance of mAbs is typically more challenging due to the variability associated with target characteristics. A few other studies have shown species differences in off-target binding, such as anti-FGFR (off-target binding to mouse complement component 3) and anti-amyloid beta (off-target binding to fibrinogen in cynomolgus monkey), but these molecules do not have corresponding human data to draw similar conclusions.[80, 81]

Efforts to optimize the use of NHPs

Leveraging toxicology studies to reduce NHP use for PKPD evaluation

One approach that can be applied to reduce the number of NHPs used in preclinical development is to gather PKPD data

from safety assessment studies. NHPs tend to be the species of choice for toxicology studies for the same reasons that they are frequently chosen for PKPD investigations.[3] In the case of targets for which the dynamics are already well understood or that present limited risk in terms of clinical efficacy, such as soluble cytokines or membrane targets where clinical data already exist on specific mode of action, safety studies can be used as the sole source of PKPD information. For more complex targets, safety assessment investigations can be complementary to PKPD studies because they allow for the investigation of a wide range of therapeutic and supra-therapeutic doses. Due to the high doses tested in toxicology studies, the maximum expected effect on biomarkers can be measured. In addition, mechanistic or empirical models developed in PKPD experiments can be validated and/or refined with the independent dataset generated in toxicology studies. This is exemplified in the preclinical development of rozanolixizumab, an anti-FcRn mAb, already discussed above. Following single dose IV administrations to cynomolgus monkeys at potential therapeutic doses (5, 10 and 30 mg/kg), levels of endogenous IgG were reduced by 69% compared to baseline. When rozanolixizumab was dosed to cynomolgus monkeys at 150 mg/kg every 3 days via IV or SC administrations for 13 weeks in a toxicology study, endogenous IgG levels were further reduced by ~85% of baseline levels. The fact that both routes of administration led to similar PD effects despite the IV routes reaching exposures (AUC) 1.7-fold greater than the SC route indicates that these doses led to the maximum possible effect in this biological system.[47] This observation was supported by reports of FcRn deficiency in human also leading to up to 85% decrease in endogenous IgG.[82] FcRn-deficient mice, which are impaired in their ability to recycle IgG and thus have accelerated IgG clearance, also show a > 85% decrease in IgGs compared to wild-type mice.[83] The extension of the effect range investigated in cynomolgus monkeys ensured the validity of the mechanistic model developed to predict the effect of rozanolixizumab in human. An alternative approach to performing separate PKPD and safety assessment studies is to include a low therapeutic dose level in the safety assessment study or to include a single low dose as a run in for these toxicology studies (with some risk for loss of exposure due to immunogenicity in cases where immunogenicity is not known). In this case, a full PKPD model can be built including data over a wide range of therapeutic and supra-therapeutic doses.

Finally, safety assessment studies present a valuable opportunity to assess the turnover of soluble targets such as cytokines if that has not been done in dedicated PKPD studies previously. Indeed, cytokines tend to have much shorter half-lives in the systemic circulation than mAbs.[84, 85] As a result, the target cytokine (free + bound to antibody) accumulates in the systemic circulation.[85] Applying a simple model to the total target and antibody concentrations allows PKPD scientists to estimate the turnover of the targeted cytokine.[84, 86] This in turn helps in the design of early clinical trials by indicating the frequency of dosing required to effectively neutralize the target. Estimation of the turnover of cytokines can be done in toxicology studies as effectively as in dedicated PKPD studies since the maximum accumulation of total target is dependent on the

target turnover but not on the amount of drug in the systemic circulation. Measurement of total target can also be required in toxicology studies proving the engagement of the target by the antibody tested. Thus, assessing target turnover in toxicology studies does not necessarily require any additional resources. The main hurdle to measuring total target in toxicology studies is the need for assays that are drug tolerant. The model assumes that the rate of synthesis of the cytokine remains constant over time following administration of the drug. This assumption can easily be tested in longer term safety assessment studies. Whether target turnover is measured in PKPD or toxicological studies, one of the key challenges remains the measurement of baseline level of cytokines in healthy animals.

One of the key parameters that drives the need for dedicated PKPD studies is often the timing of the safety assessment studies. Toxicology studies by definition need to investigate supra-therapeutic doses whilst one of the aims of PKPD studies is to explore the therapeutic range to be tested in early clinical development. As such, a level of PKPD understanding and a preliminary prediction of the maximum exposure to be tested in early clinical development is required to ensure that the toxicology studies are designed appropriately to support the first trials in human. The need for PKPD and/or toxicology studies will depend on the need for an early derisking of the target and the mechanism of action of the therapeutics. PKPD and safety assessment colleagues should cooperate effectively to maximize the information gathered from every study involving NHPs.

Strategic re-use of NHPs

There is a strong rationale for re-using NHPs in PKPD and toxicology studies. First and foremost, ethical concerns motivate all researchers to reduce animal use, in particular the use of NHPs. The availability of NHPs, and more acutely, certain types of NHPs (e.g., disease models, sexually mature) can be highly limited, which can affect the ability to conduct the right experiment at the right time and lead to delays in bringing new therapies to patients. Using non-naïve animals could reduce the use of NHPs. However, there are some physiological and operational considerations that currently limit our ability to reuse NHPs for biotherapeutic studies.

While immunogenicity within a study can complicate study conduct and interpretation, it also has implications for the continued use of animals for further study.[64, 87] In particular, animals (including NHPs) can develop immunogenicity against structural elements of human biotherapeutics that are foreign to the NHP, but common to many biotherapeutic test articles (e.g., IgG Fc, light chain, and heavy chain, endogenous proteins either as therapeutics or as components of fusion proteins; see the human anti-IL17 antibody example). This immunogenicity can manifest itself upon NHP re-use as anti-drug antibodies against the test article or as other forms of immunological response (e.g., hypersensitivity reactions) that can compromise study conduct and interpretation. For these reasons, most investigators prefer to use naïve NHPs for PKPD and toxicology studies to mitigate the risks outlined above. In addition, the non-

terminal nature of PKPD studies means that a population of biotherapeutic treatment-experienced NHPs can grow and pose ethical and operational difficulties for NHP users.

Strategies for screening and selecting other animals that may be suitable for biotherapeutic retreatment are still undergoing development. It is important not to underestimate the difficulty associated with developing and implementing such a strategy. The immunogenic response in each animal can differ in epitope(s), strength, and durability. The presence of a positive ADA response may not indicate that the animal is not suitable for treatment with a related, or even unrelated, biotherapeutic. The absence of measurable ADA that could cross-react with a biotherapeutic of interest may not indicate whether an animal will generate ADA to a different biotherapeutic upon dosing.

Beyond these scientific aspects, there are other practical matters that also need to be considered. There are costs associated with holding animals during a washout period, particularly if this extends for several months after prior treatment. Before re-use, it will be important to know what biotherapeutic(s) (e.g., IgG, endogenous protein) the animals were previously administered; testing strategies will need to be in place to confirm that drug and pharmacologic washout is sufficient and ADA testing for the to-be-dosed test article will need to be conducted. This will pose challenges to sponsors and CROs eager to use non-naïve animals for additional biotherapeutic studies.

Despite these challenges, investigators are looking at ways to allow NHP re-use, as is described in the human anti-IL17 antibody example. In addition, Hey et al. describe a decision tree used to determine if NHPs previously dosed with a biotherapeutic can be reused.[88] Animals that have been treated with an immunomodulatory drug have exhibited irreversible pathological or immunological changes, or have measurable drug concentrations or pharmacologic effects persisting from a previous study are excluded from re-use. A three-month washout is considered standard but may be adjusted accordingly depending on the circumstances. Non-naïve animals are excluded from GLP toxicology studies due to existing uncertainties about potential residual effects of previous treatment. Animals that pass the above criteria can be included in small molecule, non-terminal studies (e.g., PK, PKPD, safety pharmacology) without further screening. If the intended use is for evaluation of biotherapeutics, then animals are further screened using a generic ADA assay. If they are found to be negative, they can be used for non-terminal biotherapeutic studies, including those evaluating PK, PKPD, mechanistic, tolerability (including local tolerance), and also for some terminal non-GLP toxicology studies (e.g., dose range finding studies).

Hey et al. described the use of within-animal dose escalations to characterize the PK of mAbs that are thought to exhibit TMDD.[88] Typically, studies to evaluate the dose-dependent PK of such molecules rely on testing groups of animals at different dose levels to gain sufficient information to inform PK modeling that is used to identify safe and effective drug concentration levels. The risk of immunogenicity and the protracted time required make serial dosing of NHPs with sufficient washout across a dose range very challenging. Using a rapid within-subject up-titration where the low dose is predicted to be partially pharmacologically active and the high dose achieves 100% response can provide the exposure information across the wide range of concentrations required to meet the needs of PK modeling and still be completed in a period (approximately 10–14 days) that reduces the risk of ADA and also meets the timelines of drug development, all with a single cohort of animals. Hey et al. presented an example where an up-titration was conducted in three animals covering a 20X dose range with 3 days between dosing. The data obtained, when integrated with data obtained from toxicology studies, allowed robust estimation of the PK parameters describing TMDD.

There are also strategies being explored that can reduce or maximize the value of NHP use by using ADA-positive animals for alternative purposes and by dosing multiple test articles simultaneously (cassette dosing), which are discussed in the next two sections below.

Use of ADA-positive monkeys from PKPD studies to generate reagents for immunogenicity assays

As mentioned previously, most investigators prefer not to reuse non-naive NHPs for PKPD and toxicology studies due to the risks associated with immunogenicity (i.e., ADAs). Consequently, the growing population of biotherapeutic treatment-experienced NHPs produced from non-terminal PKPD studies may pose ethical and operational issues. Hey et al. has explored an ingenious approach to re-use such biotherapeutic-treated NHPs and harvested the anti-drug antibodies in the monkey for bioanalytical purposes.[88] Purified polyclonal antibodies specific to the respective biotherapeutics that are derived from immunized animals are often used as reference standard(s) in ligand-binding assays to assess immunogenicity in nonclinical or clinical studies (as a surrogate positive control in the latter). To generate positive controls in ADA assays, hyper-immunized rabbits are typically the species of choice because the relatively high amount of somatic hypermutation leads to the production of high-affinity antibodies. Although both rabbit polyclonal antibodies and monkey polyclonal antibodies can be used as positive control for human ADA assays, harvesting the polyclonal antibodies that are specific to the dosed biotherapeutic protein in monkeys offer two advantages. First, the ADA-positive monkeys are already available for harvesting as part of PKPD studies, which negates the dedicated rabbit immunization campaign for the purpose of generating ADA-positive control, and second, monkey polyclonal antibodies may be a closer representative of human ADA that are reactive toward the dosed biotherapeutic protein can be used in both bridging and non-bridging assay formats. Instead of using naive NHPs, ADA-positive monkeys from non-terminal PKPD studies can be further hyper-immunized with the same test article to generate reference reagent(s) for immunogenicity assays. In such hyperimmunization studies, the previous dose(s) of biotherapeutic in PKPD studies serve as initial dose(s) and subsequent doses with the same biotherapeutic are administered repeatedly (generally through the SC route) to those NHPs at appropriate dosing intervals for 2 to 3 months or until a robust immunogenic response against the

administered biotherapeutic is evident. Finally, serum samples are collected multiple times toward the end of treatment and post treatment to ensure sufficient volume for use as reference standard(s) in both preclinical and clinical studies. The reuse of non-naive NHPs with preexisting ADAs to produce positive controls for ADA assays can be established as a routine practice in biotech and pharmaceutical companies to maximize the benefits from the usage of NHPs.

Cassette dosing as a way of reducing primate usage

The use of cassette dosing, i.e., concomitant dosing of several compounds, to reduce animal numbers is well established in the PK characterization of small molecules. For biotherapeutics, cassette dosing was hampered in the past by lack of specific bioanalytical assays to quantify multiple analytes in the same sample. The availability of highly sensitive, specific liquid chromatography-mass spectrometry (LC-MS) assays enables cassette dosing also for biotherapeutics. If specific binding assays are available for all cassette components, these may be used as well. Test compounds are to be selected so that there is no PK interference between the components of a dosing cassette. For instance, cassette dosing is not applicable for compounds undergoing TMDD at the same target. Nagayasu and Oziki combined cassette dosing and microsampling to reduce the numbers of animals (NHPs and mice) in PK studies.[89] The PK of three test mAbs (cetuximab, denosumab, and infliximab) was studied after both cassette dosing and dosing of the individual mAbs. Bioanalytics were performed with specific binding assays. The PK of all three antibodies was well characterized. Prior to onset of accelerated clearance due to ADA formation, plasma concentration-time curves from cassette dosing and dosing of individual mAbs were virtually superimposable. These data demonstrate that cassette dosing can be an option to reduce primate usage. In addition, cassette dosing allows the intra-individual PK comparison of test compounds. This may allow a better differentiation of test compounds compared to the conventional parallel group approach. Drawbacks of the cassette dosing approach include the need for time-consuming bioanalytical method development (specific binding assays or specific LC-MS assay for all components) and potentially an increased risk of PK alteration due to ADA formation.

Efforts to replace and minimize the use of NHP

Several efforts are underway to reduce the use of NHP consistent with the thinking around the 3R strategy, which aims to reduce, refine, and replace the use of animals in research. For PKPD evaluation and screening of protein therapeutics, similar efforts are being explored to reduce NHP use and have shown promise. Some of the approaches and tools are outlined in the sections below.

Development of in silico, in vitro and in vivo methods to replace NHPs

Screening candidate molecules to select a lead molecule with optimal PK characteristics in humans is important in ensuring the clinical success of antibody therapeutics. Due to its proven predictive power, PK evaluation in cynomolgus monkeys has

been a common step in antibody selection. However, several in silico, in vitro and in vivo tools have been developed or are being evaluated for use for PK screening to enable reduction or perhaps even future elimination of the use of NHP. It is worth noting that the use of NHPs for screening purposes is already banned in Europe.

Non-animal testing strategies can be developed through a combination of various in vitro techniques, which complement each other, and work in concert with in silico knowledge management and predictive modeling. This way, early on in drug development quantitative structure–activity relationship models, -omics, and translational database mining may serve to inform and mature a heuristic computational model and the simplistic understanding of the potential safety profile of the drug. This initial model could then inform and refine in vitro assays, which focus on the identified key concerns by using new stem cell technologies, label-free cell assays, micro-scale systems, new safety biomarker like circulating omics, or other alternative approaches. These data in turn will again mature predictive modeling before studies in humans are started.[90]

A recent review by Dostalek et al. outlined the various tools available to use for screening antibody candidates.[91] Some of the promising in vitro tools being used are assays to evaluate the nonspecific binding of antibodies to reduce the likelihood of off-target-binding of antibodies in vivo, as off-target binding can lead to faster clearance and possibly unintended pharmacology (see the section "An example where NHP did not successfully predict non-specific clearance in humans" above).[92] These binding assays include binding to baculovirus particles, heparin-coated plates, Chinese hamster ovary cells, human embryonic kidney cells, and human-derived extracellular matrix using ELISA or flow cytometry-based detection methods.[91] The mechanisms of action of these assays are not known and are likely associated with hydrophobic and electrostatic interactions between the antibody and the assay systems. Recently, Chung et al. developed a cell-based FcRn-dependent transcytosis assay, evaluated 53 mAbs, and showed correlation between their transcytosis readouts and clearance in humans.[93] Kraft et al. developed a heparin chromatography tool assay and proposed using it in combination with a FcRn chromatography assay to allow identification of antibodies with abnormal PK by covering the major causes for nonspecific and off-target binding of Fc-containing therapeutic proteins.[94]

In silico approaches being evaluated include methods such as sequence-based or three-dimensional structure modeling and provide some early ways to select candidates.[91] As mentioned above, several groups are also exploring the use of human FcRn transgenic mice to evaluate the nonspecific PK of mAbs with some promising results.[23–25] However, more work needs to be done to use these in silico tools and human FcRn transgenic mice for prediction of human clearance. One key limitation of these alternative approaches to predicting human PK is that they do not typically assess the risk and magnitude of TMDD.

Physiologically based PK modeling as an approach to minimize NHP use

PBPK models have become prevalent in the development of small molecules, mostly for prediction of drug–drug

interactions and PK in special populations.[95] In recent years, PBPK models for therapeutic proteins, especially mAbs, have been greatly advanced by considering specific mechanisms of mAb absorption, distribution, and elimination at tissue and cell levels. Since the first PBPK model of IgG published by Covell et al. in 1986, the concepts of target-specific tissue distribution, transcapillary convective transport, endosomal transit, and FcRn recycling have been incorporated during the development of a mechanistic PBPK model for mAb.[96] A PBPK model consists of anatomically and physiologically relevant tissue compartments linked together by flow of circulating blood and lymph system. Each tissue compartment is subdivided into several compartments to describe vascular to interstitial exchange, FcRn binding and lysosomal trafficking. PBPK models have been used in various applications, including target identification, lead optimization, PK prediction for preclinical species in pharmacological and toxicological studies, tissue distribution, and drug interaction potential.[97, 98, 99] In many cases, PBPK models for therapeutic proteins were developed for the purpose of human PK prediction using system-dependent properties from human physiology and drug-specific properties from in vitro physicochemical measurement. The drug-specific parameters are found correlating to certain drug properties, such as electrostatic interactions, glycosylation, or large patches of charges within the variable domain contributing to the variability of different mAb PK. Glassman and Balthasar developed a PBPK model using human PK data from 11 mAbs and estimated drug-specific parameters using the rates of pinocytosis and convection. The proposed model was able to predict well the clinical PK of 3 mAbs (cetuximab, dalotuzumab, trastuzumab) which were not used in the model development.[100] Hu and D'Argenio used plasma concentration-time data from 12 mAbs following SC and IV administration in humans to develop a PBPK model where drug-specific parameters were estimated by establishing regression relationship based on biophysical properties of the patches of positive charge at complementarity-determining regions of mAbs.[101] The model was able to predict well human PK of four mAbs (omalizumab, tildrakizumab, ixekizumab, lanadelumab) not used in the model development. Jones et al. developed a PBPK using PK data from mice and humans with a mechanistic parameter from a particular in vitro assay and species-specific FcRn affinity to accurately provide a priori prediction of the terminal half-life for 90% of the mAbs evaluated within a two-fold error.[102] Bae et al. developed a whole-body PBPK model using PK and biodistribution data from mice and predicted human PK of trastuzumab with the ratio of simulated versus observed AUC and Cmax being 1.02 and 0.72, respectively.[103] Taken together, these studies have demonstrated that there is a potential to use PBPK models for human PK prediction, which will avoid the use of NHPs when the sole purpose is to predict human PK without TMDD assessment.

Glassman and Balthasar developed a PBPK model using physiological parameters and plasma data of mAbs associated with linear PK from the literature.[104] Kinetics of target binding and turnover were added to predict non-linear mAb disposition in plasma and in tissues in monkeys. Prediction for two mAbs (2F8 and tocilizumab) was performed a priori and found successful in predicting dose-dependencies in clearance and the areas under plasma concentration versus time curves. Such a model can be very useful in predicting tissue to plasma concentration ratios and saturation of RO to support preclinical and toxicological studies in NHP. Shah and Betts used antibody biodistribution coefficient to estimate the mathematical relationship between the plasma and various tissue concentrations and developed a PBPK model across mouse, rat, monkey, and human species to demonstrate that predicted concentrations were within 2-fold of the observed data.[105] Niederalt et al. developed a PBPK model by revising the previous PBPK model developed for small-molecule drugs within the software PK-Sim.[106] Beside good human PK prediction, the model characterized well the differences in clearance for a wild-type mAb and a high-affinity Fc variant in monkeys. The results demonstrated its ability to assist mAb selection and optimization during translational research and potentially reduce the use of NHPs for extensive drug screening and mechanistic studies.

PBPK models for mAbs have limitations due to the availability of tissue-level information and mathematical complexity of the model.[107] Some researchers proposed the minimal PBPK models to simplify the number of compartments with less complexity.[108–111] Several commercially available PBPK models have been developed in recent years, including Simcyp, GastroPlus, and PK-Sim. All these modeling tools have demonstrated their potentials in reducing the number of animals, studies, and the scale of each study when using NHPs during drug development.

Leveraging existing data to reduce NHP use in ocular drug development

Posterior eye diseases such as age-related macular degeneration, diabetic retinopathy, and diabetic macular edema are major causes of visual impairment and blindness.[112] The underlying mechanism of these diseases is neoangiogenesis and neovascularization. Intravitreal (IVT) administration of anti-angiogenesis therapies is highly effective and has become the main therapeutic intervention of these diseases.[113, 114] Due to the invasive nature of IVT injections, protein therapies with longer ocular t½ (days to weeks) are much more desirable than small-molecule drugs. Currently marketed therapies include ranibizumab, aflibercept, and brolucizumab, which have human ocular half-life between 5 and 12 days.[113,115–117]

Predicting human PK, including ocular t½, has traditionally been achieved using animal studies (including NHPs).[118] Del Amo and Urtti have demonstrated that the molecular weight of protein therapeutics is considered one of the most important factors in determining the ocular half-life in rabbits.[119] Based on this finding, it is reasonable to make the argument that protein therapeutics with similar molecular weights are expected to have similar ocular t½ in animal species as well as in human. This will potentially eliminate the use of animals, including NHPs, in the prediction of ocular t½ of a therapeutic protein if its molecular weight is similar to that of an already approved therapeutic protein (e.g., an IgG vs. bevacizumab, an antibody-binding fragment (Fab) vs. ranibizumab). If it is desirable to obtain some ocular t½ in preclinical species for

a therapeutic protein before dosing it in humans, a preclinical species such as rabbits is preferred over NHPs because rabbits can predict human ocular t½ as well as NHPs. In fact, rabbits are commonly used for comparing and predicting ocular t½ for therapeutic proteins,[118] although rabbits may not be a cross-reactive species for the therapeutic protein while an NHP is. However, since the concentration of the drug in the vitreous humor after IVT injection is significantly higher that of the target(s), the impact of the target on PK is expected to be negligible.

There are ample examples of using rabbits to predict human ocular t½ in literature. For example, Gadkar et al. has used rabbits to study the key factors in the determination of ocular and systemic PK of various antibody and antibody fragment-based drugs.[120] Igney and Fuchs also used rabbits to assess the impact of half-life extension principle of a therapeutic protein that binds to human serum albumin.[121] Caruso et al. performed a model-based meta-analysis in humans and nonclinical species (rat, rabbit, monkey, and pig) to determine consensus values for the ocular t½ of IgG antibodies and Fabs.[117] Their study has demonstrated that the ocular t½ increases with molecule size and eye size. They also derived a formula which describes a proportional relationship between ocular t½ and the product of the hydrodynamic radius of the macromolecule (3.0 nm for Fab and 5.0 nm for IgG) and the square of the radius of the vitreous globe. Using this formula, they predicted ocular t½ values of aflibercept, brolucizumab, and PEGylated Fabs in multiple species, which matched reasonably well with those experimentally determined ocular t½ values. Since their results demonstrate that ocular t½ can be predicted accurately based on molecular size (hydrodynamic radius) and vitreous globe radius of an animal species, there exists a good opportunity to use their predicting model and eliminate or reduce the use of animals (including NHPs) in ocular drug development. For a protein drug intended for ocular diseases via IVT injection, two approaches may be taken to predict its human ocular t½. One is to assume its hydrodynamic radius is similar to that of an approved protein drug with a similar molecular weight; therefore, the human ocular t½ can be calculated based on the formula proposed by Caruso et al.[117] The other is to conduct an ocular PK study in rabbits to determine the ocular t½, followed by multiplying the rabbit ocular t½ with the square of the ratio between the human and rabbit vitreous globe radii to obtain the human ocular t½ (based on the Caruso model). With either approach, elimination or reduction of use of NHPs is achieved.

Discussion

NHP offers relevance in understanding and prediction of PKPD of mAbs in humans because of similarity of relevant characteristics between NHP and humans. Generally, NHP (cynomolgus monkey) has similar specific (target mediated) and nonspecific pathways (mostly FcRn mediated) involved in mAb PK to those in humans. Cynomolgus monkeys share target sequence homology and similar tissue cross-reactivity profiles with humans as a result of a high genetic similarity between NHP and humans. Similar binding affinity of human

IgG to cynomolgus monkey and human FcRn also makes NHP a relevant species to evaluate nonspecific clearance of mAbs.

The physiologic similarities between humans and NHPs render NHPs a critical part of an integrated approach to determining the safe and effective exposure of a candidate drug in humans. The PKPD data obtained from NHPs are critical in predicting the PKPD of humans, which can be translated to critical decision-making data such as MRSD, dose escalation scheme, and maximum dose in early clinical trials. Some of the highlighted examples include prediction of MRSD for lulizumab and rozanolixizumab, predicting PKPD of mAbs with half-life extension, and deciding to discontinue the development of a compound or preventing the discontinuation of a compound. In the lulizumab example, use of NHPs was essential during its development given the inherent high risk of targeting CD28 pathway and the lack of cross-reactivity in other nonclinical species. Lulizumab showed comparable binding affinity, RO, and inhibitory effect between human and monkey.

This PKPD relationship established in NHPs laid the groundwork in defining MABEL and selecting a subsequent FIH dose.[43] In the rozanolixizumab example, this human FcRn-targeted mAb was developed to decrease the levels of circulating pathological IgGs in auto- and allo-immune diseases. It exhibits similar affinity toward cynomolgus monkey and human FcRn.[47] The PKPD of rozanolixizumab in cynomolgus monkeys were integrated with the affinity and transcytosis in vitro measurements in a semi-mechanistic PKPD model to predict the decrease in circulating IgG in cyno, which was subsequently used to predict human PKPD and played a critical role in designing a concise Phase 1 trial, limiting exposition of healthy volunteers and expediting clinical development for the benefit of patients. In the example of predicting half-life extension, increasing half-life of mAbs and decreasing dosing frequency in humans of antibodies through protein engineering is often of great value. One promising approach is to increase half-life of antibodies by increasing affinity to FcRn. Both cynomolgus monkey and human FcRn have similar binding affinity to human IgG, making cynomolgus monkey an ideal species to evaluate the impact of changing binding to FcRn on antibody PK and predict human PK, as seen in the example of the MEDI-524, a mAb with a triple mutation M252Y/S254T/T256E into the Fc portion.[15] In the example of PKPD in deciding the fate of further development of drug candidates, QBP359, a human IgG1 mAb that binds specifically to human CCL21 and cross-reacts with cynomolgus monkey, has similarity in binding between NHP and humans CCL21. The physiologic comparability between same species made the NHP a suitable system to explore the PKPD of this novel biotherapeutic for the purposes of estimating human efficacious dose. The target RO data in the monkey and the PKPD model predicted QBP359 would be highly unlikely to achieve required suppression of CCL21 at manageable human doses, thus leading the discontinuation of this program and minimizing further use of animals and other resources.

Although NHPs play a critical role in predicting human PKPD, there are multiple challenges to overcome in the use of these animals. First, there are rare examples where NHPs

actually do not predict the human PK accurately as a result of unexpected differences in off-target binding between NHP and human, as seen in the case of MNRP1685A.[79] Second, immunogenicity can complicate the use of NHP data. Human or humanized mAbs are foreign to the immune system of the NHP and anti-drug antibodies can be formed when these human/humanized antibodies are administered to the NHP in PKPD and safety studies. ADA can affect the PK of the drug and neutralize its ability to bind to the target. These complications are generally manageable, which allows valuable information to be derived from these studies. In the cases of lenercept and obinutuzumab, ADA increased their clearance and the PK could be followed only over 10 days. Although not ideal, the monkey PK data from the first 10 days predicted the human PK accurately in both examples. In the case of the anti-IL17 mAb, non-naïve monkeys were divided into two groups – one with pre-dose ADA positive against the anti-IL17 mAb and another with pre-dose ADA negative against the anti-IL 17 mAb. Animals that tested ADA negative before the dosing remained negative at the end, with no individual animals showing evidence of an impact of ADA on PK. In the animals with preexisting ADA, accelerated clearance was observed in approximately 57% of those animals. Although more studies similar to this one are needed before we can fully understand the risks of re-dosing animals, it is encouraging to see that screening of pre-dose ADA status of non-naïve animals could help guide the re-use of these animals for PKPD purposes.

The NHP is often the species of choice for PKPD and safety assessment for therapeutic mAbs. However, every effort should be made to adhere to the 3R principles of NHP use. Multiple approaches have already been tested to reduce the use of NHP, which includes leveraging toxicology studies to reduce NHP use for PKPD evaluation, cassette dosing, PBPK modeling, and strategic re-use. Another important approach of re-use of NHPs is to use ADA-positive monkeys from PKPD studies to harvest the ADA and use them as reagents for immunogenicity assays. The replacing approach include development of in silico, in vitro and in vivo methods to replace NHPs and use of rabbits for human ocular PK predication. PK and PD data from safety assessment studies can be used as either the sole source of PKPD information or complementary to data from dedicated PKPD studies. PKPD and safety assessment colleagues should cooperate effectively to maximize the information gathered from every study involving NHPs. The more prevalent use of highly sensitive and specific LC-MS in bioanalysis of therapeutic proteins has enabled cassette dosing of several mAbs together in the same animals, which has the advantage of reducing NHP use as well as obtaining valuable PK data for individual mAbs. PBPK modeling has also found more applications in predicting human PK of mAbs. Several commercially available modeling tools have demonstrated the potential to reduce the use of NHPs. Strategic re-use of NHPs is another important approach to reduce the use of NHPs. As demonstrated in the example of the re-use of non-naïve NHPs for PK study of the anti-IL17 mAb, strategic re-use of NHPs (with appropriate testing of pre-dose ADA status to overcome potential complications) not only reduces the overall use of NHPs but also accelerates study initiations. As mentioned previously, most investigators prefer not to reuse non-naive NHPs for

PKPD and toxicology studies due to the risks associated with immunogenicity (i.e., ADAs). For NHPs that developed positive ADA responses in PKPD studies, purified polyclonal antibodies specific to the respective biotherapeutics that are derived from immunized animals are often used as reference standard(s) in ligand-binding assays to assess immunogenicity in nonclinical or clinical studies, which is a way to maximize the use of NHPs. Beside reducing and reusing of NHPs, replacing NHPs with in silico/in vitro and in vivo tools or with lower species has also become popular. Several in silico, in vitro and in vivo tools have been developed to replace NHPs for PK prediction, including in vitro tools to assess nonspecific binding of mAbs to reduce off-target binding and FcRn-dependent transcytosis cellular assays. Rabbits are frequently used to evaluate the ocular PK of mAbs, typically side-by-side with already marketed drug with known human ocular PK (reference compound, e.g., bevacizumab). By comparing the rabbit ocular PK between the mAb in development and the reference compound, we can reasonably predict the ocular PK of the investigational mAb.

As described above, the value of NHPs in studying PKPD of mAbs is based on its physiological relevance to human, which often is not achieved in other species. Van Meer and colleagues question the use of in vivo, and especially NHP studies in biologics development based on the impact of ADAs on the interpretability of the results generated.[63, 122, 123] Although it is a general consensus that the use of animals (including NHPs) should be minimized, there are clear evidences that NHPs are often necessary to predict human PKPD and safe starting doses to ensure the safety of patients and volunteers who participate in clinical trials of mAbs, as outlined above in the Relevance of NHP and Value of NHP sections. At the same time, different approaches have been implemented to reduce the use of NHPs. Whenever feasible, lower species that can replace NHPs while achieving the same objective to ensure human safety should be considered.

For many years, there has been a conscious effort throughout the pharmaceutical industry to optimize and reduce the use of NHPs in drug discovery and development. Primarily driven by ethical concerns, the industry is further motivated in their efforts by additional pressure from the European Union, a decrease in supply due to the limitations on export of NHPs from China, and an increase in demand by novel therapeutic modalities such as gene therapy.[124] As a result, interest in re-using NHPs for several studies has increased, as we illustrated in the human anti-IL17 antibody and Strategic re-use of NHPs sections. The re-use of animals dosed with modalities that do not elicit an immunogenic response (e.g., new chemical entities) to understand the PKPD of mAbs already occurs. However, for the re-use of NHPs already dosed with biologics to happen routinely, the immune status of the animals toward the new biologics to be tested needs to be understood. As illustrated in the human anti-IL17 example, it is feasible for companies that own a colony of NHPs. In that case, the key requirement is to have an ADA assay in place to test the animals before each experiment. For groups relying on CROs to perform experiments in NHPs on their behalf, the process is more complex. Indeed, a framework is needed for companies to share information regarding the nature of the test

articles that have already been tested on the NHPs without divulging any proprietary or confidential information. In addition, since the transfer of serum from NHPs is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulations, appropriate ADA tests need to be available in the country or continent where the animals are kept to avoid potentially lengthy logistical issues.

In vitro models to predict the PK behavior of new chemical entities have been used for years, spanning from isolated hepatocytes to predict metabolic clearance in human to complex 3D liver systems to understand the interplay between metabolism and active transport.[125–128] In addition, 3D models are also being developed to determine the distribution of small molecules through the blood-brain barrier and into the brain.[129] As illustrated in the "Development of in silico, in vitro and in vivo methods to replace NHPs" section, the field of biologics PKPD is naturally following a similar path. In vitro assays are being developed to predict the clearance of mAbs in human. Efforts in this area have focused on FcRn-dependent transcytosis assays since FcRn is a key determinant of mAb PK. Recently, Chung et al. reported a correlation between results in their transcytosis assay and human clearance for a set of 53 mAbs.[93] It is worth nothing that previous attempts to correlate FcRn-driven transcytosis with human clearance had not been so successful.[130] Based on their results, this second group had rationalized the lack of correlation by the presence of other processes driving human PK that were not fully recapitulated in a simple transcytosis assay.

As mentioned previously, an important aim of the studies performed in NHPs is to develop a better understanding of the concentration/effect relationship of candidate drugs. This aspect is much more complex to recapitulate accurately in vitro in a manner that can be quantitatively extrapolated to human in isolation from data generated in animals. However, the dynamics of specific membrane receptors can be studied very accurately in human cells when combined with mechanistic mathematical model. For instance, Khailaie et al. managed to quantify the rate of synthesis, internalization, recycling, and degradation of CTLA4, an immune checkpoint expressed on T cells, in human cells through a set of in vitro experiments blocking these specific cellular processes.[131] These in vitro results can in turn be included in mathematical models describing TMDD-driven PK behavior of mAbs.[132] In vitro data generated in human cells combined with the appropriate mathematical model have been successfully used to predict the effect of T-cell redirecting anti-tumor bispecific agents.[133] Blinatumomab is a bispecific T-cell engager directed against CD19 that aims to reduce malignant B cells in blood and bone marrow. Jiang et al. developed a mathematical model describing the interaction of the target cells, the biologics, and the effector cells.[127] Model parameters were estimated in in vitro incubations with varying ratios of effector and target cells over a range of timepoints. The model effectively predicted the efficacious concentrations of blinatumomab in both blood and bone marrow in patients with acute lymphoblastic leukemia.

Beyond this success, once validated such models can be applied to optimize novel biologics against similar targets or be applied to move toward patient-specific dose and dosing regimen based on in vitro data generated in patient cells or

tissues.[134] This is of course beyond the realm of possibilities available from results generated in NHPs. This review highlights the importance of NHP studies in drug discovery and development due to the key PKPD information that can be gathered from such studies. However, the use of NHPs should be driven by a strong scientific rationale. Throughout the industry, scientists recognize the need to limit the use of NHPs to a minimum. Alternative in vivo models as well as in vitro and in silico tools are being developed to reduce our reliance on NHPs in drug development. Where NHPs cannot be replaced yet, efforts are made to ensure that animals are used in the most optimal way and, where possible re-used for the investigation of several biological entities. In the future, integration of all available data from cell assays or patient information in mathematical models is likely to supersede the power of data generated in NHPs and provide more patient-specific predictions which remain out of reach from most experiments performed in animals.

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References

1. ICH. Preclinical safety evaluation of biotechnology-derived pharmaceuticals s6 (r1). In. Preclinical safety evaluation of biotechnology-derived pharmaceuticals s6 (r1). Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2011.
2. ICH. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals m3 (r2). In. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals m3 (r2). International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use; 2009.
3. Brennan FR, Cavagnaro J, McKeever K, Ryan PC, Schutten MM, Vahle J, Weinbauer GF, Marrer-Berger E, Black LE. Safety testing of monoclonal antibodies in non-human primates: case studies highlighting their impact on human risk assessment. MAbs. 2018;10(1):1–17. doi:10.1080/19420862.2017.1389364.
4. Baumann A, Prabhu S, Kanodia J. 2016. Use of pk/pd knowledge in guiding bispecific biologics research and development. In: Zhou I, Theil FP, editors. Use of pk/pd knowledge in guiding bispecific biologics research and development. Adme and translational pk/pd of thereapeutic proteins. Hoboken NJ, USA: Wiley; p. 1–13.
5. Horvath C, Andrews L, Baumann A, Black L, Blanset D, Cavagnaro J, Hastings KL, Hutto DL, MacLachlan TK, Milton M, et al. Storm forecasting: additional lessons from the cd28 super-agonist tgn1412 trial. Nat Rev Immunol. 2012;12(10):740. author reply 740. doi:10.1038/nri3192-c1.
6. ICH. Nonclinical evaluation for anticancer pharmaceuticals s9. In. Nonclinical evaluation for anticancer pharmaceuticals s9. International Conference on Harmonization; 2009.
7. EMA. 2017. Guideline on strategies to identify and mitigate risks for first inhuman and early clinical trials with investigational medicinal products. In. Guideline on strategies to identify and mitigate

risks for first-in-human and early clinical trials with investigational medicinal products. London, UK: Committee for Medicinal Products for Human Use (CHMP).

8. USFDA. 2019. Guidance for industry: severely debilitating or life-threatening hematologic disorders: nonclinical development of pharmaceuticals. In. Guidance for industry: severely debilitating or lifethreatening hematologic disorders: nonclinical development of pharmaceuticals. Silver Spring, MD: Center for Drug Evaluation and Research (CDER).

9. USFDA. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. In. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Center for Drug Evaluation and Research (CDER). 2005. https://www.fda.gov/media/72309/download

10. Elmeliegy M, Udata C, Liao K, Yin D. Considerations on the calculation of the human equivalent dose from toxicology studies for biologic anticancer agents. Clin Pharmacokinet. 2021;60 (5):563–67. doi:10.1007/s40262-021-00987-2.

11. Leach MW, Clarke DO, Dudal S, Han C, Li C, Yang Z, Brennan FR, Bailey WJ, Chen Y, Deslandes A, et al. Strategies and recommendations for using a data-driven and risk-based approach in the selection of first-in-human starting dose: an international consortium for innovation and quality in pharmaceutical development (iq) assessment. Clin Pharmacol Ther. 2021;109(6):1395–415. doi:10.1002/cpt.2009.

12. Muller PY, Milton M, Lloyd P, Sims J, Brennan FR. The minimum anticipated biological effect level (mabel) for selection of first human dose in clinical trials with monoclonal antibodies. Curr Opin Biotechnol. 2009;20(6):722–29. doi:10.1016/j.copbio.2009.10.013.

13. Saber H, Gudi R, Manning M, Wearne E, Leighton JK. An fda oncology analysis of immune activating products and first-in-human dose selection. Regul Toxicol Pharmacol. 2016;81:448–456.

14. Saber H, Del Valle P, Ricks TK, Leighton JK. An fda oncology analysis of cd3 bispecific constructs and first-in-human dose selection. Regul Toxicol Pharmacol. 2017;90:144–152.

15. Kamath AV. Translational pharmacokinetics and pharmacodynamics of monoclonal antibodies. Drug Discov Today Technol. 2016;21–22:75–83.

16. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther. 2008;84 (5):548–58. doi:10.1038/clpt.2008.170.

17. Deng R, Jin F, Prabhu S, Iyer S. Monoclonal antibodies: what are the pharmacokinetic and pharmacodynamic considerations for drug development? Expert Opin Drug Metab Toxicol. 2012;8 (2):141–60. doi:10.1517/17425255.2012.643868.

18. Roopenian DC, Akilesh SF. The neonatal fc receptor comes of age. Nat Rev Immunol. 2007;7(9):715–25. doi:10.1038/nri2155.

19. Garg A, Balthasar JP. Investigation of the influence of fcrn on the distribution of igg to the brain. Aaps j. 2009;11(3):553–57. doi:10.1208/s12248-009-9129-9.

20. Hansen RJ, Balthasar JP. Intravenous immunoglobulin mediates an increase in anti-platelet antibody clearance via the fcrn receptor. Thromb Haemost. 2002;88(6):898–99. doi:10.1055/s-0037-1613331.

21. Ober RJ, Radu CG, Ghetie V, Ward ES. Differences in promiscuity for antibody-fcrn interactions across species: implications for therapeutic antibodies. Int Immunol. 2001;13(12):1551–59. doi:10.1093/intimm/13.12.1551.

22. Andersen JT, Daba MB, Berntzen G, Michaelsen TE, Sandlie I. Cross-species binding analyses of mouse and human neonatal fc receptor show dramatic differences in immunoglobulin g and albumin binding. J Biol Chem. 2010;285(7):4826–36. doi:10.1074/jbc.M109.081828.

23. Avery LB, Wang M, Kavosi MS, Joyce A, Kurz JC, Fan YY, Dowty ME, Zhang M, Zhang Y, Cheng A, et al. Utility of a human fcrn transgenic mouse model in drug discovery for early assessment and prediction of human pharmacokinetics of monoclonal antibodies. MAbs. 2016;8(6):1064–78. doi:10.1080/19420862.2016.1193660.

24. Proetzel G, Roopenian DC. Humanized fcrn mouse models for evaluating pharmacokinetics of human igg antibodies. Methods. 2014;65(1):148–53. doi:10.1016/j.ymeth.2013.07.005.

25. Betts A, Keunecke A, van Steeg Tj, van der Graaf Ph, Avery LB, Jones H, Berkhout 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(5):751–64. doi:10.1080/19420862.2018.1462429.

26. Jefferis R. Glycosylation of natural and recombinant antibody molecules. Adv Exp Med Biol. 2005;564:143–148.

27. Sinclair AM, Elliott S. Glycoengineering:The effect of glycosylation on the properties of therapeutic proteins. J Pharm Sci. 2005;94 (8):1626–35. doi:10.1002/jps.20319.

28. Jones AJ, Papac DI, Chin EH, Keck R, Baughman SA, Lin YS, Kneer J, Battersby JE. Selective clearance of glycoforms of a complex glycoprotein pharmaceutical caused by terminal n-acetylglucosamine is similar in humans and cynomolgus monkeys. Glycobiology. 2007;17(5):529–40. doi:10.1093/glycob/cwm017.

29. 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 (1):61–66. doi:10.4161/mabs.3.1.13799.

30. Ling J, Zhou H, Jiao Q, Davis HM. Interspecies scaling of therapeutic monoclonal antibodies: initial look. J Clin Pharmacol. 2009;49(12):1382–402. doi:10.1177/0091270009337134.

31. Oitate M, Masubuchi N, Ito T, Yabe Y, Karibe T, Aoki T, Murayama N, Kurihara A, Okudaira N, Izumi T. Prediction of human pharmacokinetics of therapeutic monoclonal antibodies from simple allometry of monkey data. Drug Metab Pharmacokinet. 2011;26(4):423–30. doi:10.2133/dmpk.DMPK-11-RG-011.

32. Dong JQ, Salinger DH, Endres CJ, Gibbs JP, Hsu CP, Stouch BJ, Hurh E, Gibbs MA. Quantitative prediction of human pharmacokinetics for monoclonal antibodies: retrospective analysis of monkey as a single species for first-in-human prediction. Clin Pharmacokinet. 2011;50(2):131–42. doi:10.2165/11537430-000000000-00000.

33. Kamath AV, Lu D, Gupta P, Jin D, Xiang H, Wong A, Leddy C, Crocker L, Schaefer G, Sliwkowski MX, et al. Preclinical pharmacokinetics of mehd7945a, a novel egfr/her3 dual-action antibody, and prediction of its human pharmacokinetics and efficacious clinical dose. Cancer Chemother Pharmacol. 2012;69(4):1063–69. doi:10.1007/s00280-011-1806-6.

34. 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 disposition. Aaps j. 2015;17(2):389–99. doi:10.1208/s12248-014-9690-8.

35. Figueroa I, Leipold D, Leong S, Zheng B, Triguero-Carrasco M, Fourie-O'Donohue A, Kozak KR, Xu K, Schutten M, Wang H, et al. Prediction of non-linear pharmacokinetics in humans of an antibody-drug conjugate (adc) when evaluation of higher doses in animals is limited by tolerability: case study with an anti-cd33 adc. MAbs. 2018;10(5):738–50. doi:10.1080/19420862.2018.1465160.

36. Crowley AR, Ackerman ME. Mind the gap: how interspecies variability in igg and its receptors may complicate comparisons of human and non-human primate effector function. Front Immunol. 2019;10(697). doi:10.3389/fimmu.2019.00697.

37. Grunst MW, Grandea AG 3rd, Janaka SK, Hammad I, Grimes P, Karl JA, Wiseman R, O'Connor DH, Evans DT. Functional interactions of common allotypes of rhesus macaque fcγr2a and fcγr3a with human and macaque igg subclasses. J Immunol. 2020;205 (12):3319–32. doi:10.4049/jimmunol.2000501.

38. Zheng Y, Tesar DB, Benincosa L, Birnböck H, Boswell CA, Bumbaca D, Cowan KJ, Danilenko DM, Daugherty AL, Fielder PJ, et al. Minipig as a potential translatable model for monoclonal antibody pharmacokinetics after intravenous and subcutaneous administration. MAbs. 2012;4(2):243–55. doi:10.4161/mabs.4.2.19387.

39. Richter WF, Grimm HP, Gouy MH, Søgaard S, Kreuzer C, Wessels U, Draganov D, Muenzer C, Hoche T. Subcutaneous site-of-absorption study with the monoclonal antibody tocilizumab in minipigs: administration behind ear translates best to humans. Aaps j. 2020;22(3):63. doi:10.1208/s12248-020-00446-z.

40. Horvath CJ, Milton MN. The tegenero incident and the duff report conclusions: a series of unfortunate events or an avoidable event? Toxicol Pathol. 2009;37(3):372–83. doi:10.1177/0192623309332986.

41. Duff G. 2006. Final report; 2006. Expert scientific group on phase one clinical trials: final report. In Expert Scientific Group on Phase One Clinical Trials. Hoboken NJ, USA; 1–106. https://repository.library.georgetown.edu/handle/10822/969183

42. Suchard SJ, Davis PM, Kansal S, Stetsko DK, Brosius R, Tamura J, Schneeweis L, Bryson J, Salcedo T, Wang H, et al. A monovalent anti-human cd28 domain antibody antagonist: preclinical efficacy and safety. J Immunol. 2013;191(9):4599–610. doi:10.4049/jimmunol.1300470.

43. Yang Z, Wang H, Salcedo TW, Suchard SJ, Xie JH, Schneeweis LA, Fleener CA, Calore JD, Shi R, Zhang SX, et al. Integrated pharmacokinetic/pharmacodynamic analysis for determining the minimal anticipated biological effect level of a novel anti-cd28 receptor antagonist bms-931699. J Pharmacol Exp Ther. 2015;355(3):506–15. doi:10.1124/jpet.115.227249.

44. Shi R, Honczarenko M, Zhang S, Fleener C, Mora J, Lee SK, Wang R, Liu X, Shevell DE, Yang Z, et al. Pharmacokinetic, pharmacodynamic, and safety profile of a novel anti-cd28 domain antibody antagonist in healthy subjects. J Clin Pharmacol. 2017;57(2):161–72. doi:10.1002/jcph.791.

45. Gable KL, Guptill JT. Antagonism of the neonatal fc receptor as an emerging treatment for myasthenia gravis. Front Immunol. 2019:10:3052.

46. Li X, Kimberly RP. Targeting the fc receptor in autoimmune disease. Expert Opin Ther Targets. 2014;18(3):335–50. doi:10.1517/14728222.2014.877891.

47. Smith B, Kiessling A, Lledo-Garcia R, Dixon KL, Christodoulou L, Catley MC, Atherfold P, D'Hooghe LE, Finney H, Greenslade K, et al. Generation and characterization of a high affinity anti-human fcrn antibody, rozanolixizumab, and the effects of different molecular formats on the reduction of plasma igg concentration. MAbs. 2018;10(7):1111–30. doi:10.1080/19420862.2018.1505464.

48. Dall'Acqua WF, Kiener PA, Wu H. Properties of human igg1s engineered for enhanced binding to the neonatal fc receptor (fcrn). J Biol Chem. 2006;281(33):23514–24. doi:10.1074/jbc.M604292200.

49. Neuber T, Frese K, Jaehrling J, Jäger S, Daubert D, Felderer K, Linnemann M, Höhne A, Kaden S, Kölln J, et al. Characterization and screening of igg binding to the neonatal fc receptor. MAbs. 2014;6(4):928–42. doi:10.4161/mabs.28744.

50. Kim J, Hayton WL, Robinson JM, Anderson CL. Kinetics of fcrn-mediated recycling of igg and albumin in human: pathophysiology and therapeutic implications using a simplified mechanism-based model. Clin Immunol. 2007;122(2):146–55. doi:10.1016/j.clim.2006.09.001.

51. Chen Y, Balthasar JP. Evaluation of a catenary pbpk model for predicting the in vivo disposition of mabs engineered for high-affinity binding to fcrn. Aaps j. 2012;14(4):850–59. doi:10.1208/s12248-012-9395-9.

52. Sharma A, Jusko WJ. Characterization of four basic models of indirect pharmacodynamic responses. J Pharmacokinet Biopharm. 1996;24(6):611–35. doi:10.1007/BF02353483.

53. Lledo-Garcia R, Dixon K, Shock A, Oliver R. Pharmacokinetic-pharmacodynamic modelling of the anti-fcrn monoclonal antibody rozanolixizumab: translation from preclinical stages to the clinic. CPT Pharmacometrics Syst Pharmacol. 2021;11(1):116–28. doi:10.1002/psp4.12739.

54. Kiessling P, Lledo-Garcia R, Watanabe S, Langdon G, Tran D, Bari M, Christodoulou L, Jones E, Price G, Smith B, et al. The fcrn inhibitor rozanolixizumab reduces human serum igg concentration: a randomized phase 1 study. Sci Transl Med. 2017;9(414). doi:10.1126/scitranslmed.aan1208.

55. Kontermann RE. Strategies to extend plasma half-lives of recombinant antibodies. BioDrugs. 2009;23(2):93–109. doi:10.2165/00063030-200923020-00003.

56. Yeung YA, Leabman MK, Marvin JS, Qiu J, Adams CW, Lien S, Starovasnik MA, Lowman HB. Engineering human igg1 affinity to human neonatal fc receptor: impact of affinity improvement on pharmacokinetics in primates. J Immunol. 2009;182(12):7663–71. doi:10.4049/jimmunol.0804182.

57. Zalevsky J, Chamberlain AK, Horton HM, Karki S, Leung IW, Sproule TJ, Lazar GA, Roopenian DC, Desjarlais JR. Enhanced antibody half-life improves in vivo activity. Nat Biotechnol. 2010;28(2):157–59. doi:10.1038/nbt.1601.

58. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A novel investigational fc-modified humanized monoclonal antibody, motavizumab-yte, has an extended half-life in healthy adults. Antimicrob Agents Chemother. 2013;57(12):6147–53. doi:10.1128/AAC.01285-13.

59. Valente D, Mauriac C, Schmidt T, Focken I, Beninga J, Mackness B, Qiu H, Vicat P, Kandira A, Radošević K, et al. Pharmacokinetics of novel fc-engineered monoclonal and multispecific antibodies in cynomolgus monkeys and humanized fcrn transgenic mouse models. MAbs. 2020;12(1):1829337. doi:10.1080/19420862.2020.1829337.

60. Dudal S, Subramanian K, Flandre T, Law WS, Lowe PJ, Skerjanec A, Genin JC, Duval M, Piequet A, Cordier A, et al. Integrated pharmacokinetic, pharmacodynamic and immunogenicity profiling of an anti-ccl21 monoclonal antibody in cynomolgus monkeys. MAbs. 2015;7(5):829–37. doi:10.1080/19420862.2015.1060384.

61. Bardroff M, Ewert S, Jaeger U, Jarai G, Carsten C, Kunz S, Wiesehan K. Ccl21 binding molecules. In Ccl21 Binding Molecules. 2010. https://patents.google.com/patent/WO2011080050A3/en

62. Bugelski PJ, Treacy G. Predictive power of preclinical studies in animals for the immunogenicity of recombinant therapeutic proteins in humans. Curr Opin Mol Ther. 2004;6:10–16.

63. van Meer Pj, Kooijman M, van der Laan Jw, Moors EH, Schellekens H. The value of non-human primates in the development of monoclonal antibodies. Nat Biotechnol. 2013;31(10):882–83. doi:10.1038/nbt.2709.

64. Ponce R, Abad L, Amaravadi L, Gelzleichter T, Gore E, Green J, Gupta S, Herzyk D, Hurst C, Ivens IA, et al. Immunogenicity of biologically-derived therapeutics: assessment and interpretation of nonclinical safety studies. Regul Toxicol Pharmacol. 2009;54(2):164–82. doi:10.1016/j.yrtph.2009.03.012.

65. Wierda D, Smith HW, Zwickl CM. Immunogenicity of biopharmaceuticals in laboratory animals. Toxicology. 2001;158(1–2):71–74. doi:10.1016/S0300-483X(00)00410-8.

66. Thway TM, Magana I, Bautista A, Jawa V, Gu W, Ma M. Impact of anti-drug antibodies in preclinical pharmacokinetic assessment. Aaps j. 2013;15(3):856–63. doi:10.1208/s12248-013-9484-4.

67. USFDA. 2013. Albiglutide #125431. Center for Drug Evaluation and Research (CDER). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/125431Orig1s000PharmR.pdf.

68. Rehlaender BN, Cho MJ. Antibodies as carrier proteins. Pharm Res. 1998;15(11):1652–56. doi:10.1023/A:1011936007457.

69. Gunn H. Immunogenicity of recombinant human interleukin-3. Clin Immunol Immunopathol. 1997;83(1):5–7. doi:10.1006/clin.1996.4302.

70. Koren E, Zuckerman LA, Mire-Sluis AR. Immune responses to therapeutic proteins in humans–clinical significance, assessment and prediction. Curr Pharm Biotechnol. 2002;3(4):349–60. doi:10.2174/1389201023378175.

71. Aston R, Cowden WB, Ada GL. Antibody-mediated enhancement of hormone activity. Mol Immunol. 1989;26(5):435–46. doi:10.1016/0161-5890(89)90103-X.

72. Sailstad JM, Amaravadi L, Clements-Egan A, Gorovits B, Myler HA, Pillutla RC, Pursuhothama S, Putman M, Rose MK, Sonehara K, et al. A white paper–consensus and recommendations of a global harmonization team on assessing the impact of immunogenicity on pharmacokinetic measurements. Aaps j. 2014;16 (3):488–98. doi:10.1208/s12248-014-9582-y.

73. Herzyk D. Anti-drug antibody testing in toxicity studies. In: NIoHS J, editor. Anti-drug antibody testing in toxicity studies; 2010. https://www.nihs.go.jp/dec/rs/hyouka/2010/PM6_Immunogenicity_Scientific.pdf

74. Brock WJ, Mounho B, Fu L. 2014. The role of the study director in nonclinical studies: pharmaceuticals, chemicals, medical devices, and pesticides. Hoboken NJ, USA: John Wiley & Sons.

75. Richter WF, Gallati H, Schiller CD. Animal pharmacokinetics of the tumor necrosis factor receptor-immunoglobulin fusion protein lenercept and their extrapolation to humans. Drug Metab Dispos. 1999;27:21–25.

76. Rau R, Sander O, van Riel P, van de Putte L, Hasler F, Zaug M, Kneer J, van der Auwera P, Stevens RM. Intravenous human recombinant tumor necrosis factor receptor p55-fc igg1 fusion protein ro 45-2081 (lenercept): a double blind, placebo controlled dose-finding study in rheumatoid arthritis. J Rheumatol. 2003;30:680–90.

77. Grimm HP, Schick E, Hainzl D, Justies N, Yu L, Klein C, Husar E, Richter WF. Pkpd assessment of the anti-cd20 antibody obinutuzumab in cynomolgus monkey is feasible despite marked anti-drug antibody response in this species. J Pharm Sci. 2019;108 (11):3729–36. doi:10.1016/j.xphs.2019.07.013.

78. Han C, Gunn GR, Marini JC, Shankar G, Han Hsu H, Davis HM. Pharmacokinetics and immunogenicity investigation of a human anti-interleukin-17 monoclonal antibody in non-naïve cynomolgus monkeys. Drug Metab Dispos. 2015;43(5):762–70. doi:10.1124/dmd.114.062679.

79. Xin Y, Bai S, Damico-Beyer LA, Jin D, Liang WC, Wu Y, Theil FP, Joshi A, Lu Y, Lowe J, et al. Anti-neuropilin-1 (mnrp1685a): unexpected pharmacokinetic differences across species, from preclinical models to humans. Pharm Res. 2012;29(9):2512–21. doi:10.1007/s11095-012-0781-x.

80. Bumbaca D, Wong A, Drake E, Reyes AE 2nd, Lin BC, Stephan JP, Desnoyers L, Shen BQ, Dennis MS. Highly specific off-target binding identified and eliminated during the humanization of an antibody against fgf receptor 4. MAbs. 2011;3(4):376–86. doi:10.4161/mabs.3.4.15786.

81. Vugmeyster Y, Szklut P, Wensel D, Ross J, Xu X, Awwad M, Gill D, Tchistiakov L, Warner G. Complex pharmacokinetics of a humanized antibody against human amyloid beta peptide, anti-abeta ab2, in nonclinical species. Pharm Res. 2011;28 (7):1696–706. doi:10.1007/s11095-011-0405-x.

82. Waldmann TA, Terry WD. Familial hypercatabolic hypoproteinemia. A disorder of endogenous catabolism of albumin and immunoglobulin. J Clin Invest. 1990;86(6):2093–98. doi:10.1172/JCI114947.

83. Junghans RP, Anderson CL. The protection receptor for igg catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. Proc Natl Acad Sci U S A. 1996;93(11):5512–16. doi:10.1073/pnas.93.11.5512.

84. Tiwari A, Kasaian M, Heatherington AC, Jones HM, Hua F. A mechanistic pk/pd model for two anti-il13 antibodies explains the difference in total il-13 accumulation observed in clinical studies. MAbs. 2016;8(5):983–90. doi:10.1080/19420862.2016.1172151.

85. Meno-Tetang GM, Lowe PJ. On the prediction of the human response: a recycled mechanistic pharmacokinetic/pharmacodynamic approach. Basic Clin Pharmacol Toxicol. 2005;96 (3):182–92. doi:10.1111/j.1742-7843.2005.pto960307.x.

86. Vugmeyster Y, Tian X, Szklut P, Kasaian M, Xu X. Pharmacokinetic and pharmacodynamic modeling of a humanized anti-il-13 antibody in naive and ascaris-challenged cynomolgus monkeys. Pharm Res. 2009;26(2):306–15. doi:10.1007/s11095-008-9739-4.

87. Leach MW, Rottman JB, Hock MB, Finco D, Rojko JL, Beyer JC. Immunogenicity/hypersensitivity of biologics. Toxicol Pathol. 2014;42(1):293–300. doi:10.1177/0192623313510987.

88. Hey A, Baumann A, Kronenberg S, Blaich G, Mohl S, Fagg R, Ulrich P, Rattel B, Richter WF, Kiessling A, et al. Nonclinical development of biologics: integrating safety, pharmacokinetics, and pharmacodynamics to create smarter and more flexible nonclinical safety programs optimizing animal use. Int J Toxicol. 2021;40(3):270–84. doi:10.1177/1091581821994288.

89. Nagayasu M, Ozeki K. Combination of cassette-dosing and microsampling for reduced animal usage for antibody pharmacokinetics in cynomolgus monkeys, wild-type mice, and human fcrn transgenic mice. Pharm Res. 2021;38(4):583–92. doi:10.1007/s11095-021-03028-6.

90. Ker T, Blaich G, Baumann A, Kronenberg S, Hey A, Kiessling A, Schmitt PM, Driessen W, Carrez C, Kramer D, et al. Challenges of non-clinical safety testing for biologics: a report of the 9th biosafe european annual general membership meeting. MAbs. 2021;13 (1):1938796. doi:10.1080/19420862.2021.1938796.

91. Dostalek M, Prueksaritanont T, Kelley RF. Pharmacokinetic de-risking tools for selection of monoclonal antibody lead candidates. MAbs. 2017;9(5):756–66. doi:10.1080/19420862.2017.1323160.

92. Hötzel I, Theil FP, Bernstein LJ, Prabhu S, Deng R, Quintana L, Lutman J, Sibia R, Chan P, Bumbaca D, et al. A strategy for risk mitigation of antibodies with fast clearance. MAbs. 2012;4 (6):753–60.

93. Chung S, Nguyen V, Lin YL, Lafrance-Vanasse J, Scales SJ, Lin K, Deng R, Williams K, Sperinde G, Li JJ, et al. An in vitro fcrn-dependent transcytosis assay as a screening tool for predictive assessment of nonspecific clearance of antibody therapeutics in humans. MAbs. 2019;11(5):942–55. doi:10.1080/19420862.2019.1605270.

94. Kraft TE, Richter WF, Emrich T, Knaupp A, Schuster M, Wolfert A, Kettenberger H. Heparin chromatography as an in vitro predictor for antibody clearance rate through pinocytosis. mAbs. 2020;12(1):1683432. doi:10.1080/19420862.2019.1683432.

95. Rostami-Hodjegan A, Tamai I, Pang KS. Physiologically based pharmacokinetic (pbpk) modeling: it is here to stay! Biopharm Drug Dispos. 2012;33(2):47–50. doi:10.1002/bdd.1776.

96. Covell DG, Barbet J, Holton OD, Black CD, Parker RJ, Weinstein JN. Pharmacokinetics of monoclonal immunoglobulin g1, f(ab')2, and fab' in mice. Cancer Res. 1986;46:3969–78.

97. Glassman PM, Balthasar JP. Physiologically-based modeling of monoclonal antibody pharmacokinetics in drug discovery and development. Drug Metab Pharmacokinet. 2019;34(1):3–13. doi:10.1016/j.dmpk.2018.11.002.

98. Hu L, Hansen RJ. Issues, challenges, and opportunities in model-based drug development for monoclonal antibodies. J Pharm Sci. 2013;102(9):2898–908. doi:10.1002/jps.23504.

99. Wang J, Iyer S, Fielder PJ, Davis JD, Deng R. Projecting human pharmacokinetics of monoclonal antibodies from nonclinical data: comparative evaluation of prediction approaches in early drug development. Biopharm Drug Dispos. 2016;37(2):51–65. doi:10.1002/bdd.1952.

100. Glassman PM, Balthasar JP. Physiologically-based pharmacokinetic modeling to predict the clinical pharmacokinetics of monoclonal antibodies. J Pharmacokinet Pharmacodyn. 2016;43 (4):427–46. doi:10.1007/s10928-016-9482-0.

101. Hu S, D'Argenio DZ. Predicting monoclonal antibody pharmacokinetics following subcutaneous administration via whole-body physiologically-based modeling. J Pharmacokinet Pharmacodyn. 2020;47(5):385–409. doi:10.1007/s10928-020-09691-3.

102. Jones HM, Zhang Z, Jasper P, Luo H, Avery LB, King LE, Neubert H, Barton HA, Betts AM, Webster R. A physiologically-based pharmacokinetic model for the prediction of monoclonal antibody pharmacokinetics from in vitro data. CPT Pharmacometrics Syst Pharmacol. 2019;8(10):738–47. doi:10.1002/psp4.12461.

103. Bae DJ, Kim SY, Bae SM, Hwang AK, Pak KC, Yoon S, Lim HS. Whole-body physiologically based pharmacokinetic modeling of trastuzumab and prediction of human pharmacokinetics. J Pharm Sci. 2019;108(6):2180–90. doi:10.1016/j.xphs.2019.01.024.

104. Glassman PM, Chen Y, Balthasar JP. Scale-up of a physiologically-based pharmacokinetic model to predict the disposition of monoclonal antibodies in monkeys. J Pharmacokinet Pharmacodyn. 2015;42(5):527–40. doi:10.1007/s10928-015-9444-y.

105. Shah DK, Betts AM. Antibody biodistribution coefficients: inferring tissue concentrations of monoclonal antibodies based on the plasma concentrations in several preclinical species and human. mAbs. MAbs 2013;5(2):297–305. doi:10.4161/mabs.23684.

106. Niederalt C, Kuepfer L, Solodenko J, Eissing T, Siegmund HU, Block M, Willmann S, Lippert J. A generic whole body physiologically based pharmacokinetic model for therapeutic proteins in pk-sim. J Pharmacokinet Pharmacodyn. 2018;45(2):235–57. doi:10.1007/s10928-017-9559-4.

107. Sager JE, Yu J, Ragueneau-Majlessi I, Isoherranen N. Physiologically based pharmacokinetic (pbpk) modeling and simulation approaches: a systematic review of published models, applications, and model verification. Drug Metab Dispos. 2015;43 (11):1823–37. doi:10.1124/dmd.115.065920.

108. Varkhede N, Forrest ML. Understanding the monoclonal antibody disposition after subcutaneous administration using a minimal physiologically based pharmacokinetic model. J Pharm Pharm Sci. 2018;21(1s):130s–148s. doi:10.18433/jpps30028.

109. Zhao J, Cao Y, Jusko WJ. Across-species scaling of monoclonal antibody pharmacokinetics using a minimal pbpk model. Pharm Res. 2015;32(10):3269–81. doi:10.1007/s11095-015-1703-5.

110. Cao Y, Balthasar JP, Jusko WJ. Second-generation minimal physiologically-based pharmacokinetic model for monoclonal antibodies. J Pharmacokinet Pharmacodyn. 2013;40(5):597–607. doi:10.1007/s10928-013-9332-2.

111. Li L, Gardner I, Dostalek M, Jamei M. Simulation of monoclonal antibody pharmacokinetics in humans using a minimal physiologically based model. Aaps j. 2014;16(5):1097–109. doi:10.1208/s12248-014-9640-5.

112. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, et al. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health. 2017;5(12):e1221–e1234. doi:10.1016/S2214-109X(17)30393-5.

113. Stewart MW. Pharmacokinetics, pharmacodynamics and pre-clinical characteristics of ophthalmic drugs that bind vegf. Expert Rev Clin Pharmacol. 2014;7(2):167–80. doi:10.1586/17512433.2014.884458.

114. Peyman GA, Lad EM, Moshfeghi DM. Intravitreal injection of therapeutic agents. Retina. 2009;29(7):875–912. doi:10.1097/IAE.0b013e3181a94f01.

115. Krohne TU, Eter N, Holz FG, Meyer CH. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. Am J Ophthalmol. 2008;146(4):508–12. doi:10.1016/j.ajo.2008.05.036.

116. Moisseiev E, Waisbourd M, Ben-Artsi E, Levinger E, Barak A, Daniels T, Csaky K, Loewenstein A, Barequet IS. Pharmacokinetics of bevacizumab after topical and intravitreal administration in human eyes. Graefes Arch Clin Exp Ophthalmol. 2014;252(2):331–37. doi:10.1007/s00417-013-2495-0.

117. Caruso A, Füth M, Alvarez-Sánchez R, Belli S, Diack C, Maass KF, Schwab D, Kettenberger H, Mazer. Ocular half-life of intravitreal biologics in humans and other species: meta-analysis and model-based prediction. Mol Pharm. NA;17:695–709.

118. Agrahari V, Mandal A, Agrahari V, Trinh HM, Joseph M, Ray A, Hadji H, Mitra R, Pal D, Mitra AK. A comprehensive insight on ocular pharmacokinetics. Drug Deliv Transl Res. 2016;6(6):735–54. doi:10.1007/s13346-016-0339-2.

119. Del Amo EM, Urtti A. Rabbit as an animal model for intravitreal pharmacokinetics: clinical predictability and quality of the published data. Exp Eye Res. 2015;137:111–124.

120. Gadkar K, Pastuskovas CV, Le Couter JE, Elliott JM, Zhang J, Lee CV, Sanowar S, Fuh G, Kim HS, Lombana TN, et al. Design and pharmacokinetic characterization of novel antibody formats for ocular therapeutics. Invest Ophthalmol Vis Sci. 2015;56 (9):5390–400. doi:10.1167/iovs.15-17108.

121. Fuchs H, Igney F. Binding to ocular albumin as a half-life extension principle for intravitreally injected drugs: evidence from mechanistic rat and rabbit studies. J Ocul Pharmacol Ther. 2017;33 (2):115–22. doi:10.1089/jop.2016.0083.

122. van Meer Pj, Ebbers HC, Kooijman M, Wied Gispen-de CC, Silva-Lima B, Moors EH, Schellekens H. Contribution of animal studies to evaluate the similarity of biosimilars to reference products. Drug Discov Today. 2015;20(4):483–90. doi:10.1016/j.drudis.2014.11.009.

123. van Meer Pj, Graham ML, Schuurman HJ. The safety, efficacy and regulatory triangle in drug development: impact for animal models and the use of animals. Eur J Pharmacol. 2015;759:3–13.

124. Epstein MM, Vermeire T. An opinion on non-human primates testing in Europe. Drug Discov Today Dis Models. 2017;23:5–9.

125. Houston JB. Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. Biochem Pharmacol. 1994;47 (9):1469–79. doi:10.1016/0006-2952(94)90520-7.

126. Kanebratt KP, Janefeldt A, Vilén L, Vildhede A, Samuelsson K, Milton L, Björkbom A, Persson M, Leandersson C, Andersson TB, et al. Primary human hepatocyte spheroid model as a 3d in vitro platform for metabolism studies. J Pharm Sci. 2021;110(1):422–31. doi:10.1016/j.xphs.2020.10.043.

127. Foster AJ, Chouhan B, Regan SL, Rollison H, Amberntsson S, Andersson LC, Srivastava A, Darnell M, Cairns J, Lazic SE, et al. Integrated in vitro models for hepatic safety and metabolism: evaluation of a human liver-chip and liver spheroid. Arch Toxicol. 2019;93(4):1021–37. doi:10.1007/s00204-019-02427-4.

128. Riede J, Wollmann BM, Molden E, Ingelman-Sundberg M. Primary human hepatocyte spheroids as an in vitro tool for investigating drug compounds with low clearance. Drug Metab Dispos. 2021;49(7):501–08. doi:10.1124/dmd.120.000340.

129. Ahn SI, Sei YJ, Park HJ, Kim J, Ryu Y, Choi JJ, Sung HJ, MacDonald TJ, Levey AI, Kim Y. Microengineered human blood-brain barrier platform for understanding nanoparticle transport mechanisms. Nat Commun. 2020;11(1):175. doi:10.1038/s41467-019-13896-7.

130. Jaramillo CAC, Belli S, Cascais AC, Dudal S, Edelmann MR, Haak M, Brun ME, Otteneder MB, Ullah M, Funk C, et al. Toward in vitro-to-in vivo translation of monoclonal antibody pharmacokinetics: application of a neonatal fc receptor-mediated transcytosis assay to understand the interplaying clearance mechanisms. MAbs. 2017;9(5):781–91. doi:10.1080/19420862.2017.1320008.

131. Khailaie S, Rowshanravan B, Robert PA, Waters E, Halliday N, Badillo Herrera JD, Walker LSK, Sansom DM, Meyer-Hermann M. Characterization of ctla4 trafficking and implications for its function. Biophys J. 2018;115(7):1330–43. doi:10.1016/j.bpj.2018.08.020.

132. Krippendorff BF, Kuester K, Kloft C, Huisinga W. Nonlinear pharmacokinetics of therapeutic proteins resulting from receptor mediated endocytosis. J Pharmacokinet Pharmacodyn. 2009;36 (3):239–60. doi:10.1007/s10928-009-9120-1.

133. Jiang X, Chen X, Carpenter TJ, Wang J, Zhou R, Davis HM, Heald DL, Wang W. Development of a target cell-biologics-effector cell (tbe) complex-based cell killing model to characterize target cell depletion by t cell redirecting bispecific agents. MAbs. 2018;10(6):876–89. doi:10.1080/19420862.2018.1480299.

134. Karolak A, Markov DA, McCawley LJ, Rejniak KA. Towards personalized computational oncology: from spatial models of tumour spheroids, to organoids, to tissues. J R Soc Interface. 2018;15 (138):20170703. doi:10.1098/rsif.2017.0703.