Integration of Consortia Recommendations for Justification of Animal Use Within Current and Future Drug Development Paradigms

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Abstract

The pharmaceutical and biotechnology industries continually review the requirements for, and relevance of, safety assessment strategies. Various industry consortia are currently discussing and reviewing data on a range of topics with respect to regulatory toxicology programs. These consortia are charged with critical evaluation of data and the identification of opportunities to promote best practice and to introduce improved approaches to safety assessment. Such improvements may include enhanced predictivity, more efficient ways of working, and opportunities for promoting and implementing the 3Rs (replacement, refinement, or reduction). As each consortium is considering a distinct question, individual outputs and recommendations could be perceived to be conflicting. However, a common theme embraced by the consortia represented here is exploration of the most appropriate use of animals for the safety assessment of new medicinal products. This short review summarizes presentations and discussions from a symposium describing the work of four industry consortia and considers whether their recommendations can be aligned into realistic approaches to improve future toxicology testing strategies, highlighting justification for the appropriate use of different animal species and opportunities for reductions in animal use without compromising patient safety.

Keywords

safety assessment, 3Rs, drug development

Introduction

It is important that the requirements for the safety assessment of new medicines are regularly reviewed and, if necessary revised, to ensure that they reflect advances in technology and a growing appreciation of mechanistic toxicology, but also to ensure that opportunities to replace, refine, and reduce the use of laboratory animals are realized. Such reviews are arguably conducted most effectively when pharmaceutical companies work in concert, and there is a long history of companies working together to share and critically review the available data and to recommend realistic changes in practices and regulations.[1-4] Some of these consortia have also highlighted opportunities for the more appropriate use of animals for the safety assessment of novel drugs and biopharmaceuticals.[5-8] The work of these consortia is not a trivial undertaking. A substantial investment of time and resources is required for data collation and analysis, and in the formulation of recommendations. However, the benefits that can derive from monitoring and, when appropriate, revising, the practice of safety assessment and regulatory requirements justify that investment. The sharing of experience and challenging current practices in an open environment provides a larger evidence base to draw upon, more relevant study designs derived from approaches developed by individual companies, improved confidence, a shared and reduced risk, and improved interactions with regulators. More tangible business advantages may include reduced costs through improved efficiencies or streamlined processes, a requirement for fewer animals/studies, and reduced time to reach decision-making milestones.

A symposium was held in November 2018 as part of the 39th Annual Meeting of the American College of Toxicology. This symposium was designed to bring together scientists and regulators contributing to four active industry consortia:

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IQ-DruSafe, European Federation of Pharmaceutical Industries and Associations (EFPIA), Biosafe, and the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). The speakers and audience explored outcomes of large data sharing projects and a variety of case studies, to consider whether separate consortia recommendations could be aligned into complementary and practical approaches for future toxicology testing strategies. One aspect, justification for the appropriate use of different species of animals in nonclinical toxicology studies, provided an important theme for the symposium. The individual presentations and resulting discussions are summarized briefly below.

The IQ Consortium Nonclinical to Clinical Translational Database and Predictive Value (Thomas Monticello)

DruSafe, the Preclinical Safety Leadership Group in the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ), has created and analyzed an industry-wide database to determine how safety assessment in animals translates into clinical risk. Mangipudy and colleagues published a commentary addressing the debate on the utility of animal toxicology studies to evaluate human safety and, following a literature review, concluded that animal testing provides value for ensuring patient safety.[9] The same conclusion was reached by the DruSafe analysis of their translational database.[10]

The database contained animal toxicology and safety pharmacology data coupled with clinical observations from completed phase I human studies for 182 molecules. Concordance statistics were performed by organ system and test species. Sensitivity (the proportion of positive clinical findings that had positive nonclinical findings) was 48% with a 43% positive predictive value (PPV; the proportion of positive nonclinical findings that had positive clinical findings). When the same target organ was identified in both the rodent and nonrodent, the PPV increased. Specificity (the proportion of negative clinical findings that had negative nonclinical findings) was 84% with a negative predictive value (NPV; the proportion of negative nonclinical findings that had negative clinical findings) of 86%. If no target organ toxicity was observed in either test species, the NPV increased.

The safety pharmacology data (central nervous system [CNS], cardiovascular [CV], and respiratory end points) obtained from the animal studies and phase I clinical trials were categorized by organ system and concordance statistics determined.[11] The PPV for CNS, CV, and respiratory end points were 42%, 41%, and 0%, respectively (with the respiratory PPV attributed to a lack of a respiratory clinical observations). The NPV for CNS, CV, and respiratory findings was 99%, 97% and 100%, respectively. These results support the current regulatory guideline to include CNS and CV assessments in phase I nonclinical packages. However, it was also concluded that a stand-alone safety pharmacology respiratory study may not be needed, due to the lack of impact and low predictive value (a 3Rs opportunity to reduce the animals required).

Aligned with other published reports,[12] the clinical adverse event (AE) prevalence in the database was low, which was attributable to earlier attrition of less optimal candidates based on in silico, in vitro, and animal toxicology data. For any diagnostic test, changes in prevalence are directly correlated with changes in PPV and inversely to changes in NPV, so when prevalence is low, the PPV will also be low,[10] even if both sensitivity and specificity are high.[13] It is important to be cognizant of the prevalence of the outcome to be determined. The low prevalence identified in this database for clinical outcomes emphasizes the importance of the NPV of nonclinical testing to ensure patient safety.[10]

Another observation from the database related to the impact of false positives. Animal study designs are biased toward these type 1 errors (false positives) since high doses in animal studies aim to achieve a maximum tolerate dose, an exposure saturation, a maximal feasible dose, or a mean exposure margin of 50 times the clinical exposure (ICHM3(R2)[14]). It was also noted that animal models do not predict more subjective constitutional signs such as headache, fatigue, or anxiety. Animal models would not be expected to predict such clinical signs,[10,15] but this still leads to false-negative results. Finally, it was concluded from the database results that the value of testing in two species goes beyond just expanding the breadth of target organ identification.

In summary, while nonclinical studies can demonstrate value in the PPV for certain species and organ categories, the NPV was the stronger predictive performance measure across test species and target organs indicating that an absence of toxicity in animal studies strongly predicts a similar outcome in the clinic.[10] These results support the current regulatory paradigm of animal testing in ensuring safe entry to clinical trials and provide context for emerging alternative models.

The Dog as a Second Species for Toxicology Testing Provides Value to Drug Development (Virginie Boulifard)

In many cases, the accepted regulatory standard to support the development of new pharmaceuticals involves toxicity testing in two species. However, the use of a second species to establish the safety of new pharmaceuticals has been the subject of much scrutiny in recent years, and the industry has been repeatedly challenged to reduce, refine, or replace some or all of the animals used. In particular, the value of the dog in this testing paradigm has been questioned. Publications reviewing available data for marketed drugs suggest that for many of these, the dog does not uniquely identify toxicities critical to human safety.[16] The weakness of this approach, however, is that many of the cases where the dog (or any other species) has the greatest impact on drug development decisions may not have been shared publically, as potential drugs were stopped during the development prior to marketing. The EFPIA Preclinical Development Expert Group

collected case studies from both its membership and the literature to explore the value of the dog in drug development decision-making and clinical monitoring practices to protect the safety of trial subjects. The project was not intended to be fully comprehensive, but instead sought to collect representative cases illustrating the value of the dog in drug development decision-making, rather than an overall industry perspective for when the dog did or did not provide value.

Preclinical Development Expert Group members submitted 19 cases for consideration. Of these, 18 were based on toxicology data and 1 on safety pharmacology data. These cases encompassed 9 therapeutic areas, and all cases were for small molecules. Study durations ranged from 2 to 52 weeks, but the majority of the examples were based on studies of 4 weeks' duration or less. Two additional cases were obtained from the literature and product labeling. Together, these cases provided examples of the influence of the results of dog studies on early project development decisions, clinical monitoring and safety measures, late project termination decisions, and product approval and labeling.

Six case studies were shared during the symposium (5 small molecules in development and 1 marketed small molecule), which presented the following scenarios:

1. The dog was the only pharmacologically responsive species. In this case, the lack of significant adverse findings in the dog supported the ongoing clinical development of the compound.
2. Notable effects in the dog predicted the observed human toxicity in early clinical trials. In this case, clinically relevant findings were noted in the dog, but not the rat 4-week toxicology studies. Similar findings were noted in the clinical phase I study at exposure levels required for pharmacodynamic (PD) effects, and as a result, clinical development was terminated.
3. Metabolite qualification could only be achieved in the dog. The highest achievable exposure of a major metabolite in the chronic rat study was less than one-fold the human exposure at the maximum recommended human dose. In contrast, the highest achievable exposure of a major metabolite in the chronic dog study was greater than 1-fold the human exposure at the maximum recommended human dose. The dog study was therefore critical for supporting the phase III clinical trials in the United States and to address questions about the safety of the metabolite that arose during the review of the marketing application.
4. Notable effects observed in the chronic dog study resulted in additional clinical monitoring in trials for a rare but frequently life-threatening disease with a predominately pediatric onset. Axonal degeneration was noted in the chronic dog study, but similar findings were not observed in the rat. The dog was considered the most sensitive species, and as a result, additional monitoring was added to an ongoing clinical trial to screen for potential effects.
5. Notable effects not predicted from the pharmacologic basis of action were observed only in the dog toxicity studies and impacted clinical development and product labeling for a compound that is currently marketed globally for a variety of urogenital and CV diseases. Testicular changes, indicative of reduced spermatogenesis, were observed in the dog toxicity studies (13-52 weeks' duration), and in the 52-week dog study, these changes were noted at systemic exposures similar to that at the maximum recommended human dose. Additional clinical trials were conducted to assess the risk to male reproduction in humans, and the effects of the compound on dog testes were noted in the impairment of fertility section of the product label.
6. A review of the available product approval summary documents and product labeling suggests that findings observed in the dog toxicity studies for various marketed statins identified potential human AEs and impacted product labeling. Cerebral hemorrhage and cataracts, 2 findings which were identified in dog toxicity studies, have been reported in humans for 4 to 6 different statins, respectively. These findings were not observed in mice or the rat. As a class, the product labeling for statins describes the CNS effects observed in the dog toxicity studies, as well as a description of dose-dependent optic nerve degeneration noted in dogs.

The other cases collected by the EFPIA serve to illustrate further the value of the dog in drug development decision-making. These include cases where the results of dog toxicology or safety pharmacology studies influenced early- or mid-stage project development decisions, clinical monitoring, and implementation of safety measures during clinical trials, or product labeling. In many situations, the value of the dog toxicity studies is not necessarily apparent because study results for compounds that are not continued in development, or for which the development process is still in progress, are often not disclosed. However, when the data from the dog toxicity studies are viewed in combination with all of the other nonclinical data that support the safety of the patient, these results play a critical role in internal decision-making and the protection of patients.

The Value of Non-human Primates for Human Risk Assessment of Monoclonal Antibodies (Frank Brennan)

A recently published Biosafe White Paper,[17] examining the value of non-human primates (NHPs) for human risk assessment, was presented. This was prepared in response to recent publications[18,19] highlighting the deficiencies of NHPs for monoclonal antibody (mAb) safety testing. Those papers analyzed approved mAbs, using clinical AEs of varying nature and incidence as starting points and asked whether human AEs were predicted by animal toxicology studies. The authors argued that almost all AEs of mAbs are predictable because they are mediated by exaggerated pharmacology, and that in

silico and in vitro studies, as well as target knockout (KO)/ knockin mouse data, can predict the majority of AEs with mAbs, making NHPs redundant for routine toxicology. In those publications, it was also argued that the formation of anti-drug antibodies (ADAs) to human mAbs in NHPs compromises safety assessment. Biosafe convened a task force to review current approaches to the safety assessment of mAbs, collecting and analyzing examples from member companies where NHP safety studies had meaningful impacts on clinical development of mAbs. Some of these case studies were presented during the symposium and the main conclusions discussed.

For mAbs against novel targets or with novel mechanisms of action, and for novel mAb scaffold and structures (eg, bi-/tri-functional mAbs) where the pharmacology is less well known or less predictable, assessing safety impact of target modulation in NHPs (if pharmacological relevance has been confirmed) can be critical. Some cases highlighted that although a target/pathway had safety liabilities identified from multiple sources (including target biology, in vitro data, KO mouse data, and other), which would have stopped development, the availability of NHP data provided confidence that the mAb could be dosed safely in humans. Studies in NHPs were also shown to be useful in defining safe starting dose levels and dose frequency, as well as informing clinical safety monitoring and risk management. Although KO mice can identify potential toxicities for further investigation, they may overpredict effects in humans because the target is often completely missing for the full life span. They may not accurately reflect the risk of dosing an mAb to adult humans where the role of the target in development is not manifested, nor account for physiological differences in pathways between rodents and humans, nor the fact that an mAb dosing regimen may not induce complete target blockade in all tissues all of the time. Other cases showed pathways, molecules, and routes of administration with human safety liabilities where NHP data supported mAb and/or target termination or identified safety/PD biomarkers for clinical monitoring. In no case were ADA found to compromise safety assessment.

It is necessary also to acknowledge the limitations of NHP studies. These include differences in expression/biology of certain pathways compared with humans and the use of normal animals with often low target expression, function, and activation status compared with higher levels in disease (such as immune checkpoint molecules). Often there is no biomarker of activity to build a human PK/PD model and it is hard to assess the potential for human relevant toxicity. In many instances, the level/function of the target in normal NHPs was equivalent to patients, probably increasing predictive power. When there is low target expression/function in normal animals, there is a need to supplement the toxicology data with safety/PK/PD data from in vitro systems and animal disease models to determine the relationship between dose/exposure and pharmacology/toxicology, allowing translational modeling of human doses. This requires the development of critical in vivo activity and safety markers using genomic, proteomic, and immunological assays in NHPs and humans to increase the chance of detecting early signs of toxicity and to allow comparisons to be made between nonclinical and clinical data.

Despite the limitations of toxicology studies in healthy NHPs, they can be good predictors of PD in humans and should identify the majority of severe AEs induced in the majority of dosed subjects (ie, overt pharmacology-based hazards), provided pharmacological relevance is confirmed. NHPs are, however, poor predictors of infrequent downstream effects of mAb pharmacology such as infusion reactions, cytokine release, infection, autoimmunity, and cancer, many of which are governed by patient- and disease-specific factors, comedication, and so on, and can only addressed in large clinical trials. Others (headache, pain, fatigue, nausea) are subjective end points not measurable in NHPs, or using in vitro methods. Risk mitigation relies on a weight-of-evidence approach, benefit:risk assessment, and clinical risk management based on the unique characteristics of product, target, and patient.

In conclusion, NHP use in drug development of mAbs is increasing due to a great number of novel multifunctional drugs with perceived increased safety risk requiring rigorous assessment, as well as conservatism by toxicologists, company management, and Health Authorities. This must be challenged and NHP studies should not be performed as default to satisfy a standard development and regulatory path. We must use rational, science-based decision-making in the ethical and scientific use of NHPs based upon the specific attributes of each product. When this is practiced, safety assessment studies in NHPs can provide critical information for human risk assessment and safe guarding of participants in clinical trials.

When Would Data From a Single Species Be Sufficient for Safe Progression in Humans? (Helen Prior)

The NC3Rs is a UK-based independent scientific organization funding innovation and technological developments that replace or reduce the need for animals in research and testing and lead to improvements in welfare where animals continue to be used. Since its inception in 2004, a collaboration funded by the Association of the British Pharmaceutical Industry has facilitated close interactions with international pharmaceutical, biotechnology, and contract research organizations (CROs), as well as regulatory agencies. Many of the projects have depended on cross-company data sharing of pre-competitive nonclinical information to provide evidence bases for new opportunities for applying the 3Rs across the drug development pipeline.[4,20,21]

The most recent collaboration aimed to review how and when two species were used within regulatory toxicology studies, and specifically to investigate if data from a single species could be sufficient for safe progression in humans.[22] The main focus was to explore whether a rodent and a non-rodent species are still required for toxicology testing in the current industry landscape and whether existing opportunities to use a single species are being fully exploited and/or could be expanded.

There is already widespread adoption of a single-species approach for biotherapeutics following ICHS6 guidelines (as there is often only one pharmacologically relevant species, frequently the NHP), as illustrated in the previous presentation.[17] Nevertheless, if biotherapeutic molecules are cross-reactive with multiple species, testing in two species is expected in a similar manner to molecules following ICHM3 and ICHS9 guidelines.[14,23] There are options within ICHS6[24] for the use of a single species (preferably the rodent) for the longer term chronic toxicity studies to support phase II/III clinical trials, if toxicities observed in two species are similar in short-term studies (generally accepted as Investigational New Drug (IND)-enabling studies to support phase I clinical trials). The work presented focused on the incidence of one or two species use across current portfolios (for multiple molecule types), the frequency for a reduction to one species within the package of toxicology studies, and potential opportunities for expansion of ICHS6 principles to different molecule types (eg, small molecules following ICHM3 or oncology products following ICHS9) or more widely for mAbs and other molecules following ICHS6.

Data were collected within a survey devised by an international working group (consisting of representatives from pharmaceutical and biotechnology companies, CROs, consultancies, academia, and regulatory bodies). Questions focused on current practices with regard to species use within regulatory toxicology studies, including details of the studies conducted within the package and the toxicities observed (target organ level only) in each study. Data were received for 172 molecules (from 18 different organizations) consisting of 92 small molecules, 46 mAbs, 15 recombinant proteins, 13 synthetic peptides, and 6 antibody–drug conjugates (ADCs).

For molecules following ICHM3 and/or ICHS9 guidelines (all 92 small molecules and 3 synthetic peptides), toxicology studies were generally performed in two species, as expected. However, there were three examples of small molecules that only used one species: two of these conducted early rodent toxicity studies only, stopping development prior to the start of planned non-rodent studies; the other had stopped development after performing IND-enabling toxicity studies using the non-rodent only (ie, this was a considered decision to use one species only). For another small molecule, IND-enabling toxicity studies were conducted in two species, with rodent studies then dropped when progressing to longer term chronic dosing studies (this molecule was still active in development). For molecules following ICHS6 guidelines (all 46 mAbs, all 15 recombinant proteins, 10 synthetic peptides, and all 6 ADCs), toxicology studies in one (NHP) species were performed for 30 mAbs, 3 recombinant proteins, and 1 ADC. However, there were two examples of mAbs that used a single rodent species: one mAb was in a late stage of active development, having completed the data package using a transgenic mouse model only, while the other mAb was in active development using the rat only for the IND-enabling toxicity package. Two species were used for the remaining mAbs, recombinant proteins, synthetic peptides, and ADCs (41 molecules in total). Five of these

molecules (2 ADCs and 3 mAbs) reduced to a single species (the NHP) for the IND-enabling package, while two further mAbs reduced to a single rodent species for longer term chronic dosing studies. Nine other molecules following ICHS6 guidelines retained both species for longer term chronic dosing studies (4 mAbs, 2 recombinant proteins, and 3 synthetic peptides).

A key decision for progression of longer term chronic dosing studies in one or two species revolves around the "similarity" of toxicities between the species. A review of target organ toxicities identified in the IND-enabling toxicity study package was performed to determine how often toxicities were similar or different in the two species. For the 115 molecules within the database that used two species for IND-enabling toxicity studies (75 small molecules, 13 mAbs, 11 recombinant proteins, 12 synthetic peptides, and 4 ADCs), toxicities were similar in both species for 32%, 85%, 36%, 42%, and 25% per molecule type, respectively. Therefore, there may be opportunities for more or different molecules currently following ICHS6 guidelines, as well as molecules following other guidelines, to reduce to one species for longer term chronic dosing studies in the future.

Concluding Comments

The presentations at this symposium describing the work of substantial international consortia clearly illustrate the importance that the global pharmaceutical industry give to considering the relevance of safety testing paradigms. One common theme running through these contributions has been to question whether opportunities exist to replace, refine, or reduce the use of animals in the safety assessment process without compromising human health or the effectiveness of new medicines. Although current regulatory requirements for toxicity testing in animals remain in place, opportunities for complete replacement presently appear limited. However, there is flexibility within current guidelines to reduce and refine animal use that should be exploited fully (eg, the use of a single species for longer term chronic toxicity studies within ICHS6). The sharing of case examples (anonymized when appropriate) where individual companies have successfully adopted an innovative approach (eg, the small molecule using a single species, mentioned in presentation 4) is important for building confidence to move away from standard regulatory packages of studies.

Some of the data presented during the symposium were based on retrospective evaluation of established ways of working, illustrating that data from a particular species had been critical for decision-making and contributed to human safety by identification of a potential risk that either stopped development prior to human trials or facilitated effective clinical monitoring. The challenge is to use the information gained from retrospective evaluations to better predict and select the most appropriate species without the benefit of hindsight. Were we able to predict upfront which nonclinical species would provide the most useful/relevant data, then we would be able to restrict toxicity studies to the most appropriate species.

Experience with the approach taken by mAbs and other new modalities (toxicity testing in pharmacologically relevant species, which could be two, one, or no species) could influence the future of toxicity testing toward a case-by-case approach for all molecules. An initial approach could be for the IND-enabling toxicity package to be conducted in two species, then to reduce to one species for the longer term chronic toxicity studies in conjunction with emerging human data from the clinic. This could be an area for the different industry consortia to work together in the future.

It must be acknowledged that changing the paradigm regarding the use of animals is not a trivial undertaking, especially around building confidence in new/different approaches and/or changing regulations. Further progress demands an increased understanding of mechanisms resulting in adverse health effects and a willingness to exploit fully new developments in science and technology. As our understanding of toxic mechanisms increases further, and as the battery of methods at the disposal of toxicologists expands, there will no doubt be new and exciting opportunities to further refine approaches to safety assessment of new drugs and opportunities also to refine and reduce our reliance on animals for this purpose. Consortia such as those that contributed to the symposium are well placed to ensure that safety assessment paradigms evolve and improve as the science advances.

Author Contributions

H.P. contributed to conception and design, contributed to analysis and interpretation, and drafted the manuscript. I.K. contributed to analysis and interpretation, drafted the manuscript, and critically revised the manuscript. T.M., V.B., and F.R.B. contributed to interpretation and drafted the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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