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Comprehensive Review

Implementation of the principles of the 3Rs of animal testing at CDER: Past, present and future

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ABSTRACT

The safety testing of pharmaceutical candidates has traditionally relied on data gathered from studies in animals, and these sources of information remain a vital component of the safety assessment for new drug and biologic products. However, there are clearly ethical implications that attend the use of animals for safety testing, and FDA fully supports the principles of the 3Rs, as it relates to animal usage; these being to replace, reduce and refine. We provide an overview of some of the events and activities (legal and programmatic) that have had, and continue to have, the greatest impact on animal use in pharmaceutical development, and highlight some ongoing efforts to further meet the challenge of achieving our mission as humanely as possible.

1. Introduction

CDER's core mission is to ensure the availability of safe and effective medicines to improve the health and well-being of people in the United States, and to assure that individuals enrolled in clinical trials for pharmaceuticals are not placed at unnecessary risk of injury as a result of pharmaceutical toxicity. An important element of this process is Agency review of Sponsor-submitted nonclinical data intended to: 1) establish under what conditions (e.g., dose, population, clinical monitoring) a new pharmaceutical can be safely administered to humans, and 2) whether a new therapy carries an increased risk for developmental and reproductive toxicity or an increased cancer risk (Table 1). A key component of these assessments, in particular the general toxicity assessment, are endpoints that cannot be ethically obtained in humans, such as histopathologic examination of all major organs and tissues. Assessment of other endpoints (e.g., phototoxicity, immunotoxicity) can also be important on a case-by-case basis. To this end, Sponsors are required[1] to provide the pharmacology and toxicology data from which they have concluded that it is reasonably safe to conduct clinical trials (21CFR312) and ultimately to support marketing (21CFR314; 21CFR601). In satisfying these statutory requirements, CDER has a long-standing commitment to uphold the principles of the "3Rs" of animal testing (Russell, 1959), which holds that the use of animals in product testing should be replaced, reduced, and refined to the greatest extent that is consistent with achieving our core public health mission. This paper attempts to capture ongoing Center efforts to support the 3Rs, while also documenting past successes in this important area.

The studies discussed here, and the efforts at implementing the 3Rs, are focused on those studies conducted to satisfy regulatory requirements, and encompass studies typically categorized as "toxicity testing." Animal studies conducted for all regulatory purposes (i.e., not just pharmaceuticals) are estimated to account for approximately one quarter of all animals used for scientific purposes (European Commission, 2020). A larger number of animals are used in basic and applied research settings not directly associated with regulatory purposes. CDER is aware that Sponsors take steps to reduce, refine, or replace animal use in the early stages of product development (e.g., research, discovery, candidate selection) but CDER generally does not play a role in this stage of product development and thus cannot assess the impact of alternatives in this area.

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[1] We have attempted to limit the use of terms such as 'required' and 'necessary' to mandates established by law or regulation. Agency statements in guidance generally reflect recommendations on how to satisfy the requirements promulgated in regulation.

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Table 1
Typical nonclinical assessments and their significance in pharmaceutical safety assessment.

Assessment	Key Issues Addressed
Safety Pharmacology	o Identifies potential adverse pharmacological effects of the drug in vitro and/or in vivo o Identifies specific parameters to monitor more closely in clinical trials
General Toxicity	o Estimates safe "first in human" (FIH) starting dose o Estimates safe maximum doses in early clinical trials o Identifies possible consequences of chronic exposure o Identifies specific parameters to monitor more closely in clinical trials
Carcinogenicity	o Predicts long-term risks that are difficult to assess or are unethical to assess in humans
Developmental and Reproductive Toxicity	o Predicts risks that are difficult to assess or are unethical to assess in humans o Identifies risks for special populations
Special Toxicity	o Identifies specific parameters to monitor more closely in clinical trials o Allows the mechanistic understanding of an adverse biological change observed in animals or humans

2. Past successes

2.1. The Hatch-Waxman Act (Public Law 98–417, 1984)

Although not strictly speaking a CDER-initiated effort, the creation of the 505(j) and 505(b)(2) NDA approval pathways by passage of *The Drug Price Competition and Patent Term Restoration Act* (often referred to as the Hatch-Waxman Amendments) in 1984 has had a tremendous and continuing impact on the number of animals used to support drug development, by eliminating the need for duplicative animal studies.

The 505(j) Abbreviated New Drug Application (ANDA) pathway for generic drugs allowed the FDA to rely upon its prior conclusions regarding the safe use of the innovator's active pharmacological ingredient (API), eliminating the need to conduct animal studies to assess the safety of the API. Indeed, the regulation directly prohibits the FDA from requiring such studies on the API for an ANDA approval (21 U.S.C. 355). The impact of the availability of the ANDA pathway on reducing animal usage in drug approvals should not be underestimated. FDA statistics indicate that 9 out of every 10 prescriptions in the US are for generic drugs (FDA, 2020c).

The 505(b)(2) provisions of the Act define a pathway whereby an applicant can rely upon information that it does not own, or have right of reference to, in supporting its NDA marketing application (21 U.S.C. 355). This allows an applicant to leverage what is already known about a drug, including FDA's determination of safety and efficacy, without having to conduct duplicative studies in animals or humans. This pathway could be used, for example, for a new formulation of a previously approved active ingredient being used by a new route. In many cases, this translates into a markedly reduced non-clinical development program, often involving only a short-term 'bridging' toxicology study to confirm that there are no toxicologically significant differences between the new drug product and the drug product being relied upon that would preclude reliance upon the safety determination for the listed drug product. The savings in animals from not having to repeat chronic toxicity, reproductive and developmental toxicity and carcinogenicity studies is substantial.

While captured here as past successes, the 505(j) and 505(b)(2) NDA pathways continue to produce substantial reductions in animal use. In 2019 there were 1014 generic drug approvals (FDA, 2020c), and more than half of all NDA approvals relied on the 505(b)(2) pathway (FDA, 2020a).

2.2. FDA modernization act (FDAMA) of 1997 (Public Law 105–115, 1997)

Section 119 of FDAMA (paraphrasing) directed the FDA to put into place a process whereby binding agreements could be made between the Agency and pharmaceutical developers regarding the design of clinical studies intended to demonstrate pharmaceutical efficacy. In the implementation of FDAMA, this principle was also expanded to the review of animal toxicology studies to assess for the carcinogenic potential of new products, as captured in FDA's Guidance for Industry on Special Protocol Assessment (FDA, 2018b). As a result of this process, Sponsors who conduct their rodent carcinogenicity studies, with appropriate quality, and according to the recommendations of CDER's Executive Carcinogenicity Assessment Committee (ECAC) are assured that their study will be judged acceptable for complying with the regulations for nonclinical carcinogenicity assessment.

As noted above for the provisions of the Hatch-Waxman Act, although FDAMA is documented here as a past accomplishment, the practices established in response to this Act continue to save animals by ensuring that an agreed upon protocol will be accepted by the Agency for carcinogenicity assessment. Moreover, the ECAC continues to support efforts to reduce the number of animals used in carcinogenicity assessments. For example, ECAC discourages Sponsors from including satellite animals for toxicokinetic (TK) analysis in carcinogenicity studies conducted in transgenic mice (since these models are viewed to provide hazard identification, not risk assessment), and in the 2-year rodent carcinogenicity study, when relevant TK data are already available from other toxicology studies.

2.3. Biologics price competition and innovation act (BPCIA) (Public Law 111–148, 2010)

The passage of the BPCIA in 2009 created for the first time a pathway, the 351(k) pathway, which allows for an abbreviated marketing application for protein-based therapeutic agents (biologics) that are "biosimilar" to an approved biologic (Public Law 111–148, 2010). As with the Hatch-Waxman Act, the BPCIA supports substantial reductions in the number of animal studies required to support the licensing of a biosimilar product. In some instances, no animal studies may need to be conducted at all.

While captured as a past success, the BPCIA-created 351(k) pathway continues to produce substantial reductions in animal use by reducing the need to conduct duplicative nonclinical studies. As of the end of 2020, CDER had approved 29 biosimilars (FDA, 2021a), ten of which were approved in 2019 alone, accounting for one-half of CDER's biologic approvals that year (FDA, 2020a).

An analysis of the approval packages for biosimilar products licensed in the EU and the US found that while most of these programs did include animal studies, the number of studies were generally far fewer than for a standard biologic development program, and when conducted, repeated dose toxicity studies were generally of shorter duration (Pipalava et al., 2019). Neither Developmental and Reproductive Toxicity (DART) studies nor carcinogenicity studies were conducted in support of approval of any of the products captured in this analysis, consistent with a 2015 FDA biosimilar guidance, which states: "In general, nonclinical safety pharmacology, reproductive and developmental toxicity, and carcinogenicity studies are not warranted …" (FDA, 2015b).

2.4. International council for harmonization of technical requirements for pharmaceuticals for human use (ICH)

It is difficult to overstate the impact that the creation of the ICH, and in particular the promulgation of harmonized guidance on the recommendations for nonclinical development programs for human pharmaceuticals, has had on laboratory animal use worldwide. CDER played a

key role in the inception of ICH, which was founded in 1990 in an attempt to achieve greater regulatory harmonization of drug development requirements between the United States, Japan and Europe (van der Laan and DeGeorge, 2013). With its recent reorganization, ICH is now truly international in scope, with scores of additional countries now contributing towards the ICH's mission to ensure that safe, effective, and high-quality medicines are developed and registered in the most resource-efficient manner across the globe (ICH).

The recent expansion in the number of ICH member nations reflects in many ways what has become the reality of modern drug development. Pharmaceutical development in the 21st century is largely a multinational business, with most pharmaceuticals being developed with the intention of being marketed around the world, not just in a single country. In the absence of harmonization, a pharmaceutical company is likely to conduct animal studies that will meet the requirements of the most restrictive regulatory authority. These restrictive requirements may dictate the conduct of more studies, earlier in development, and with more animals per study. So, while the initiatives of any given nation to replace, reduce, or refine animal use are important, it is the international agreements that have delivered, and will continue to deliver, the greatest impact on animal use in pharmaceutical development.

Prior to the inception of ICH, each region had its own specific recommendations regarding the design of toxicology studies, and when these studies should be conducted, relative to clinical development. This lack of harmony between regions often led to the need for drug Sponsors to repeat animal studies, or to conduct animal studies earlier in clinical development in some regions. Under the auspices of the ICH, a number of harmonized guidances on the general requirements for the types of nonclinical data recommended to support clinical development of human pharmaceuticals have been developed. Of equal, or perhaps greater importance with regard to reduction in animal usage, has been the international harmonization of the timing of when particular nonclinical assessments need to be submitted to drug regulatory authorities to support particular stages of clinical drug development. The primary ICH guidances for nonclinical assessments are (ICH M3(R2), 2009; ICH S6(R1), 2011; ICH S9, 2009). These guidances and their impacts are discussed briefly below. Please note that the date in the citation for ICH guidances refer to when it was approved by the ICH Assembly for adoption, not necessarily the date of implementation by the FDA.

Notably, for every ICH guidance with a nonclinical component, consideration of inclusion of alternative approaches and limiting unnecessary animal use is an active part of the guidance development process. These efforts are science-driven, often relying on analyses of large datasets regarding the predictive reliability of current and new approaches. These analyses form the basis for the discussions that lead to harmonization of recommendations regarding the nonclinical assessment of pharmaceutical safety.

2.4.1. ICH M3 – guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals

First adopted in 1997, this guidance has undergone two revisions since then, most recently in 2009 (ICH M3(R2), 2009). This guidance also has a Q & A document associated with it (ICH M3(R2) Q&A(R2), 2011). ICH M3 applies to most small molecule pharmaceutical development programs, excluding drugs intended to treat advanced cancer. The timing elements of the guidance also apply to protein-based drugs (biologics). As illustrated in the examples that follow, this guidance has resulted in a significant reduction in animal use, compared to the aggregate pre-ICH practices of individual nations:

- Standardized the timing of nonclinical studies to support clinical development
  - o For example, the guidance outlines when nonclinical fertility, embryofetal development and pre- and postnatal development studies should be submitted. By delaying the submission of fertility and pre- and postnatal toxicity studies to the time of marketing application, many such studies are obviated by the large percentage of drugs whose development is discontinued due to lack of efficacy or unexpected adverse events discovered during clinical Phase 2/3 testing (Wong et al., 2019).
  - o The guidance also indicates that it can be appropriate to delay submission of animal embryofetal development toxicity studies until the time of the marketing application under some circumstances (e.g., monoclonal antibodies, since there is negligible human fetal exposure during embryogenesis).
- Established an exposure-based high-dose selection criterion in animal toxicity studies
  - o Previously it was often necessary to repeat animal toxicology studies in which no target organs of toxicity were identified, even at very high multiples of intended human exposures. With the most recent revision of M3, any dose that achieves a 50-fold ratio of exposure (typically AUC) to the human exposure, at the maximum clinical dose, is considered to be an adequate high dose for the toxicology assessment for most toxicity studies.
- Explicitly supports, whenever possible, inclusion of Safety Pharmacology endpoints in the general toxicology studies, rather than conducting separate, stand-alone, Safety Pharmacology studies.
- Defined and limited the situations under which additional toxicology studies would be recommended to characterize human metabolites formed at lower levels in animals treated with the parent drug.
- Indicated that stand-alone, single-dose acute toxicity studies are not warranted, when appropriately conducted dose-escalation studies or short-duration dose-ranging studies are available to inform on the acute toxicity risk.
- Created exploratory clinical trial criteria whereby data from a relatively small number of animals can be used to support low dose human exposures to drug candidates.
  - o These approaches allow for collection of human PK and pharmacology data, which can aid efforts to select the most appropriate drug candidate for clinical development, potentially further reducing animal use, by avoiding animal studies to support a drug candidate that would be unlikely to achieve efficacious levels in humans.
- Encourages inclusion of local tolerance endpoints into the general animal toxicology studies, rather than running stand-alone local tolerance studies.
- Clarified that juvenile animal toxicology studies should only be conducted when human adult data and data from general animal toxicology studies are not adequate for assessing risk to the intended pediatric population.
- In conjunction with ICH S8 (Immunotoxicity Studies for Human Pharmaceuticals)(ICH S8, 2006), stipulates that stand-alone animal immunotoxicity studies should only be conducted if the weight-of-evidence (WoE) from the general animal toxicology studies, knowledge of the target biology and/or PK/PD data suggest a risk that should be better characterized.
- For product development programs where two drugs are intended to be co-administered, defines the scenarios under which combination toxicity studies are recommended. Generally, recommends against combination toxicity studies when two "late stage" (i.e., Phase 3 or marketed) drugs are to be combined.
- Recommends a WoE approach to phototoxicity assessments, and notes that in vitro or clinical assessments for phototoxic potential can be considered instead of animal studies.
- Indicates that currently available models (e.g., hairless rodent) are not considered useful in support of risk assessment for pharmaceuticals that have an identified potential photocarcinogenicity hazard, and recommends risk mitigation by limiting sunlight exposure, and communicating the potential risk to the subject/patient.

In addition, ICH M3(R2) explicitly supports consideration of in vitro alternative methods:

- "Although not discussed in this guidance, consideration should be given to use of new in vitro alternative methods for safety evaluation. These methods, if validated and accepted by all ICH regulatory authorities, can be used to replace current standard methods."

2.4.2. ICH S6 – guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals

First adopted in 1997, ICH S6 was subsequently revised in 2011 (ICH S6(R1), 2011). Biotechnology-derived pharmaceuticals (biologics) are highly selective in their actions and are generally not prone to the off-target toxicities that are commonly seen with small molecule drugs. Consequently, not all assessments recommended under the ICH M3 guidance are appropriate for nonclinical testing of biologics. Elements of the initially adopted guidance that help to appropriately limit animal use include:

- Specific recognition that "Both in vitro and in vivo studies can contribute to this [preclinical safety] characterization."
- Recognition that less extensive toxicity testing may be warranted for biologics that are structurally and pharmacologically comparable to well-characterized products.
- Stipulation that only pharmacologically relevant species should be used in safety assessments.
- Recommendation that Safety Pharmacology endpoints be included in the general toxicology studies.

The 2011 update of the guidance has these additional recommendations that can contribute towards a reduction in animal use:

- In situations where both a rodent and nonrodent are pharmacologically relevant, it recommends that chronic toxicology studies be conducted in a single species, if no material species differences are observed in shorter term toxicology studies.
  - o It further stipulates that rodents are the preferred species, if there is no scientific rationale for using non-rodents (typically monkeys), resulting in use of phylogenetically lower species.
- Doses achieving a 10-fold exposure margin to the highest anticipated clinical exposure, or that provide a maximum intended pharmacological effect are suitable high-doses for toxicology studies (i.e., toxicology studies need not be repeated due to failure to identify target organs or dose-limiting toxicity if either of these criteria are met).
- In a refinement of animal use, chronic toxicity studies with biologics need only be of 6-months duration, rather than the 9 months typically recommended for small molecule drugs.
- The inclusion of recovery animals is not necessarily warranted, or, if warranted, they only need to be included in one toxicology study.
- It strongly discourages the use of nonhuman primates (NHP) for studies of fertility and early embryonic development.
- It encourages that Developmental and Reproductive Toxicity (DART) studies be conducted in rodents and rabbits, rather than NHPs, when the former are pharmacologically relevant.
- For biologics that are only active in the NHP, it recommends conducting a single study, the enhanced Pre-/Post-natal Development study, to cover all endpoints from organogenesis through birth and postnatal development.
- It recognizes that NHP DART studies are only intended to identify hazard (as opposed to characterize risk).
  - o Therefore, only a limited number of NHPs (6–8 evaluable pregnancies per dose group) need to be included in the study.
  - o With appropriate justification, a study can be conducted that only has a control group and a single dose group.
- For biologics pharmacologically active only in NHPs, where there are sufficient precautions to prevent pregnancy in clinical studies enrolling women of child-bearing potential (WOCBP), an embryofetal development (EFD) or ePPND study can be conducted during Phase III, and the report submitted at the time of marketing application.
- It indicates that nonclinical DART studies may not be warranted when the WoE suggests that there will be an adverse effect on fertility or pregnancy outcome, as the hazard has already been identified.
- Indicates that 2-year rodent carcinogenicity studies are generally of only limited value in the assessment of carcinogenic risk for biologics, and suggests a WoE approach, based on what is known about target biology and chronic toxicology study findings.
  - o The ECAC has reviewed over 100 of these WOE carcinogenicity assessments, and in the vast majority of cases (84%) has found the assessments to be an adequate evaluation of carcinogenic potential such that rodent carcinogenicity studies were not recommended.

2.4.3. ICH S9 – guidance on nonclinical evaluation for anticancer pharmaceuticals

ICH S9 describes the nonclinical support recommended for clinical development of pharmaceuticals for the treatment of advanced cancers that otherwise have limited treatment options (ICH S9, 2009). The guidance was adopted by ICH in 2009, and was updated in 2018 with a Question and Answer document (ICH S9 Q&A, 2018). Given that malignant tumors are life-threatening in the absence of effective treatment, the patients, and physicians treating them, are generally willing to accept a greater risk of injury from an incompletely characterized toxicity than would be appropriate for the treatment of more benign diseases. The tradeoff being faster access to potentially efficacious treatments. The net effect is that this guidance allows for a substantial reduction in the number of animals needed to characterize a pharmaceutical prior to first-in-human exposures and marketing, and adopts a generally more abbreviated approach to toxicity assessment compared to ICH M3(R2). Of particular note:

- General Toxicology studies of 3-months duration are adequate to support Phase 3 trials and marketing, meaning chronic toxicology studies are not generally warranted.
- Carcinogenicity studies are not generally warranted.
- DART assessments are generally not warranted to support Phase III clinical trials, and only embryofetal development studies are expected to support a marketing application for non-cytotoxic agents.
- Combination toxicity studies are generally not warranted.
- The Q & A document explicitly states that alternative assessments may be used to aid in the safety assessment for reproductive risk, and that an NHP study to assess a hazard to EFD should not be considered a default approach for agents that are only pharmacologically active in the NHP.
- For genotoxic drugs, targeting rapidly dividing cells, that have antiproliferative effects, toxicity studies of 3-months in a single, relevant rodent species will support Phase III and marketing.

2.4.4. ICH S10 – photosafety evaluation of pharmaceuticals

The ICH S10 guidance was finalized in 2015. It describes the internationally harmonized nonclinical approach to assess the photosafety of human pharmaceuticals (ICH S10, 2015). The guidance includes assessment strategies, employing physiochemical and in vitro methods, that can be completed without the use of any animal studies. Prior to implementation of this guidance, phototoxicity assessments were frequently conducted in rodents. It is anticipated that the availability of this guidance will continue to reduce animal use for phototoxicity assessment.

3. Recent advances and ongoing efforts

3.1. ICH

As noted above, it is through continued international harmonization that we are likely to achieve the greatest reductions in animal usage in pharmaceutical testing. In this section we consider some of the ongoing or recently completed ICH efforts that have served to better harmonize nonclinical testing for pharmaceutical development, which will lead to further reductions and refinements in animal use.

3.1.1. Potential revision of ICH S1

ICH S1 provides guidance on the need for, and conduct of, rodent carcinogenicity studies for human pharmaceuticals. The current version of ICH S1 has three components: S1A) which provides guidance as to which drug development programs require rodent carcinogenicity assessments (ICH S1A, 1996), S1B) which defines what rodent carcinogenicity studies should be conducted, and provides guidance on how they should be conducted (ICH S1B, 1998), and S1C) which establishes the criteria by which the pharmaceutical doses tested in the carcinogenicity studies should be selected (ICH S1C(R2), 2008). The three components of the S1 guidance were all initially implemented in the mid-1990s. Of note, prior to adoption of ICH S1B, it was expected that drugs being developed for chronic indications would be tested in lifetime bioassays in two rodent species, typically the rat and the mouse. ICH S1B allowed for a refinement in animal use, in that it indicates that alternatives to the 2-year mouse study can be appropriate. In particular, it indicates that one can conduct a 6-month study in certain transgenic strains of mice. In addition to being of a shorter duration, these transgenic mouse studies employ typically half as many mice as the 2-year bioassay (Bourcier et al., 2015).

Currently, the ICH S1(R1) Expert Working Group (EWG) is evaluating whether it would be possible, on a case-by-case basis, to use a WoE approach to identify small molecule drugs for which the 2-year rat bioassay would not meaningfully contribute to the overall carcinogenicity assessment of the drug, and could therefore be waived without compromising patient safety (ICH). As part of this effort, the S1(R1) EWG, via its component drug regulatory authorities (DRAs), have solicited Carcinogenicity Assessment Documents (CADs) from drug Sponsors. In the CADs, Sponsors predict, based on the totality of available data on a compound and its target, whether a drug fits into one of 4 categories:

- *Category 1: Highly likely to be carcinogenic in humans, such that rodent carcinogenicity studies would not add value.*
- *Category 2: Uncertain carcinogenic potential, such that rodent carcinogenicity studies are likely to add value.*
- *Category 3a: Highly likely to be carcinogenic in rats through prior established and well-recognized mechanisms known to be human irrelevant, such that a rat carcinogenicity study would not add value.*
- *Category 3b: Highly unlikely to be carcinogenic in both rats and humans, such that a rat carcinogenicity study would not add value.*

Each of the participating DRAs also evaluates the evidence presented in the CADs, and makes their own determination of the appropriate category for the drug. For each drug, the 2-year rat bioassay is conducted, and then the results of the bioassay are compared to the Sponsor's and DRAs' predictions. If there is broad concordance between the predictions and results for even a subset of the category 3 predictions, then it should be possible to identify drug characteristics that may obviate the need to conduct the 2-year rat bioassay for these drugs, with attendant reduction in animal use. Notably each 2-year rat bioassay uses between 500 and 700 animals, so the potential reduction in animals from even a 20% reduction in the number of studies needing to be conducted would be substantial.

The ICH S1(R1) EWG is also involved in an exploratory investigation of whether it would be scientifically appropriate to allow for dose selection in the 6-month transgenic mouse model on the basis of an exposure multiple to the highest anticipated human exposure in clinical use. (This approach to dose selection was not previously validated in this model.) Should this effort prove fruitful, this would likely further reduce the use of the 2-year mouse bioassay, based on industry reports (personal communication) that Sponsors choose to use the 2-year mouse bioassay rather than run the transgenic mouse assay at the higher doses necessary to achieve an MTD for the 6-month study or at the high limit dose or maximal feasible dose when an MTD is not identified. The concern of Sponsors being that such high doses may not be clinically relevant.

3.1.2. ICH S3A Q & A

The question and answer document for ICH S3A (ICH S3A Q&A, 2018) introduces support for the use of microsampling in toxicity studies. This is a method to collect a very small amount of blood (typically $\leq$50 μL) to measure TK parameters of the drug and/or its metabolites. The guidance describes the advantages (and limitations) of the method and provides technical considerations for conducting such assays. The intent is to encourage the use of microsampling techniques whenever appropriate. The impact on the 3Rs would be to 1) minimize the pain and distress that may attend collection of larger blood samples, as well as 2) reduce the need for additional (satellite) animals in rodent toxicology studies that are used solely to assess the toxicokinetic parameters of a drug and/or its metabolites.

3.1.3. Revision of ICH S5

ICH S5 provides guidance on approaches that can be used to assess the potential of a pharmaceutical to adversely affect human fertility, normal embryofetal development, parturition and postnatal development (ICH S5(R3), 2020). The core component of this guidance was adopted in 1993. Despite its numerical designation, this was the first of the safety topics to be approved and implemented under ICH (Bass et al., 2013). Indeed, the lack of harmonization of DART testing requirements in the US, Europe and Japan was arguably one of the driving forces that ultimately led to the creation of the ICH.

Systematic, routine DART testing was only required following the thalidomide tragedy of the late 1950s and early 1960s, in which maternal exposures to thalidomide, during critical windows of embryofetal development, led to a large number of children born with limb defects. In reaction to this tragedy, each country established its own legal and regulatory requirements in order to protect against a recurrence of similar tragedies (Bass et al., 2013). Unsurprisingly, given the rapidity with which these regulations were developed, there was little opportunity for scientific exchange between the different regions, and each country's regulations ended up being sufficiently different, such that they were essentially mutually incompatible. As a consequence, a company looking to market a drug in each of the three regions found that it had to repeat DART studies multiple times. The implementation of ICH S5, which established study designs that would be recognized in all regions, thus substantially reduced the number of animals used in drug development (Bass et al., 2013).

It is recognized that in the more than 20 years since ICH S5 was first implemented that there have been substantial gains in experience with the testing of pharmaceuticals in the standard and novel testing paradigms, and that scientific, technological and regulatory knowledge has evolved over this time. This experience and evolution of knowledge offered the opportunity to modernize the testing paradigm to better protect human health, while at the same time offering an opportunity to assess whether animal use can be replaced, reduced or refined. With regard to this latter goal, some of the concepts that were adopted in the recent revision of the guidance include:

- The revised guidance includes a section discussing the potential use of alternative in vitro, ex vivo, and non-mammalian in vivo assays

(alternative assays) to reduce animal use while preserving the ability to detect relevant reproductive risks. The revised guidance does not recommend specific assays; rather, basic principles are included to assist in assay qualification for potential regulatory use.

- The revised guidance expands the criteria for high dose selection for embryofetal development toxicity studies to include an exposure-based criterion. The prior guidance indicated that the high dose should either represent a limit dose or a maximally tolerated dose. Previously, when a dose range-finding study failed to identify an MTD, this study would typically need to be repeated at higher doses, in order to identify an MTD. Under the current guidance, as long as the high dose in the definitive EFD study achieves an exposure that is greater than 25× that which is expected to be achieved in WOCBP, the study would be considered acceptable in all regions.
- The revised guidance provides new approaches that allow for deferral of definitive EFD studies to later in clinical development in all regions. This change brings all regions into better alignment with the US in not requiring definitive EFD studies until Phase 3. This revision will likely result in substantial reductions in animals use, due to attrition of drug candidates prior to Phase 3 (Wong et al., 2019).
- The revised guidance clarifies that non-GLP dose range finding DART studies can, on a case-by-case basis, be sufficient to communicate risk without the conduct of a definitive DART study, if a clear clinically significant signal of DART risk is identified at clinically relevant exposures.

3.1.4. ICH E14/S7B Q&A and the comprehensive in vitro proarrhythmia assay (CiPA) initiative

The objective of the CiPA initiative is to facilitate the adoption of a new paradigm for assessment of clinical potential of Torsades de pointes that is not measured exclusively by potency of hERG block or by QT prolongation. Towards this goal, the initiative seeks to develop better nonclinical tools and more standardized protocols for this assessment. The intent is to establish a test paradigm driven by a suite of mechanistically based in vitro assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity, with verification of the model's predictions via observed responses in human-derived cardiac myocytes (Cavero and Holzgrefe, 2015; Vicente et al., 2018; Wallis et al., 2018). CDER is a member of the steering committee for CiPA and plays key roles in multiple workstreams related to this effort.

The CiPA effort is being combined with an ICH effort to issue a revised Q&A document for ICH E13 (Guidance on The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs) (ICH E14, 2005), which would also apply to ICH S7B (Guidance on The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) By Human Pharmaceuticals) (ICH S7B, 2005). The currently proposed Q&As would focus on nonclinical in vitro and in silico methods. In November 2018, the ICH Assembly endorsed the establishment of the E14/S7B Implementation Working Group (IWG) for this task (ICH). A draft Q&A document was endorsed in August 2020.

While the main thrust of this effort is to improve the quality of the nonclinical data used to assess drugs for proarrhythmic potential, it is anticipated that further reliance on cultured human cardiomyocytes (primary or from induced pluripotent stem cells) will lead to a reduction in animal use.

3.2. Interagency coordination and collaboration

3.2.1. ICCVAM

In addition to CDER's international harmonization work impacting the 3Rs, CDER also plays an active role in organizations that coordinate the activity of US government agencies that have an impact on experimental animal usage. A good example of this is our longstanding membership and active participation in the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) which serves to coordinate activities within the federal government relevant to new test method evaluation, acceptance, and use. The goal of ICCVAM is "to establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness."

3.2.1.1. ICCVAM—Integrated testing strategies to identify potential skin sensitizers. This working group has been assessing an integrated strategy that uses only validated non-animal tests to predict human skin sensitization. The strategies assessed thus far have high predictivity for compounds detected by animal and human studies (Kleinstreuer et al., 2018; Strickland et al., 2016, 2017; Zang et al., 2017). Additional work is being conducted to establish open source software for the prediction models. Adoption of an integrated testing strategy to identify potential skin sensitizers could allow assessment of this potential in topical drug products without the use of animals.

3.2.1.2. ICCVAM—Ocular corrosivity and irritation testing. In vitro methods to assess ocular corrosivity and irritation have been reviewed by ICCVAM. FDA concurred in 2008 with the ICCVAM finding that the bovine corneal opacity and permeability test method and the isolated chicken eye test method were appropriate tests for ocular corrosion and severe irritation. CDER accepts the results of the in vitro assays recommended by ICCVAM and does not request in vivo eye irritation studies for topical drug products. These assays use eye tissues from animals harvested for food purposes, so no additional, dedicated, animal use results from conducting these assays. Additional refinements to these assays continue to be developed and assessed by ICCVAM (ICCVAM, 2017).

3.2.2. NCATS/DARPA—Tissue chips for drug screening

The National Center for Advancing Translational Sciences (NCATS) at NIH and the Defense Advanced Research Projects Agency (DARPA) have funded research into the development of tissues chips for drug screening. FDA has been an active partner in this program since its inception and has supported the development and integration of a number of tissue/organ models. Additional efforts at testing and validating the platforms are occurring through Tissue Chip Testing Centers. More recent funding from NCATS also includes developing tissue chips as specific disease models (NCATS (National Center for Advancing Translational Sciences)).

The collaborative nature of this effort allows for platform developers to gain a clearer perception of the FDA regulatory perspective, and the attributes that would be expected for regulatory acceptance of tissue chip data, hopefully facilitating the movement of such technologies more quickly into the drug development process. In addition, through the collaboration, the FDA gains experience with the platforms, which can promote confidence in the use of such technologies. Should particular tissue chips (or tissue chip systems) be shown to be valid for particular uses, it is anticipated that the data from those chips could be submitted to the FDA to support regulatory decision making. Although long term impact is difficult to predict, potential reduction in animal use is envisioned.

3.2.3. Toxicology in the 21st century (Tox21)

Tox21 is a US federal research collaboration intended to foster the evolution of toxicology in the 21st Century by developing methods intended to efficiently evaluate the safety of commercial chemicals, pesticides, food additives/contaminants, and medical products (Tox21). FDA is an active member of this partnership. It is generally expected that the efforts of the Tox21 partners will lead to implementation of approaches to toxicology assessment that will further limit the need for

toxicology studies in animals.

3.3. CDER involvement in FDA-Specific efforts impacting 3Rs

3.3.1. FDA Toxicology Working Group and the Predictive Toxicology Roadmap

As is true for our international- and national-level efforts, CDER recognizes the importance of working towards FDA-wide adoption of new approaches to toxicity assessment. Toward this goal, CDER was a key partner in the formation of the standing FDA Toxicology Working Group (TWG). The TWG serves as an internal forum for enhancing scientific discussions between toxicologists across all of FDA's Centers, and also helps coordinate cross-cutting toxicology activities at FDA as well as coordinating FDA's participation in interagency activities related to toxicology. The TWG also serves as a resource for FDA toxicologists on current and emerging science and technologies, including non-animal assessment methodologies. In 2018, FDA published "FDA's Predictive Toxicology Roadmap," which was drafted by the TWG (FDA, 2019a). To further efforts to modernize toxicology testing, the Predictive Toxicology Roadmap 1) identifies the FDA Toxicology Working Group as an appropriate entity for coordinating Agency efforts to assess the scientific validity of novel toxicology assessments; 2) promotes the concept of "qualification for context of use" as an appropriate metric for assessing the utility and regulatory acceptability of novel toxicology assessments, rather than requiring the more time-consuming and expensive "validation" model; 3) emphasizes the need to ensure that Agency toxicologists have the opportunity to learn about the strengths and limitations of new approaches; 4) emphasizes the need for continued engagement with external stakeholders regarding new technologies; 5) emphasizes the importance of FDA-directed research efforts to identify data gaps associated with current and novel approaches to toxicity assessment, as well as the importance of continued international- and national-level research collaborations.

Notably, FDA has already held two public meetings on the implementation of the Predictive Toxicology Roadmap. The first, in 2018, sought public comment regarding the Roadmap. In the second, in 2019, FDA provided an update to our efforts in regard to implementing the Roadmap. Information about these workshops and an annual report from the TWG can be found at the FDA webpage on the Predictive Toxicology Roadmap (FDA, 2019a).

3.3.2. FDA alternative methods working group

FDA recently launched its Alternative Methods Working Group as part of its long-standing commitment to promote the development and use of new technologies to better predict human and animal responses to substances relevant to its regulatory mission. This group focuses on opportunities for evolving and innovative technologies to advance useful tools as well as new areas of science to support alternative methods to traditional toxicity and efficacy testing that extend across FDA's product areas. The Group supports research, training and communication related to alternative methods within the Agency.

The Group also acts as a catalyst to foster the development and potential application of alternative systems (in vitro, in vivo, in silico, and systems toxicology modeling), such as microphysiological systems, to support decision-making in regulatory toxicology. The Group facilitates interactions with global regulatory bodies interested in implementing alternative methods in toxicology. Additionally, it examines opportunities and viable ways by which emerging methods and new technologies can support regulatory review of risk, safety, and efficacy of FDA-regulated products. As part of this effort, the Group established a subgroup to explore performance standards for microphysiologic test systems intended to support regulatory decision making.

Importantly, the activities of FDA's Alternative Methods Working Group are informational and do not serve as official regulatory guidance.

The Group is inviting developers to showcase their cutting-edge technologies in an FDA Webinar Series on Alternative Methods. Information about the Group including contact information and how to propose a topic for a webinar are on the FDA website on Advancing Alternative Methods at FDA (https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda). A report entitled Advancing New Alternative Methodologies at FDA that describes agency efforts related to alternative methods was published in January 2021 and is also available on the website (FDA, 2021b).

With regard to supporting alternative assay development and implementation, it is important to note that the primary goal, given CDER's mission, is to improve the ability of the nonclinical assessment to predict clinical safety. While most of the effort in alternative assay development is focused on in vitro or in silico approaches, in principle, an alternative assay could also be a novel in vivo model or endpoint. Notably, an alternative assay need not necessarily replace an existing in vivo model to reduce animal use. Any assay that increases the ability of the nonclinical program to identify compounds at high risk of causing intolerable adverse effects in the clinic can decrease animal use by obviating the need to conduct late-stage, animal-intensive, studies (i.e., DART and carcinogenicity).

3.4. CDER-specific efforts impacting 3Rs

3.4.1. Regional guidance

In addition to CDER-lead efforts to develop internationally harmonized guidance, CDER also generates regional-level guidances to aid Sponsor's efforts at pharmaceutical development. These guidances also clearly have the potential to significantly impact the 3Rs. This is perhaps illustrated most clearly by the fact that recently published guidances pertaining to nonclinical testing all contain language emphasizing that the Agency supports the principles of the 3Rs and encourages Sponsors to consult with the Agency on the applicability of alternative, nonanimal, methods, when available. An example from a recent nonclinical guidance (FDA, 2020b) reads:

"We support the principles of the 3Rs (replace/reduce/refine) for animal use in testing when feasible. FDA encourages Sponsors to consult with review divisions when considering a nonanimal testing method believed to be suitable, adequate, validated, and feasible. FDA will consider if the alternative method is adequate to meet a nonclinical regulatory need."

Selected CDER guidances with particular impact on the 3Rs are listed below.

- *Guidance for Industry: Severely Debilitating or Life-Threatening Hematologic Disorders [SDLTHD]: Nonclinical Development of Pharmaceuticals* (FDA, 2019e)
  - o Recognizes that due to the severity of the diseases covered by the scope of the guidance (similarly to treatment of advanced cancer) that patients with these diseases, their caregivers and their physicians, are generally willing to accept a greater risk of injury from a less thoroughly characterized toxicity profile than would be appropriate for the treatment of more benign diseases. Consequently, a more streamlined nonclinical program may be appropriate for development of pharmaceuticals intended to treat patients with SDLTHDs, where speed to therapeutic benefit is of the essence. As with ICH S9, such an approach can lead to a substantial reduction in animal use.
- *Guidance for Industry and Review Staff: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route* (FDA, 2015a)
  - o This guidance provides recommendations for the nonclinical evaluation of previously approved drug substances, when a new formulation or a new route of administration for a previously approved formulation is being proposed by a Sponsor.

- Notes that the need for additional nonclinical data generally will be limited when a new formulation is to be used in a manner similar to previous formulations.
- Indicates that, for reformulated drugs administered by the same route as the approved product, systemic toxicity assessments, if recommended, can be of a shorter duration than that indicated in ICH M3 or S9 (a refinement of animal use).
- Specifically states that the in vivo rabbit ocular irritation test method is no longer recommended for ocular irritation testing of topical drug products.

- *Guidance for Industry: Investigational Enzyme Replacement Therapy Products: Nonclinical Assessment* (FDA, 2019b)
  - o Describes considerations for nonclinical development programs supporting clinical development of replacement enzymes. Most typically, these agents are intended to treat inborn errors of metabolism, which often cause rapid progression to severe morbidity or death. The guidance notes that for indications of this nature, that general toxicology studies of 1-month in duration in two species (a rodent and a nonrodent) are generally sufficient to allow for initiation of patient treatment. These studies are also sufficient to support continued treatment beyond one month. For the marketing application, a 3-month study in a single species (typically the rodent) is considered sufficient for assessing general toxicity.
  - o Indicates that flexibility in the timing of the conduct of DART studies is appropriate. These are often conducted as a post-approval requirement.
  - o Carcinogenicity studies are typically not warranted.
- *Guidance for Industry: Microdose Radiopharmaceutical Diagnostic Drugs: Nonclinical Study Recommendations* (FDA, 2018a)
  - o This guidance provides guidance with regard to what nonclinical data are needed to support approval of a microdose radiopharmaceutical intended to be used diagnostically.
    - Safety Pharmacology studies are generally not warranted.
    - Repeat dose toxicity studies are generally not warranted.
    - DART studies are waived because of the inherent radiation risk to the fetus from the radiopharmaceutical drug, which would be reflected in labeling.
- *Guidance for Industry: Oncology Therapeutic Radiopharmaceuticals: Nonclinical Studies and Labeling Recommendations* (FDA, 2019c)
  - o Notes that it is generally not warranted to conduct general toxicology studies with radiopharmaceuticals, since the toxicity due to the radionuclide can be adequately determined from an animal biodistribution study and knowledge of the effect of the particular radiation type on the target organs.
  - o Notes that since the toxicity associated with the targeting ligand is generally minor, compared to that of the toxicity induced by the radiation emitted by the radionuclide, it is generally acceptable to conduct a general toxicology study in a single species, with the targeting ligand by itself (or with a stable, "cold," isotope). Moreover, the guidance identifies scenarios under which no general toxicology study may be warranted.
  - o The guidance indicates that no reproductive toxicity or carcinogenicity studies with the radiopharmaceutical or the cold pharmaceutical is warranted during drug development or for approval. Since alpha, beta, and gamma radiation cause deoxyribonucleic acid damage, are inherently carcinogenic, and damage male and female germ cells and the developing conceptus, these risks are assumed and should be communicated in product labeling.
- *Rare Diseases: Common Issues in Drug Development Guidance for Industry (Draft)(FDA, 2019d)*
  - o This Rare Disease guidance notes that it is appropriate for the Agency to exercise flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness, for drugs to treat serious and life-threatening diseases. This flexibility includes determining the nonclinical data necessary to support clinical development programs. Factors that FDA evaluates when determining areas of nonclinical flexibility include the pharmacological and chemical characteristics of the drug, the design and objectives of the proposed clinical investigations, the anticipated risks to humans, and the existing accumulated nonclinical toxicology and human data. In some cases, for serious or life-threatening diseases where current treatments, if any, are inadequate, clinical trials can often proceed with a modified nonclinical development program.
  - o The guidance notes that toxicology testing in an animal model of disease may contribute to the nonclinical support for clinical trials in rare diseases but usually will not substitute for toxicology testing in healthy animals. However, safety evaluation in an animal model may be particularly valuable when drug toxicity is predicted to be more severe in the presence of disease pathophysiology.
  - o FDA encourages Sponsors to communicate early in the drug development process with the review division to discuss an appropriate nonclinical development program for an investigational drug intended to treat a rare disease.
- *Nonclinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics Guidance for Industry (Draft) (FDA, 2020b)*
  - o With regard to the assessment of dermal sensitization, an assay typically conducted in animals, FDA has indicated that it is open to considering the use of a battery of studies (e.g., in silico, in chemico, in vitro) in place of the in vivo assessment, assuming that the proposed assessment has been shown to adequately predict human skin sensitization with an accuracy similar to existing in vivo methods.

3.4.2. Innovative science and Technology Approaches for new drugs (ISTAND) pilot program

- The 21st Century Cures legislation authorized the Agency to develop Drug Development Tools (DDTs) that are intended to facilitate drug development. DDTs include biomarkers, clinical outcome assessments, and other methods, materials, or measures that aid drug development and regulatory review. To support DDT development efforts, FDA has established qualification programs for biomarkers, clinical outcome assessments, and for animal models for use under the Animal Rule. In November 2020, FDA announced a new pilot program, The Innovative Science and Technology Approaches for New Drugs (ISTAND) Pilot Program, which is intended to expand DDT types by encouraging development of DDTs that are out of scope for existing DDT qualification programs but may still be beneficial for drug development. Examples cited on the website include: the use of tissue chips (i.e., microphysiologic systems) to assess safety or efficacy questions, and the development of novel nonclinical pharmacology/toxicology assays (FDA, 2021c).

3.5. OND nonclinical review staff-directed efforts

Additional CDER/OND efforts and their anticipated impacts are listed below:

- OND Pharmacology and Toxicology Coordinating Committee (PTCC) Emerging Technology Subcommittee
  - o This subcommittee was created with the primary goal of identifying regulatory needs (e.g., where non-clinical models are lacking, or existing models are inadequate) and to evaluate emerging technologies that have the potential to impact these areas. The subcommittee also seeks to understand how new toxicology approaches are being applied to regulatory submissions. This group also organizes opportunities whereby other nonclinical reviewers can learn about the newly emerging technologies, and how they

may impact the way in which nonclinical reviews are conducted in the future.

- Most significantly, this group has recently published a paper that describes the CDER perspective on what information needs to be provided by nonclinical testing strategies, and the perceived strengths and weakness of the classical in vivo toxicology assessment approach and currently described New Approach Methodologies (NAMs)(Avila et al., 2020). This paper also conveys the Center's openness to regulatory acceptance of NAMs, which will hopefully help to spur further refinements to these new approaches, so that they will be able to live up their promise of improving regulatory predictive toxicology and reducing animal usage.

4. Challenges and future directions

CDER, via its international, national, FDA-wide and internal efforts, has made great strides in reducing the number of animals used in support of pharmaceutical development; however, there is always more that can be done. It is hoped that the ongoing efforts described above, such as the recent revisions to ICH S5 and the proposed revisions to ICH S1 will serve to further replace, reduce and refine how animals are used in supporting clinical development of pharmaceuticals.

There remain, of course, significant challenges though that, if addressed, could substantially aid in the efforts to use animals only when clearly needed.

In working to implement provisions from ICH and FDA regional guidances that rely on WoE scientific assessments for obviating the need for product-specific animal data to satisfy given nonclinical endpoints, it can occasionally be difficult to clearly identify what information falls within the domain of generally accepted scientific knowledge (GASK), as opposed to sponsor-specific data, the latter being potentially subject to intellectual property protections. The inability to use sponsor-specific data more broadly (e.g., extrapolating data across pharmacologic classes) impairs the Agency's opportunities to avoid recommending potentially redundant studies. Finding ways to better define GASK and allow its use may facilitate a reduction in animal use in drug development.

One of the largest challenges to increasing implementation of the 3Rs in drug safety assessment is the relative lack of alternative models able to efficiently provide all the information obtained from some of the traditional animal models. Substantial progress has been made in generating alternative approaches to relatively straightforward toxicity endpoints. When an adverse outcome pathway can be determined that is linear and consists of a small number of key events, it has been occasionally possible to develop alternative approaches based on this understanding. Examples include alternative approaches to ocular irritation, skin irritation and skin sensitization. However, testing a drug in a traditional general toxicity study takes advantage of all the complex physiological interactions that occur in a whole animal. These studies are conducted, in part, to identify unpredicted effects, particularly those effects that would cause significant injury to human subjects, and histopathological effects that cannot be ethically studied in humans. It is currently not possible to use a defined set of in vitro assays to address all of the possible mechanisms of toxicity. Even a large array of in vitro approaches is currently unable to recapitulate all physiological and anatomic aspects of a whole animal. These in vivo aspects such as tissue innervation, immune function, metabolism, etc. can be critically responsible for the toxicity of a drug. The creation of ever more complex in vitro models is beginning to reassemble some of these in vivo interactions; however, faithfully recreating the fully integrated systems that are the hallmark and strength of in vivo models for general toxicity screening of pharmaceuticals is a daunting task, whose timeline, if possible, is likely measured in decades, rather than years.

That is not to say that we should stop striving to, wherever practical and without reducing patient safety, reduce the numbers of animals used in pharmaceutical testing, and support the development of new approach methodologies (NAMs). Toward this latter goal, the FDA recently announced a pilot program through which interested parties can seek FDA review and comment (outside of a regulatory pathway) on NAMs – the ISTAND Pilot Program described above. The program is intended to expand DDT types to include NAMs. As described on the website, the pilot program is intended to support the use of microphysiologic systems to assess safety or efficacy questions, and the development of novel nonclinical pharmacology and toxicology assays (FDA, 2021c). It is hoped that successful adoption of this program will stimulate the development of novel in vitro approaches that will allow us to further reduce the number of animals used in pharmaceutical development. In addition, Sponsors are always encouraged to discuss with review divisions whether they think a new approach would be sufficient to address a particular scientific issue. Early discussion (such as in a preIND meeting) can facilitate a better understanding of expectations for both the FDA and the Sponsor and may allow incorporation of a new approach in subsequent submissions.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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