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Preclinical development of monoclonal antibodies

Considerations for the use of non-human primates

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Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; EFD, embryo fetal development; EMEA, european medicines agency; GA, genetically altered; FDA, food and drug administration; FIH, first in human; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; IFNγ, interferon gamma; IL-6, interleukin-6; IL-12, interleukin-12; KO, knock-out; mAb, monoclonal antibody; NCE, new chemical entity; NHP, non-human primate; NK, natural killer; NOAEL, no observed adverse effect level; PD, pharmacodynamic; PK, pharmacokinetic; PBMC, peripheral blood mononuclear cell; PPND, pre and post natal development; TNFα, tumor necrosis factor alpha; WOCBP, women of child bearing potential

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The development of mAbs remains high on the therapeutic agenda for the majority of pharmaceutical and biotechnology companies. Often, the only relevant species for preclinical safety assessment of mAbs are non-human primates (NHPs), and this raises important scientific, ethical and economic issues. To investigate evidence-based opportunities to minimize the use of NHPs, an expert working group with representatives from leading pharmaceutical and biotechnology companies, contract research organizations and institutes from Europe and the USA, has shared and analyzed data on mAbs for a range of therapeutic areas. This information has been applied to hypothetical examples to recommend scientifically appropriate development pathways and study designs for a variety of potential mAbs. The addendum of ICHS6 provides a timely opportunity for the scientific and regulatory community to embrace strategies which minimize primate use and increase efficiency of mAb development.

Introduction

The unrivalled growth in the monoclonal antibody (mAb) sector is predicted to continue unabated.[1] Currently, there are over 200 mAbs in clinical studies, and the number entering clinical trials is increasing year on year, reaching 34 in 2006.[2] Regulatory approval rates for mAbs remain higher than that for new chemical entities (NCEs).[2,3] With expansion into new therapeutic areas and technological advances to tailor and improve their efficiency, mAbs offer unprecedented opportunities for the pharmaceutical and biotechnology industries over the next decade. There have been major changes in the development of mAbs since the first antibody was approved in 1986,[4,5] and with the increasing prominence of mAbs in the drug pipeline, the current addendum of the ICHS6 regulatory guidelines on '*Preclinical safety evaluation of biotechnology-derived pharmaceuticals*' is an important step in ensuring an expedient path to the clinic.[6]

One major challenge in the development of mAbs is the choice of species for preclinical safety assessment studies. Due to the high target and species specificity of mAbs, the use of rodents is often precluded and in some instances there may be no appropriate species to select. Frequently, the only pharmacologically relevant species is a non-human primate (NHP), usually the cynomolgus or rhesus macaque, but occasionally the common marmoset or African Green monkey, and this has a significant impact on the number of NHPs used in preclinical testing. Note in this paper we use the term NHP to refer to the commonly used monkey species and not chimpanzees.

The use of NHPs is likely to increase further as a result of the expansion of therapeutic mAbs for chronic debilitating diseases with the concomitant requirement for preclinical risk assessment of

Table 1. Key attributes for relevance of preclinical species

Species relevance
• Comprehensive comparative data on orthologous binding affinity and functional potency or avidity compared with human.
• Comparative target tissue distribution profile between orthologue and human e.g., immunohistochemical analysis of animal and human tissue.
• Equivalent mechanism of action to that of target modulation in the human.
• Absence of immune response that limits exposure.

(see also Tabrizi et al. 2007, Martin et al. 2009, and ICH S6 guidelines).

reproductive effects and long term treatment, and the selection of new molecules on the basis of potency in NHPs to ensure availability of a pharmacologically relevant species for preclinical testing. In addition, factors such as the post-TGN1412 environment and lack of confidence in, or acceptance of, other approaches such as homologous proteins or genetically altered (GA) models have the potential to reduce the flexibility previously adopted in mAb development, thereby driving an increase in the use of NHPs. In this paper, a homologous protein/homologue is defined as an anti-mouse, rat or primate antibody that recognises the appropriate mouse, rat or primate target with similar potency to the intended clinical molecule. Homologues are also frequently referred to as surrogates.

While NHPs are currently necessary to provide risk assessment data for many mAbs, the very characteristics of mAb biology that have enhanced their success as potential therapeutic agents, such as high target specificity, predictable metabolic stability (e.g., the lack of metabolic activation into reactive species) and low toxicity, also provide opportunities to minimize the use of NHPs in safety testing and to increase the efficiency of mAb development. To examine and promote these opportunities, the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research has established an expert working group, with representatives from 15 leading pharmaceutical and biotechnology companies, contract research organizations and institutes from Europe and the USA. The working group has shared and analyzed anonymized data on safety studies from preclinical development pathways for mAbs, including information on potency, availability and use of homologous proteins, study design and the number of studies carried out to support clinical trials. Information was provided on over 100 mAbs in development for a range of therapeutic areas.

The data sharing has shown that there is wide variation in approach to mAb development, particularly in the design of in vivo toxicity studies, and that opportunities exist to minimize NHP use without compromising human safety. To avoid issues of a proprietary and confidential nature, the working group have used this information to design scientifically robust preclinical development pathways for six hypothetical mAbs, based on real antibodies, for a variety of therapeutic areas and targets. The examples fall into three categories where, (1) NHPs are not relevant species for preclinical safety testing, (2) NHPs are only relevant for some studies and (3) NHPs are relevant. A species is considered relevant if the mAb has affinity and sufficient functional potency to modulate the target antigen in a manner comparable to that in human. Relevance should be based on pharmacological activity rather than binding affinity (see **Table 1**). Our discussion is limited to safety evaluation studies, with a specific focus on identifying potential toxicity and minimising NHP use. In publishing these examples, we aim to provide an educational tool that will promote wider consideration within companies of the opportunities that exist to minimize NHP use and increase discussion in the broader scientific and regulatory communities.

Selecting a Relevant Species for Preclinical Safety Studies

Designing a preclinical development strategy for either NCEs or mAbs involves generating a body of evidence to assess the risk to human safety. Much of this is carried out prior to in vivo experimental work and includes consideration of the therapeutic area, patient population, treatment regime (e.g., dosing schedule, route of administration) and historical information on the target. Review of relevant regulatory guidance or discussion with the appropriate regulatory agency is also important. Based on this, studies are then conducted to provide information on target validation, efficacy, dose response relationships, potential toxicities, starting and maximum dose in man, safety parameters (i.e., biomarkers) that can be monitored in clinical trials and drug interactions for combination therapy.

Preclinical in vivo safety studies for NCEs are designed to identify a broad range of potential adverse effects that may or may not be related to the pharmacological action of the drug. However, for mAbs, the high target specificity, the lack of metabolite toxicity (e.g., catabolic elimination) and the fact that toxicity is usually the result of exaggerated pharmacology, allows better prediction of the adverse effects that may be observed. This means that studies can often be tailored accordingly, allowing a more flexible approach to be taken than is the case with NCEs. The types of preclinical, in vivo studies that are usually considered for the safety assessment of mAbs are described in **Figure 1** and refs. 7–11. Key regulatory guidelines covering mAb development are shown in **Table 2**.

The primary consideration in designing safety studies for mAbs is the selection of a pharmacologically relevant species. Species differences in target affinity, expression pattern, mechanism of action, pharmacological activity and immunogenicity can all potentially reduce the translation of preclinical studies to human. A thorough understanding of species differences at the outset is essential for designing appropriate studies using a relevant species and subsequent interpretation of results. Using a 'non-relevant' species can confound or potentially delay translation into the clinic by providing information which can be scientifically misleading or offer no value in assessing risk to humans.

The question of what is a relevant species for safety testing is complex; for instance, binding of a mAb to its target

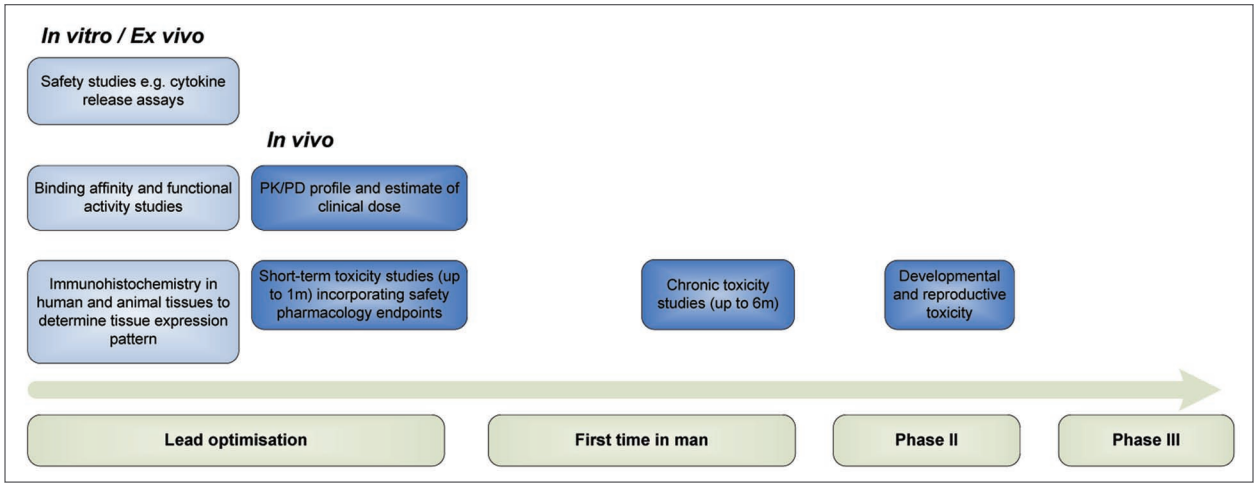

Figure 1. Scheme showing the studies carried out for a general mAb development program. Adapted from Chapman et al. 2007.

at normal tissue expression levels does not necessarily mean the species is relevant or that there is pharmacological activity. Our data showed that 10% of mAbs that did cross-react with the NHP species used showed less than 10% potency. In this paper, we have defined a species as relevant if the mAb is pharmacologically active as demonstrated by functional potency assays. Ideally, the target should be modulated in a manner similar to that of man, and, to fully assess species relevance, activation of downstream signalling pathways or effector function may require investigation.[7]

Important considerations for determining species relevance and how these might be used to minimize NHP use are shown in **Table 3** and **Figure 2**. The NHP should not be used simply as a 'default' with the assumption that they will be the most appropriate species, as a screen, or because they have been used previously. A scientific approach should always be taken when designing toxicity studies taking account of all available approaches, including the use of rodents with the clinical molecule even when the NHP is relevant. Our data show that when the rodent is relevant, NHP use can be reduced by using rodents for some aspects of hazard detection or for making go/no go decisions. Where there is more than one relevant species, the use of two species in parallel should be avoided in line with ICHS6 guidelines. In this instance, it is possible to justify the use of only one species for chronic toxicology studies if the subchronic studies (e.g., one month) show a comparable toxicity profile. The potential therefore exists for the use of rodents only in long-term toxicology studies. Our data shows that by adopting this strategy, e.g., by using rodents for reproductive toxicology studies, it is possible to reduce NHP use by up to 60 animals. This is consistent with ICHS5(R2) guidelines which states that 'If it can be shown-by means of kinetic, pharmacological and toxicological data-that the species selected is a relevant model for the human, a single species may be sufficient'.

There is not a single solution for mAbs, and there should be clear rationale for the development program and the use of NHPs. When NHPs are relevant based on pharmacology, studies should be designed to provide the required safety information using the minimum number of animals. While it is difficult to prescribe a 'typical' or generic mAb preclinical development program, there are common principles that can be used as summarised in **Table 4** and **Figure 3**. These depend on the patient population, intended dosing regimen, the risk of toxicity as evaluated from early in vivo studies and the level of knowledge of the target and response. Specific opportunities to minimize NHP use are described in the section entitled "opportunities to minimize NHP use where the NHP is the only relevant species" on page 512.

Table 2. Regulatory guidelines supporting preclinical mAb development

•ICH S6: Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals (CPMP/ICH/302/95) (update ongoing)
•ICH S8: Note for guidance on immunotoxicity studies for human pharmaceuticals (CHMP/167235/2004)
•ICH S9: Note for guidance on nonclinical evaluation for anticancer pharmaceuticals (EMEA/CHMP/ICH/646107/2008) (in preparation)
•ICH S5a: Note for guidance on the detection of toxicity to reproduction for medicinal products including toxicity to male fertility (CPMP/ICH/386/95)
•Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (CDER/FDA 2007)
•Guideline on development, production, characterization and specification for monoclonal antibodies and related products (EMEA/CHMP/BWP/157653/2007)
•Concept paper on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use (EMEA/CHMP/BMWP/114720/2009)
•Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins (EMEA/CHMP/BMWP/14327/2006)

(see also Muller and Brennan, 2009).

Table 3. Considerations for relevance of NHPs

	Impact on animal use—examples
NHP not relevant	
•No potency/pharmacological activity	•No studies in NHP. Minimal studies (e.g., basic pharmacology) in animal model of disease. Explore potential of homologous protein.
•Target not expressed in normal tissue	•No studies in NHP. Minimal studies (e.g., basic pharmacology) in animal model of disease.
NHP has reduced relevance	
•Mechanism of therapeutic action not modelled e.g., no ADCC activity	•Fewer studies in NHP; explore potential of homologous protein.
•Activation of equivalent pathways not demonstrated in preclinical species.	•Fewer studies in NHP.
•Equivalent tissue expression pattern of target not observed in preclinical species.	•Fewer studies in NHP.
•Reduced potency e.g., 30 fold less pharmacological activity	•Fewer studies in NHP; explore potential of homologous protein.

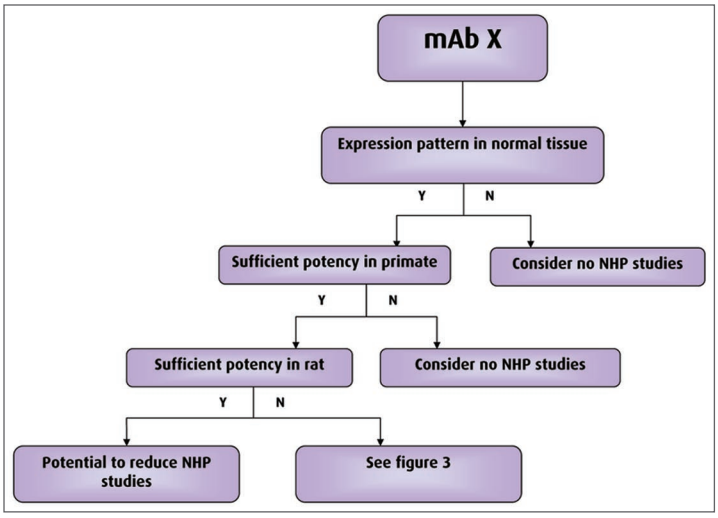

Figure 2. Flow diagram illustrating important considerations for determining species relevance and how these might be used to inform decisions on NHP studies.

Program Development and Species Selection in Practice

The following examples, structured by therapeutic area, illustrate the factors that should be considered when designing preclinical safety studies. For each example, consideration is given as to whether NHPs are relevant to assess safety, whether other approaches can be used, and when the NHP is used, how the numbers of animals can be minimized. The use of homologous proteins and GA rodents (see **Table 5**) is discussed. These case studies are intended to illustrate the flexibility that can be used to assess the safety of mAbs; the approaches described are not intended to be prescriptive and will differ depending on the specific product. For mAbs where there is no relevant preclinical species and therefore limited in vivo data, emphasis should be placed on the clinical plan which will usually consist of a very low starting dose and slow dose escalation scheme. Early discussion with regulators on the acceptability of individual programs is recommended.

Oncology mAbs. The success of mAbs is most apparent for anticancer agents where they have led to major advances in treating common malignancies, such as breast cancer (e.g., trastuzumab), colorectal cancer (e.g., cetuximab, bevacizumab), lymphoma (e.g., rituximab) and leukemia (e.g., alemtuzumab). The majority of mAbs, both approved and in clinical trials, are primarily intended for oncology indications, and there are currently nine approved in the US and other countries.[2] Designing a preclinical strategy for oncology mAbs is particularly demanding; therapeutic efficacy is often dependent on recruitment of host effector function in addition to binding to the target.[12-15] Frequently, the target protein is expressed endogenously but is upregulated in tumor tissue or is only expressed in tumor tissue. The preclinical safety evaluation packages for approved oncology mAbs and our data for those in development show variation in species choice and the number and the design of studies undertaken. Rationale for these differences includes low binding affinity of the mAb in preclinical species, expression pattern of the target, disease indication and patient population. Flexibility in approach is particularly relevant for mAbs for oncology indications where there is a significant unmet medical need and the mAb is likely to be given in combination with, or subsequent to, cytotoxic drugs.

Example A. An IgG1 isotype mAb that targets a tumor antigen is being developed for a life-threatening oncology indication in a patient population that includes women of child bearing potential (WOCBP). Chronic intravenous administration is required for the primary indication. The mAb is effective against a number of human solid tumors in vitro and in xenograft models. The tumor antigen is not expressed in normal tissue and there is no specific or nonspecific cross-reactivity with any normal human or animal tissue. Additionally, there is evidence of tumor lysis via antibody-dependent cellular cytotoxicity (ADCC) from in vitro studies.

Ideally, the safety evaluation studies for this mAb should be designed to detect potential toxicity resulting from both on-target binding and activation of ADCC, which is an intended therapeutic function. However, the lack of expression

Table 4. Considerations to minimize NHP use

Chronic toxicology	Potential impact on animal use
•Preclinical dosing regime should be designed according to the intended clinical dosing regime. e.g., single dose indication.	•Fewer repeat dose studies.
•Immune response prevents high enough levels of exposure.	•Fewer long-term studies.
•Information from initial toxicology studies. e.g., no immediate or delayed toxic effects at high doses in early studies.	•Fewer dose groups and recovery animals on subsequent studies.
•Drug intended to be dosed in combination with other drugs that are known to cause toxicity.	•Fewer long-term studies.
•Life-threatening diseases where drug will be given to patients in spite of toxic effects observed with chronic treatment.	•Fewer long-term studies.
•Dose response. e.g., 100% saturation achieved at low dose.	•Fewer dose groups.
Reproductive toxicology	
•Patient population. Drug will not be given to women of child bearing potential e.g., post-menopausal females, males only.	•No reproductive toxicity studies.
•Historical information available on target from other drugs in class.	•No NHP studies, explore potential of homologue.

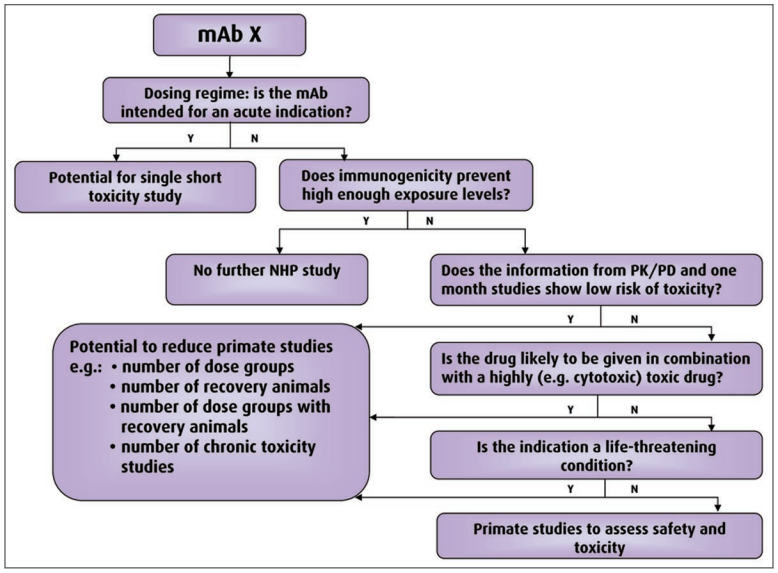

Figure 3. Flow diagram incorporating general principles that inform decisions around NHP use, e.g., the therapeutic area and intended clinical dosing regime. Areas where there are potential to minimize NHP use are illustrated.

in any animal tissue, demonstrated by immunohistochemistry where the disease tissue is positive, means that it is not possible to select a relevant species with which to do this. Clinical studies of mAbs have been conducted without conventional in vivo toxicity data for certain cancer indications (e.g., alemtuzumab[16]). Such an approach is also possible in this example, as safety information can be provided using data from in vitro cytotoxicity studies to demonstrate potential ADCC activity, and long-term (e.g., over one month duration) safety data from the pharmacological xenograft model. Although adherence to good laboratory practice cannot usually be claimed for such efficacy studies, the mAb should be of clinically comparable material allowing the data obtained to be useful in assessing human safety.

If there is an additional need for in vivo safety data, a targeted two week toxicity study in the rodent only should be sufficient to provide information on potential off-target or intrinsic formulation toxicity—both of which are unlikely based on experience with other IgG1 mAbs. Given the dosing schedule in the rodent, it is unlikely immunogenicity will limit exposure and NHP studies should not be necessary.

As the patient population includes WOCBP, the value of a reproductive toxicology study, for understanding potential risk to the developing fetus, should be considered. The lack of expression in animals precludes doing a meaningful study in any species, and instead historical information on the target combined with the lack of expression in normal human tissue should be used in the assessment of risk to reproduction. For a life-threatening condition, the harm/benefit assessment is likely to favor administration in man as there is high probability that the therapy will be given anyway, and in combination with a cytotoxic drug.

Example B. An IgG1 isotype mAb that binds a cell surface expressed antigen is being developed for an oncology indication for which it will be administered intravenously. The product recognises the human and NHP target, but the mAb is very immunogenic (e.g., causes production of neutralising or clearing antibodies which reduce the amount of circulating active mAb) in the NHP. A mouse homologous protein is available.

As this mAb binds a cell-surface target, there is a greater risk of cytotoxicity than with many other mAbs based on the propensity for IgG1 to activate ADCC pathways. In this example, the NHP may be suitable for short-term toxicity studies of up to one month duration if it can be demonstrated by pharmacokinetic and/or pharmacodynamic (PK/PD)

Table 5. Homologous proteins and GA rodents

Desired characteristics
• Equivalent pharmacological activity to clinical molecule in man (e.g., binding affinity [K_d], potency)
• Equivalent isotype and function (e.g., IgG2a mouse = IgG1 in humans, binding to Fc receptors and demonstration of ADCC, CDC)
• Confirmation of overlapping tissue binding
• Equivalent PK/PD profile and translation of mechanism of action between rodent and human target
Additional characteristics required for GA rodents
• KO representative of drug treatment (e.g., compensation by other gene products during development does not under-predict risk)
• Absence of exposure-limiting neutralising antibodies to the clinical molecule
• No significant effects of presence of the human protein in terms of secondary interactions between human and mouse proteins
Occasions where alternative rodent approaches may be useful
• Human and chimpanzee specific product
• Reduced potency of the clinical molecule in the NHP (e.g., 30 fold)
• Specific studies (e.g., reproductive toxicology and fertility)
• Testing mAb in disease models
Additional opportunities for use of the homologous protein
• Immunogenicity prevents exposure in the NHP

(see also Bussiere et al. in press).

biomarker(s) that the animals are exposed to active drug. The one month toxicity study should provide sufficient data to support FIH and further development if immunogenicity prevents exposure for long-term studies and dosing through the antibody response using higher dose levels is excluded.[17]

In this example, the initial clinical trial is likely to be conducted in patients rather than healthy volunteers, and will therefore provide information on efficacy and safety. To address specific safety issues arising from long-term clinical dosing, further preclinical studies (e.g., three or six month duration) may be necessary. Given the likelihood of immunogenicity preventing exposure in longer term studies, the use of the mouse homologous protein should be considered. It should be noted that there may be instances when the rodent mAb is also immunogenic. As in example A, reproductive toxicology studies may not be necessary depending on the severity of the indication, likelihood of combination therapy with cytotoxic drugs and whether a hazard has already been identified (e.g., anti-growth factor mAbs).

mAbs for immune-related diseases. As for oncology indications, mAbs with an immunomodulatory function have also been approved to treat autoimmune and inflammatory diseases such as rheumatoid arthritis (e.g., adalimumab, rituximab), multiple sclerosis (e.g., natalizumab), Crohn disease (e.g., infliximab, certolizumab), psoriasis (e.g., efalizumab), asthma (e.g., omalizumab) and transplant rejection (e.g., muromonab, daclizumab, basiliximab). There are ten approved immune-related mAbs on the market and many more in development.[2] Whereas for oncology mAbs, T cells or natural killer cells are recruited to stimulate an immune response against tumor cells, mAbs for immune-related diseases are typically designed to suppress immune function. IgG2 and IgG4 isotype mAbs which show no or limited complement activity, are often used as they do not elicit effector function and therefore avoid cell depletion. Species differences in complement activation between isotypes must be taken into consideration for toxicology studies.

To exert their pharmacological effect, immunomodulatory mAbs either (1) bind to targets on cells of the immune system, or (2) bind and sequester soluble cytokines. Specific toxicological concerns for immunomodulatory mAbs vary depending on the type of immune modulation but can include cytokine release, autoimmunity (e.g., eliciting immunity to endogenous proteins) and immunosuppression. Although the human and NHP immune systems have many similarities there are some significant functional differences, particularly with regard to T-cell activation which differs even between human and chimpanzee.[18,19] Consequently, the preclinical assessment of adverse immune-mediated reactions such as cytokine release syndrome, which is a potential risk for mAbs that bind to targets on cells of the immune system, can be challenging, and may not be predicted by studies in NHPs. To overcome some of these difficulties, a variety of preclinical packages have been used to develop mAbs in this therapeutic area, including the use of homologous proteins when there is no relevant species for the clinical molecule.[20-22] In addition, in vitro assays are currently being developed and modified to better predict immune-mediated reactions.[23]

Example C. An IgG2 isotype mAb is being developed for rheumatoid arthritis. The target is a soluble cytokine and the product will be administered subcutaneously. Binding potency of the mAb for the NHP target is approximately 30-fold less than for the human target. A mouse homologous protein is available which has similar potency to the therapeutic mAb for the human target. Homozygous knock-out (KO) mice are also available which show impaired immune function and altered lymphoid tissue development.

The primary challenge for the preclinical safety evaluation of this mAb is the lack of a relevant model. To investigate further whether high enough exposure is achievable in the NHP with a 30-fold difference in potency, an appropriate PK/PD study should be undertaken. This should use as few NHPs as possible to meet the study objective—to determine the translation of the in vitro binding data in vivo. If the NHP is shown not to be relevant, i.e., appropriate exposure or target saturation cannot be reached, then

the mouse homologous protein could be used for chronic and reproductive toxicology. Useful information can be gained from studies with the homologue, rather than the use of the clinical mAb in a species where it does not have sufficient potency for the target. In this example, data from the homologous protein can be further supported by the information from the KO mouse.

An appropriate strategy would be to conduct a one month toxicity study in the mouse with the homologous protein. If a toxicological effect is seen which is consistent with the KO mouse data, then further studies with the homologue could be conducted (e.g., three or six month studies, reproductive toxicology). Due to the relative lack of in vivo studies with the clinical molecule, particular emphasis should be given to detecting impaired immune function in the clinic. Depending on the target biology, immune function can be monitored by profiling lymphocyte activation markers such as CD25 and CD69, monitoring natural killer (NK) cell function and other assays such as chemotaxis, phagocytosis and mitogen or recall antigen assays.[19]

A major challenge with this strategy exists if data from the homologue are inconsistent with that of the KO mouse. This can occur when the target is absent during development leading to KO mice being either too sensitive or not sensitive enough to determine toxicity depending on the ability of other gene products to compensate. If there is inconsistency, NHP data may be required. To determine if this is likely to provide any additional data on safety, information from studies of other mAbs targeting the same protein should be evaluated. Historical data should be used with caution as occasionally differences between mAbs to the same target exist. If the mAb is first in class, a PD study to investigate impaired immune function in the NHP should be considered provided that a sufficient level of exposure can be achieved.

Example D. An IgG4 isotype mAb that targets an immune system cell is being developed to reduce acute rejection of transplanted organs with a potential secondary indication for the treatment of newly diagnosed type I diabetes with residual insulin function also being investigated. The mAb will be administered by intravenous infusion. Specific, predicted cross-reactivity with human and NHP tissue and cells is seen. The expected pharmacology of the antibody is class switching (Th2 to Th1; characterized by raised levels of IL-12 and IFNγ) but may be accompanied by short-lived T cell activation (stimulating production of TNFα and IL6).

In this example, the NHP is the only relevant species. However, NHP use can be minimized by providing safety information for one therapeutic indication at a time and building on previous data to provide information for the secondary indication. Historical information from mAbs which target the same pathways suggests that the major risk with the single acute dose required for the primary indication is cytokine release from short-term T cell activation. The limitations of translating findings in the NHP to humans with respect to cytokine release, as seen with TGN1412,[24-27] should be recognised and alternative strategies (e.g., homologous proteins, humanised mice) explored. However, no alternatives e.g., homologous rodent protein, exist for this mAb and therefore a one month toxicity study in the NHP and assessment of NHP T cell activation and release of pro-inflammatory cytokines should be undertaken. In vitro studies with NHP and human peripheral blood mononuclear cells PBMCs (PMBCs) should also be conducted[23] with the understanding that any transient increase in cytokine release may be amplified in human trials. The number of dose groups and recovery animals should take account of the considerations in the section entitled "opportunities to minimize NHP use where the NHP is the only relevant species" on page 512. Data from the one month study should provide sufficient information for first in human (FIH) trials for acute organ rejection provided that a robust safety monitoring plan is in place.

Assuming the human safety data was acceptable to progress the mAb for the secondary indication, further information on chronic administration will be necessary for longer clinical trials. A six month chronic NHP toxicity study which focuses on assessing the long-term effects of immune system modulation should be carried out. Toxicity studies should include extended histopathology of lymphoid organs, immunophenotyping of lymphocytes and other specific immune parameters (see example C). Safety pharmacology endpoints can also be included. Further information on specific safety considerations for immunmodulatory mAbs can be found in ref. 19.

The timing of reproductive toxicology studies can be used to minimize NHP use in this example. Due to the patient population and the IgG4 isotype of the mAb, which does not cross the placenta as readily as IgG1,[28] the requirement for reproductive toxicology for the primary indication should be reviewed. For the secondary indication, recruiting clinical trial volunteers that do not include WOCBP means that the reproductive toxicology can be delayed until later in development (e.g., concurrent to phase 3 clinical trials) when there is greater assurance that the mAb will provide clinical benefit.

Anti-infective mAbs. Currently, there is only one approved mAb for a viral antigen, although there are a number in development, for example for hepatitis C virus and human immunodeficiency virus.[29,30] Palivizumab targets respiratory syncytial virus (RSV), and is used as treatment for lower respiratory tract illness in patients at high risk of experiencing severe symptoms, such as premature infants. The preclinical package for palivizumab was limited to PK (NHP), single dose toxicity (rat and NHP) and tolerance studies (rabbit).[31] The NHP was not used in repeat dose toxicity studies, including reproductive toxicology, due to immunogenicity and the absence of RSV in normal animals.

Example E. An IgG1 isotype mAb that targets a viral antigen is being developed for a patient population that includes WOCBP. The primary indication requires a single dose. The virus is not permissive in any species other than human and there is no specific or non-specific cross-reactivity seen with any human or animal tissue. The only relevant disease model is the chimpanzee.

This example provides similar challenges to example A in terms of selecting a suitable preclinical species to use. In order to establish on-target toxicity an animal

which can be infected with the virus is required. Uninfected animals would be pharmacologically irrelevant. Unlike in example A, in this case there is a relevant disease model available, however for many companies the use of chimpanzees is unacceptable on ethical grounds aside from issues of supply and availability. In some countries the use of the chimpanzee is not permitted.

One potential risk for a viral antigen is cytotoxicity in the target organ, for example, hepatocytotoxicity arising from the mAb binding to the target organ cells expressing the viral antigen. Therefore, in the first instance, an investigation of whether in vitro assays exist or could be developed to monitor this should be conducted. It is possible that some in vivo safety data may be required to support development and regulatory approval even though the only information that can obtained from in vivo studies, in species other than the chimpanzee, is off-target toxicity. Assuming exposure can be achieved in the rodent, the use of NHPs should be avoided. Given that the candidate drug is intended to be a single dose, a two week toxicity study in the mouse as described in example A, could potentially be used to support clinical trials.

In the unlikely event of an adverse effect being identified in the two week mouse study, consideration should be given to further investigative work rather than a toxicology study in a second species. In rare circumstances where a second toxicology study would be scientifically justified, a two week rat study should be sufficient.[32]

mAbs for other therapeutic areas. There are an increasing number of mAbs in development for chronic, life-debilitating conditions. Currently 18% of humanised mAbs under clinical study are outside of the top two therapeutic areas.[2] Whilst the majority of approved mAbs to date (86%) are in oncology or immune-related disease areas, this is likely to change as mAbs become more complex and tailored to improve efficacy for example, by manipulation of the constant regions to reduce or increase effector functions and modification of the variable regions to increase target binding affinity. Some of these modifications are aimed at overcoming the effect of individual patient polymorphisms on efficacy in the clinic. While potentially increasing the regulatory approval rate, the effects of these modifications may be difficult to assess in preclinical species.

Example F. An IgG4 isotype mAb is being developed for osteoarthritis. The dosing regime is chronic with subcutaneous administration. The mAb targets a novel cell-surface expressed antigen which is upregulated on chondrocytes in cartilage tissue from patients. Specific cross-reactivity with normal human tissue is observed in the myocardium in healthy individuals. Pharmacological activity of the clinical molecule has been demonstrated in the NHP but not the rat.

In this example, the pharmacology suggests that the NHP is a relevant species for safety studies. However, as the target is expressed in the human myocardium, in addition to comparing target affinity and pharmacological potency across species, it is important to demonstrate that this expression pattern exists in the NHP. Although cardiotoxicity with mAbs is rare, it is the greatest potential risk for this mAb and specific safety studies would be necessary to address this, not least because this would be an important factor in decisions regarding the further development of the mAb. Significant cardiovascular side effects, particularly for a non-life threatening indication such as osteoarthritis, would be unacceptable.

Although binding has been demonstrated in the tissue cross-reactivity studies, this does not necessarily imply in vivo activity. Initially, in vitro studies on human and NHP myocytes and purkinje fibres; and ex vivo studies with isolated hearts should be undertaken to aid in dose selection for in vivo studies. These results, in conjunction with tissue cross-reactivity studies would indicate whether target binding in the NHP myocardium is comparable to that in human and therefore relevant for assessment of cardiac risk. If so, an appropriate dose range finding study in NHPs (one male and one female at three dose levels) followed by necropsy and pathology should give information on any changes in the myocardium, determine the dose at which cardiotoxicity or other toxic effects are observed and the most appropriate dose for further toxicity studies. Generally, if there were no specific mechanism-related concerns, safety pharmacology studies, including electrocardiogram and blood pressure measurements, should be incorporated into the repeat-dose studies. However, if there are adverse effects detected in the initial studies, more detailed analysis using telemetry may be required to determine the exact nature of any cardiovascular effects.

In this example, if no cardiovascular effects are observed, a preclinical safety development program would be continued using a study design which minimizes the number of NHPs used. The program should be based on the intended duration of the clinical trial and should incorporate the general principles shown in **Table 4**, **Figure 3** and the section entitled "opportunities to minimize NHP use where the NHP is the only relevant species."

The design and timing of clinical trials can be used to minimize NHP use in this example. Consideration should be given to running clinical trials in post-menopausal women, therefore delaying the requirement for reproductive toxicology studies. This is particularly relevant, as osteoarthritis is generally an age-related disease. By moving the reproductive studies later in the development program, they will only be performed when there is enough information to indicate that the mAb is efficacious and safe, thus avoiding unnecessary animal use. For this example, there is no indication from historical data that the mAb will have an effect on the reproductive system and there is no homologue available. Therefore, reproductive studies in the NHP are likely to be required before registration. However, primate use can be minimized by an 'enhanced' pre and post natal development (PPND) study design (reviewed in refs. 33–35 and the following section).

Opportunities to Minimize NHP Use Where the NHP is the Only Relevant Species

A typical development program for mAbs is hard to describe, but it may include a dose range-finding study and a one month study to enable dosing for the FIH (Phase I). Subsequently, a combination of repeat dose toxicity studies (i.e., three, six, nine and/or 12 month) will be conducted to

support different phases of clinical development. Therefore, it is possible that up to four studies may be carried out for a chronic indication; for example, a one month study to support Phase I clinical trials, a 3 month study to support Phase II, a 6 month study to support Phase III and depending on duration of dosing, a 12 month study may be needed for registration.

Species selection should be based on the biology of the mAb and in particular its pharmacological activity. If the NHP is considered relevant (see **Tables 1 and 3**) and there are no potential alternative options to the NHP (e.g., rodent with the clinical or homologous protein), preclinical studies in NHPs are likely to be required for mAb development. A general study design, using NHPs, for toxicity studies of one month and longer duration involves three treatment groups, of low, medium and high dose levels and one control group, each consisting of eight animals (four male and four female) (see **Table 6**). Additional animals (up to two males and two females), used to assess recovery from any toxic effects would be included in some or all of these groups. A development package of three studies with recovery animals uses up to 144 animals. Our data shows that that many companies typically undertake three studies to support their development programs and use an average of 100 -120 NHPs for each mAb (excluding reproductive toxicology studies).

Provided that there is evidence to show the mAb is at low risk of toxicity, (e.g., from a well-characterized class, no toxic effects at high doses in early studies) the working group has identified opportunities where this development program can be adapted to halve the animals used by reducing the number of chronic studies, dose groups and the need for recovery animals (see **Table 7**). As described later, there is also the opportunity to reduce the number of animals used by conducting a reduced reproductive toxicology program.

Reducing the number of toxicology studies, to a one month study and one further study. Planning in advance what studies are necessary to support the clinical need can help reduce the number of toxicology studies without compromising human safety. The number of studies should be based on understanding of the target, the intended clinical population and dosing regimen. For some mAbs, a one month study plus one further study may be sufficient to provide information for marketing authorisation.

A six month study is described in ICHS6 guidelines as being generally sufficient to identify long-term toxicity for biotechnology products, including mAbs. However, on occasion the regulators have asked for longer studies, although the reason for this has been unclear (authors' personal experience). A six month duration is scientifically justified based on the specific metabolic, PK and toxicological characteristics of large molecular weight proteins and is supported by a retrospective review of biotechnology-derived pharmaceuticals.[36] In rare cases, a longer duration study may be necessary, for example, if an entirely new toxicity presents in the six month study that was not observed in studies of shorter duration. However, for all mAbs, the formation of anti-mAb antibodies can limit the utility of longer studies and this should be taken into account when considering the need for extended studies.

Reducing the number of dose groups to two treatment (low and high) and one control. Dose selection for toxicity studies for mAbs is different to that for NCEs as any adverse effects are typically target related and due to exaggerated pharmacology rather than off-target toxicity. Generally, dose levels should be selected to cover the range between the No Observed Adverse Effect Level (NOAEL), the projected clinical dose equivalent and an overtly toxic dose. The high dose is either based on a multiple of the intended clinical dose or is the maximum feasible dose based on animal welfare considerations for large dose volumes rather than observed

Table 6. Typical study design for main repeat dose toxicity study

Dose group	Low	Medium	High	Control
No. of animals	4M + 4F	4M + 4F	4M + 4F	4M + 4F
No. of recovery	Up to 2M + 2F	Up to 2M + 2F	Up to 2M + 2F	Up to 2M + 2F
Total for one study				48
Maximum total per program (three studies)				144

*There is variation in approach between companies; not all companies carry out the studies exactly as described in this table.

Table 7. Impact of minimized study design

Dose group	Low	Medium	High	Control
(1) Reduction of dose groups to two treatment and one control				
No. of animals	3M + 3F		3M + 3F	3M + 3F
No. of recovery	Up to 2M + 2F		Up to 2M + 2F	Up to 2M + 2F
Total for one study				30
(2) Recovery animals only on the high dose group				
No. of animals	3M + 3F	3M + 3F	3M + 3F	3M + 3F
No. of recovery			Up to 2M + 2F	Up to 2M + 2F
Total for one study				32
(3) Combination of 1 and 2				
No. of animals	3M + 3F		3M + 3F	3M + 3F
No. of recovery			Up to 2M + 2F	Up to 2M + 2F
Total for one study				26
(4) Reduction of toxicology studies to a one month and one further study				
Total for minimized program (two studies)				52

toxicity.[10] For mAbs where toxicity is not pharmacologically mediated, the low dose (NOAEL) will often show 100% saturation of the target receptor and be equivalent to, or higher than, the intended clinical dose. The high dose is then selected to identify toxic effects above saturation point from high levels of circulating, unbound mAb. Based on the kinetics of the low and high doses, the additional scientific value of a mid-dose level group in the safety assessment of many mAbs is questionable.

There are also opportunities to reduce primate use for mAbs where the risk of toxicity is unknown, for instance a novel target. Accumulating toxicity information from early studies can be used to inform decisions to reduce primate use in later studies. For instance, the initial (two week or one month) study may be conducted with three dose levels in order to assess whether all three dose levels are scientifically valuable in longer term toxicity studies.

Only including recovery animals on the high dose group. For mAbs, recovery animals have been included on toxicity studies to evaluate the presence of anti-drug antibodies, the reversibility of an observed adverse effect and the potential for delayed toxicity. Where the liability for anti-mAb antibodies is understood and there is no demonstration of toxicity in early studies, the need for recovery animals should be questioned. Where they are necessary, the use of one recovery animal on the high dose and control groups only should be considered. Where a risk of delayed toxicity exists or reversibility of an adverse effect is an issue consideration should be given to including additional animals in the high dose group only, instead of up to two animals/sex/dose group as may currently be used.

Conducting a single pre- and post-natal development study. If reproductive toxicology studies are shown to be necessary and no potential alternative options to the NHP exist (e.g., rodent with the clinical or homologous protein[33]) the approach proposed by Jarvis et al. could be undertaken.[34,35] The paper describes an 'enhanced' PPND study design, which is conducted as a single study to replace the embryo fetal development (EFD) and the traditional PPND, which have historically been conducted as two separate studies. Additionally, this strategy incorporates fertility end-points in the chronic safety studies and reduces the numbers of dose groups in the pre- and post-natal development study (PPND).

This rationalized approach can be further enhanced by combining short term acute studies with PK/PD analysis and incorporating safety pharmacology endpoints into toxicology studies. Additionally, if the reproductive hazard has clearly been identified from other studies e.g., GA rodents, the opportunity to avoid a developmental toxicity study in NHPs be should be considered. This should include assessment of the added value and impact of the NHP study (e.g., labelling/contraindication in pregnancy).

Discussion

The working group has considered hypothetical examples of mAbs, based on real antibodies in development, as a tool for considering how the number of NHPs can be minimized during preclinical safety studies without compromising human safety. The examples have encapsulated the important and complex challenges faced when developing mAbs, such as no target antigen expression in normal tissue, low potency and high levels of immunogenicity in the NHP. These, in combination with factors linked to therapeutic area, such as immune modulation or ADCC/complement-dependent cytotoxicity (CDC) activity, illustrate that a flexible approach is required for the preclinical development of mAbs. This flexibility is also critical for minimising NHP use.

The use of the NHP plays an important role in assessing the safety of mAbs. Nevertheless, the close relatedness of the NHP to man does not necessarily guarantee that they are the most appropriate species for the development of mAbs as illustrated by the TGN1412 clinical trial in 2006 where the NHP had limited utility in detecting the cytokine storm subsequently observed in the phase 1 clinical trials.[26] Species selection should be based on the biology of the mAb and in particular its pharmacological activity. The NHP should not be used as 'default' species, as a screen or because NHP studies have previously been conducted.

The challenges of species selection and the use of NHPs have changed considerably, even over the last few years. Discussions in the working group have shown that screening for cross-reactivity in the NHP and making early decisions using primate data is now more commonplace. This, together with the increase in mAbs where the only relevant preclinical species is the NHP due to the development of humanised and fully human mAbs places further demands on NHP use. Recently, there has also been a trend towards larger and increased numbers of NHP studies due to the development of mAbs for chronic indications and non-life-threatening conditions and trials for chronic secondary indications of mAbs already in the clinic. In addition, there is increasing variability in the cell lines used for manufacturing mAbs. Changes in manufacturing (e.g., cell line) can trigger a whole new program of safety studies potentially using large numbers of NHPs. The need for such extensive studies should be questioned unless a PK or PD change is demonstrated in in vivo studies with the new product.

The use of homologous proteins, both rodent and NHP, has significant implications for the use of NHPs.[11] Our data shows that rodent homologous proteins are available for approximately 30% of mAbs, are being produced for a further 13% and are being used to provide safety data for 25% of mAbs in development. All companies which provided data agreed that homologous proteins would be more widely developed if there was regulatory agency support for the use of homologues to replace studies in NHPs. Currently, regulatory acceptance of the use of homologues is more likely if there is no relevant species to test the clinical molecule (e.g., infliximab, efalizumab).[37-39] Murine homologous proteins are the most frequently used. Wider exploitation of these tools could reduce the use of NHPs where there are issues with potency or immunogenicity. Similarly the use of homologous proteins when the NHP is relevant, for example in an integrated strategy where rodent homologous proteins are used for some studies (e.g., reproductive toxicology)

and the NHP for others, requires further investigation. However, due to some of the scientific and practical limitations of homologous proteins, such as the length of time taken to generate and characterize them, the use of murine homologous proteins in mAb development is controversial.[11,22] Nevertheless, their application remains firmly on the agenda as a result of specific advantages of using the mouse for pharmacology and toxicology studies. These include genetically homogeneous populations which can be powered to statistical significance, well-established toxicology endpoints (e.g., immunotoxicology, fertility) and the ability to test the mAb in disease models.[22]

The use of NHP homologous proteins is less common but two recent examples have been described.[22] However, the major limitation of homologous proteins, rodent or NHP, is that the clinical molecule is not being tested. The advantages of using the rodent may provide an incentive to use the homologue where it is pharmacologically comparable to the clinical molecule. However, the use of the NHP homologue does not provide these advantages, and carries with it the additional limitations of NHP studies in general (e.g., time, expense).

GA rodents have also been used to provide safety data (e.g., PK/PD, single and repeat dose toxicity studies) for some mAbs.[40-43] A variety of GA models exist; full KOs are used to assess the effect of absence of the target, conditional KOs, where the target is removed either later in development or post-natally, may be more representative of drug action and not complicated by redundancy or compensation, and KO/human knock-in models can enable studies to be conducted with the clinical molecule. There is potential for these models to be used to predict suitable doses for clinical trials, for reproductive toxicology studies and to answer specific mechanism-based safety questions. Currently, as for homologous proteins, the use of GA rodents is more acceptable to regulators when NHPs are not relevant.

Future use of homologous proteins and GA rodents is dependent on clear guidance on what makes these alternative models 'fit for purpose.' A description of the studies necessary to demonstrate comparability is included in a recently published review of the benefits and limitations of homologous proteins.[20] In the meantime, the use of homologues and GA rodents remains contentious and is further complicated by the undesirable potential for a two-species approach, where a full package of preclinical studies is conducted in parallel in both the NHP and the rodent. The scientific value of a parallel set of studies with a homologous protein or clinical molecule in the GA rodent is questionable and not consistent with ICHS6 guidelines.

The new generation of mAbs is highly engineered, and this is likely to add further challenges to assessing safety in preclinical development. From a clinical perspective, antibody engineering brings exciting potential, both the variable and constant regions can be tailored to increase specificity, efficiency and affinity of binding to improve therapeutic efficacy. Recent work has focussed on the specific mAb isotype used (i.e., IgG1, 2, 3 or 4) and the manipulation of amino acids or glycosylation patterns in the constant (Fc) region of the mAb to elicit greater ADCC and CDC function. For instance, mAbs that have backbones comprised of IgG1 and IgG3 domains have been shown in vitro and in vivo to demonstrate improved effector function and cytotoxicity.[44] It is known that there is individual patient variation in response to some mAbs, and this is due to their ability to elicit effector function. mAbs with amino acid changes to overcome these individual differences could increase the patient population that could benefit from these drugs. These attractive advances have the potential to exploit mAbs further and to contribute to personalized medicine. However, understanding these subtle differences preclinically is likely to be complicated, and the relevance of even closely related species must be demonstrated prior to embarking on a full set of toxicity studies in animals.

Currently, there is increased pressure on the use of NHP for the development of mAbs. Nevertheless, there are scientific, ethical and economic drivers for minimizing their use. Opportunities exist through the use of scientifically-based rationalized development programs, which take into account issues of species selection based on pharmacology, potency and immunogenicity, and the design and timing of preclinical studies based on the patient population, dosing regimen, and the availability of other approaches. To translate these findings into practice wider discussion is required both within companies and with the regulatory bodies (e.g., FDA, EMEA). The addendum of the ICHS6 guidelines provides a platform to do this.

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Disclaimer

The content of this paper is not official FDA opinion.

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