INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

DRAFT CONSENSUS GUIDELINE

ADDENDUM TO ICH S6: PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS S6(R1)

Current *Step 2* version
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At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Steering Committee to the regulatory authorities of the three ICH regions (the European Union, Japan and the USA) for internal and external consultation, according to national or regional procedures.

S6
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Draft ICH Consensus Guideline
Released for Consultation on 29 October 2009, at *Step 2* of the ICH Process

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADA	Anti-drug antibodies
ADC	Antibody drug conjugate
ePPND	Enhanced pre- and post-natal development
EU	European Union
GD	Gestational Day
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
i.v.	Intravenous
NHP	Non Human Primate
PK	Pharmacokinetics
PD	Pharmacodynamics
WOCBP	Women of Childbearing Potential

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S6 (R1)

1.   INTRODUCTION

1.1   Purpose of the Addendum

The purpose of the addendum is to provide clarification on and an update of the following topics discussed in the original ICH S6 guidance: species selection, study design, immunogenicity, reproductive and developmental toxicity and assessment of carcinogenic potential. Scientific advances and experience gained since publication of the original ICH S6 guidance call for this addendum.

This harmonised addendum will help to define the current recommendations and reduce the likelihood that substantial differences will exist among regions.

This guidance should facilitate the timely conduct of clinical trials, reduce the use of animals in accordance with the 3Rs (reduce/refine/replace) principles and reduce the use of other drug development resources. 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.

This guidance promotes safe and ethical development and availability of new pharmaceuticals.

1.2   Background

The recommendations of this addendum further harmonise the nonclinical safety studies to support the various stages of clinical development among the regions of European Union (EU), Japan, and the United States. The present guidance represents the consensus that exists regarding the safety evaluation of biotechnology-derived pharmaceuticals.

1.3   Scope of the Guideline

This addendum does not alter the scope of the original ICH S6 guideline.

2.   SPECIES SELECTION

2.1   General Principles

A number of factors should be taken into account when determining species relevancy. Comparisons of target sequence homology between species can be an appropriate starting point, followed by cell-based assays to make qualitative and quantitative cross-species comparisons of relative target binding affinities and receptor/ligand occupancy and kinetics. Assessments of functional activity are also recommended. Functional activity can be demonstrated in species-specific cell-based systems and/or *in vivo* pharmacology or toxicology studies. Modulation of a known biologic response or of a pharmacodynamic marker can provide evidence for functional activity to support species relevance.

Consideration of cross-species differences in target binding and functional activity in the context of the intended dosing regime should provide confidence that a model is capable of demonstrating any potentially adverse consequences of target modulation. When the target is not constitutively expressed in typical preclinical species, binding affinity and activity in cell-based systems can be sufficient to guide species selection.

For monoclonal antibodies and related products directed at foreign targets (i.e., bacterial, viral, etc.), it is desirable to evaluate safety in an animal model of disease. Where this is not feasible, a short-term safety study (see ICH S6) in one species (choice of species to be justified by the sponsor) can be considered and appropriate risk mitigation strategies should be adopted for clinical trials. No additional toxicity studies are appropriate.

As described in ICH S6, when no relevant species can be identified because the biopharmaceutical does not interact with the orthologous target in any species, use of homologous molecules, transgenic models and/or animal models of disease can be considered.

The potential for toxicity arising from a novel toxin or toxicant incorporated as an antibody-drug/toxin conjugate (ADC) is likely to occur in a target-independent manner and should be assessed in two species (one rodent and one non-rodent). Where possible, preference should be given to species that exhibit target-specific binding. For toxins or toxicants which are not novel and for which there is a sufficient body of scientific information available, safety evaluation of ADC in a single relevant species should suffice.

2.2   Tissue Cross-Reactivity

Based on recent scientific data, the text in ICH S6, Section 3.3 paragraph 2 is no longer appropriate. Immunohistochemical examination of potential binding of monoclonal antibodies and related products to the target epitope (tissue cross reactivity) should not be used for selection of relevant species for safety evaluation. Other techniques that assess target expression (e.g., *in situ* hybridization, flow cytometry) can provide supportive information for species selection.

However, tissue cross-reactivity data with human tissues can provide useful information to supplement knowledge of target distribution and can provide information on unexpected epitope binding. Tissue cross reactivity studies in nonclinical species are considered to have limited value and therefore are not generally recommended.

Binding to areas not typically accessible to the biopharmaceutical *in vivo* i.e., cytoplasm might not be relevant.

For bi-specific antibodies, evaluating each binding site separately in this assay is not called for.

2.3   One or Two Species

If there are two pharmacologically relevant species for the clinical candidate (one rodent and one non-rodent), then both species should be used for short-term (up to 1 month duration) toxicology studies. If the toxicological findings from these studies are similar in both species, then longer-term studies in one species are usually considered sufficient; the rodent species should be considered unless there is a rationale for using non-rodents. Studies in two non-rodent species are not appropriate.

The use of one species is justified when the biological activity of the biopharmaceutical is well understood or the clinical candidate is pharmacologically active in only one species.

Studies in a second species with a homologous product are not considered to add further value for risk assessment and are not recommended.

2.4 Use of Homologous Proteins, Transgenic Models, KOs and Disease Models

In some cases, alternative approaches to evaluating safety in a pharmacologically-relevant animal species should be used. ICH S6 Section 3.3 can be referenced for further information.

Homologous proteins can be used for hazard detection and understanding the potential for adverse effects due to exaggerated pharmacology, not for exposure-based quantitative risk assessment. It can be possible to conduct safety evaluation studies with homologous proteins using a control group and one dose group provided there is a scientific justification for the dose level selected.

3. STUDY DESIGN

3.1 Dose Selection and application of PK/PD Principles

The toxicity of most biopharmaceuticals is related to their targeted mechanism of action; therefore, relatively high doses can elicit adverse effects which are apparent as exaggerated pharmacology.

A rationale should be provided for high dose selection. PK-PD approaches can assist in high dose selection by identifying i) a dose which gives the maximum intended pharmacological effect in the preclinical species and ii) a dose which gives an up to 10-fold exposure multiple over the maximum exposure to be achieved in the clinic. The highest of these two doses should be chosen as the high dose group in pre-clinical toxicity studies unless scientific data supports a lower dose.

Where PD endpoints are not available, then an up to 10-fold multiple over the highest anticipated clinical exposure is sufficient, provided that corrections are made for differences in target binding and *in vitro* pharmacologic activity between the nonclinical species and humans. For example, a large relative difference in binding affinity and/or *in vitro* potency might suggest the need for higher doses in the nonclinical studies. In the event that toxicity cannot be demonstrated by this approach, then additional toxicity studies at higher multiples of human dosing are unlikely to provide additional useful information.

3.2 Duration of Studies

For chronic use products, the adequacy of 6-month chronic studies is supported by the scientific experience with biopharmaceuticals to date. Studies of longer duration are not anticipated to provide useful information to change the clinical course of development.

3.3 Recovery

Recovery of pharmacological and toxicological effects with potential adverse clinical impact should be understood. This information can be obtained by including a non dosing period in at least one study. The purpose of the non dosing period is to examine reversibility of these effects, not to assess delayed toxicity. The demonstration of complete recovery is not considered essential. An evaluation of recovery is not warranted if there are no adverse effects at the end of the dosing period or sufficient scientific justification can be provided (e.g., evidence that an adverse effect is generally reversible, or an adequate margin of safety exists for the proposed clinical population).

The addition of a recovery period just to assess for immunogenicity is not appropriate.

3.4 Exploratory Clinical Trials

The flexible approaches to support exploratory clinical trials as outlined in ICH M3(R2) can be applicable to biopharmaceuticals. It is recommended that these approaches be discussed and agreed upon with the appropriate regulatory authority.

4. IMMUNOGENICITY

Immunogenicity assessments are conducted to assist in the interpretation of the study results and design of subsequent studies. Such analyses in nonclinical animal studies are not relevant in terms of predicting potential immunogenicity of human or humanized proteins in humans.

Measurement of anti-drug antibodies (ADA) in nonclinical studies is not routinely warranted if there is evidence of sustained pharmacodynamic activity, no unexpected changes in the pharmaco/toxicokinetics of the test article during the dosing or recovery phase, and/or no evidence of immune-mediated reactions (immune complex-related, vasculitis, anaphylaxis, etc.). However, it is difficult to predict whether such analysis will be called for prior to completion of the in-life phase of the study; therefore, it is often useful to obtain appropriate samples during the course of the study, which can subsequently be analyzed if needed to aid in interpretation of the study results. When study results suggest there is a need to understand immunogenicity to interpret study data, potential for immunogenicity antibody detection assays should be conducted to evaluate the presence of ADAs. When ADAs are detected, the effect on the study results should be assessed, including effects on PK and drug clearance, pharmacology effects, and toxicity. Characterization, specifically of neutralizing potential, is generally not warranted, particularly if adequate exposure and pharmacological effect can be demonstrated by a pharmacodynamic marker of activity in the *in vivo* toxicology studies. In the event that neutralizing antibody assessment is deemed appropriate to interpret the study findings, assessment of neutralizing activity can be addressed indirectly with *ex-vivo* bioactivity assay, a combination of assay formats for PK-PD, or directly in a specific neutralizing antibody assay.

5. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

5.1 General Comments

Reproductive toxicity studies should be conducted in accordance with the principles outlined in with ICH S5(R2); however, the specific study design and dosing schedule can be modified based on an understanding of species specificity, the nature of the product and mechanism of action, immunogenicity and/or pharmacokinetic behaviour and embryo-fetal exposure.

Note 2 of ICH S6 still applies with respect to products where there is public knowledge regarding toxicity to reproduction.

The evaluation of toxicity to reproduction should be conducted only in pharmacologically relevant species. When the clinical candidate is pharmacologically active in rodents and rabbits, these species should be used unless there is a scientific reason to use a NHP. As per ICH S5 Note 5 (2.1), one species can be sufficient to address effects on embryo-fetal development if there is more than one relevant species (see Section on species selection). The sponsor should provide a scientific rationale for selection of species for assessment of effects on embryo-fetal development.

When the clinical candidate is pharmacologically active only in NHP, an appropriate assessment of reproductive toxicity in NHPs is generally preferred over alternative approaches such as studies with homologous products in other species. However, the sponsor should provide a scientific justification when these alternative approaches are proposed. When the weight of evidence (e.g., mechanism of action, phenotypic data from KO mice, class effects) suggests that there will be an adverse effect on pregnancy outcome, these data might provide adequate information to communicate risk, and additional nonclinical studies might not be warranted.

When no relevant animal species exists for the clinical candidate, the use of transgenic mice expressing the human target or homologous protein in a species expressing the human ortholog should be considered.

5.2   Fertility

For products where rodents are a relevant species, an assessment of fertility can be made in a rodent species. The design of the study should be amended as appropriate, for example, to address the nature of the product and potential for immunogenicity.

When the NHP is the only relevant species, the potential for effects on male and female fertility can be assessed by standard histopathological evaluation and assessment of menstrual cyclicity in repeat dose toxicity studies of at least 3 months duration using sexually mature NHPs. If there is a specific cause for concern, specialized assessments such as sperm count, sperm morphology/motility, testicular volume, and male or female reproductive hormone levels should be evaluated in the repeat dose toxicity study.

It is recognized that mating studies are not practical for NHPs. If there is a specific concern from the pharmacological activity about potential effects on conception / implantation and the NHP is the only relevant species, the concern should be addressed experimentally. A homologous product or transgenic model could be the only practical means to assess potential effects on conception or implantation when those are of specific concern. However, it would not be appropriate to produce a homologous product or transgenic model only to conduct mating studies in rodents.

5.3   Embryo-fetal and Pre/Post-Natal Development

The type of molecule and potential differences in placental transfer should be considered in the choice of species for testing – see note 1.

For products pharmacologically active only in NHPs, one well-designed study in NHPs (stage C to E ICH S5a) which includes dosing from day 20 of gestation to birth can be considered. It is also possible for the sponsor to provide a scientific justification for the evaluation of effects on embryo-fetal and postnatal development using alternative study designs or a homologous product in rodents.

For the single NHP study design addressing ICH S5a stages C to E, no caesarian Section group is warranted, but assessment of pregnancy outcome at natural delivery should be performed. This study should also evaluate offspring viability and survival, external malformations, skeletal effects (e.g., by X-ray) and, ultimately, visceral morphology at necropsy. Ultrasound is useful to track maintenance of pregnancy but not for monitoring embryo-fetal development or detecting malformations. Other endpoints in the offspring can also be evaluated if relevant for the pharmacological activity (e.g., immune function or neurobehavioural assessment). The duration of the postnatal phase will be dependent on which additional endpoints are considered relevant for the pharmacological activity.

The numbers of confirmed pregnant cynomolgus monkeys per group should be sufficient to detect 3-fold increase in pregnancy/parturition-associated losses with 80% power with 95% confidence interval (Jarvis, P. *et al*, abstract European Teratology Society Meeting, 2009). The sponsor should justify the study design if other NHP species are used.

Because the developmental toxicity study in NHP as outlined above is a hazard identification study, it might be possible to conduct these studies using a control group and one dose group, provided there is a scientific justification for the dose level selected. An example of an appropriate scientific justification would be a monoclonal antibody which binds a soluble target and the clinical dosing regimen is intended to saturate target binding. If such a saturation of target binding can be demonstrated in the animal species selected and there is an up to 10-fold exposure multiple over therapeutic drug levels, a single dose level and control group would provide adequate evidence of hazard to embryo-fetal development.

These study design principles can also be more appropriate than separate ICH S5a C and D to E studies for biopharmaceuticals other than monoclonal antibodies.

5.4 Timing of studies

For monoclonal antibodies for which embryo-fetal exposure during organogenesis is understood to be low in humans based on current scientific knowledge, the embryo-fetal development toxicity study can be conducted during Phase III (see ICH M3(R2)). The completed reports should be available to support submission of a marketing application. For other biological products where embryo-fetal exposure is demonstrated to be low during organogenesis, the same timing for testing can be applicable. Where there is embryo-fetal exposure during organogenesis and the product is pharmacologically active only in NHPs and a sponsor elects to use the ePPND study design, an interim report (see note 2) for data to day 7 post-partum for all animals is called for to support Phase III.

If the rodent or rabbit is a relevant species and embryo-fetal exposure is demonstrated, see ICH M3(R2) for timing of reproductive toxicity studies. ICH M3(R2) should also be followed for the timing of data on fertility for products where rodents are relevant species.

For oncology products which fall within the scope of ICH S9, see this guidance for aspects relating to timing.

6. CARCINOGENICITY

The need for a product-specific assessment of the carcinogenic potential for biopharmaceutical should be determined with regard to the intended clinical population and treatment duration (see ICH S1a). When an assessment is warranted, the sponsor should design a strategy to address the issue.

This strategy could be based on a review of relevant data from a variety of sources. The data sources can include published data (e.g., information from transgenic, knock-out or animal disease models, human genetic diseases), information on class effects, detailed information on target biology, *in vitro* data, data from chronic toxicity studies and clinical data.

The product specific assessment of carcinogenic potential is used to communicate risk and provide input to the risk management plan along with labelling proposals, clinical monitoring, post-marketing surveillance, or a combination of these approaches.

In some cases, the available information can be considered sufficient to address carcinogenic potential and inform clinical risk without warranting additional nonclinical studies. For example, immunomodulators and growth factors pose a potential carcinogenic risk which can best be evaluated by post-marketing clinical surveillance rather than further nonclinical studies.

The mode of action of some biopharmaceuticals might raise concern regarding potential for neoplasm induction or tumour promotion. Rodent bioassays are not warranted if data from *in vitro* or chronic toxicity studies support the concern regarding carcinogenic potential. When *in vitro* and chronic toxicity studies do not support this theoretical risk but the sponsor prefers not to label on this basis, the sponsor can propose additional studies that could mitigate the concern.

For products where there is insufficient knowledge about specific product characteristics and mode of action in relation to carcinogenic potential, a more extensive assessment might be appropriate. The sponsor should consider the inclusion of additional endpoints in toxicity studies. If the weight of evidence from the product-specific assessment (e.g., *in vitro* and chronic toxicity studies) does not suggest carcinogenic potential, a rodent bioassay is not recommended. If the weight of evidence suggests a concern about carcinogenic potential, then the label should reflect the concern or the sponsor can propose additional nonclinical studies that could mitigate the concern.

Rodent bioassays or short-term carcinogenicity studies with homologous products are generally of limited value to assess carcinogenic potential of the clinical candidate.

There is clearly a need for better assessment tools. Alternative approaches can be considered as new strategies / assays are developed.

7.   ENDNOTES

Note 1: High molecular weight proteins (>5,000 D) do not cross the placenta by simple diffusion. For monoclonal antibodies with molecular weight as high as 150,000 D, there exists a specific transport mechanism, (FcRn) which determines fetal exposure and varies across species.

In the NHP and human, IgG does not begin to cross the placenta until early second trimester and increases to higher levels late in the third trimester; thus, in NHPs and humans, IgG crosses the placenta only after organogenesis (Pentsuk and Van der Laan, 2009). Therefore, standard embryo-fetal studies in NHPs, which are dosed from early pregnancy up to Gestation Day 50, are not representative of human fetal exposure throughout pregnancy for a parenterally administered therapeutic IgG and might assess only indirect effects on embryo-fetal development. Therefore, this might not be the optimal study design to assess indirect and direct effects of treatment throughout gestation.

IgG crosses the yolk sac in rodent/rabbits by FcRn transport mechanism and exposure will occur during late organogenesis. In addition, offspring of rat/mouse dams dosed during lactation will be exposed via the milk.

Note 2: Endpoints to be included in interim report of ePPND study in non human primates;

Dam data: survival, clinical observations, bodyweight, gestational exposure data (if available), any specific PD endpoints;

Pregnancy data: number of pregnant animals started on study, pregnancy status at end of organogenesis (GD50) and at GD100 as a minimum, abortions and timing of abortions. There is no need for ultrasound observations of fetal size in the interim report; these are not considered essential since actual birth weight will be available;

Pregnancy outcome data: number of live births / still births, infant birth weight, infant survival and bodyweight at day 7 post-partum, qualitative external morphological assessment (i.e., confirming appearance is within normal limits), infant exposure data (if available), any specific PD endpoints in the infant if appropriate.

8. REFERENCES

1. ICH S6 Guideline: Safety Studies for Biotechnological Products; July 1997.

2. ICH S5(R2) Guideline: Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility; June 1993.

3. ICH S1A Guideline: Guideline on the Need for Carcinogenicity Studies for Pharmaceuticals; November 1995.

4. ICH M3(R2) Guideline: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals; June 2009.

5. ICH S9 Guideline: Nonclinical Evaluation for Anticancer Pharmaceuticals; November 2008.

6. Pentsuk N, Van der Laan JW. An interspecies comparison of placental antibody transfer: new insights into developmental toxicity testing of monoclonal antibodies. Birth defects research (Part B) 2009; 86: 328-344.

7. Jarvis P et al. abstract European Teratology Society Meeting, 2009.