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

ICH HARMONISED TRIPARTITE GUIDELINE

PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS
S6

Current *Step 4* version

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This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

S6
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PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS

ICH Harmonised Tripartite Guideline

Having reached Step 4 of the ICH Process at the ICH Steering Committee meeting on 16 July 1997, this guideline is recommended for adoption to the three regulatory parties to ICH

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PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS

1. INTRODUCTION

1.1 Background

Biotechnology-derived pharmaceuticals (biopharmaceuticals) were initially developed in the early 1980s. The first marketing authorisations were granted later in the decade. Several guidelines and points-to-consider documents have been issued by various regulatory agencies regarding safety assessment of these products. Review of such documents, which are available from regulatory authorities, may provide useful background in developing new biopharmaceuticals.

Considerable experience has now been gathered with submission of applications for biopharmaceuticals. Critical review of this experience has been the basis for development of this guidance that is intended to provide general principles for designing scientifically acceptable preclinical safety evaluation programs.

1.2 Objectives

Regulatory standards for biotechnology-derived pharmaceuticals have generally been comparable among the European Union, Japan and United States. All regions have adopted a flexible, case-by-case, science-based approach to preclinical safety evaluation needed to support clinical development and marketing authorisation. In this rapidly evolving scientific area, there is a need for common understanding and continuing dialogue among the regions.

The primary goals of preclinical safety evaluation are: 1) to identify an initial safe dose and subsequent dose escalation schemes in humans; 2) to identify potential target organs for toxicity and for the study of whether such toxicity is reversible; and 3) to identify safety parameters for clinical monitoring. Adherence to the principles presented in this document is intended to improve the quality and consistency of the preclinical safety data supporting the development of biopharmaceuticals.

1.3 Scope

This guidance is intended primarily to recommend a basic framework for the preclinical safety evaluation of biotechnology-derived pharmaceuticals. It applies to products derived from characterised cells through the use of a variety of expression systems including bacteria, yeast, insect, plant, and mammalian cells. The intended indications may include *in vivo* diagnostic, therapeutic, or prophylactic uses. The active substances include proteins and peptides, their derivatives and products of which they are components; they could be derived from cell cultures or produced using recombinant DNA technology including production by transgenic plants and animals. Examples include but are not limited to: cytokines, plasminogen activators, recombinant plasma factors, growth factors, fusion proteins, enzymes, receptors, hormones, and monoclonal antibodies.

The principles outlined in this guidance may also be applicable to recombinant DNA protein vaccines, chemically synthesised peptides, plasma derived products, endogenous proteins extracted from human tissue, and oligonucleotide drugs.

This document does not cover antibiotics, allergenic extracts, heparin, vitamins, cellular blood components, conventional bacterial or viral vaccines, DNA vaccines, or cellular and gene therapies.

2. SPECIFICATION OF THE TEST MATERIAL

Safety concerns may arise from the presence of impurities or contaminants. It is preferable to rely on purification processes to remove impurities and contaminants rather than to establish a preclinical testing program for their qualification. In all cases, the product should be sufficiently characterised to allow an appropriate design of preclinical safety studies.

There are potential risks associated with host cell contaminants derived from bacteria, yeast, insect, plants, and mammalian cells. The presence of cellular host contaminants can result in allergic reactions and other immunopathological effects. The adverse effects associated with nucleic acid contaminants are theoretical but include potential integration into the host genome. For products derived from insect, plant and mammalian cells, or transgenic plants and animals there may be an additional risk of viral infections.

In general, the product that is used in the definitive pharmacology and toxicology studies should be comparable to the product proposed for the initial clinical studies. However, it is appreciated that during the course of development programs, changes normally occur in the manufacturing process in order to improve product quality and yields. The potential impact of such changes for extrapolation of the animal findings to humans should be considered.

The comparability of the test material during a development program should be demonstrated when a new or modified manufacturing process or other significant changes in the product or formulation are made in an ongoing development program. Comparability can be evaluated on the basis of biochemical and biological characterisation (i.e., identity, purity, stability, and potency). In some cases additional studies may be needed (i.e., pharmacokinetics, pharmacodynamics and/or safety). The scientific rationale for the approach taken should be provided.

3. PRECLINICAL SAFETY TESTING

3.1 General principles

The objectives of the preclinical safety studies are to define pharmacological and toxicological effects not only prior to initiation of human studies but throughout clinical development. Both *in vitro* and *in vivo* studies can contribute to this characterisation. Biopharmaceuticals that are structurally and pharmacologically comparable to a product for which there is wide experience in clinical practice may need less extensive toxicity testing.

Preclinical safety testing should consider:

1) selection of the relevant animal species;
2) age;
3) physiological state;
4) the manner of delivery, including dose, route of administration, and treatment regimen; and
5) stability of the test material under the conditions of use.

Toxicity studies are expected to be performed in compliance with Good Laboratory Practice (GLP); however, it is recognised that some studies employing specialised test systems which are often needed for biopharmaceuticals, may not be able to comply fully with GLP. Areas of non-compliance should be identified and their significance evaluated relative to the overall safety assessment. In some cases, lack of full GLP compliance does not necessarily mean that the data from these studies cannot be used to support clinical trials and marketing authorisations.

Conventional approaches to toxicity testing of pharmaceuticals may not be appropriate for biopharmaceuticals due to the unique and diverse structural and biological properties of the latter that may include species specificity, immunogenicity, and unpredicted pleiotropic activities.

3.2 Biological activity/ pharmacodynamics

Biological activity may be evaluated using *in vitro* assays to determine which effects of the product may be related to clinical activity. The use of cell lines and/or primary cell cultures can be useful to examine the direct effects on cellular phenotype and proliferation. Due to the species specificity of many biotechnology-derived pharmaceuticals, it is important to select relevant animal species for toxicity testing. *In vitro* cell lines derived from mammalian cells can be used to predict specific aspects of *in vivo* activity and to assess quantitatively the relative sensitivity of various species (including human) to the biopharmaceutical. Such studies may be designed to determine, for example, receptor occupancy, receptor affinity, and/or pharmacological effects, and to assist in the selection of an appropriate animal species for further *in vivo* pharmacology and toxicology studies. The combined results from *in vitro* and *in vivo* studies assist in the extrapolation of the findings to humans. *In vivo* studies to assess pharmacological activity, including defining mechanism(s) of action, are often used to support the rationale of the proposed use of the product in clinical studies.

For monoclonal antibodies, the immunological properties of the antibody should be described in detail, including its antigenic specificity, complement binding, and any unintentional reactivity and/or cytotoxicity towards human tissues distinct from the intended target. Such cross-reactivity studies should be carried out by appropriate immunohistochemical procedures using a range of human tissues.

3.3 Animal species/model selection

The biological activity together with species and/or tissue specificity of many biotechnology-derived pharmaceuticals often preclude standard toxicity testing designs in commonly used species (e.g., rats and dogs). Safety evaluation programs should include the use of relevant species. A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies). A variety of techniques (e.g., immunochemical or functional tests) can be used to identify a relevant species. Knowledge of receptor/epitope distribution can provide greater understanding of potential *in vivo* toxicity.

Relevant animal species for testing of monoclonal antibodies are those that express the desired epitope and demonstrate a similar tissue cross-reactivity profile as for human tissues. This would optimise the ability to evaluate toxicity arising from the binding to the epitope and any unintentional tissue cross-reactivity. An animal species which does not express the desired epitope may still be of some relevance for assessing toxicity if comparable unintentional tissue cross-reactivity to humans is demonstrated.

Safety evaluation programs should normally include two relevant species. However, in certain justified cases one relevant species may suffice (e.g., when only one relevant species can be identified or where the biological activity of the biopharmaceutical is well understood). In addition even where two species may be necessary to characterise toxicity in short term studies, it may be possible to justify the use of only one species for subsequent long term toxicity studies (e.g., if the toxicity profile in the two species is comparable in the short term).

Toxicity studies in non-relevant species may be misleading and are discouraged. When no relevant species exists, the use of relevant transgenic animals expressing the human receptor or the use of homologous proteins should be considered. The information gained from use of a transgenic animal model expressing the human receptor is optimised when the interaction of the product and the humanised receptor has similar physiological consequences to those expected in humans. While useful information may also be gained from the use of homologous proteins, it should be noted that the production process, range of impurities/contaminants, pharmacokinetics, and exact pharmacological mechanism(s) may differ between the homologous form and the product intended for clinical use. Where it is not possible to use transgenic animal models or homologous proteins, it may still be prudent to assess some aspects of potential toxicity in a limited toxicity evaluation in a single species, e.g., a repeated dose toxicity study of $\leq$ 14 days duration that includes an evaluation of important functional endpoints (e.g., cardiovascular and respiratory).

In recent years, there has been much progress in the development of animal models that are thought to be similar to the human disease. These animal models include induced and spontaneous models of disease, gene knockout(s), and transgenic animals. These models may provide further insight, not only in determining the pharmacological action of the product, pharmacokinetics, and dosimetry, but may also be useful in the determination of safety (e.g., evaluation of undesirable promotion of disease progression). In certain cases, studies performed in animal models of disease may be used as an acceptable alternative to toxicity studies in normal animals (*Note 1*). The scientific justification for the use of these animal models of disease to support safety should be provided.

3.4 Number/ gender of animals

The number of animals used per dose has a direct bearing on the ability to detect toxicity. A small sample size may lead to failure to observe toxic events due to observed frequency alone regardless of severity. The limitations that are imposed by sample size, as often is the case for non-human primate studies, may be in part compensated by increasing the frequency and duration of monitoring. Both genders should generally be used or justification given for specific omissions.

3.5 Administration/dose selection

The route and frequency of administration should be as close as possible to that proposed for clinical use. Consideration should be given to pharmacokinetics and bioavailability of the product in the species being used, and the volume which can be safely and humanely administered to the test animals. For example, the frequency of administration in laboratory animals may be increased compared to the proposed schedule for the human clinical studies in order to compensate for faster clearance rates or low solubility of the active ingredient. In these cases, the level of exposure of the test animal relative to the clinical exposure should be defined. Consideration should also be given to the effects of volume, concentration, formulation, and site of administration. The use of routes of administration other than those used clinically

may be acceptable if the route must be modified due to limited bioavailability, limitations due to the route of administration, or to size/physiology of the animal species.

Dosage levels should be selected to provide information on a dose-response relationship, including a toxic dose and a no observed adverse effect level (NOAEL). For some classes of products with little to no toxicity it may not be possible to define a specific maximum dose. In these cases, a scientific justification of the rationale for the dose selection and projected multiples of human exposure should be provided. To justify high dose selection, consideration should be given to the expected pharmacological/physiological effects, availability of suitable test material, and the intended clinical use. Where a product has a lower affinity to or potency in the cells of the selected species than in human cells, testing of higher doses may be important. The multiples of the human dose that are needed to determine adequate safety margins may vary with each class of biotechnology-derived pharmaceutical and its clinical indication(s).

3.6 Immunogenicity

Many biotechnology-derived pharmaceuticals intended for human are immunogenic in animals. Therefore, measurement of antibodies associated with administration of these types of products should be performed when conducting repeated dose toxicity studies in order to aid in the interpretation of these studies. Antibody responses should be characterised (e.g., titer, number of responding animals, neutralising or non-neutralising), and their appearance should be correlated with any pharmacological and/or toxicological changes. Specifically, the effects of antibody formation on pharmacokinetic/pharmacodynamic parameters, incidence and/or severity of adverse effects, complement activation, or the emergence of new toxic effects should be considered when interpreting the data. Attention should also be paid to the evaluation of possible pathological changes related to immune complex formation and deposition.

The detection of antibodies should not be the sole criterion for the early termination of a preclinical safety study or modification in the duration of the study design unless the immune response neutralises the pharmacological and/or toxicological effects of the biopharmaceutical in a large proportion of the animals. In most cases, the immune response to biopharmaceuticals is variable, like that observed in humans. If the interpretation of the data from the safety study is not compromised by these issues, then no special significance should be ascribed to the antibody response.

The induction of antibody formation in animals is not predictive of a potential for antibody formation in humans. Humans may develop serum antibodies against humanised proteins, and frequently the therapeutic response persists in their presence. The occurrence of severe anaphylactic responses to recombinant proteins is rare in humans. In this regard, the results of guinea pig anaphylaxis tests, which are generally positive for protein products, are not predictive for reactions in humans; therefore, such studies are considered of little value for the routine evaluation of these types of products.

4. SPECIFIC CONSIDERATIONS

4.1 Safety pharmacology

It is important to investigate the potential for undesirable pharmacological activity in appropriate animal models and, where necessary, to incorporate particular monitoring for these activities in the toxicity studies and/or clinical studies. Safety pharmacology studies measure functional indices of potential toxicity. These functional indices may be investigated in separate studies or incorporated in the design of toxicity studies. The aim of the safety pharmacology studies should be to reveal any functional effects on the major physiological systems (e.g., cardiovascular, respiratory, renal, and central nervous systems). Investigations may also include the use of isolated organs or other test systems not involving intact animals. All of these studies may allow for a mechanistically-based explanation of specific organ toxicities, which should be considered carefully with respect to human use and indication(s).

4.2 Exposure assessment

4.2.1 Pharmacokinetics and toxicokinetics

It is difficult to establish uniform guidelines for pharmacokinetic studies for biotechnology-derived pharmaceuticals. Single and multiple dose pharmacokinetics, toxicokinetics, and tissue distribution studies in relevant species are useful; however, routine studies that attempt to assess mass balance are not useful. Differences in pharmacokinetics among animal species may have a significant impact on the predictiveness of animal studies or on the assessment of dose response relationships in toxicity studies. Alterations in the pharmacokinetic profile due to immune-mediated clearance mechanisms may affect the kinetic profiles and the interpretation of the toxicity data. For some products there may also be inherent, significant delays in the expression of pharmacodynamic effects relative to the pharmacokinetic profile (e.g., cytokines) or there may be prolonged expression of pharmacodynamic effects relative to plasma levels.

Pharmacokinetic studies should, whenever possible, utilise preparations that are representative of that intended for toxicity testing and clinical use, and employ a route of administration that is relevant to the anticipated clinical studies. Patterns of absorption may be influenced by formulation, concentration, site, and/or volume. Whenever possible, systemic exposure should be monitored during the toxicity studies.

When using radiolabeled proteins, it is important to show that the radiolabeled test material maintains activity and biological properties equivalent to that of the unlabeled material. Tissue concentrations of radioactivity and/or autoradiography data using radiolabeled proteins may be difficult to interpret due to rapid *in vivo* metabolism or unstable radiolabeled linkage. Care should be taken in the interpretation of studies using radioactive tracers incorporated into specific amino acids because of recycling of amino acids into non-drug related proteins/peptides.

Some information on absorption, disposition and clearance in relevant animal models should be available prior to clinical studies in order to predict margins of safety based upon exposure and dose.

4.2.2 Assays

The use of one or more assay methods should be addressed on a case-by-case basis and the scientific rationale should be provided. One validated method is usually considered sufficient. For example, quantitation of TCA-precipitable radioactivity

following administration of a radiolabeled protein may provide adequate information, but a specific assay for the analyte is preferred. Ideally the assay methods should be the same for animals and humans. The possible influence of plasma binding proteins and/or antibodies in plasma/serum on the assay performance should be determined.

4.2.3 Metabolism

The expected consequence of metabolism of biotechnology-derived pharmaceuticals is the degradation to small peptides and individual amino acids. Therefore, the metabolic pathways are generally understood. Classical biotransformation studies as performed for pharmaceuticals are not needed.

Understanding the behaviour of the biopharmaceutical in the biologic matrix, (e.g., plasma, serum, cerebral spinal fluid) and the possible influence of binding proteins is important for understanding the pharmacodynamic effect.

4.3 Single dose toxicity studies

Single dose studies may generate useful data to describe the relationship of dose to systemic and/or local toxicity. These data can be used to select doses for repeated dose toxicity studies. Information on dose- response relationships may be gathered through the conduct of a single dose toxicity study, as a component of pharmacology or animal model efficacy studies. The incorporation of safety pharmacology parameters in the design of these studies should be considered.

4.4 Repeated dose toxicity studies

For consideration of the selection of animal species for repeated dose studies see section 3.3. The route and dosing regimen (e.g., daily versus intermittent dosing) should reflect the intended clinical use or exposure. When feasible, these studies should include toxicokinetics.

A recovery period should generally be included in study designs to determine the reversal or potential worsening of pharmacological/toxicological effects, and/or potential delayed toxic effects. For biopharmaceuticals that induce prolonged pharmacological/toxicological effects, recovery group animals should be monitored until reversibility is demonstrated. The duration of repeated dose studies should be based on the intended duration of clinical exposure and disease indication. This duration of animal dosing has generally been 1-3 months for most biotechnology-derived pharmaceuticals. For biopharmaceuticals intended for short-term use (e.g., $\leq$ to 7 days) and for acute life-threatening diseases, repeated dose studies up to two weeks duration have been considered adequate to support clinical studies as well as marketing authorisation. For those biopharmaceuticals intended for chronic indications, studies of 6 months duration have generally been appropriate although in some cases shorter or longer durations have supported marketing authorisations. For biopharmaceuticals intended for chronic use, the duration of long term toxicity studies should be scientifically justified.

4.5 Immunotoxicity studies

One aspect of immunotoxicological evaluation includes assessment of potential immunogenicity (see section 3.6). Many biotechnology-derived pharmaceuticals are intended to stimulate or suppress the immune system and therefore may affect not only humoral but also cell-mediated immunity. Inflammatory reactions at the injection site may be indicative of a stimulatory response. It is important, however, to recognise that simple injection trauma and/or specific toxic effects caused by the formulation vehicle may also result in toxic changes at the injection site. In addition,

the expression of surface antigens on target cells may be altered, which has implications for autoimmune potential. Immunotoxicological testing strategies may require screening studies followed by mechanistic studies to clarify such issues. Routine tiered testing approaches or standard testing batteries, however, are not recommended for biotechnology-derived pharmaceuticals.

4.6 Reproductive performance and developmental toxicity studies

The need for reproductive/developmental toxicity studies is dependent upon the product, clinical indication and intended patient population (*Note 2*). The specific study design and dosing schedule may be modified based on issues related to species specificity, immunogenicity, biological activity and/or a long elimination half-life. For example, concerns regarding potential developmental immunotoxicity, which may apply particularly to certain monoclonal antibodies with prolonged immunological effects, could be addressed in a study design modified to assess immune function of the neonate.

4.7 Genotoxicity studies

The range and type of genotoxicity studies routinely conducted for pharmaceuticals are not applicable to biotechnology-derived pharmaceuticals and therefore are not needed. Moreover, the administration of large quantities of peptides/proteins may yield uninterpretable results. It is not expected that these substances would interact directly with DNA or other chromosomal material (*Note 3*).

Studies in available and relevant systems, including newly developed systems, should be performed in those cases where there is cause for concern about the product (e.g., because of the presence of an organic linker molecule in a conjugated protein product). The use of standard genotoxicity studies for assessing the genotoxic potential of process contaminants is not considered appropriate. If performed for this purpose, however, the rationale should be provided.

4.8 Carcinogenicity studies

Standard carcinogenicity bioassays are generally inappropriate for biotechnology-derived pharmaceuticals. However, product-specific assessment of carcinogenic potential may still be needed depending upon duration of clinical dosing, patient population and/or biological activity of the product (e.g., growth factors, immunosuppressive agents, etc.) When there is a concern about carcinogenic potential a variety of approaches may be considered to evaluate risk.

Products that may have the potential to support or induce proliferation of transformed cells and clonal expansion possibly leading to neoplasia should be evaluated with respect to receptor expression in various malignant and normal human cells that are potentially relevant to the patient population under study. The ability of the product to stimulate growth of normal or malignant cells expressing the receptor should be determined. When *in vitro* data give cause for concern about carcinogenic potential, further studies in relevant animal models may be needed. Incorporation of sensitive indices of cellular proliferation in long term repeated dose toxicity studies may provide useful information.

In those cases where the product is biologically active and non-immunogenic in rodents and other studies have not provided sufficient information to allow an assessment of carcinogenic potential then the utility of a single rodent species should be considered. Careful consideration should be given to the selection of doses. The use of a combination of pharmacokinetic and pharmacodynamic endpoints with consideration of comparative receptor characteristics and intended human exposures

represents the most scientifically based approach for defining the appropriate doses. The rationale for the selection of doses should be provided.

4.9 Local tolerance studies

Local tolerance should be evaluated. The formulation intended for marketing should be tested; however, in certain justified cases, the testing of representative formulations may be acceptable. In some cases, the potential adverse effects of the product can be evaluated in single or repeated dose toxicity studies thus obviating the need for separate local tolerance studies.

NOTES

Note 1 Animal models of disease may be useful in defining toxicity endpoints, selection of clinical indications, and determination of appropriate formulations, route of administration, and treatment regimen. It should be noted that with these models of disease there is often a paucity of historical data for use as a reference when evaluating study results. Therefore, the collection of concurrent control and baseline data is critical to optimise study design.

Note 2 There may be extensive public information available regarding potential reproductive and/or developmental effects of a particular class of compounds (e.g., interferons) where the only relevant species is the non-human primate. In such cases, mechanistic studies indicating that similar effects are likely to be caused by a new but related molecule, may obviate the need for formal reproductive/developmental toxicity studies. In each case, the scientific basis for assessing the potential for possible effects on reproduction/development should be provided.

Note 3 With some biopharmaceuticals there is a potential concern about accumulation of spontaneously mutated cells (e.g., via facilitating a selective advantage of proliferation) leading to carcinogenicity. The standard battery of genotoxicity tests is not designed to detect these conditions. Alternative *in vitro* or *in vivo* models to address such concerns may have to be developed and evaluated.