PRECLINICAL TOXICOLOGY

GUIDANCE FOR INDUSTRY - ICH M3 AND S6



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PRECLINICAL TOXICOLOGY

GUIDANCE FOR INDUSTRY – ICH GUIDANCES

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PACIFIC BIOLABS – YOUR PARTNER FOR PRECLINICAL SAFETY TESTING

As ***The Service Leader in Bioscience Testing***, Pacific BioLabs (PBL) strives to help our clients deliver safe and effective pharmaceuticals to the patients who need them. Well designed and executed preclinical studies are critical to the success of any drug development program. They must reliably assess the safety of a new drug entity, laying the groundwork for clinical trials and ultimately, regulatory approval.

Pacific BioLabs interacts closely with our clients, providing quality nonclinical testing results to meet regulatory requirements and guide your drug development decisions. As part of our commitment to our clients, we have prepared a pair of publications that will assist you in planning your preclinical testing program. This publication presents two major ICH guidance documents that directly address safety testing of new pharmaceuticals:

- Guidance M3 – *Nonclincal Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals*
- Guidance S6 – *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*

Both documents are presented in their entirety and can be used as references on questions regarding requirements for safety testing of new chemical entities (NCEs).

The FDA website http://www.fda.gov/cder/guidance contains these two documents along with a variety of other references on regulatory expectations for the nonclinical development of NCEs. Topics include various aspects of safety (e.g. reproductive, carcinogenicity and genotoxicity), ADME (e.g. bioanalytical, pharmacokinetics and toxicokinetics), and safety pharmacology.

PBL's companion publication – *Preclinical Toxicology - Points to Consider in Program Design*, is available on our website at PacificBioLabs.com. We hope you find these resources useful, and we wish you the best in your continuing quest to develop safe and effective new medicines.

Tom Spalding
President
Pacific BioLabs

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GUIDANCE FOR INDUSTRY – M3

NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS FOR PHARMACEUTICALS (July 1997 ICH)

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GUIDANCE FOR INDUSTRY – M3

NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS FOR PHARMACEUTICALS

I. INTRODUCTION (1)

A. Objectives of the Guidance (1.1)

The purpose of this document is to recommend international standards for and to promote harmonization of the nonclinical safety studies needed to support human clinical trials of a given scope and duration.

Harmonization of the guidance for nonclinical safety studies will help to define the current recommendations and reduce the likelihood that substantial differences will exist between regions.

This guidance should facilitate the timely conduct of clinical trials and reduce the unnecessary use of animals and other resources. This should promote safe and ethical development and availability of new pharmaceuticals.

B. Background (1.2)

The recommendations for the extent of nonclinical safety studies to support the various stages of clinical development differ among the regions of Europe, the United States, and Japan. This raises the important question of whether there is scientific justification for these differences and whether it would be possible to develop a mutually acceptable guidance.

The present guidance represents the consensus that exists among the ICH regions regarding the scope and duration of nonclinical safety studies to support the conduct of human clinical trials for pharmaceuticals.

C. Scope of the Guidance (1.3)

The nonclinical safety study recommendations for the marketing approval of a pharmaceutical usually include single and repeated dose toxicity studies, reproduction toxicity studies, genotoxicity studies, local tolerance studies, and for drugs that have special cause for concern or are intended for a long duration of use, an assessment of carcinogenic potential. Other nonclinical studies include pharmacology studies for safety assessment (safety pharmacology) and pharmacokinetic (absorption, distribution, metabolism, and excretion (ADME)) studies. These types of studies and their relation to the conduct of human clinical trials are presented in this guidance.

This guidance applies to the situations usually encountered during the conventional development of pharmaceuticals and should be viewed as providing general guidance for drug development. Animal safety studies and human clinical trials should be planned and designed to represent an approach that is scientifically and ethically appropriate for the pharmaceutical under development.

There have been marked changes in the kinds of therapeutic agents being developed (e.g., biotechnology-derived products), and the existing paradigms for safety evaluation may not always be appropriate or relevant. The safety evaluation in such cases should be considered on a case-by-case basis as described in the ICH guidance "Safety Studies in Biotechnological Products" (Ref. 1). Similarly, pharmaceuticals under development for indications in life-threatening or serious diseases without current effective therapy may also warrant a case-by-case approach to both the toxicological evaluation and clinical development to optimize and expedite drug development. In these cases, particular studies may be abbreviated, deferred, or omitted.

D. General Principles (1.4)

The development of a pharmaceutical is a stepwise process involving an evaluation of both the animal and human safety information. The goals of the nonclinical safety evaluation include: A characterization of toxic effects with respect to target organs, dose dependence, relationship to exposure, and potential reversibility. This information is important for the estimation of an initial safe starting dose for the human trials and the identification of parameters for clinical monitoring for potential adverse effects. The nonclinical safety studies, although limited at the beginning of clinical development, should be adequate to characterize potential toxic effects under the conditions of the supported clinical trial.

Human clinical trials are conducted to demonstrate the efficacy and safety of a pharmaceutical, starting with a relatively low exposure in a small number of subjects. This is followed by clinical trials in which exposure usually increases by dose, duration, and/or size of the exposed patient population. Clinical trials are extended based on the demonstration of adequate safety in the previous clinical trial(s) as well as additional nonclinical safety information that is available as the clinical trials proceed. Serious adverse clinical or nonclinical findings may influence the continuation of clinical trials and/or suggest the need for additional nonclinical studies and a reevaluation of previous clinical adverse events to resolve the issue.

Clinical trials are conducted in phases for which different terminology has been utilized in the various regions. This document uses the terminology as defined in the ICH guidance" General Considerations for Clinical Trials" (Ref. 2). Clinical trials may be grouped by their purpose and objectives. The first human exposure studies are generally single dose studies, followed by dose escalation and short-term repeated dose studies to evaluate pharmacokinetic parameters and tolerance (Phase I studies — Human Pharmacology studies). These studies are often conducted in healthy volunteers but may also include patients. The next phase of trials consists of exploratory efficacy and safety studies in patients (Phase II studies — Therapeutic Exploratory studies). This is followed by confirmatory clinical trials for efficacy and safety in patient populations (Phase III studies— Therapeutic Confirmatory studies).

II. SAFETY PHARMACOLOGY (2)

Safety pharmacology includes the assessment of effects on vital functions, such as cardiovascular, central nervous, and respiratory systems, and these should be evaluated prior to human exposure. These evaluations may be conducted as additions to toxicity studies or as separate studies.

III. TOXICOKINETIC AND PHARMACOKINETIC STUDIES (3)

Exposure data in animals should be evaluated prior to human clinical trials (Ref. 3). Further information on ADME in animals should be made available to compare human and animal metabolic pathways. Appropriate information should usually be available by the time the Phase I (Human Pharmacology) studies have been completed.

IV. SINGLE DOSE TOXICITY STUDIES (4)

The single dose (acute) toxicity for a pharmaceutical should be evaluated in two mammalian species prior to the first human exposure (Note 1). A dose escalation study is considered an acceptable alternative to the single dose design.

V. REPEATED DOSE TOXICITY STUDIES (5)

The recommended duration of the repeated dose toxicity studies is usually related to the duration, therapeutic indication, and scale of the proposed clinical trial. In principle, the duration of the animal toxicity studies conducted in two mammalian species (one nonrodent) should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated dose toxicity studies (Tables 1 and 2).

In certain circumstances, where significant therapeutic gain has been shown, trials may be extended beyond the duration of supportive repeated dose toxicity studies on a case-by-case basis.

A. Phase I and II Studies (5.1)

A repeated dose toxicity study in two species (one nonrodent) for a minimum duration of 2-4 weeks (Table 1) would support Phase I (Human Pharmacology) and Phase II(Therapeutic Exploratory) studies up to 2 weeks in duration. Beyond this, 1-, 3-, or 6-month toxicity studies would support these types of human clinical trials for up to 1, 3, or 6 months, respectively. Six-month rodent and chronic nonrodent studies (Ref. 11) would support clinical trials of longer duration than 6 months.

Table 1.—Duration of Repeated Dose Toxicity Studies to Support Phase I and II Trials in the EU and Phase I, II, and III Trials in the United States and Japan[1]

Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies	
	Rodents	Nonrodents
Single Dose	2-4 Weeks[2]	2 Weeks
Up to 2 Weeks	2-4 Weeks[2]	2 Weeks
Up to 1 Month	1 Month	1 Month
Up to 3 Months	3 Months	3 Months
Up to 6 Months	6 Months	6 Months[3]
> 6 Months	6 Months	Chronic

B. Phase III Studies (5.2)

For the Phase III (Therapeutic Confirmatory) studies, the recommendations for the United States and Japan are the same as those in

[1] In Japan, if there are no Phase II clinical trials of equivalent duration to the planned Phase III trials, conduct of longer1duration toxicity studies should be considered as given in Table 2.

[2] In the EU and the United States, 2-week studies are the minimum duration. In Japan, 2-week nonrodent and 4-week2rodent studies are needed (Also see Note 2). In the United States, as an alternative to 2-week studies, single dose toxicity studies with extended examinations can support single dose human trials (Ref. 4).

[3] See Ref. 11. Data from 6 months of administration in nonrodents should be available before the initiation of clinical3trials longer than 3 months. Alternatively, if applicable, data from a 9-month nonrodent study should be available before the treatment duration exceeds that which is supported by the available toxicity studies.

Table 1. In the EU, a 1-month toxicity study in two species (one non-rodent) would support clinical trials of up to 2 weeks duration (Table2). Three-month toxicity studies would support clinical trials for up to 1 month duration, while 6-month toxicity studies in rodents and 3-month studies in nonrodents would support clinical trials of a duration up to 3 months. For longer term clinical trials, a 6-month study in rodents and a chronic study in nonrodents are recommended.

Table 2.—Duration of Repeated Dose Toxicity Studies to Support Phase III Trials in the EU and Marketing in All Regions[1]

Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies	
	Rodents	Nonrodents
Up to 2 Weeks	1 Month	1 Month
Up to 1 Month	3 Months	3 Months
Up to 3 Months	6 Months	3 Months
> 3 Months	6 Months	Chronic[2]

VI. LOCAL TOLERANCE STUDIES (6)

Local tolerance should be studied in animals using routes relevant to the proposed clinical administration. The evaluation of local tolerance should be performed prior to human exposure. The assessment of local tolerance may be part of other toxicity studies.

VII. GENOTOXICITY STUDIES (7)

Prior to first human exposure, in vitro tests for the evaluation of mutations and chromosomal damage are generally needed. If an equivocal or positive finding occurs, additional testing should be performed (Ref. 5).The standard battery of tests for genotoxicity (Ref. 6) should be completed prior to the initiation of Phase II studies.

[1] The above table also reflects the marketing recommendations in the three regions except that a chronic nonrodent1study is recommended for clinical use > 1 month.

[2] See Ref. 11.

VIII. CARCINOGENICITY STUDIES (8)

Completed carcinogenicity studies are not usually needed in advance of the conduct of clinical trials unless there is cause for concern. Conditions relevant for carcinogenicity testing are discussed in the ICH document (Ref. 7).

For pharmaceuticals developed to treat certain serious diseases, carcinogenicity testing, if needed, may be concluded postapproval.

IX. REPRODUCTION TOXICITY STUDIES (9)

Reproduction toxicity studies (Refs. 8 and 9) should be conducted as is appropriate for the population that is to be exposed.

A. Men (9.1)

Men may be included in Phase I and II trials prior to the conduct of the male fertility study since an evaluation of the male reproductive organs is performed in the repeated dose toxicity studies (Note 2).

A male fertility study should be completed prior to the initiation of Phase III trials (Refs. 8and 9).

B. Women Not of Childbearing Potential (9.2)

Women not of childbearing potential (i.e., permanently sterilized, postmenopausal) may be included in clinical trials without reproduction toxicity studies provided the relevant repeated dose toxicity studies (which include an evaluation of the female reproductive organs) have been conducted.

C. Women of Childbearing Potential (9.3)

For women of childbearing potential there is a high level of concern for the unintentional exposure of an embryo/fetus before information is available concerning the potential benefits versus potential risks. There are currently regional differences in the timing of reproduction toxicity studies to support the inclusion of women of childbearing potential in clinical trials.

In Japan, assessment of female fertility and embryo-fetal development should be completed prior to the inclusion of women of childbearing potential using birth control in any type of clinical trial. In the EU, assessment of embryo-fetal development should be completed prior to Phase I trials in women of childbearing potential and female fertility studies prior to Phase III trials.

In the United States, women of childbearing potential may be included in early, carefully monitored studies without reproduction toxicity studies provided appropriate precautions are taken to minimize risk. These precautions include pregnancy testing (for example, based on the b-subunit of HCG), use of a highly effective method of birth control (Note3), and entry after a confirmed menstrual period. Continued testing and monitoring during the trial should be sufficient to ensure compliance with the measures not to become pregnant during the period of drug exposure (which may exceed the length of study). To support this approach, informed consent should include any known pertinent information related to reproductive toxicity, such as a general assessment of potential toxicity of pharmaceuticals with related structures or pharmacological effects. If no relevant information is available, the informed consent should clearly note the potential for risk.

In the United States, assessment of female fertility and embryo-fetal development should be completed before women of childbearing potential using birth control are enrolled in Phase III trials.

In the three regions, the pre- and postnatal development study should be submitted for marketing approval or earlier if there is cause for concern. For all regions, all female reproduction toxicity studies (Ref. 8) and the standard battery of genotoxicity tests (Ref.6) should be completed prior to the inclusion, in any clinical trial, of women of childbearing potential not using highly effective birth control (Note 3) or whose pregnancy status is unknown.

D. Pregnant Women (9.4)

Prior to the inclusion of pregnant women in clinical trials, all the reproduction toxicity studies (Refs. 8 and 9) and the standard battery of genotoxicity tests (Ref. 6) should be conducted. In addition, safety data from previous human exposure are generally needed.

X. SUPPLEMENTARY STUDIES (10)

Additional nonclinical studies may be needed if previous nonclinical or clinical findings with the product or related products have indicated special safety concerns.

XI. CLINICAL TRIALS IN PEDIATRIC POPULATIONS (11)

When pediatric patients are included in clinical trials, safety data from previous adult human exposure would usually represent the most relevant information and should generally be available before pediatric clinical trials. The necessity for adult human data would be determined on a case-by-case basis.

In addition to appropriate repeated dose toxicity studies, all reproduction toxicity studies (Ref. 8) and the standard battery of genotoxicity tests (Ref. 6) should be available prior to the initiation of trials in pediatric populations. Juvenile animal studies should be considered on an individual basis when previous animal data and human safety data are insufficient.

The need for carcinogenicity testing should be addressed prior to long term exposure in pediatric clinical trials considering the length of treatment or cause for concern (Ref. 7).

XII. CONTINUING EFFORTS TO IMPROVE HARMONIZATION (12)

It is recognized that significant advances in harmonization of the timing of nonclinical safety studies for the conduct of human clinical trials for pharmaceuticals have already been achieved and are detailed in this guidance. However, differences remain in a few areas. These include toxicity studies to support first entry into man and the recommendations for reproduction toxicity studies for women of childbearing potential. Regulators and industry will continue to consider these differences and work towards further improving the drug development process.

XIII. ENDNOTES (13)

Note 1 For the conduct of single dose toxicity studies, refer to the ICH-1 recommendations (Ref.10) and the regional guidances.

Note 2 There are currently regional differences for the minimum duration of repeated dose toxicity studies; 2 weeks in the EU and the United States, and 2 weeks nonrodent and 4 weeks rodent in Japan. In Japan, unlike the EU and the United States, the male fertility study has usually been conducted prior to the inclusion of men in clinical trials. However, an assessment of male fertility by careful histopathological examination in the rodent 4-week repeated dose toxicity study has been found to be more sensitive in detecting effects on male reproductive organs than fertility studies (Ref. 9), and is now recommended to be performed prior to the first clinical trial in Japan. In the EU and the United States, 2-week repeated dose studies are considered adequate for an overall assessment of the potential toxicity of a drug to support clinical trials for a short duration.

Note 3 A highly effective method of birth control is defined as one that results in a low failure rate (i.e., less than 1 percent per year) when used consistently and correctly, such as implants, injectables, combined oral contraceptives, some intrauterine contraceptive devices (IUDs), sexual abstinence, or a vasectomized partner. For subjects using a hormonal contraceptive method, information regarding the product under evaluation and its potential effect on the contraceptive should be addressed.

XIV. REFERENCES (14)

1. ICH Topic S6 Document "Preclinical Testing of Biotechnology-Derived Pharmaceuticals."

2. ICH Topic E8 Document "General Considerations for Clinical Trials."

3. ICH Harmonised Tripartite Guideline (S3A) Note for "Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies."

4. FDA, "Single Dose Acute Toxicity Testing for Pharmaceuticals; Revised Guidance," 61 FR43934 to 43935, August 26, 1996.

5. ICH Harmonised Tripartite Guideline (S2A) "Guidance on Specific Aspects of Regulatory Genotoxicity Tests."

6. ICH Topic S2B document "Standard Battery of Genotoxicity Tests."

7. ICH Harmonised Tripartite Guideline (S1A) "Guideline on the Need for Carcinogenicity Studies for Pharmaceuticals."

8. ICH Harmonised Tripartite Guideline (S5A) "Detection of Toxicity to Reproduction for Medicinal Products."

9. ICH Harmonised Tripartite Guideline (S5B) "Toxicity to Male Fertility."

10. Arcy, P. F., and D. W. G. Harron, "Proceeding of The First International Conference on Harmonisation, Brussels 1991," Queen´s University of Belfast, pp 183-184 (1992).

11. ICH Topic S4 Document "Duration of Chronic Toxicity Testing in Animals (Rodent and Nonrodent Toxicity Testing)."

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GUIDANCE FOR INDUSTRY – S6

PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS (July 1997 ICH)

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GUIDANCE FOR INDUSTRY – S6

PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS

I. INTRODUCTION (1)

A. Background (1.1)

Biotechnology-derived pharmaceuticals (biopharmaceuticals) were initially developed in the early1980's. The first marketing authorizations 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, which is intended to provide general principles for designing scientifically acceptable preclinical safety evaluation programs.

B. Objectives (1.2)

Regulatory standards for biotechnology-derived pharmaceuticals have generally been comparable among the European Union, Japan, and the United States. All three regions have adopted a flexible, case-by-case, science-based approach to preclinical safety evaluation needed to support clinical development and marketing authorization. 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 should improve the quality and consistency of the preclinical safety data supporting the development of biopharmaceuticals.

C. Scope (1.3)

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 characterized 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 deoxyribonucleic acid (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 synthesized 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.

II. SPECIFICATION OF THE TEST MATERIAL (2)

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 characterized 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 is developed 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 characterization (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.

III. PRECLINICAL SAFETY TESTING (3)

A. General Principles (3.1)

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 characterization. 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 recognized that some studies employing specialized test systems, which are often needed for biopharmaceuticals, may not be able to comply fully with GLP. Areas of noncompliance 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 authorizations.

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.

B. Biological Activity/Pharmacodynamics (3.2)

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.

C. Animal Species/Model Selection (3.3)

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 optimize the ability to evaluate toxicity arising from the binding to the epitope and any unintentional tissue cross-reactivity. An animal species that 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 characterize 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 nonrelevant 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 optimized when the interaction of the product and the humanized 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 < 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.

D. Number/Gender of Animals (3.4)

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 nonhuman 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.

E. Administration/Dose Selection (3.5)

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 to 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).

F. Immunogenicity (3.6)

Many biotechnology-derived pharmaceuticals intended for humans 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 characterized (e.g., titer, number of responding animals, neutralizing or non-neutralizing) 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 neutralizes 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 humanized 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.

IV. SPECIFIC CONSIDERATIONS (4)

A. Safety Pharmacology (4.1)

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).

B. Exposure Assessment (4.2)

1. Pharmacokinetics and Toxicokinetics (4.2.1)

It is difficult to establish uniform guidances for pharmacokinetic studies for biotechnology-derived pharmaceuticals. Single and multiple dose pharmacokinetics, toxiccokinetics, 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, utilize preparations that are representative of those 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 nondrug 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.

2. Assays (4.2.2)

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.

3. Metabolism (4.2.3)

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 behavior 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.

C. Single Dose Toxicity Studies (4.3)

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 or 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.

D. Repeated Dose Toxicity Studies (4.4)

For consideration of the selection of animal species for repeated dose studies, see section III.C (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., < to 7 days)and for acute life-threatening diseases, repeated dose studies up to 2 weeks duration have been considered adequate to support clinical studies as well as marketing authorization. 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 authorizations. For biopharmaceuticals intended for chronic use, the duration of long-term toxicity studies should be scientifically justified.

E. Immunotoxicity Studies (4.5)

One aspect of immunotoxicological evaluation includes assessment of potential immunogenicity (see section III.F (3.6)). Many biotechnology-derived pharmaceuticals are intended to stimulate or suppress the immune system and, therefore, may affect not only humeral but also cell-mediated immunity. Inflammatory reactions at the injection site may be indicative of a stimulatory response. It is important, however, to recognize 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.

F. Reproductive Performance and Developmental Toxicity Studies (4.6)

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.

G. Genotoxicity Studies (4.7)

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.

H. Carcinogenicity Studies (4.8)

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 nonimmunogenic 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.

I. Local Tolerance Studies (4.9)

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 optimize 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 nonhuman 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.

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Pacific BioLabs conducts its operations in a stunning 32,000 square foot facility in Hercules, CA overlooking the San Francisco Bay. The building houses a 12,000 square foot vivarium with a surgery suite, necropsy lab, radiation lab, procedure rooms, and ample support areas. The semi-barrier SPF rodent suite has a HEPA filtered air supply and dedicated procedure space. Animal facilities and critical equipment are monitored 24/7. Emergency power is supplied by an on-site generator. The site can accommodate a planned 20,000 square foot facility expansion.

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