

M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals

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

ICH HARMONISED TRIPARTITE GUIDELINE

**GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT
OF HUMAN CLINICAL TRIALS AND
MARKETING AUTHORIZATION FOR PHARMACEUTICALS**

M3(R2)

Current *Step 2* version
dated 15 July 2008

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HUMAN CLINICAL TRIALS AND
MARKETING AUTHORIZATION FOR PHARMACEUTICALS**

Draft ICH Consensus Guideline

Released for Consultation on 15 July 2008, at *Step 2* of the ICH Process

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LIST OF ABBREVIATIONS

AUC	Area Under the Curve
EU	European Union
GLP	Good Laboratory Practices
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IUD	Intra-Uterine Device
MFD	Maximum Feasible Dose
MTD	Maximum Tolerated Dose
NOAEL	No Observed Adverse Effect Level
PET	Positron Emission Tomography
PK	Pharmacokinetics
PD	Pharmacodynamics
SAR	Structure-Activity Relationship
t _{1/2}	Half Life
WOCBP	Women of Childbearing Potential

GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS

1. INTRODUCTION

1.1 Objectives of the Guideline

The purpose of this document is to recommend international standards for, and promote harmonisation of, the nonclinical safety studies recommended to support human clinical trials of a given scope and duration and marketing authorization.

Harmonisation 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, reduce the use of animals in accordance with the 3R (reduce/refine/replace) principles and reduce the use of other drug development resources. This should promote safe and ethical development and availability of new pharmaceuticals.

1.2 Background

The recommendations of this revised guidance further harmonize the nonclinical safety studies to support the various stages of clinical development among the regions of Europe, USA, and Japan. The present guideline represents the consensus that exists regarding the scope and duration of nonclinical safety studies to support the conduct of human clinical trials and marketing authorization for pharmaceuticals.

1.3 Scope of the Guideline

The nonclinical safety study recommendations for the marketing approval of a pharmaceutical usually include safety pharmacology studies, repeated dose toxicity studies, toxicokinetic and nonclinical pharmacokinetic studies, reproduction toxicity studies, genotoxicity 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 including phototoxicity studies, immunotoxicity studies, juvenile animal toxicity studies, and abuse potential studies should be conducted on a case-by-case basis as appropriate. These types of studies and their relation to the conduct of human clinical trials are presented in this guideline.

This guideline 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.

It is generally recognized that the types of safety studies conducted in the evaluation of biotechnology-derived products (as defined in Ref. 1) are varied and should be determined in accordance with the ICH guideline for biotechnology-derived products. The present ICH guideline (M3) can provide general insight for biotechnology-derived

products only with regard to timing of nonclinical studies relative to clinical development stage.

Pharmaceuticals under development for indications in life threatening or serious diseases (e.g., advanced cancer, resistant HIV infection, and congenital enzyme deficiency disease) without current effective therapy might also warrant a case-by-case approach to both the toxicological evaluation and clinical development to optimise and expedite drug development. In these cases and for products using innovative therapeutic modalities (e.g., siRNA), as well as vaccine adjuvants, particular studies can/might be abbreviated, deferred, omitted, or added.

1.4 General Principles

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 generally include a characterisation of toxic effects with respect to target organs, dose dependence, relationship to exposure, and when appropriate, potential reversibility. This information is helpful for the estimation of an initial safe starting dose and dose range 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 characterise potential toxic effects that might occur 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 duration and/or size of the exposed patient population. Clinical trials should be extended based on the demonstration of adequate safety in the previous clinical study(ies), as well as, additional nonclinical safety information that become available as clinical development proceeds.

Serious adverse clinical or nonclinical findings can influence the continuation of clinical trials and/or suggest the appropriateness of additional nonclinical studies. These nonclinical findings should be evaluated in light of the clinical results in determining the appropriateness of additional nonclinical studies and the nature of those studies.

Clinical trials are conducted in phases for which different terminology has been utilised in the various regions. This document generally uses the terminology as defined in the ICH guideline “General Considerations for the Clinical Trials” (Ref. 2). However, as there is a growing trend to merge phases of clinical development, in some cases this document also relates the nonclinical studies to the duration and size of clinical trials and the nature of the subjects included.

2. SAFETY PHARMACOLOGY

The core battery of safety pharmacology studies includes the assessment of effects on cardiovascular, central nervous and respiratory systems, and should generally be conducted prior to human exposure in accordance with ICH guidelines S7A and S7B (Refs. 3 and 4). If warranted, supplemental and follow-up safety pharmacology studies can be conducted during later clinical development. Consideration should be given to inclusion of any *in vivo* evaluations as additions to general toxicity studies, to the extent feasible, in order to reduce animal use.

3. TOXICOKINETIC AND PHARMACOKINETIC STUDIES

In vitro metabolic data for animals and humans, and exposure data (Ref. 5) in animals should be evaluated prior to initiating human clinical trials. Further information on absorption, distribution, metabolism and excretion in animals should be available prior to exposing large numbers of human subjects or treating for long duration (generally prior to Phase 3). These data can be used to compare human and animal metabolites and for determining if any additional testing is warranted.

4. ACUTE TOXICITY STUDIES

Historically, acute toxicity information has been obtained from single dose toxicity studies in two mammalian species using both the clinical and a parenteral route of administration. However, such information can be obtained from appropriately conducted dose escalation studies or short duration dose ranging studies that define a maximum tolerated dose in the general toxicity test species (Refs. 6 and 7). Other equally appropriate studies include those that achieve large exposure multiples (e.g., 50-fold the clinical C_{max} or AUC at the intended human dose), achieve saturation of exposure, or use the maximum feasible dose. In all cases a limit dose of 2000 mg/kg/day in rodents and 1000 mg/kg/day in non-rodents is considered appropriate for acute, subchronic, and chronic toxicity studies (See Note 1). When this acute toxicity information is available from any study, separate single dose studies are not recommended. Studies providing acute toxicity information can be limited to the clinical route only and such data can be obtained from non-GLP studies if clinical administration is supported by appropriate GLP repeated dose toxicity studies.

In some specific situations (e.g., microdose studies; see Section 7) acute toxicity or single dose studies can be the primary support for single dose studies in humans. In these situations, because these nonclinical studies are conducted to support human safety, the high dose selection can be different than that described above, but should be adequate to support the intended clinical dose and route. These studies should be performed in compliance with GLP and lethality should not be an intended endpoint.

Information on the acute toxicity of pharmaceutical agents could be useful to predict the consequences of human overdose situations and should be available prior to Phase 3. An earlier assessment of acute toxicity could also be important for therapeutic indications for which patient populations are at higher risk for overdosing (e.g., depression, pain, and dementia) in out-patient clinical trials.

5. REPEATED DOSE TOXICITY STUDIES

The recommended duration of the repeated dose toxicity studies is usually related to the duration, therapeutic indication and scope of the proposed clinical trial. In principle, the duration of the animal toxicity studies conducted in two mammalian species (one non-rodent) 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 (Table 1). See Note 1 for limit doses/exposures that are considered appropriate in repeated dose toxicity studies.

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

5.1 Clinical Development Trials

Repeated dose toxicity studies in two species (one non-rodent) for a minimum duration of 2 weeks (Table 1) would generally support any clinical development trial up to 2 weeks in duration.

Clinical trials of longer duration should be supported by repeated dose toxicology studies of at least equivalent duration. Six month rodent and 9 month non-rodent studies would generally support dosing for longer than 6 months in clinical trials (for exceptions see Table 1 footnotes).

Table 1 Duration of Repeated Dose Toxicity Studies to Support the Conduct of Clinical Trials in All Regions

Maximum Duration of Clinical Trial	Minimum Duration of Repeated Dose Toxicity Studies to Support Clinical Trials	
	Rodents	Non-rodents
Up to 2 weeks	2 weeks ^a	2 weeks
Between 2 weeks and 6 months	Same as clinical trial	Same as clinical trial
> 6 months	6 months	9 months ^{b, c, d}

- a. In Japan, WOCBP can be included in clinical trials if repeated dose toxicity studies of at least 2 weeks in at least one species (generally rodents) have been completed with careful histopathological evaluation of the ovary.
- b. In the EU, studies of 6 month duration in non-rodents are considered acceptable. However, where studies with a longer duration have been conducted, it would not be considered appropriate to conduct an additional study of 6 months.

The following are examples where non-rodent studies of up to 6 month duration can also be appropriate for Japan and US:

- When immunogenicity or tolerance problems confound conduct of longer term studies;
 - Repeated short-term drug exposure even if clinical trial duration exceeds 6 months, such as intermittent treatment of migraine, erectile dysfunction, or herpes simplex;
 - Drugs intended for indications for life-threatening diseases, such as cancer chemotherapy in advanced disease or in adjuvant use.
- c. There can be cases where a pediatric population is the primary population, existing animal studies do not adequately address developmental concerns for target organs of toxicology or pharmacology, and long-term juvenile animal toxicity testing in a non-rodent would be valuable. In this case, a chronic study initiated in the appropriate age and species of juvenile animals that can address this developmental concern (e.g., 12 months duration in dog) can be appropriate. This could replace the standard chronic non-rodent and separate juvenile animal study in non-rodents.
 - d. Clinical trials of longer duration than 6 months can be initiated providing the data are available from a 3 month rodent and a 3 month non-rodent study, and complete data from the chronic rodent and non-rodent study are made

available, consistent with local clinical trial procedures, before 3 months of dosing is exceeded in the clinical trial.

If the chronic rodent data are available, for serious or life-threatening indications or on a case-by-case basis, this extension can be based on in-life and necropsy data in cases where the complete histopathology data are available within 3 additional months.

5.2 Marketing Authorization

Because of the size of the population at risk and the relatively less controlled conditions in clinical practice in contrast to clinical trials, longer durations of nonclinical testing can be valuable (see Table 2). For durations of continuous use of less than or equal to 3 months see Table 2 below. For a small number of conditions in which the indicated use is between 2 weeks and 3 months, but for which there is extensive clinical experience of both widespread and long-term use beyond that recommended (e.g., anxiety, seasonal allergic rhinitis, pain), the duration of testing might more appropriately be equivalent to that recommended for treatment of greater than 3 months.

Table 2 Duration of Repeated Dose Toxicity Studies to Support Marketing in all Regions

Duration of Indicated Treatment	Rodent	Non-rodent
Up to 2 weeks	1 month	1 month
>2 weeks to 1 month	3 months	3 months
>1 month to 3 months	6 months	6 months
>3 months	6 months	9 months ^{b, c d}

N.B. See footnotes **b**, **c**, and **d** in Table 1.

6. ESTIMATION OF THE FIRST DOSE IN HUMAN

The estimation of the first dose in humans is an important element to safeguard the safety of subjects participating in first-in-human studies. All of the relevant nonclinical data, including the pharmacological dose response, the pharmacological/toxicological profile, and pharmacokinetics, should be considered when determining the recommended starting dose in humans.

In general, the No Observed Adverse Effect Level (NOAEL) determined in nonclinical safety studies performed in the most sensitive and relevant animal species gives the most important information. The relevant dose can then be modified by various factors, including pharmacodynamics, particular aspects of the molecule, and the design of the clinical trials. See available Regional guidance for specific approaches that can be used.

Exploratory clinical trials (see Section 7) in humans can be initiated with less, or different, nonclinical support than is generally warranted for traditional clinical studies and, therefore, the estimation of the clinical starting (and maximal) dose can differ. The recommended criteria for starting doses for the various exploratory clinical trial designs are described in Table 3.

7. EXPLORATORY CLINICAL STUDIES

It is recognized that in some cases insight on human physiology/pharmacology, knowledge of drug candidate characteristics and therapeutic target relevance to disease are benefited by earlier access to human data. Streamlined early exploratory approaches can accomplish this end. Exploratory clinical studies for the purpose of this guidance are those intended to be conducted early in Phase 1, involve limited human exposure, have no therapeutic or diagnostic intent, and are not intended to examine maximum tolerated dose. They can be used to investigate a variety of parameters such as pharmacokinetics, pharmacodynamics and other biomarkers, which could include PET receptor binding and displacement.

The amount of nonclinical supporting data that is appropriate in these situations will be dependent on the extent of proposed human exposure, both with respect to the maximum clinical dose used and the duration of dosing. Five different exploratory clinical approaches are described as examples below (and in more detail in Table 3), together with the nonclinical testing programs that would be recommended in these particular approaches. However, other approaches not described in this guidance can also be used. These should be discussed and agreed upon with the appropriate regulatory authority. The use of these approaches is expected to reduce overall animal use in drug development.

Appropriate starting doses and stopping doses for each approach are included in Table 3. In all cases, characterization of pharmacodynamics and pharmacology using *in vivo* and/or *in vitro* models is important and should be used in support of human dose selection.

7.1 Microdose Studies

Two different microdose approaches are described below.

The first approach would involve not more than a total dose of 100 µg that can be divided among up to five doses in any subject. This could be useful to investigate target receptor binding or tissue distribution in a PET study. A second use could be to assess pharmacokinetics with or without the use of an isotopically labelled agent. These uses could be supported by an extended single dose toxicity study in one species, usually rodent, by the clinical route of administration, together with appropriate characterization of pharmacology.

The second microdose approach is one that involves ≤ 5 administrations of a maximum of 100 µg per administration (a total of 500 µg per subject). This can be useful for similar applications as for the first microdose approach described above, but with less active PET ligands. This approach could be supported by a 7 day toxicity study in one species, usually rodent, by the clinical route of administration, together with SAR assessment of the genotoxic potential of the unlabeled compound and appropriate characterisation of pharmacology.

In some situations it could be appropriate to carry out a clinical microdose study using the i.v. route on a product intended for oral administration and for which an oral nonclinical toxicology package already exists. In this case the i.v. microdose can be qualified by the existing oral repeated dose toxicity studies in cases where adequate exposure margins have been achieved. It is not recommended to investigate i.v. local tolerance in this situation because the administered dose is very low (i.e., 100 µg maximum).

7.2 Single Dose Studies up to Sub-therapeutic or Intended Therapeutic Range

The third approach involves a single dose up to sub-therapeutic (pharmacological) or therapeutic range in subjects (see Table 3). The maximum allowable dose should be derived from the nonclinical data. It can be further limited based on clinical information obtained during the single dose study in humans. This approach could allow determination of pharmacokinetic parameters (such as $t_{1/2}$ or bioavailability) with non-radiolabeled drug at or near the predicted pharmacodynamically active dose. This approach could be supported by extended single dose toxicity studies in both a rodent and non-rodent species, an assessment of genotoxic potential (Ames test), appropriate characterisation of pharmacology and core battery of Safety Pharmacology studies.

7.3 Multiple Dose Studies

The fourth and fifth approach involve up to 14 days of dosing for determination of pharmacokinetics and pharmacodynamics in human, and are not intended to support the determination of maximum tolerated clinical dose.

The fourth approach involves 2 week repeated dose toxicity studies in rodents and non-rodents where dose selection is based on exposure multiples of anticipated AUC at the maximum clinical dose. If these studies adequately characterise the toxicity of the molecule in both species, the maximum dose in the exploratory clinical study would be based on a standard safety/risk assessment taking into account the nature, severity and monitorability of the nonclinical findings. In the absence of toxicity in both species, clinical dosing up to 1/10th the lower exposure in either species at the highest dose tested in the animal studies would be considered appropriate. Where only one species demonstrates toxicity, then the maximum clinical dose should be based on the lower of the above two paradigms.

The fifth approach involves a 2-week toxicity study in a rodent species up to a maximum tolerated dose, and a confirmatory non-rodent study that seeks to demonstrate that the NOAEL in the rodent is also not a toxic dose in the non-rodent. The confirmatory study in non-rodent can involve

repeatedly administering a dose that yields the NOAEL exposure in the rodent, usually estimated on the basis of body surface area or actual or modelled exposure. The duration of the non-rodent study should be a minimum of 3 days and at least equivalent to the number of intended administrations in the clinical trial.

Alternatively, an escalating dose study in the non-rodent can be conducted where, in the end, at least 3 days of dosing are administered to the animals at the exposure intended to be the NOAEL in the rodent. Should the non-rodent prove to be more sensitive than the rodent, clinical administration should be deferred until further nonclinical studies in this species have been conducted, usually a standard toxicity study.

Both of these approaches would also call for assessment of genotoxic potential (Ames test and an assay for clastogenicity), and the core battery of Safety Pharmacology studies, which could be conducted as stand-alone studies or included in one of the repeated dose toxicity studies.

Table 3 Non-Clinical Studies to Support Exploratory Clinical Investigations

Clinical:		Non clinical:		
Dose to be Administered	Start and Maximum Doses	Pharmacology	General Toxicity Studies	Genotoxicity / Other
<p>Approach 1: Total dose \leq 100 μg; maximum of 5 administrations (no inter- dose interval limitations) AND Total dose \leq 1/100th NOAEL and \leq1/100th pharmacologically active dose (scaled on mg/kg for i.v. and mg/m² for oral).</p>	<p>Maximal and starting doses can be the same but not exceed 100 μg.</p>	<p><i>In vitro</i> target/ receptor profiling should be conducted. Appropriate characterization of pharmacology in a pharmacodynamically relevant species should be available to support human dose selection.</p>	<p>Extended single dose toxicity study^a in one species, usually rodent, by intended route of administration with toxicokinetic profile or via the i.v. route. A limit dose of 10 mg/kg in rats (~6000 times the 100 μg clinical dose on a mg/ kg comparison basis) can be used.</p>	<p>Genotoxicity studies are generally not conducted, but any studies or SAR assessments conducted should be included in the clinical trial application. For highly radioactive agents, appropriate pharmacokinetics and dosimetry estimates should be submitted.</p>
<p>Approach 2: Total cumulative dose \leq 500 μg, maximum of 5 administrations with a washout between doses (6 or more actual or predicted half-lives) AND each dose \leq 100 μg AND each dose $<$ 1/100th of the NOAEL and $<$1/100th of the pharmacologically active dose.</p>	<p>Maximal daily and starting doses can be the same, but not exceed 100 μg.</p>	<p><i>In vitro</i> target/receptor profiling should be conducted. Appropriate characterization of pharmacology in a pharmacodynamically relevant species should be available to support human dose selection.</p>	<p>7 day toxicology study in one species, usually rodent, by i.v. route or intended route of administration, with toxicokinetics, haematology, clinical chemistry, necropsy data and histopathology. A limit dose of 10 mg/kg in rats (~6000 times the 100 μg clinical dose) can be used.</p>	<p>Genotoxicity studies are generally not conducted, if SAR assessments are negative. No genotoxicity assay is recommended for PET ligands. For highly radioactive agents, appropriate pharmacokinetics and dosimetry estimates should be submitted.</p>

Table 3 Non-Clinical Studies to Support Exploratory Clinical Investigations

Clinical:		Non clinical:		
Dose to be Administered	Start and Maximum Doses	Pharmacology	General Toxicity Studies	Genotoxicity
<p>Approach 3: Single sub-therapeutic or intended therapeutic dose.</p>	<p>Starting dose should be based on the types of toxicity findings observed in the most sensitive species and a consideration of the pharmacologically active dose. Regional guidance concerning starting dose selection, as available, should be consulted.</p> <p>Maximum dose can be that yielding up to ½ NOAEL exposure in the more sensitive species, in cases where any relevant toxicity observed in animals is anticipated to be monitorable and reversible in human.</p>	<p>Appropriate characterization of pharmacology in a pharmacodynamically relevant species should be available to support human dose selection.</p> <p>Core battery of safety pharmacology.</p>	<p>Extended single dose toxicity studies in both the rodent and non-rodent^b by intended clinical route of administration with toxicokinetics, haematology, clinical chemistry, necropsy data and histopathology. For this situation the top dose should be MTD, MFD or limit dose (see Note 1).</p>	<p>Ames assay (or appropriate alternative assay).</p>

Table 3 Non-Clinical Studies to Support Exploratory Clinical Investigations

Clinical:		Non clinical:		
Dose to be Administered	Start and Maximum Dose	Pharmacology	General toxicity studies	Genotoxicity
<p>Approach 4: Single or repeated dose (up to 14 days) exploratory studies into the therapeutic range but not intended to evaluate clinical maximum tolerated dose.</p>	<p>Starting dose predicted exposures should not exceed 1/50th the NOAEL in the more sensitive species on a mg/m² basis. Regional guidance, as available, should be consulted.</p> <p>With toxicity in both species, the maximum clinical dose should be based on standard risk assessment considering the nature, severity, monitorability of the nonclinical findings, but typically would not exceed the lowest NOAEL AUC ^c.</p> <p>Without toxicity in both species, clinical dosing up to 1/10th the lower exposure in either species at the highest dose tested in the animal is recommended.</p> <p>When only one species demonstrates toxicity, the maximum clinical dose would be based on the lower of the above two paradigms.</p>	<p>Core safety pharmacology battery using doses similar to those used for the toxicity studies.</p>	<p>Standard 2-week repeated dose toxicity studies in rodent and non-rodent where dose selection is based on exposure multiples of anticipated clinical AUC at maximum dose.</p>	<p>Ames assay(or appropriate alternative assay) and an assay for clastogenicity.</p>

Table 3 Non-Clinical Studies to Support Exploratory Clinical Investigations

Clinical:		Non clinical:		
Dose to be Administered	Start and Maximum Doses	Pharmacology	General Toxicity Studies	Genotoxicity
<p>Approach 5:</p> <p>Single or repeated dose up to duration of dosing in non-rodent up to maximum of 14 days; into therapeutic range but not intended to evaluate clinical maximum tolerated dose.</p>	<p>Starting dose predicted exposures should not exceed 1/50th the NOAEL in the more sensitive species on a mg/m² basis. Regional guidance, as available, should be consulted.</p> <p>The maximum exposure in human should not be higher than the AUC at NOAEL in the non-rodent species or than ½ the AUC at the NOAEL in the rodent species, whichever is lower ^c.</p>	<p>Core battery of safety pharmacology.</p>	<p>Standard 2-week repeated dose toxicity study in rodent (with justification of the rodent as an appropriate species).</p> <p>Confirmatory study in non-rodent (n=3) at rodent NOAEL exposure with duration of a minimum of 3 days and at least the intended clinical study duration.</p>	<p>Ames assay (or appropriate alternative assay) and an assay for clastogenicity.</p>

- a. Generally, extended single dose study designs fully evaluate haematology, clinical chemistry, necropsy data and histopathology in 10 rodents/sex/group for all groups on day 2 and 5 rodents/sex/group on day 14 in the group that is planned to support the clinical dose.
- b. For rodent, see a above. For non-rodent 3/sex/group for all groups on day 2 and 2 /sex/group for only the high dose on day 14.
- c. In the absence of clinical adverse effects, escalation above this AUC can be appropriate if the findings in the toxicology studies are anticipated to be monitorable, reversible, and of low severity in human.

8. LOCAL TOLERANCE STUDIES

It is preferable to evaluate local tolerance by the intended therapeutic route as part of the general toxicity studies; stand alone studies are not recommended.

To support limited human administration by non-therapeutic routes (e.g., a single i.v. dose to assist in the determination of absolute bioavailability of an oral drug), a local tolerance study in a single species is considered appropriate. In cases where the anticipated systemic exposure (AUC and C_{max}) from the non-therapeutic administration is covered by the existing toxicology package, the endpoints in the local tolerance study can be confined to clinical signs and macroscopic and microscopic examination of the injection site.

For an i.v. microdose study which is supported by an oral toxicology package (see Section 7), local tolerance does not need to be evaluated unless a novel vehicle is being employed.

For parenteral products, evaluation for local tolerance at unintended injection sites, when appropriate, should be conducted prior to exposure of large numbers of patients (e.g., Phase 3 clinical trials). The approach to such studies differs in the various regions. Such studies are not recommended in the US. In Japan they are recommended for i.v. drugs and for other parenterals on a case-by-case basis. In the EU, such studies are recommended for all parenteral products.

9. GENOTOXICITY STUDIES

The genotoxicity studies recommended supporting Exploratory Clinical Study approaches are discussed in Section 7.

An assay for gene mutation is generally considered sufficient to support all single dose clinical development trials. In support of multiple dose clinical development trials, two batteries of tests, Option 1 and Option 2, are described in the ICH S2(R1) document (Ref. 8). Option 2, if selected, should be completed prior to first human use in multiple dose studies. The *in vitro* components of Option 1, if selected, should be completed prior to first multiple dose human studies. The *in vivo* component of Option 1 should be completed prior to Phase 2.

If a positive finding occurs, an assessment, and then possibly additional testing (Ref. 8), should be conducted to determine if further administration to human is still appropriate.

10. CARCINOGENICITY STUDIES

If carcinogenicity studies are recommended for the clinical indication, they should be conducted for a/the marketing application. Only in circumstances where there is a significant cause for concern for carcinogenic risk should the study results be submitted to support clinical trials.

Conditions relevant for carcinogenicity testing are discussed in the ICH document (Ref. 9). For pharmaceuticals developed to treat certain serious diseases, for adults or pediatric patients, carcinogenicity testing, if recommended, can be concluded post-approval.

11. REPRODUCTION TOXICITY STUDIES

Reproduction toxicity studies (Refs. 10 and 11) should be conducted as is appropriate for the population that is to be exposed.

11.1 Men

Men can be included in Phase 1 and 2 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 large scale or long duration clinical trials (e.g., Phase 3 trials) (8, 9).

11.2 Women Not of Childbearing Potential

Women not of childbearing potential (i.e., permanently sterilised, postmenopausal) can be included in clinical trials without reproduction toxicity studies if the relevant repeated dose toxicity studies (which include an evaluation of the female reproductive organs) have been conducted. Postmenopausal is defined as 12 months with no menses without an alternative medical cause.

11.3 Women of Childbearing Potential

For women of childbearing potential (WOCBP) there is a high level of concern for the unintentional exposure of an embryo or fetus before information is available concerning the potential benefits versus potential risks. The recommendations on timing of reproduction toxicity studies to support the inclusion of WOCBP in clinical trials are similar in all ICH regions.

It is imperative to minimize the risk to the embryo or fetus when including WOCBP in clinical trials. There are several approaches to achieve this objective. One approach is to conduct reproduction toxicity studies to understand the inherent risk of a drug and take appropriate precautions during potential exposures. A second approach is to limit the risk by taking precautions to prevent pregnancy during clinical trials.

Precautions can include pregnancy testing (for example, based on the β -subunit of HCG), use of a highly effective method of birth control and study entry only after a confirmed menstrual period. Testing for pregnancy during the trial and subject education should be sufficient to ensure compliance with the measures designed to prevent pregnancy during the period of drug exposure (which could exceed the length of study). To support these approaches, informed consent should be based on any known pertinent information related to reproduction toxicity, such as a general assessment of potential toxicity of pharmaceuticals with related structures or pharmacological effects. If no relevant reproductive information is available, the potential for risks should be communicated.

In all ICH regions, WOCBP can be included in clinical trials without non-clinical developmental toxicity studies (e.g., embryo-fetal studies) in certain circumstances. One circumstance could be intensive control of pregnancy risk over short duration clinical trials (such as 2 weeks). Another circumstance, where longer duration clinical trial and exposure could be appropriate, is where there is a predominance of the disease in women, the objectives of the clinical trial cannot be effectively met without

inclusion of WOCBP, and there is sufficient control over pregnancy risk. Additional considerations for conduct of early studies in WOCBP without the non-clinical developmental toxicity studies include knowledge of the mechanism of action of the agent, the type of pharmaceutical agent (e.g., an antibody), half-life, and difficulty of conducting developmental toxicity studies in an appropriate animal model.

Generally, where appropriate preliminary reproduction toxicity data are available (see Note 3) from two species, and where adequate birth control methods are used (see Note 4), inclusion of WOCBP (up to 150) receiving investigational treatment for a relatively short duration (up to 3 months) can occur prior to completion of definitive reproduction toxicity testing. This is based on the very low rate of pregnancy in controlled clinical trials of this size and duration (see Note 5), and the ability of adequately designed preliminary studies to detect most developmental toxicity findings that could raise concern for enrollment of WOCBP in clinical trials. The number of WOCBP and the duration of the study can be influenced by characteristics of the population that alter pregnancy rates (e.g., age, disease).

In the US, assessment of embryo-fetal development can be deferred until prior to Phase 3 for WOCBP using highly effective contraceptive methods. In the EU and Japan, other than the above described situations, definitive nonclinical developmental toxicity studies should be completed prior to exposure to WOCBP.

In all ICH regions, nonclinical studies that specifically address female fertility should be completed to support inclusion of WOCBP in Phase 3 trials.

In all ICH regions, the pre- and post-natal development study should be submitted for marketing approval or earlier if there is cause of concern.

All female reproduction toxicity studies (Ref. 10) and the standard battery of genotoxicity tests (Ref. 8) should be completed prior to the inclusion, in any clinical trial, of WOCBP not using highly effective birth control (see Note 4) or whose pregnancy status is unknown.

11.4 Pregnant Women

Prior to the inclusion of pregnant women in clinical trials, all the reproduction toxicity studies (Refs. 10 and 11) and the standard battery of genotoxicity tests (Ref. 8) should be conducted. In addition, safety data from previous human exposure should be evaluated.

12. OTHER TOXICITY STUDIES

Additional nonclinical studies (e.g., to identify potential biomarkers, to provide mechanistic understanding) can be useful if previous nonclinical or clinical findings with the product or related products have indicated special safety concerns.

13. CLINICAL TRIALS IN PEDIATRIC POPULATIONS

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 initiation of pediatric clinical trials. The appropriateness and extent of adult human data should be determined on a case-by-

case basis. Extensive adult experience might not be appropriate prior to pediatric exposures (e.g., for pediatric-specific indications).

Results from repeated dose toxicity studies of appropriate duration in adult animals, the core safety pharmacology package, and the standard battery of genotoxicity tests should be available prior to the initiation of trials in pediatric populations. Reproduction toxicity studies relevant to the age and gender of the pediatric patient populations under study can also be important to provide information on direct toxic or developmental risks (e.g., fertility and peri-post natal developmental studies). Embryo-fetal developmental studies are not critical to support clinical studies for males or prepubescent females.

The appropriateness of juvenile animal toxicity studies should be considered only when previous animal data and human safety data are judged to be insufficient to support pediatric studies. One rodent species is generally considered adequate, although studies in non-rodent species can be appropriate when justified. If a juvenile animal study is considered important for conduct of a specific trial, it should be available prior to initiation of that pediatric clinical trial.

Generally, juvenile animal toxicity studies are not considered important for short term pharmacokinetic studies (e.g., 1 to 3 doses) in pediatric populations.

Depending on the indication of the drug, age of the pediatric population, and safety data from adult animal and human exposure, the appropriateness of juvenile animal study results prior to initiation of short duration multiple dose efficacy and safety trials should be considered.

The age of the study participants in relation to the duration of clinical study is among the most important considerations with respect to the timing of the juvenile animal toxicity study.

In all cases where an assessment of juvenile animal toxicity is recommended, the studies should be completed prior to initiation of long-term clinical trials in pediatric populations.

There can be cases where a pediatric population is the primary population; existing animal studies do not adequately address developmental concerns for target organs, and long term juvenile toxicity testing in a non-rodent would be valuable. In this case, a study initiated in the appropriate age and species of juvenile animals that covers the developmental period of concern can be appropriate. A non-rodent chronic study (e.g., 12 months duration in dog to cover the full development period in this species) combining the objectives of the standard chronic and separate juvenile animal toxicity study can be appropriate.

The appropriateness of carcinogenicity testing should be addressed prior to long-term exposure in pediatric clinical trials. However, unless there is a significant cause for concern (e.g., evidence of genotoxicity in multiple tests, or concern for pro-carcinogenic risk based on mechanistic consideration or findings from general toxicity studies), carcinogenicity studies are not recommended to support the conduct of pediatric clinical trials.

14. IMMUNOTOXICITY

As stated in the ICH S8 guidance (Ref. 12), all new human pharmaceuticals should be evaluated for the potential to produce immunotoxicity using standard toxicity studies and additional immunotoxicity studies conducted as appropriate based on a weight of evidence review, including immune-related signals from standard toxicity studies. If there is an indication for additional immunotoxicity studies, these should be completed before exposure of a large population of patients (e.g., Phase 3).

15. PHOTOTOXICITY

The appropriateness of or timing of photosafety testing in relation to human exposure should be influenced by: 1) the photochemical properties (photoabsorption and photostability) of the molecule, 2) information on the phototoxic potential of chemically-related compounds, 3) tissue distribution, and 4) clinical or nonclinical findings indicative of phototoxicity.

An initial assessment of phototoxic potential based on a drug's physical/chemical properties for photoreactivity, spectral absorption properties, and pharmacologic class / SAR should be performed. If the assessment indicates a potential phototoxicity risk, it could be appropriate to undertake protective measures during subsequent clinical studies.

Thereafter, an evaluation of the nonclinical drug distribution to skin and eye should be undertaken to further inform on the human risk. If appropriate, an experimental evaluation of phototoxic potential should be undertaken prior to exposures of large numbers of subjects (Phase 3).

Alternatively, a direct assessment of phototoxic potential in nonclinical or clinical studies can be undertaken to obviate the need for early assessment of eye/skin distribution studies and clinical protective measures.

Testing for photocarcinogenicity in rodents generally is not considered useful in support of pharmaceutical development and generally is not recommended. If the photocarcinogenic risk can be adequately managed in patients, specific testing is not recommended and a warning statement can be included in the informed consent or labelling. If testing is considered appropriate, methods other than rodent bioassays should be considered in assessing photocarcinogenic potential and completed prior to marketing.

16. NONCLINICAL ABUSE LIABILITY

The evaluation of abuse liability should be considered for drugs that are distributed into the brain and produce central nervous system activity, regardless of therapeutic indication. Nonclinical studies should support the design of clinical evaluations of abuse potential, classification/scheduling by regulatory agencies, and drug labeling.

Nonclinical data collected early in the drug development process can be useful in identification of early indicators of abuse potential. These early indicators would typically be available prior to first human dose and include the PK/PD profile to identify duration of action, similarity of chemical structure to known drugs of abuse, receptor binding profile, and behavioural pharmacology/clinical signs from *in vivo* nonclinical studies. When no abuse potential is apparent from these early studies,

extensive testing in nonclinical abuse liability models might not be warranted. If the active substance shows signals associated with known abuse liability patterns or when the active substance has a novel mechanism of action on the central nervous system, further nonclinical studies are recommended to support large clinical trials (e.g., Phase 3).

When the metabolite profile, the target for drug activity, and drug toxicity in rodent are consistent with that of human, the nonclinical abuse liability evaluations should be conducted in rodents. Primates should be reserved only for those limited cases where there is clear evidence that the primate would be predictive of human abuse liability and the rodent model is inadequate. Three studies are often completed to evaluate the potential for abuse liability: drug discrimination, self-administration of the compound, and an assessment of dependence/withdrawal. When conducted, studies of drug discrimination and self-administration should be stand-alone. Assessments of dependence/withdrawal can be incorporated within the design of the reversibility arm of a repeated dose toxicity study. A dose that produces a maximum plasma concentration several-fold the highest intended clinical exposure is considered appropriate for these nonclinical abuse studies. There are regional guidance documents on the conduct of nonclinical abuse liability assessment that can be helpful in designing specific abuse liability packages.

17. FIXED COMBINATION DRUG NONCLINICAL TESTING

This section of the guidance only covers combination drugs that are intended to be co-packaged or in a single dosage form. It does not cover adjunctive or concomitant administration, for which nonclinical combination studies are generally not recommended. Combinations covered might involve: (1) two or more late stage entities (defined as compounds with significant late stage (i.e., Phase 3 or greater clinical experience); (2) one or more late stage entity(ies) and one or more early stage entities (defined as compounds with limited clinical experience, i.e., Phase 2 or less); or (3) more than one early stage entity. Depending on the situation, combination studies in animals can be recommended to support clinical studies.

The nonclinical studies recommended to characterize the combination will depend on the toxicological and pharmacokinetic profiles of the individual entities, treatment indication or indications, and the intended patient population. In general, for those studies that are appropriate, their timing would follow the timing for the analogous studies in Table 1.

For most combinations involving two late stage entities, for which there is adequate clinical experience with co-administration, no nonclinical studies would be recommended for the fixed dose combination unless there are causes for concern related to pharmacokinetic interactions, toxicological interactions, or narrow margins of safety. Where there are two late stage

products for which there is inadequate clinical experience with co-administration, but there are no causes for concern based on the available data, nonclinical combination studies generally are not recommended to support small scale, short duration clinical studies. Nonclinical combination studies, however, are recommended prior to large scale or long-term combination trials in this case.

For combinations of a late stage entity(ies) with an early stage entity(ies), or two or more early stage entities, repeated-dose combination studies in animals are

recommended following the same timing in drug development as would be recommended to support clinical studies for products of new active ingredients (see Table 1). If nonclinical studies have already been conducted for each individual component, then a combination study of duration equivalent to that of the clinical trial, up to a maximum of 90 days, is recommended. For marketing, where the individual components have been fully assessed in nonclinical studies, a combination study of 90 days maximum duration generally is appropriate. Depending on the duration of the intended clinical use, combination toxicity studies shorter than 90 days can support marketing authorization.

Combination nonclinical studies should generally be limited to a single species.

An alternative approach of a complete nonclinical toxicology program with the combination only can also be appropriate.

Combination genotoxicity, safety pharmacology, or carcinogenicity studies generally are not recommended to support clinical trials or marketing, if the individual agents have been tested to current standards. In those cases where the patient population includes WOCBP and studies with the individual agent(s) have shown findings indicative of embryo-fetal risk, combination studies are not recommended as a potential human developmental hazard has already been identified. If nonclinical embryo-fetal studies have indicated that neither agent poses a potential human developmental risk, combination studies are not recommended unless concerns exist, based on the properties of individual components, that their combination could give rise to a hazard for humans. Embryo-fetal studies of the drug combination, if recommended, should be available for the marketing application, if the individual agents have been tested in embryo-fetal studies.

18. CONTINUING EFFORTS TO IMPROVE HARMONIZATION

It is recognised that significant advances in harmonisation 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 guideline. However, differences remain in a few areas. Regulators and industry will continue to consider these differences and work towards further improving the drug development process.

19. ENDNOTES

Note 1: Limit doses for general toxicity studies of 2000 mg/kg/day for rodents and 1000 mg/kg/day for non-rodents are considered appropriate if there are significant margins to the clinical exposure and the clinical dose does not exceed 1 g per day. Alternatively, doses providing a 50-fold margin of exposure to the clinical exposure generally are considered acceptable as the maximum dose for general toxicity studies in any species.

These limit doses are being added to prevent use of doses in animals that would not add value to understanding clinical safety. In addition the limit doses are being added to provide consistency with recommendations for acute, reproduction, and carcinogenicity study designs that already have defined limit doses and exposures (Refs. 10 and 13).

Note 2: In Japan, unlike the EU and US, the male fertility study usually has been conducted prior to the inclusion of men in clinical trials. An assessment of male

fertility by careful histopathological examination in a repeated dose toxicity in rodent of at least 2-week duration has been found to be more sensitive in detecting toxic effects on male reproductive organs than fertility studies (Refs. 11, 14). If this assessment is conducted, it is no longer recommended to conduct the male fertility study prior to the first clinical trial in Japan.

Note 3: A preliminary embryo-fetal study useful for this purpose is one with adequate dose levels; that includes external, visceral and skeletal examinations; that uses a minimum of six dams per group; and that has dams treated over the period of organogenesis. This nonclinical study should be conducted under GLP conditions and deviations, if any, should be discussed.

Note 4: A highly effective method of birth control is defined as one which results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly, such as implants, injectables, combined oral contraceptives, some IUDs, sexual abstinence or vasectomised partner. For subjects using a hormonal contraceptive method, information regarding the product under evaluation and its potential effect on the contraceptive should be addressed.

Note 5: The pregnancy rate of women initially attempting to become pregnant is ~17% per menstrual cycle. Pregnancy rates estimated from Phase 3 studies conducted in WOCBP were observed to be <0.1% per menstrual cycle. During these studies, subjects were encouraged to avoid pregnancy and measures were instituted to prevent pregnancy. Survey information from earlier Phase 2 studies suggests that the pregnancy rates were lower than Phase 3 studies but the extent of further reduction could not be estimated due to the limited number of women enrolled.

20. REFERENCES

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