LIST OF ABBREVIATIONS

AUC	Area Under the Curve
C_{max}	Maximum Plasma Concentration
EU	European Union
GLP	Good Laboratory Practices
HCG	Human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
i.v.	Intravenous
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
siRNA	Small Interfering RNA
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 as well as marketing authorization for pharmaceuticals.

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

This guidance should facilitate the timely conduct of clinical trials, reduce the use of animals in accordance with the 3R (reduce/refine/replace) principles and reduce the use of other drug development resources. Although not discussed in this guidance, consideration should be given to use of new *in vitro* alternative methods for safety evaluation. These methods, if validated and accepted by all ICH regulatory authorities, can be used to replace current standard methods.

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

1.2 Background

The recommendations of this revised guidance further harmonise the nonclinical safety studies to support the various stages of clinical development among the regions of European Union (EU), Japan, and the United States. The present guidance represents the consensus that exists regarding the type and duration of nonclinical safety studies and their timing to support the conduct of human clinical trials and marketing authorization for pharmaceuticals.

1.3 Scope of the Guideline

The nonclinical safety assessment for marketing approval of a pharmaceutical usually includes pharmacology studies, general 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 to assess phototoxicity, immunotoxicity, juvenile animal toxicity and abuse liability should be conducted on a case-by-case basis. The need for

nonclinical safety studies and their relation to the conduct of human clinical trials is delineated in this guidance.

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

For biotechnology-derived products (as defined in Ref. 1), appropriate nonclinical safety studies should be determined in accordance with ICH S6. For these products, ICH M3(R2) only provides guidance with regard to timing of nonclinical studies relative to clinical development.

Pharmaceuticals under development for indications in life-threatening or serious diseases (e.g., advanced cancer, resistant HIV infection, and congenital enzyme deficiency diseases) without current effective therapy also warrant a case-by-case approach to both the toxicological evaluation and clinical development in order 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 be abbreviated, deferred, omitted, or added. Where ICH guidances for specific product areas exist, they should be consulted.

1.4 General Principles

The development of a pharmaceutical is a stepwise process involving an evaluation of both animal and human efficacy and 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 used to estimate an initial safe starting dose and dose range for the human trials and to identify parameters for clinical monitoring for potential adverse effects. The nonclinical safety studies, although usually limited at the beginning of clinical development, should be adequate to characterise potential adverse effects that might occur under the conditions of the clinical trial to be supported.

Human clinical trials are conducted to investigate the efficacy and safety of a pharmaceutical, starting with a relatively low systemic exposure in a small number of subjects. This is followed by clinical trials in which exposure to the pharmaceutical 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 trial(s), as well as on additional nonclinical safety information that becomes available as clinical development proceeds.

Serious adverse clinical or nonclinical findings can influence the continuation of clinical trials. Within the overall clinical context, these findings should be

evaluated to determine the appropriateness and design of additional nonclinical and/or clinical 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 E8 guideline (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 characteristics of the subjects included.

1.5 High Dose Selection for General Toxicity Studies

Generally, in toxicity studies, effects that are potentially clinically relevant can be adequately characterized using doses up to the maximum tolerated dose (MTD). It is not essential to demonstrate the MTD in every study. Other equally appropriate limiting doses include those that achieve large exposure multiples or saturation of exposure or use the maximum feasible dose (MFD). These limit doses (see additional details below and Figure 1) prevent the use of doses in animals that would not add value to predicting clinical safety. These recommendations are consistent with those for reproduction and carcinogenicity study designs that already have defined limit doses and/or exposures (Refs. 3 and 4).

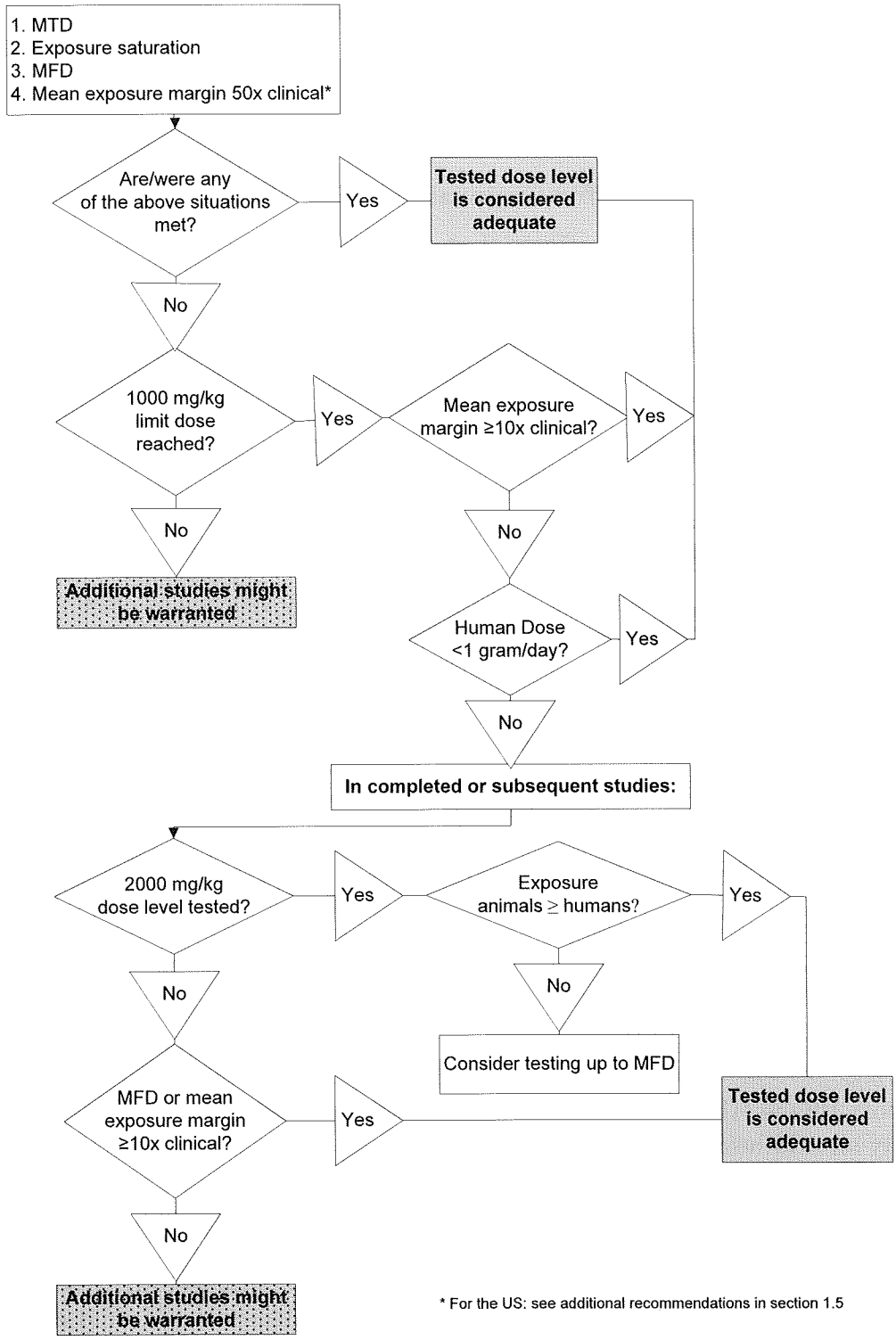
Limit doses for acute, subchronic, and chronic toxicity studies of 1000 mg/kg/day for rodents and non-rodents are considered appropriate in all cases except those discussed below. In the few situations where a dose of 1000 mg/kg/day does not result in a mean exposure margin of 10-fold to the clinical exposure and the clinical dose exceeds 1 g per day, then the doses in the toxicity studies should be limited by a 10-fold exposure margin or a dose of 2000 mg/kg/day or the MFD, whichever is lower. In those rare situations in which the dose of 2000 mg/kg/day results in an exposure that is less than the clinical exposure, a higher dose up to the MFD can be considered.

Doses providing a 50-fold margin of exposure (usually based on group mean AUC values [see Note 1] of the parent drug or the pharmacologically active molecule of a pro-drug) to the clinical systemic exposure generally are also considered acceptable as the maximum dose for acute and repeated-dose toxicity studies in any species.

To support Phase III clinical trials for the United States, dose-limiting toxicity generally should be identified in at least one species when using the 50-fold margin of exposure as the limit dose. If this is not the case, a study of one-month or longer duration in one species that is conducted at the 1000 mg/kg limit dose, MFD or MTD, whichever is lowest, is recommended. However, on a case-by-case basis this study might not be warranted if a study of a shorter duration identifies dose-limiting toxicity at doses higher than those resulting in a 50-fold exposure margin.

If genotoxicity endpoints are to be incorporated into a general toxicity study, then an appropriate maximum dose should be selected based on a MFD, MTD or limit dose of 1000 mg/kg/day.

Figure 1. Recommended high dose selection for general toxicity studies



2. PHARMACOLOGY STUDIES

Safety pharmacology and pharmacodynamic (PD) studies are defined in ICH S7A (Ref. 5).

The core battery of safety pharmacology studies includes the assessment of effects on cardiovascular, central nervous and respiratory systems, and should generally be conducted before human exposure, in accordance with ICH S7A and S7B (Refs. 5 and 6). When 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.

In addition, primary PD studies (*in vivo* and/or *in vitro*) are intended to investigate the mode of action and/or effects of a substance in relation to its desired therapeutic target. Such studies are generally conducted during the discovery phase of pharmaceutical development and as such, are not generally conducted in accordance with Good Laboratory Practices (GLP). These studies can contribute to dose selection for both nonclinical and clinical studies.

3. TOXICOKINETIC AND PHARMACOKINETIC STUDIES

In vitro metabolic and plasma protein binding data for animals and humans and systemic exposure data (ICH S3A, Ref. 7) in the species used for repeated-dose toxicity studies generally should be evaluated before initiating human clinical trials. Further information on pharmacokinetics (PK) (e.g., absorption, distribution, metabolism and excretion), in test species and *in vitro* biochemical information relevant to potential drug interactions should be available before exposing large numbers of human subjects or treating for long duration (generally before Phase III). These data can be used to compare human and animal metabolites and for determining if any additional testing is warranted.

Nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Such studies should be conducted to support Phase III clinical trials. For drugs for which the daily administered dose is <10 mg, greater fractions of the drug related material might be more appropriate triggers for testing. Some metabolites are not of toxicological concern (e.g., most glutathione conjugates) and do not warrant testing. The nonclinical characterization of metabolites with an identified cause for concern (e.g., a unique human metabolite) should be considered on a case-by-case basis.

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 an MTD in the general toxicity test species (Refs. 8 and 9).

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. Lethality should not be an intended endpoint in studies assessing acute toxicity.

In some specific situations (e.g., microdose trials; see Section 7) acute toxicity or single-dose studies can be the primary support for studies in humans. In these situations, the high dose selection can be different from that described in Section 1.5 but should be appropriate for supporting the intended clinical dose and route. These studies should be performed in compliance with GLP.

Information on the acute toxicity of pharmaceutical agents could be useful to predict the consequences of human overdose situations and should be available to support Phase III. An earlier assessment of acute toxicity could 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). Limit doses/exposures that are considered appropriate in repeated-dose toxicity studies are described in Section 1.5.

In 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 toxicity studies of at least equivalent duration. Six month rodent and 9 month non-rodent studies generally support dosing for longer than 6 months in clinical trials (for exceptions see Table 1 footnotes).

Table 1 Recommended Duration of Repeated-Dose Toxicity Studies to Support the Conduct of Clinical Trials

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

a In the United States, as an alternative to 2 week studies, extended single-dose toxicity studies (see footnote c in Table 3) can support single-dose human trials. Clinical studies of less than 14 days can be supported with toxicity studies of the same duration as the proposed clinical study.

b In some circumstances clinical trials of longer duration than 3 months can be initiated, provided that the data are available from a 3-month rodent and a 3-month non-rodent study, and that complete data from the chronic rodent and non-rodent study are made available, consistent with local clinical trial regulatory procedures, before extending dosing beyond 3 months in the clinical trial.

For serious or life-threatening indications or on a case-by-case basis, this extension can be supported by complete chronic rodent data and in-life and necropsy data for the non-rodent study. Complete histopathology data from the non-rodent should be available within an additional 3 months.

c. There can be cases where a pediatric population is the primary population, and existing animal studies (toxicology or pharmacology) have identified potential developmental concerns for target organs. In these cases, long-term toxicity testing starting in juvenile animals can be appropriate in some circumstances (see Section 12).

d. In the EU, studies of 6 months duration in non-rodents are considered acceptable. However, where studies with a longer duration have been conducted, it is not appropriate to conduct an additional study of 6 months.

The following are examples where non-rodent studies of up to 6 months duration can also be appropriate for Japan and the United States:

- When immunogenicity or intolerance confounds 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 administered on a chronic basis to reduce the risk of recurrence of cancer.
- Drugs for indications for which life expectancy is short.

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. The durations of repeated-dose toxicity studies to support marketing for different treatment durations are outlined in Table 2. However, 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 suggesting 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 Recommended Duration of Repeated-Dose Toxicity Studies to Support Marketing

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[c]	9 months [c,d]

n.b. See footnotes 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 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 appropriate animal species gives the most important information. The proposed clinical starting dose will also depend on various factors, including PD, 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 clinical development trials (see Section 5.1); therefore, the estimation of the clinical starting (and maximal) dose can differ. The recommended criteria for starting doses for various exploratory clinical trial designs are described in Table 3.

7. EXPLORATORY CLINICAL TRIALS

It is recognized that in some cases earlier access to human data can provide improved insight into human physiology/pharmacology, knowledge of drug candidate characteristics and therapeutic target relevance to disease. 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 I, involve limited human exposure, have no therapeutic intent, and are not intended to examine clinical tolerability. They can be used to investigate a variety of parameters such as PK, PD and other biomarkers, which could include PET receptor binding and displacement or other diagnostic measures. The subjects included in these studies can be patients from selected populations or healthy individuals.

The amount and type 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 examples of exploratory clinical approaches are summarized below and in more detail in Table 3, together with the nonclinical testing programs that would be recommended in these particular approaches. However, alternative approaches not described in this guidance can also be used, including strategies to support biotechnology-derived products. It is recommended that these alternative approaches be discussed and agreed upon with the appropriate regulatory authority. The use of any of these approaches can reduce overall animal use in drug development.

Recommended starting doses and maximal doses for the five approaches are included in Table 3. In all cases, characterization of PD and pharmacology using *in vivo* and/or *in vitro* models as noted in Table 3 and Section 2 is important and should be used in support of human dose selection.

7.1 Microdose Trials

Two different microdose approaches are described below with details provided in Table 3.

The first approach would involve not more than a total dose of 100 μg that can be administered as a single dose or divided 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 PK with or without the use of an isotopically labelled agent.

A 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 applications similar to the first microdose approach described above, but with less active PET ligands.

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 toxicity studies as described in Table 1 or Table 3, Approach 3, where adequate exposure margins have been achieved. It is not recommended to investigate i.v. local tolerance of the drug substance in this situation because the administered dose is very low (100 μg maximum). If a novel i.v. vehicle is being employed then local tolerance of the vehicle should be assessed.

7.2 Single-Dose Trials at Sub-therapeutic Doses or into the Anticipated Therapeutic Range

The third approach involves a single-dose clinical study typically starting at subtherapeutic doses and possibly escalating into the pharmacological or anticipated therapeutic range (see Table 3). The maximum allowable dose should be based on the nonclinical data, but could be further limited based on emerging clinical information obtained during the course of the study. This approach could allow, for example, determination of PK parameters with non-radiolabeled drug at or near the predicted pharmacodynamically active dose. Another example could be assessment of target engagement or pharmacology after a single dose. This approach is not intended to support the determination of the maximum tolerated clinical dose (see exception, Table 1, footnote a).

7.3 Multiple Dose Trials

Two different nonclinical approaches (numbers 4 and 5) to support multiple dose clinical trials are provided in Table 3. These approaches support up to 14 days of dosing for determination of PK and PD in human in the therapeutic dose range, but are not intended to support the determination of maximum tolerated clinical dose.

Approach 4 involves 2-week repeated-dose toxicity studies in rodents and non-rodents where dose selection in animals is based on exposure multiples of anticipated AUC at the maximum clinical dose.

Approach 5 involves a 2-week toxicity study in a rodent species and a confirmatory non-rodent study that is designed to investigate whether the NOAEL in the rodent is also not a toxic dose in the non-rodent. If toxic effects are

observed in the non-rodent at the rodent NOAEL exposure, clinical administration should be deferred until further nonclinical studies in this species have been conducted (usually a standard toxicity study (see Section 5)).

Table 3 Recommended Non-Clinical Studies to Support Exploratory Clinical Trials

Clinical:		Non clinical:		
Dose to be Administered	**Start and Maximum Doses**	**Pharmacology**	**General Toxicity Studies[a]**	**Genotoxicity[b] / Other**
Approach 1: Total dose ≤ 100 µg (no inter-dose interval limitations) AND Total dose ≤ 1/100th NOAEL and ≤1/100th pharmacologically active dose (scaled on mg/kg for i.v. and mg/m^2 for oral)	Maximal and starting doses can be the same but not exceed a total accumulated dose of 100 µg	*In vitro* target/ receptor profiling should be conducted Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection.	Extended single dose toxicity study (see footnotes c and d) in one species, usually rodent, by intended route of administration with toxicokinetic data, or via the i.v. route. A maximum dose of 1000-fold the clinical dose on a mg/kg basis for i.v. and mg/m^2 for oral administration can be used.	Genotoxicity studies are not recommended, but any studies or SAR assessments conducted should be included in the clinical trial application. For highly radioactive agents (e.g. PET imaging agents), appropriate PK and dosimetry estimates should be submitted.
Approach 2: Total cumulative dose ≤ 500 µg, maximum of 5 administrations with a washout between doses (6 or more actual or predicted half-lives) AND each dose ≤ 100 µg AND each dose ≤ 1/100th of the NOAEL and ≤ 1/100th of the pharmacologically active dose	Maximal daily and starting doses can be the same, but not exceed 100 µg.	*In vitro* target/receptor profiling should be conducted Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection.	7-day repeated-dose toxicity study in one species, usually rodent, by intended route of administration with toxicokinetic data, or via the i.v. route. Hematology, clinical chemistry, necropsy, and histopathology data should be included. A maximum dose of 1000-fold the clinical dose on a mg/kg basis for i.v. and mg/m^2 for oral administration can be used.	Genotoxicity studies are not recommended, but any studies or SAR assessments conducted should be included in the clinical trial application. For highly radioactive agents (e.g. PET imaging agents), appropriate PK and dosimetry estimates should be submitted.

Table 3 Recommended Non-Clinical Studies to Support Exploratory Clinical Trials

Clinical:		Non clinical:		
Dose to be Administered	**Start and Maximum Doses**	**Pharmacology**	**General Toxicity Studies[a]**	**Genotoxicity[b]**
Approach 3 Single Dose Studies at Sub-therapeutic Doses or into the Anticipated Therapeutic Range	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. For other considerations on initial dosing in humans, regional guidances should be consulted. 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 humans.	*In vitro* target/receptor profiling should be conducted Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection. Core battery of safety pharmacology (see Section 2).	Extended single dose toxicity studies in both the rodent and non-rodent (see footnote c) by intended clinical route of administration with toxicokinetics, hematology, clinical chemistry, necropsy, and histopathology data. For this situation the top dose should be MTD, MFD or limit dose (see Section 1.5).	Ames assay (or an alternative assay if Ames is inappropriate, for example, for an antibacterial product).

Table 3 Recommended Non-Clinical Studies to Support Exploratory Clinical Trials

Clinical:		Non clinical:		
Dose to be Administered	**Start and Maximum Dose**	**Pharmacology**	**General toxicity studies[a]**	**Genotoxicity[b]**
Approach 4: Dosing up to 14 days into the therapeutic range but not intended to evaluate clinical MTD	With toxicity in both species, follow appropriate regional guidance for clinical starting dose. If toxicity is not seen in either species (i.e. the NOAELs are the highest dose tested and doses used were not otherwise limited, e.g. not an MFD), or is seen only in one species, the clinical starting dose should be one that gives a predicted clinical AUC value (based on either interspecies PK modelling or mg/m^2 conversion) that is approximately 1/50th of the AUC at the NOAEL from the species yielding the lower exposure. For other considerations on initial dosing in humans, e.g. predicted PD activity, regional guidance should be consulted. Without toxicity in both species, it is recommended that the maximum clinical dose not exceed 1/10th the lower exposure (AUC) in either species at the highest dose tested in the animals. When only one species	*In vitro* target/receptor profiling should be conducted Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection. Core battery of safety pharmacology (see Section 2) using doses similar to those used for the toxicity studies.	2-week repeated-dose toxicity studies in rodent and non-rodent with standard parameters assessed and where dose selection in animals is based on exposure multiples of anticipated clinical AUC at maximum dose.	Ames assay (or an appropriate alternative assay if Ames is inappropriate, for example, for an antibacterial product) and an assay (*in vitro* or *in vivo*) capable of detecting chromosomal damage in a mammalian system

	demonstrates toxicity, the maximum clinical dose should not be higher than the NOAEL in the species showing toxicity, or 1/2 the AUC at the highest dose tested in the species not showing toxicity, whichever is lower. With toxicity in both species, the maximum clinical dose should be based on standard risk assessment approaches and, in this specific case, the clinical MTD can be explored.			

Table 3 Recommended Non-Clinical Studies to Support Exploratory Clinical Trials

Clinical:		Non clinical:		
Dose to be Administered	**Start and Maximum Doses**	**Pharmacology**	**General Toxicity Studies[a]**	**Genotoxicity[b]**
Approach 5: Dosing up to 14 days and not to exceed duration of dosing in non-rodent; into therapeutic range but not intended to evaluate clinical MTD.	Starting dose predicted exposures should not exceed 1/50th the NOAEL in the more sensitive species on a mg/m^2 basis. For other considerations on initial dosing in humans, regional guidance should be consulted. The maximum exposure in humans should not be higher than the AUC at the NOAEL in the non-rodent species or higher than ½ the AUC at the NOAEL in the rodent species, whichever is lower[e].	*In vitro* target/receptor profiling should be conducted Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection. Core battery of safety pharmacology (see Section 2) using doses similar to those used for the toxicity studies.	Standard 2-week repeated-dose toxicity study in rodents (with justification of the rodent as an appropriate species). The top dose should be the MTD, MFD or limit dose (see Section 1.5). Confirmatory study in non-rodent (n=3) at the anticipated NOAEL exposure in rodent, with duration of a minimum of 3 days and at least the intended clinical study duration. Alternatively, an escalating dose study in the non-rodent with duration of a minimum of 3 days and at least the intended clinical study duration at the anticipated NOAEL exposure in the rodent.	Ames assay (or an appropriate alternative assay if Ames is inappropriate, for example, for an antibacterial product) and an assay (*in vitro* or *in vivo*) capable of detecting chromosomal damage in a mammalian system. If an *in vivo* assessment is used then this could be part of the rodent toxicity study.

a. General toxicity studies should be conducted according to GLP regulations.

b. See Ref. 10 for genotoxicity study design and dose selection.

c. Generally, extended single dose toxicity studies should be designed to evaluate hematology, clinical chemistry, necropsy, and histopathology data (control and high dose only if no treatment-related pathology is seen at the high dose) after a single

administration, with further evaluations conducted 2 weeks later to assess delayed toxicity and/or recovery. The usual design for rodents consists of 10 animals/sex/group to be assessed on the day following dosing, and 5 animals/sex at the dose level(s) selected to be assessed on day 14 post-dose. The usual design for non-rodents consists of 3/sex/group for all groups on day 2 and 2/sex for the dose level(s) assessed on day 14.

d. A single dose level to assess reversibility/delayed toxicity on day 14 can support the microdose approach. The dose level used need not be the high dose but should be a dose that is at least 100 times the clinical dose.

e. In the absence of adverse effects in the clinical trial, escalation above this AUC can be appropriate if the findings in the toxicity studies are anticipated to be monitorable, reversible, and of low severity in humans.

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 generally 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 single dose 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 application site. The formulation delivered for local tolerance need not be identical but should be similar to the clinical formulation.

For an i.v. microdose study that is supported by an oral toxicology package (see Section 7), evaluation of local tolerance of the drug substance is not warranted. If a novel i.v. vehicle is being employed, then local tolerance of the vehicle should be assessed.

For parenteral products, evaluation for local tolerance at unintended injection sites, when appropriate, should be conducted before exposure of large numbers of patients (e.g., Phase III clinical trials). The approach to such studies differs in the various regions. Such studies are generally not recommended in the United States (an example of an exception would be intrathecal for the epidural route). Japan and the EU recommend single dose paravenous administration for the i.v. route. Other parenteral routes should be evaluated on a case-by-case basis.

9. GENOTOXICITY STUDIES

An assay for gene mutation is generally considered sufficient to support all single dose clinical development trials. To support multiple dose clinical development trials, an additional assessment capable of detecting chromosomal damage in a mammalian system(s) should be completed (Ref. 10). A complete battery of tests for genotoxicity should be completed before initiation of Phase II trials (Ref. 10).

If a positive finding occurs, an assessment, and then possibly additional testing (Ref. 10), should be conducted to determine if further administration to humans is still appropriate.

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