Guidance for Industry

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

Questions and Answers

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

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Guidance for Industry[1]

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

Questions and Answers

I. INTRODUCTION[2]

Although the ICH M3(R2) guidance is still in its early phases of implementation, the complexity of the guidance, its broader scope, and numerous changes in recommendations from the M3(R1) guidance have generated questions that have an impact on its successful implementation. This question and answer (Q&A) document is intended to clarify the key issues.

The Steering Committee has endorsed the establishment of an M3(R2) Implementation Working Group (IWG), which is currently working on the development of Q&As. This document is the first set of Q&As addressing *Limit Dose for Toxicity Studies*, *Metabolites,* and *Reversibility of Toxicity*, and was finalized at Step 4 in June 2011.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory

[1] This guidance was developed within the Expert Working Group (Multidisciplinary) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Steering Committee at *Step 4* of the ICH process, June 2011. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and the United States.

[2] Arabic numbers reflect the organizational breakdown in the document endorsed by the ICH Steering Committee at Step 4 of the ICH process, June 2011.

requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. QUESTIONS AND ANSWERS

A. Limit Dose for Toxicity Studies (1)

Q1: Can you provide a definition of a 50-fold clinical exposure margin in terms of how it is calculated and whether it relates to the intended therapeutic clinical exposure or the maximum exposure achieved in phase 1 trials?

A1: Generally, the exposure margins should be calculated using the group/cohort mean area under the curve (AUC) values for animals at the highest dose tested and for humans at the anticipated therapeutic exposure. In some special cases, based on prior knowledge of the compound class, exposure limits based on C_{max} (maximum plasma concentration) might also be appropriate (e.g., if it is suspected that the drug could cause seizures).

Using the 50-fold approach, the high dose in the toxicity studies should be selected to produce a 50-fold exposure margin over the anticipated clinical exposure at the highest dose proposed for phase 2 and 3 studies (see the exception for phase 3 trials in the United States (ICH M3(R2) guidance, section I.E High Dose Selection for General Toxicity Studies (1.5)), and the answers to Q2 and Q3 of this section). For phase 1 clinical trials, it is recognized that the therapeutic exposure generally will be exceeded and smaller margins are appropriate (for example, see answers to Q2 and Q3).

Q2: When using the 50-fold exposure approach and there are no adverse findings in the rodent and nonrodent toxicity studies, if the clinical dose is escalated up to the agreed limit (1/50th of the exposure achieved at the top dose in animal studies) and there are no adverse findings in humans, is it possible to escalate the clinical dose further?

A2: In this situation, if the clinical dose is escalated to 1/50th of the maximum exposure in the animal studies and no treatment-related adverse effects are noted in volunteers/patients, for short-term clinical studies (e.g., 14 days duration) the clinical dose could be cautiously further escalated up to 1/10th of the maximum exposure in the animal studies, or to a dose that produces adverse effects in humans, whichever occurs first. This is reasonable because exploratory trials Approach 4 (not intended to evaluate a maximum tolerated dose (MTD) supports dosing for 14 days up to 1/10th

the NOAEL (no observed adverse event level) exposure with the same First-In-Human enabling toxicity studies.

Q3: When toxicity study doses are selected by using the 50-fold exposure approach and there are adverse findings in at least one of the toxicity studies, but the findings are not dose-limiting, what is the limitation for clinical exposure?

A3: Doses might be escalated in the clinical studies based on the NOAEL for the adverse findings identified in the toxicity studies. The clinical doses should not be limited by the 50-fold margin in this case but should be based on standard risk assessment approaches (e.g., whether the findings are reversible and/or monitorable, the severity of the indication, adverse effects in clinical studies). Note the exception for phase 3 trials in the United States (section I.E (1.5) of ICH M3(R2)).

Q4: Does the 50-fold exposure limit only apply to small molecules?

A4: Yes, the 50-fold margin of exposure limit dose applies to small molecules only. As stated in section I.C (1.3) of ICH M3(R2), the guidance only applies to biologics with regard to timing of nonclinical studies relative to clinical development. High dose selection for nonclinical studies of biologics is different from that for small molecules (see ICH S6(R1)[3]).

Q5: When making a maximum feasible dose (MFD) argument, to what lengths should the sponsor go to justify the MFD?

A5: The MFD should be a dose that attempts to maximize exposure in toxicity studies, rather than maximize the administered dose. However, formulation volumes that can be administered should be based on anatomical and physiological attributes of the test species and properties of the formulation, and can have an impact on the MFD. In addition, the chemical and physical stability of the formulation are important criteria for suitability for use in toxicity studies and could limit the selection of vehicles for determining the MFD. Solubility limits can restrict the dose for some routes, such as intravenous. Solubility limits are not usually considered sufficient to justify the MFD for some other routes of administration, such as inhalation or oral. The characteristics of multiple formulations of the test article, with a range of properties (e.g., aqueous and non-aqueous and various viscosities), should be investigated before

[3] The ICH guidances referenced in this document are available on the FDA Drugs guidance Web page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. We update guidances periodically. To make sure you have the most recent version of a guidance, check the Drugs guidance Web page.

dosing in animals. The most promising formulations (generally three) should be evaluated in animals to determine which formulation produces maximal exposure. The vehicles used should be well characterized in the scientific literature or selected based on experience (sponsor or regulatory agency information) to provide confidence that they will not cause significant toxicity under conditions of use.

Q6: What if dose-limiting toxicity is not identified in any species and there is only one nonclinical toxicity study in each species before the phase 3 study (regarding phase 3 recommendation for the United States)?

A6: The guidelines for high dose selection for general toxicity studies apply irrespective of the length or complexity of the drug development paradigm. In accord with the recommendation to support phase 3 studies in the United States (see section I.E (1.5) of ICH M3(R2)), an assessment of doses up to an MTD, MFD or limit dose should be conducted in an attempt to identify toxicity.

Q7: Does the guidance on high dose selection and the 50-fold margin of clinical AUC apply to routes other than oral (e.g., dermal, inhalation)?

A7: For any drug intended to provide systemic exposure (including transdermal), the 50-fold approach is considered appropriate. For topical drugs intended to produce local effects, the high dose in topical toxicity studies should generally be based on the MFD or MTD and might not achieve high local concentrations or high systemic exposures compared to those achieved clinically. In this case, a 50-fold systemic margin is not relevant.

For inhaled drugs with intended systemic action, the high dose in an inhalation toxicity study could be one that produces an AUC value of greater than or equal to 50-fold the clinical systemic exposure and a 10-fold margin over the calculated deposited lung dose. For inhaled drugs that are designed to work locally in the lungs, the high dose could be one that achieved a calculated deposited lung dose of 50 times the calculated clinical deposited lung dose and produced a 10-fold margin over the AUC achieved in humans at the clinical dose.

Q8: Does the 50-fold margin apply to juvenile animal studies? Can the 50-fold margin be used to select the top dose for reproductive toxicity studies?

A8: Similar principles of reliance on exposure margins to limit the top dose should be applicable to some other types of toxicity testing, such as

juvenile animal toxicity studies where toxicity is not anticipated. Use of a 50-fold margin for top doses in reproductive toxicity studies has not been addressed; however, current ICH guidance states that minimal toxicity is expected to be induced in the high dose dams although other factors can also limit the dose (see ICH S5(R2)).

B. Metabolites (2)

Q1: In the M3(R2) guidance, what does "significantly greater" mean in the following statement: "Nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10 percent of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies"?

A1: The term *significantly greater* is not meant to imply a statistically greater level. Differences of ≥ 2-fold in (mean) AUC are generally considered meaningful in toxicokinetic evaluations. Thus, characterization of metabolite toxicity would generally be considered adequate when animal exposure is at least 50 percent the exposure seen in humans. In some cases, for example when a metabolite composes the majority of the total human exposure, it is appropriate for exposure to the metabolite in animals to exceed that in humans (see also Q12 of this section). In this latter case it is important to achieve a higher exposure to the metabolite in animals because this metabolite constitutes the bulk of human exposure.

Q2: What is the definition and calculation method of* 10 percent*?

A2: The 10 percent threshold refers to when a human metabolite comprises greater than 10 percent of the measured total exposure to drug and metabolites, usually based on group mean AUC (e.g., $AUC_{0\text{-}inf}$).

Q3: When characterization of metabolite toxicity is warranted, in what type(s) of in vivo nonclinical studies is it important that adequate systemic exposure to a metabolite be achieved?

A3: It is important to have adequate exposure to the metabolite in one species used in the general toxicity evaluation, one species used in a carcinogenicity study when carcinogenicity evaluation is warranted (or one species used in an in vivo micronucleus study when carcinogenicity evaluation is not warranted), and one species used in an embryo-fetal development study.

Q4: Are in vitro genotoxicity studies recommended for metabolites?

When genotoxicity assessment is warranted for a metabolite, is QSAR (quantitative structure-activity relationship) assessment sufficient or should genotoxicity studies be conducted?

A4: This topic is outside the scope of ICH M3(R2).

Q5: Is the metabolite exposure data provided from the single-dose radiolabeled human ADME (absorption, distribution, metabolism, and excretion)) study sufficient for comparison to the exposures observed in animal toxicity studies without evaluation of steady state levels, which cannot be done with radiolabel clinically?

A5: An evaluation of whether a metabolite is 10 percent of the total drug-related exposure can be based on single-dose data in humans. It is not generally feasible to measure AUC of all metabolites by non-radiolabeled methods, particularly for those drugs that have many metabolites. In these cases, a single-dose radiolabeled study provides a reasonable estimate of human total drug-related exposure and is an adequate basis for calculating whether a metabolite exceeds 10 percent. (A metabolite cannot be more than 10 percent of the total drug-related material if non-radiolabeled methods indicate that a metabolite is less than 10 percent of the parent or of any drug-related component(s). For example, $P+M_1+M_2+\ldots M_n$ = total; if M_1 is less than 10 percent of P or M_1 is less than 10 percent of any M, then M_1 is less than 10 percent of the total. In this case, no further assessment of that metabolite is warranted.)

If during development exposure data normally collected from multiple-dose human studies indicate that steady state levels of a metabolite exceed 10 percent, then additional nonclinical evaluation of the metabolite should be considered.

Generally, exposure data from nonclinical studies and single-dose clinical studies can be compared to determine whether further metabolite toxicity characterization is warranted. For those metabolites that have been determined to exceed 10 percent of drug-related material in humans only after repeated dosing, steady state levels (clinical and nonclinical) should be used to assess the adequacy of the exposure margins.

Q6: The M3(R2) guidance says: "Nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10 percent of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies."

When a human metabolite exposure is compared to the maximum exposure of that metabolite in toxicity studies, should it always be to the highest exposure achieved in the animal studies or is it more appropriate in some cases to use the exposure at the NOAEL, NOEL (no observed effect level), or MTD?

A6: Because the parent drug and metabolites contribute to the target organ toxicity profile observed in animals at the MTD, the exposure comparisons across species should be conducted at the MTD in the animal compared to the maximum exposure in humans at the therapeutic dose, assuming the toxicity of concern can be adequately monitored in humans and does not pose an unacceptable risk. If the toxicity at the MTD is not monitorable in humans or poses an unacceptable risk, then the exposure comparison should be conducted at the NOAEL for the toxicity of concern.

Q7: When in development, should data on nonclinical metabolites be available*?*

A7: As described in ICH M3(R2), section III Toxicokinetic and Pharmacokinetic Studies (3), paragraph 1, in vitro metabolism data for animals and humans should be evaluated before initiating human clinical trials. Data on in vivo metabolism in test species and humans should be available before exposing large numbers of human subjects or treating for long duration (generally before phase 3).

Q8: Clarification is sought on metabolites that may not be of toxicological concern. In ICH M3(R2), what is meant by "most" in the phrase "most glutathione conjugates"*? Would acyl glucuronides that can undergo chemical rearrangement be an example of a concern? What should we do about chemically reactive metabolites?***

A8: Although there are relatively rare exceptions, most glutathione conjugates are formed by conjugation with reactive metabolites to form excretory metabolites that are not of toxicological concern. Most glucuronides are not of concern, except those that undergo chemical rearrangement (e.g., reactive acyl glucuronides). Highly chemically reactive metabolites, although of toxicological concern, do not generally accumulate in plasma due to their short half-life. Generally, it is not feasible to test highly reactive metabolites independently because of their instability, but they are assumed to contribute to the overall nonclinical toxicity of the drug.

Q9: Should safety pharmacology studies be conducted for metabolites that warrant nonclinical characterization?

A9: Clinical studies assessing safety pharmacology endpoints are generally conducted during phase 1. These endpoints will have already been assessed in humans before a full characterization of the metabolites is conducted. Therefore, nonclinical safety pharmacology studies are generally not warranted for the characterization of metabolites. However, if a safety pharmacology signal is seen in humans that was not predicted by nonclinical studies with the parent, then additional safety pharmacology studies of these human metabolites can be considered to better understand the mechanism (see ICH S7A and ICH S7B).

Q10: What does "in vitro biochemical information" mean in section III (3), paragraph 1, of ICH M3(R2)?

A10: In vitro biochemical information includes standard in vitro metabolic evaluation (e.g., cytochrome P450 (CYP) inhibition, pregnane X receptor (PXR) activation assays). It can include studies with hepatic microsomes/hepatocytes or studies on potential interactions via drug transporters.

Q11: What should be the design of nonclinical studies for metabolites (e.g., species, duration, study type)?

A11: This level of detail is generally out of scope for ICH M3(R2); study design should be considered on a case-by-case basis using scientific judgment in consultation with regulatory agencies. Also see answers to other questions in this section (e.g., Q3 and Q9).

Q12: Does the guidance on metabolites in ICH M3(R2) apply to a prodrug (i.e., when a metabolite provides most of the pharmacologic activity)?

A12: The guidance does not specifically address prodrugs. If the animal species converts the prodrug to the active metabolite similarly to humans, then a standard testing approach as recommended in ICH M3(R2) can be used. If the active metabolite is not adequately produced in the animal species, then the target molecule for toxicological evaluation is the active metabolite and therefore additional testing beyond that recommended for metabolites can be appropriate. Timing of the nonclinical testing of the active metabolite in this case should follow the general timelines as outlined in ICH M3(R2) rather than the timing indicated for metabolite testing in section III (3) of M3(R2).

C. Reversibility of Toxicity (3)

Q1: When is assessment of reversibility considered to be appropriate and is it important to demonstrate full reversibility or is it sufficient to demonstrate the <u>potential</u> for full reversibility?

A1: ICH M3(R2) states the following in section I.D General Principles (1.4): "The goals of the nonclinical safety evaluation generally include a characterization of toxic effects with respect to target organs, dose dependence, relationship to exposure, and, when appropriate, potential reversibility."

Evaluation of the potential for reversibility of toxicity (i.e., return to the original or normal condition) should be provided when there is severe toxicity in a nonclinical study with potential adverse clinical impact. The evaluation can be based on a study of reversibility or on a scientific assessment.

The scientific assessment of reversibility can include the extent and severity of the pathologic lesion, the regenerative capacity of the organ system showing the effect and knowledge of other drugs causing the effect. Thus, recovery arms or studies are not always critical to conclude whether an adverse effect is reversible. The demonstration of full reversibility is not considered essential. A trend towards reversibility (decrease in incidence or severity), and scientific assessment that this trend would eventually progress to full reversibility, are generally sufficient. If full reversibility is not anticipated, this should be considered in the clinical risk assessment.

A toxicity study that includes a terminal non-dosing period is generally warranted if a scientific assessment cannot predict whether the toxicity will be reversible and if:

1. there is severe toxicity at clinically relevant exposures (e.g., ≤10-fold the clinical exposure); or
2. the toxicity is only detectable at an advanced stage of the pathophysiology in humans and where significant reduction in organ function is expected. (The assessment of reversibility in this case should be considered even at >10-fold exposure multiples.)

A toxicity study that includes a terminal non-dosing period is generally not warranted when the toxicity:

1. can be readily monitored in humans at an early stage before the toxicity becomes severe; or
2. is known to be irrelevant to humans (e.g., rodent Harderian gland toxicity); or
3. is only observed at high exposures not considered clinically relevant (see 2 above for exception); or

4. is similar to that induced by related agents, and the toxicity based on prior clinical experience with these related agents is considered a manageable risk.

If a study of reversibility is called for, it should be available to support clinical studies of a duration similar to those at which the adverse effects were seen nonclinically. However, a reversibility study is generally not warranted to support clinical trials of a duration equivalent to that at which the adverse effect was not observed nonclinically.

If a particular lesion is demonstrated to be reversible in a short duration (e.g., 2-week or 1-month) study, and does not progress in severity in longer term studies, repeating the reversibility assessment in longer term toxicity studies is generally not warranted.

If a reversibility study is warranted, it is efficient to conduct it as part of a chronic study so that all toxicities of concern can be assessed in a single study, provided that it is not critical to conduct it earlier to support a specific clinical trial.