Guidance for Industry

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

Questions and Answers(R2)

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)

February 2013
ICH

Revision 1

Contains Nonbinding Recommendations

Guidance for Industry

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

Questions and Answers(R2)

Additional copies are available from:
Office of Communications
Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10903 New Hampshire Ave.
Bldg. 51, Room 2201
Silver Spring, MD 20993-0002
(Tel) 301-796-3400
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

and/or

Office of Communication, Outreach and
Development, HFM-40
Center for Biologics Evaluation and Research
Food and Drug Administration
1401 Rockville Pike, Rockville, MD 20852-1448
(Tel) 800-835-4709 or 301-827-1800
http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

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)

February 2013
ICH

Revision 1

TABLE OF CONTENTS

Guidance for Industry[1]

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

Questions and Answers(R2)

I. INTRODUCTION[2]

Although the ICH M3(R2) guidance is still in its early phases of the 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 guidance is a revision of the ICH guidance of the same title dated February 2012 (M3(R2) Q&As). The February 2012 guidance addressed the first set of Q&As on *Limit Dose for Toxicity Studies*, *Metabolites,* and *Reversibility of Toxicity* that was finalized under *Step 4* in June 2011.

In December 2011, a second set of Q&As addressing *Combination Drug Toxicity Testing* was developed and approved under *Step 4* for integration in the M3(R2) Q&As. In March 2012, an additional question on *Limit Dose for Toxicity Studies* and four additional sections addressing *Safety Pharmacology*, *Exploratory Clinical Trials*, *Reproductive Toxicity*, and *Juvenile Animal*

[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, December 2011 and March 2012. 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.

Studies were approved under *Step 4* for integration in the (M3(R2) Q&As. This revised guidance incorporates the changes approved in December 2011 and March 2012.

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 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

[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.

article, with a range of properties (e.g., aqueous and non-aqueous and various viscosities), should be investigated before 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)).

Q9: What exposure limits should be applied for clinical development studies when the top dose for the nonclinical studies is the limit dose such as 1000 mg/kg or 2000 mg/kg described in section 1.5 or an MFD and no toxicity is observed at this dose?

A9: The clinical dose could be conservatively escalated up to one that produced a plasma AUC exposure of 1/2 that seen in the animal species that gives the lowest exposure at the limit dose or MFD. If there are no adverse effects in humans at this clinical exposure, further careful escalation might be justified based on risk/benefit considerations.

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 quantitative structure-activity relationship (QSAR) 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 potential 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.

D. Combination Drug Toxicity Testing (4)

Q1: If two (or more) late stage entities are combined but for one of them the human dosage/exposure will be higher than that already approved, is it important to conduct a combination toxicity study or are the existing nonclinical data and clinical experience with the lower dose considered adequate to address the nonclinical assessment?

A1: If there has been previous clinical experience with the two entities used together, a combination toxicity study would generally not be recommended for an increase in dose/exposure of one of the entities unless this gave cause for significant toxicological concern. The level of concern would depend on the new exposure margins, the established safety profile of the individual agents, the degree of experience with the co-administration, and the ability to monitor any potential adverse effects in humans. If the increase in dose/exposure does cause concern and a study is conducted to address that concern, then it should generally be completed before carrying out clinical studies with the combination. If there is no clinical experience with the entities used together, see paragraph 4 of section XVII (17) of ICH M3(R2).

Q2: Section XVII (17) of M3(R2) states, "[i]f 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." Although this statement is in line with European Medicines Agency (EMA) guidance, it contradicts FDA guidance that states that embryo-fetal development studies of the combination should be conducted unless the marketed drug substance or the new molecular entity (NME) is already known to have significant risk for developmental toxicity (e.g., the

marketed drug has been assigned a pregnancy category "D" or "X"). Please provide clarity regarding the precedence of ICH guidance over regional guidances in those areas where such differences occur.

A2: Statements made in ICH guidances represent an agreed position across the participating bodies and reflect each regulatory body's current recommendations on a given topic.

Q3: The current guidance states that for combinations of late stage products for which there is adequate clinical experience of co-administration, combination toxicity studies are generally not recommended unless there is a significant toxicological concern. In this context, what is considered "adequate clinical experience with co-administration"? Specifically, how do you get "adequate" clinical experience with the combination without having done combination toxicity testing? This guidance seems only to apply to marketed products that have been used together. Was that the intent?

A3: This section of the guidance was not intended to only apply to marketed products. *Adequate clinical experience* is defined in ICH M3(R2) as data from phase 3 clinical studies and/or postmarketing use. Adequate clinical experience can be the result of common clinical practice with drug combinations.

Co-administration of two or more late stage entities is a common practice in many therapeutic areas of clinical development where add-on therapy to the standard of care or combination therapy is common, such as with hypertension, diabetes, human immunodeficiency virus (HIV), hepatitis C, and cancer.

Q4: For non-fixed-dose combinations, if one of the agents is a member of a class containing multiple approved products, should each member of the class be tested in a combination toxicity study?

A4: Generally, combination toxicity studies are recommended when there is an intent to combine (co-package or administer in a single dosage form) specific drugs, or when the product information of one drug recommends co-use with another specified drug. There is no recommendation for combination toxicity testing in the guidance for the situation described in this question. When there is a specific cause for concern with an agent, combination toxicity testing should be done with the agent. When there is a class-related cause for concern, a combination toxicity study with a representative agent in the class could be informative (see also Q&A3 in this section). A rationale should be provided for the agent selected for testing.

Q5: How are dosage, duration, and endpoint of a combination toxicity study selected?

A5: ICH M3(R2) is intended primarily to address the timing and duration of nonclinical studies relative to clinical development. Provided a nonclinical combination toxicity study is warranted to support the combination clinical trial, the duration of the study should be equivalent to that of the clinical trial it is intended to support, up to a maximum of 90 days (which would also support marketing). A combination study of shorter duration can be used to support marketing, depending on the duration of clinical use. A combination toxicity study intended to address a particular cause for toxicological concern, based on the experience with the individual agents, should be of a duration that is appropriate to address the concern.

The combination toxicity study should incorporate endpoints to evaluate additive and synergistic effects for known toxicities that might be predicted from what is known of the pharmacological, toxicological, and pharmacokinetic (PK) profiles of the individual entities, as well as the available clinical data, and standard endpoints typically used in a general toxicity study. Detailed discussion of experimental design (e.g., choice of species, dose and dosing frequency justifications) is outside the scope of this guidance. However, dosages should be appropriate to address any identified cause for concern or to provide exposure margins that are clinically relevant (e.g., when conducting a study with two early stage agents).

Q6: When there is a cause for concern for multiple entities being used together (e.g., more than two), how should the multiple entity combinations be assessed in the toxicity studies?

A6: Because of the potential complexity of performing and interpreting a combination toxicity study with more than two entities, it is generally more practical for initial studies to evaluate combinations of no more than two entities. Additional testing would then depend on the outcome of these studies and should be considered on a case-by-case basis, and in consultation with appropriate regulatory authorities.

Q7: If a compound is being developed that aims to reduce another compound's side effect, such combination effects would be evaluated in clinical or nonclinical pharmacology studies. Do the pharmacology studies replace the combination toxicity study?

A7: When combination toxicity studies are warranted, they generally cannot be replaced by combination pharmacology studies, except for anticancer pharmaceuticals (see ICH S9). The purpose of a combination toxicity study is to evaluate toxicity endpoints that could give rise to an unanticipated hazard for humans. These toxicity endpoints are not usually adequately evaluated in the pharmacology studies. Situations where combination toxicity studies are not warranted are described in section XVII (17) of the M3(R2) guidance.

Q8: ***Section XVII (17) of M3(R2) indicates that if there is a concern for a potential human developmental risk of a combination and a combination embryo-fetal development study is warranted, such a study should be available to support the marketing application. Please clarify whether such a study should be performed before the commencement of a clinical trial that includes women of childbearing potential (WOCBP).***

A8: As described in the M3(R2) guidance, the combination embryo-fetal toxicity study should be provided to support marketing. Any trial enrolling WOCBP before completing a combination embryo-fetal development study should include appropriate precautions, including informed consent, to minimize the risk of unintentional exposure of the embryo or fetus as outlined in section XI.C (11.3).

Q9: ***In ICH M3(R2), section I.C Scope of the Guidance (1.3) states that "[p]harmaceuticals 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 optimize and expedite drug development." Although not specifically stated in the combination section of M3(R2) (see section XVII (17)), it is generally accepted that combination toxicity studies on advanced cancer and HIV products are not warranted unless there is a specific cause for concern. Can this be confirmed? Would this also extend to hepatitis C virus (HCV) products, as discussed in the recently issued FDA draft guidance for industry on Chronic Hepatitis C Virus Infection: Developing Direct-Acting Antifiral Agents for Treatment (September 2010),[4] and to other therapeutic areas where "cocktails" of drugs are standard clinical practice?***

A9: It is accepted that combination toxicity studies on advanced cancer, tuberculosis, and HIV products are generally not warranted unless there is a specific cause for concern under clinically relevant conditions. Combination toxicity studies are also not generally warranted for antiviral agents for treatment of hepatitis C. There are other situations where combinations of drugs are standard clinical practice for serious or life-threatening conditions without current effective therapies, and a similar approach might also apply.

Q10: ***In case of combinations with at least one biotechnology-derived product, does section XVII Combination Drug Toxicity Testing (17) apply as such, or only with regard to timing as suggested in section I.C (1.3) on the scope of the M3(R2) guidance? And in case of the latter, which guidance would (still) apply in deciding whether and which types of studies would be recommended?***

[4] When final, this guidance will represent FDA's current thinking on this topic.

A10: For biotechnology-derived products, appropriate nonclinical safety studies should be determined in accordance with ICH S6(R1). However, the topic of combination toxicity studies is not addressed by that guidance. When the combination consists of a biotechnology-derived component and a non-biotechnology-derived component, the design and feasibility of any nonclinical combination study are complex and should be considered on a case-by-case basis. The rationale for such a study should be clearly scientifically justified, using the principles of ICH S6(R1) and ICH M3(R2).

Q11: In the discussion of inclusion of WOCBP in combination drug development, M3(R2) states, "where . . . 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." What is meant by the phrase "have shown findings indicative of embryo-fetal risk"? FDA's guidance for industry on Nonclinical Safety Evaluation of Drug or Biologic Combinations references Pregnancy Category D and X only as yielding this exclusion. Is this the intent for the ICH as well?

A11: A finding indicative of embryo-fetal risk includes any observations for reproductive hazard at relevant exposure multiples (within approximately an order of magnitude of the clinical exposure) or directly related to the pharmacodynamics of the drug. In these cases, recommendations about patient actions to minimize the identified hazard would likely be unchanged even if data from a combination study showed an enhanced effect, because a significant risk to patients has already been identified. Therefore, combination reproductive toxicity studies are not recommended when a finding with one of the individual agents indicates embryo-fetal risk; that information would be made available to patients and physicians as part of the risk communication, irrespective of pregnancy category. For example, if studies with one of the agents showed fetal death or terata at approximately 10-fold the clinical exposure, even if observed in only one species, a combination study would not be warranted, provided that this information was present in the single agent product labeling.

Q12: There is no mention about pharmacology studies, and pharmacodynamic or pharmacokinetic drug-drug interaction studies in section XVII (17). Please indicate whether and when these studies should be conducted.

A12: Presumably, the pharmacodynamic activities and pharmacokinetic profile, including the effects on the CYP450s of the individual drugs, are known before the drugs are combined. Because potential pharmacodynamic interactions are anticipated based on the nonclinical and clinical experiences with the individual entities or their combination, no nonclinical pharmacodynamic interaction studies are warranted. If the pharmacology information indicates potential interactions that could lead to toxicity, then combination nonclinical toxicity studies might be warranted.

Concerns regarding pharmacokinetic interactions can often be addressed by lowering the initial doses administered below those that might be appropriate for the individual drugs or by conducting a clinical pharmacokinetic drug-drug interaction study.

E. Safety Pharmacology (5)

Q1: ICH M3(R2) states that including the in vivo safety pharmacology evaluations in toxicity studies to the extent feasible should be considered. Does this mean that the safety pharmacology assessment conducted as part of general toxicity studies can be less thorough than that obtained in stand-alone safety pharmacology studies?

A1: No. Assessment of safety pharmacology as part of the general toxicity studies should provide rigor similar to that in stand-alone safety pharmacology studies. This can be achieved with current technology, provided the methods have been adequately assessed.

F. Exploratory Clinical Trials (6)

Q1: To support exploratory clinical trials, why should the extended single-dose studies be done in both sexes when the clinical exploratory studies are likely to be done in one sex?

A1: Exploratory clinical studies do not represent a commitment to full development. Therefore, when intent is to conduct the exploratory clinical study in one sex only, the single-dose toxicity studies can be restricted to that sex. However, in such cases, animal group sizes for the Day 2 termination should be increased, as it is normal to combine effects from both sexes with respect to identifying and characterizing toxicities that are not sex-specific. For extended single-dose toxicity studies using a single sex, the usual animal numbers should be 15/group (rodents) or 5/group (nonrodents) for the Day 2 termination, and 7/group (rodents) or 3/group (nonrodents) for the Day 14 termination.

Q2a: Please clarify the differences between Approaches 3, 4, and 5.

A2a: Approach 3 involves just a single dose in humans supported by extended single-dose toxicity studies in rodents and nonrodents conducted up to the animal maximum tolerated dose (MTD), maximum feasible dose (MFD), or limit dose.

Approach 4 involves multi-dose clinical trials (up to 14 days) supported by 14-day toxicity studies (in rodents and nonrodents) in which dose selection for

animals is based on multiples of proposed human exposure in the exploratory clinical trial. If no toxicity is observed in either species, it is recommended that the maximum clinical dose not exceed $1/10^{th}$ the lower exposure (AUC) in either species at the highest dose tested in the animals. If toxicity is observed, see answer *A2b* below.

Approach 5 involves multi-dose clinical trials (up to 14 days) supported by a 14-day study in rodents up to the MTD, MFD, or limit dose and a nonrodent *confirmatory* study (at least equivalent to the duration of the exploratory clinical trial) that indicates that the nonrodent is not more sensitive than the rodent. In this case, the highest exposure appropriate in the exploratory clinical trial should be determined by the findings in the toxicity studies.

Thus, the differences between Approach 4 and Approach 5 include how the standard nonclinical toxicity study recommendations are modified, and how the clinical exposure limit is established. Approach 5 probably uses less drug than Approach 4, but relies heavily on the rodent for identifying safety risks. Approach 4 gives equal weight to the rodent and nonrodent, but might not identify target organ toxicity in either species. In this case, clinical progression is supported by the knowledge that a reasonable safety margin exists.

The series of examples are intended to provide sponsors flexibility in exploratory clinical trial approaches so that they can do what best fits their purpose. The approaches given are only examples, and sponsors can propose alternatives that do not fit neatly into one of the described approaches.

Q2b: Why does Approach 4 have a more stringent maximum clinical dose than Approaches 3 and 5?

A2b: Approach 4 is the only one of these approaches that does not rely on the standard high-dose criteria described in section I.E (1.5) (MTD, MFD, 50X exposure multiple, or limit dose) in at least one species.

In Approach 4, the high dose in both the rodent and nonrodent studies is based on multiples of the proposed human exposure, and thus the high-dose selection recommendations described in section I.E (1.5) are not applied to either species. This is in contrast to Approach 3, in which the standard high-dose criteria should be met in both species, and in contrast to Approach 5, in which the standard high-dose criteria should be met in rodents. In Approach 3 and Approach 5, the use of standard high-dose selection criteria reduces the uncertainty around potential unidentified toxicities that might be relevant to humans.

Because Approach 4 uses exposure multiples for the high-dose selection in both species, it is possible that potential toxicity might not be identified in either species. In this case, more conservative limits on clinical exposure (e.g., $1/10^{th}$ the exposure obtained using the lower exposure of the two species) are

recommended because the dose-limiting toxicities of potential concern for clinical monitoring have not been identified. If toxicity is identified in one species, then the limit on clinical exposure is based on the No Observed Adverse Effect Level (NOAEL) exposure in the species with toxicity or ½ the NOAEL exposure in the species without toxicity, whichever is lower. This can yield a higher limit in Approach 4 than in the case where toxicity in neither species has been observed. The limit on clinical exposure for Approach 4 when based on toxicity can be comparable to the limit on clinical exposure in Approach 5. If dose-limiting toxicity is identified in both species using Approach 4, then the high-dose recommendations of section I.E (1.5) have been met or exceeded in both species and a maximum clinical dose can be based on standard risk assessment used for phase 1 trials and a clinical MTD can be explored.

Q2c: In cases where toxicity is demonstrated (e.g., Approaches 3 and 5) why is the maximum allowable human dose (equal to or 1/2 the NOAEL) different from usual practice (i.e., (1) where toxicity is nonserious and/or monitorable, human doses above the NOAEL would normally be allowed, and (2) where toxicity is serious and non-monitorable the maximum human dose would usually be limited to 1/10th the NOAEL).

A2c: The more stringent limits on maximum exposure in exploratory clinical trials compared to standard phase 1 trials are consistent with the more limited nonclinical recommendations compared with the standard toxicity study recommendations described in section I.E (1.5) and section V (5) in M3(R2). For example, in Approach 3, extended single-dose studies are recommended rather than the typical recommendation of a study of at least 2 weeks' duration (see Table 1 and Approach 5); the nonrodent study is only confirmatory in nature and can be limited to 3 animals at a single-dose level targeted to be a NOAEL. The recommendation that the maximum human exposure allowed could be up to 1/2 the NOAEL exposure assumes that the toxicity defining the NOAEL is not severe and is monitorable. If this is not the case, it might be appropriate to adjust the exposure margin based on the nature of the dose-limiting toxicity.

Q3: Why is an MFD treated like an MTD in Approaches 3 and 5 when considering the maximum clinical exposure in the exploratory clinical trial? If no toxicity is observed in either species when using an MFD, shouldn't this be treated similarly to the case in Approach 4 when there is no toxicity in either species (i.e., limit the clinical exposure to 1/10th rather than 1/2 the exposure at the highest dose tested)?

A3: In any situation in which the MFD is used as the top dose for a toxicity study, it is simply not possible to test a higher dose/exposure. If the top dose used is the MFD and no toxicity is observed, this situation is similar to that of the limit dose when toxicity has not been identified (i.e., the limit dose is the NOAEL) where clinical exposures up to 1/2 the AUC at the NOAEL can be used (see section I.E

(1.5) of the M3(R2) guidance, and section II.A (1) of this Q&A guidance, Limit Dose for Toxicity Studies, Q&A9). The $1/10^{th}$ exposure limit is not applied when the high dose is limited by an MFD, because this could prevent adequate clinical testing of a drug under the exploratory clinical trial concept. When no toxicity is identified using Approach 4, a more stringent safety limit has been recommended because it would have been possible to test higher doses in animals to characterize the toxicity profile of the drug.

Q4: The M3(R2) guidance provides advice on establishing the maximum dose (exposure) permitted in exploratory clinical trials but provides minimal guidance for establishing the maximum dose in standard phase 1 or clinical development trials. Can the maximum dose in standard phase 1 trials be based on the principles described for exploratory clinical trials (Table 3 of M3(R2))?

A4: When the package of nonclinical studies meets the general recommendations of section V.A (5.1) of ICH M3(R2), then the maximum clinical dose for a clinical development phase 1 study can be based on standard risk assessments (e.g., whether the findings are reversible and/or monitorable, the severity of the indication, adverse effects in clinical studies; also see section VI (6) of ICH M3(R2) and regional guidances). This would normally support a higher clinical dose than that recommended for exploratory clinical trials. However, a sponsor has the option to set a lower maximum clinical dose for a phase 1 study (e.g., based on the principles described for exploratory approaches).

Q5: What are reasonable strategies for exploratory clinical trials with biotechnology-derived products?

A5: Exploratory clinical trial approaches can be applicable to biotechnology-derived products. Biotechnology-derived products include a wide variety of molecular structures and targets (e.g., peptides, polypeptides, therapeutic proteins, and monoclonal antibodies). The designs of the exploratory clinical trial and supporting toxicity studies for biotechnology-derived products should reflect their special features as described in ICH S6(R1). This includes the duration of exposure, the potential for immunogenicity in animals or humans, and the possibility that dose-limiting toxicity might be due to on-target, pharmacodynamic-related mechanisms. ICH S6(R1) recommends that exploratory clinical trial approaches be discussed with the appropriate regulatory authorities.

Note that some biotechnology-derived products, for example monoclonal antibodies, are not active in rodents, and in such cases a nonhuman primate can be used as a single relevant species for toxicity testing. In such cases, an approach analogous to Approach 5 would not be applicable because it relies on a rodent toxicity study and confirmatory nonrodent study. Also, for standard toxicity studies of biotechnology-derived products, the high dose is routinely based on

exposure multiples (i.e., 10X the maximum clinical exposure) rather than on an MTD, an MFD (unless these are lower), or a limit dose. Thus, the high dose recommendation in Approach 4 is not substantially different from the standard recommendations for biotechnology-derived products.

Q6: In exploratory Approaches 1 and 2 that use doses of < 100 micrograms (µg), why is the cross-species exposure conversion for intravenous administration based on milligram (mg)/kilogram (kg) and not mg/square meter (m^2) as it is for oral administration?

A6: The intravenous (i.v.) approach of using mg/kg and permitting dosing with $1/100^{th}$ of the NOAEL reflects a conservative risk mitigation strategy, considering the low levels of drug being administered. The use of mg/kg for i.v. and mg/m^2 for oral administration when determining dose multiples for microdose studies reflects the thinking that it is appropriate to use a more conservative scaling factor for oral versus i.v. administration. With oral administration, there is the additional complexity of potential differences in absorption between species and, therefore, the more conservative mg/m^2 basis was used rather than the mg/kg basis used for i.v. administration.

Q7: For Approach 1, the M3(R2) guidance says:

(a) Total dose ≤ 100 µg (no inter-dose interval limitations) AND Total dose ≤ 1/100th NOAEL and ≤ $1/100^{th}$ pharmacologically active dose (scaled on mg/kg for i.v. and mg/m^2 for oral).

But it also says:

(b) 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.

It is unclear whether the margin of exposure should be 100-fold the NOAEL or 1000-fold.

A7: The $1/100^{th}$ the NOAEL in the animals is one of the criteria that could limit the clinical dose. Statement Q7(b) above refers to defining a limit dose for testing in animals for the microdose approaches rather than a clinical margin based on dose.

Q8: For positron emission tomography (PET) tracers, please confirm that for Approaches 1 and 2, toxicokinetics (TK) is not needed for either oral or i.v. administration.

A8: A nonclinical toxicity study conducted to support a clinical microdose trial should include TK assessment unless the study is conducted by the intravenous route. This is to demonstrate that systemic exposure has occurred. However, it is

recognized that for some PET tracers, the clinical microdose can be very low and in such cases it might not be possible to characterize a full TK profile.

Q9: What chemistry, manufacturing, and control (CMC) information should be available for an exploratory clinical trial?

A9: CMC information for exploratory clinical trials was not addressed in ICH M3(R2). Consult appropriate regulatory authorities and regional guidances.

Q10: Does evaluation of potential mutagenic impurities (e.g., structure-activity relationship (SAR) or testing) apply to exploratory clinical trial support?

A10: The drug substance should be considered appropriate from a CMC perspective. For approaches 1 and 2 (microdose studies), SAR or genotoxicity testing is not recommended for the parent drug or for the impurities. For other exploratory clinical trial approaches where higher doses and longer treatments are used, available guidance on mutagenic impurities should be followed.

G. Reproductive Toxicity (7)

Q1a: Endnote 4: In the preliminary embryo-fetal developmental study, what is the definition of "adequate dose levels"? Does this mean maternal toxicity at least one dose level? If only one or two dose levels have surviving fetuses, would that be adequate?

A1a: The same dose selection criteria used for a definitive embryo-fetal development study should be used for the preliminary study (see ICH S5).

***Q1b: Endnote 4: The text specifies a minimum of six dams per group. Does this mean a minimum of six litters per group should be evaluated*?**

A1b: No. Sometimes pregnant females have total loss of litters. Dosing should be initiated with a minimum of six presumed pregnant females per group, with all surviving litters evaluated.

Q1a: Are embryo-fetal development studies or the demonstration that the drug and/or metabolites do not partition into semen important for male-only products?

A1a: The ICH M3(R2) guidance does not address recommendations for embryo-fetal development studies in products intended for use only in males. Embryo-fetal development studies for a male-only drug should be considered on a case-by-case basis.

Q2b: ***Should contraception be used in male-only studies until reproductive risks have been evaluated?***

A2b: It is general practice to use contraception in males until the potential for reproductive and developmental risk has been addressed.

H. Juvenile Animal Studies (8)

Q1: What is the appropriate duration of treatment for a toxicity study using juvenile animals to address a specific issue of concern?

A1: Specific aspects of the design of juvenile toxicity studies are outside the scope of ICH M3(R2). However, in general, the duration of such a study will depend on the toxicity to be addressed, the organ system involved, and the information available from previous studies. The design and duration of the study should address the concerns for the product's potential to affect the developing organ systems of the intended clinical population.

To reduce animal use, the specific issue of concern can sometimes be evaluated by incorporating developmental endpoints into a general repeated-dose toxicity study or into a pre/postnatal toxicity study in which the pups were adequately exposed to the drug.

Q2: Clarify when a second species might be important.

The guidance states that when a juvenile animal toxicity study is warranted, one relevant species (preferably rodents) is generally considered adequate. It may be difficult to prospectively describe the majority of instances in which a second study in another species is scientifically justified, but can parameters be described that are not reasonable justifications?

A2: There are few circumstances for which juvenile animal studies in two species would be recommended besides (1) an absence of adult human data (i.e., a pediatric-only indication), or (2) there are multiple specific issues of developmental concern and no one species is able to address them adequately. Some situations for which a juvenile study in a second species is not warranted include the following: solely because a therapeutic is first-in-class, when verifying adverse findings in a juvenile study in one species, or when further examining behavioral effects of agents for which such effects are known or can be expected.

Q3: Please clarify what is important for pediatric-only indications. Should a juvenile animal study be conducted to support a pharmacokinetic (PK) study in

pediatric populations if you don't have any adult data? Should a second species be studied?

A3: Generally, data from adult human volunteers and the supporting nonclinical data (in two species) should be available before pediatric clinical trials are initiated, even when the product is not intended for development in adults. Section XII (12) of ICH M3(R2), Clinical Trials in Pediatric Populations, generally provides recommendations for the situation in which adult clinical trials should precede pediatric trials and indicates that juvenile animal toxicity studies are not considered important to support short-term PK trials in pediatric populations. However, if data from adult humans are not available and the drug will be developed only for pediatric subjects, then this is a case where juvenile animal studies in two species would be appropriate to support pediatric PK trials.