To: Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, RM. 1061
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From: Eli Lilly and Company

RE: Guidance for Industry: Nonclinical Safety Evaluation of Drug Combinations

Docket Number:2005D-0004

We at Eli Lilly and Company would like to take this opportunity to comment on the "Guidance for Industry: Nonclinical Safety Evaluation of Drug Combinations" as published in the January 26, 2005 Federal Register. Specifically, we have found seven concepts in the document which we feel need greater clarity. A summary of concerns is given below followed by more in depth discussions for each.

Executive Summary

1. Greater clarity is needed for the type of information necessary to justify the evaluation of only one species in the combination toxicity study. An example or two would provide greater guidance and reduce the likelihood that the sponsor and the agency would come to different conclusions.

2. The timing of when nonclinical combination studies would be needed relative to clinical development is not addressed in the guidance. Lilly believes that early clinical development including proof-of-concept studies could be justified without conducting repeat-dose nonclinical studies as long as single agent studies are conducted according to ICH M3. The 90-day combination and embryofetal studies would be needed at the time of product registration or earlier if the available data suggest a potential interaction.

3. The selection and justification of species for embryofetal evaluation should default to the rat as the species of choice unless data with one of the FDC (fixed-dose combination) components suggests the rabbit may be the better model.

4. The goals and study design for use in nonclinical combination studies need greater clarification. It should be clear that the goal of such studies is to find new and unexpected toxicities. The determination of an NOAEL is out of scope and meaningless unless the ratio of components is a constant. It is unlikely that the optimal ratio will be determined without extensive clinical investigation by which time the nonclinical studies should have been initiated or completed.

5. Doses for nonclinical combination studies should be selected based on the animal's tolerance to the drug(s) and not the clinical dose or dose ratio that may be marketed. This is the best way to predict clinical toxicity when relatively few animals are used

per group and is the basis for dose selection and study interpretation for a single agent. We see no scientific justification for deviating from this concept for FDC nonclinical studies.

6. The document should provide some guidance on the extent of nonclinical investigation needed to support adjunctive therapy when the compound of interest will be given with any number of agents within a therapeutic class. We believe that it is unnecessary to study each possible combination especially when the primary mechanism of action for the established drug class is the same. Although differences will exist that justify multiple drugs within a class, the likely mechanisms for adverse interaction will be either common to the mechanism of the class or predictable from the known differences within the class and the add-on agent. Nonclinical combination studies with the most pharmacologically diverse compound or the agent with the greatest likelihood for interaction could serve as a model for the class without having to conduct studies with each possible combination.

7. Clarification is needed for independent metabolic studies. Specifically, the agency should explain its rationale for needing combination protein binding studies, since effects on protein are most often predictable, and only in rare cases will they have clinical significance.

Selection And Justification of One Species for General Toxicity Study

The document is unclear as to what the default situation should be for the number of species to test for the general toxicity bridging study. There are a number of instances where one species is implied while others imply two species.

While **lines 129-135** allude to criteria for justifying a single species, additional clarity is needed to delineate how a single species will be justified. **Lines 129-131** provide some guidance, however, retrospective analysis of preclinical species with respect to clinical toxicity has shown there is often poor concordance from animals to humans, rendering this point of little value in justifying a single species. In addition, the comment about the toxicity being similar between species is subject to interpretation. Does that mean toxicity within a class (GI, CNS etc) or the exact toxicity (neutropenia, tremors, etc), ? In addition, the final sentence of the paragraph allows for an additional species to be requested based on results from the first species which is likely to cause significant delays in the clinical development of new therapeutics. Lilly recommends that additional clarity be added to this section providing some examples as to its definition of similar toxicity, and for the Agency to recommend a meeting to discuss the justification of a single species early in the development process.

Timing of Nonclinical Combination Studies Relative to Clinical Trials

Comments on Section II. Nonclinical Studies for a Combination of Two (or more) Previously Marketed Drugs (Figure A)

Lines 116-118. Although implied by preceding text in the same paragraph, adding "before Phase 1" to this sentence would clarify expectations. The new sentence would read, "…FDA strongly recommends that sponsors conduct nonclinical studies of the combination prior to Phase 1 clinical studies to better evaluate the interaction potential (see Figure A).

Comments on Section III. Nonclinical Studies for a Combination of Drugs When One or More is Previously Marketed and One is a New Molecular Entity (Figure B).

1. The timing of combination nonclinical studies relative to clinical trials is unclear. Although Figure B appears to indicate that studies up to 90 days are necessary prior to *any* clinical studies, the text does not. The text appears to follow the same logic as that for 2 or more marketed compounds, which indicates nonclinical studies would be needed prior to Phase 1 clinical studies in humans only when there is concern according to the factors listed in section II.A. Figure B appears to be inconsistent with the logic as shown in both Figures A and C and should be modified.

2. If studies are needed following the considerations in Section II.A., the guidance should recommend following ICH M3 with studies up to 90 days in duration supporting chronic combination clinical trials and registration unless preneoplastic lesions are observed in a new organ or tissue site compared to studies with the marketed drug or NME alone. This approach is contingent on the completion of studies with the NME alone according to ICH M3.

Comments on Section IV. Nonclinical Studies for a Combination of Two or More Drugs When Both Are New Molecular Entities (Figure C).

1. The timing of combination nonclinical studies relative to clinical trials is unclear. Figure C, Box 1 indicates combination studies "usually" should be conducted and to "(see text for details)". However, the text does not clearly indicate when combination studies are needed in relation to the timing of clinical trials. We recommend the same algorithm for all sections, which is to consider factors in section II.A to determine whether there is cause for concern.

2. Similar to Section III above, the guidance should recommend following ICH M3 for nonclinical combination studies of up to 90 days in duration supporting chronic combination clinical trials and registration unless preneoplastic lesions are observed in a new organ or tissue site compared to studies with the marketed

drug or NME alone. This approach is contingent on the completion of studies with all NMEs alone according to the ICH M3 guidance. If only combination studies will be completed, then ICH M3 should be followed for the combination studies, including carcinogenicity studies.

Selection and Justification of Species for Embryofetal Study

Lines 121-122: "It may be important to repeat some studies, such as equivocal reproductive toxicity studies." Given the variability inherent in many reproductive toxicity endpoints, repetition of a study may again produce equivocal results. Rather than repeat studies with the individual molecular entities, Lilly recommends that an embryofetal development study be conducted with the combination, and, if necessary to aid in explaining results, that a relevant dose of the single molecular entity(ies) be included.

Lines 218-219: "Embryofetal developmental studies of the combination should be conducted unless the marketed drug substance is already known to have significant risk for developmental toxicity." Clarification is required on the number of species to be used in evaluation of embryofetal development. Lilly recommends that an embryofetal development study be conducted with the combination in rats only, unless the rabbit has been shown to be uniquely sensitive to developmental effects induced by one or more of the FDC active components.

Lines 319-320: "If developmental toxicity has been assessed only on each NME separately, then FDA recommends that developmental toxicity studies be conducted on the combination as well." Lilly recommends that an embryofetal development study in rats should be sufficient to evaluate developmental toxicity with a drug combination. However if the rabbit has been shown to be uniquely sensitive to developmental effects induced by one or more of the molecular entities, then this species should be considered instead of the rat. Also, if developmental toxicity was manifested in the pre- and postnatal development study by one or more of the molecular entities individually, but not in the embryofetal development study, then a pre- and postnatal development study with the combination should be considered instead. The appropriate developmental toxicity study should be conducted prior to treatment of women of child-bearing potential with the combination.

Nonclinical Study Goals and Design

Primary Goal

From a nonclinical drug safety perspective, the primary goal of any combination studies should be to determine whether there are unexpected effects of combining the agents on toxicity. Additive toxicity should be an expected outcome if the target organs of toxicity are the same or overlapping for the two agents. Synergism, whether positive or negative, is important to understand, but may not be easily characterized in whole animal studies.

The determination of an NOAEL is entirely dependent on the ratio of the two compounds if there is synergism or additivity. A single numerical NOAEL will be entirely dependent on the ratio of the compounds being tested, and therefore has little meaning in the understanding of combination drug safety unless the ratio of the active drug components is constant in all dosage forms. The quantitative nature of the interaction can be determined by isobolography, but is usually prohibitive for a whole animal repeat-dose study, and is not necessarily all that meaningful in the development of adjunctive therapy when trying to affect different aspects of a disease than the conventional drug being used for treatment. NOAEL determination is best left to studies involving multiple levels of the single agent and has no practical value in risk assessment when the ratio that is used clinically is different than that used in nonclinical studies (See comments on dose selection, below). Thus, the goal of combination studies should not be to generate NOAEL results, but to detect and characterize unexpected toxicities.

Study Designs

At first one might envision a complete 3 by 3 factorial design plus single agent control groups (16 groups of animals) to determine deviations from the one factor dose response curve, however, such studies would be exceedingly costly and unwieldy. If an optimal ratio of the two drugs is known, the nonclinical safety program is similar to that for a single agent but instead can use a fixed ratio mixture of the two drugs. Depending on when the program is initiated relative to what is already known about the single agents, bridging studies employing the mixture can be designed. More likely however, the sponsor will wish to embark on a combination development program when the optimal ratio of the two drugs has not been defined. In this case the nonclinical combination study designs will be driven by animal toxicity data previously established for the single agents plus any available animal or human pharmacology data which may be the basis for pursuing combination development.

By focusing on the primary question of whether or not there is a synergistic or new toxicity when combining the two agents, a simpler design can be employed by combining one or two levels of one drug with one or two of the other. At least two combinations groups along with corresponding control groups should be assessed in any toxicologic assessment. The design should also include a vehicle control group as well as single agent control groups. The power of the statistical tests used to analyze data from this design is dependent on how many groups exist for each factor. If two levels of each compound are used (as depicted in Figure 1) statistical analyses should first compare the combination treated groups to the vehicle control, and, if a significant effect is found, a second series of statistical analyses should compare the combination group(s) to the single agent control group(s).

Compound B	Compound A: Control	Compound A: Low	Compound A: High
Control	X		X
Low		X	
High	X		X

Figure 1. **A 2 X 2 matrix design looking for off-target or synergistic responses.**

In some cases, a single dose of one of the agents may be appropriate (Figure 2) such as when the agent has a steep dose response curve. In this case a 2-factor ANOVA can be employed looking for a significant interaction term. If there is a positive interaction of the single level agent (Compound B) on the response of Compound A, p values can be computed comparing each level of Compound A in the presence and absence of Compound B.

Compound B	Compound A: Control	Compound A: Low	Compound A: High
Control	X	X	X
Treated	X	X	X

Figure 2. **General study design using a 2 factor model where one is limited to 1 degree of freedom.**

These designs do not allow for the determination of an NOAEL for the combination, but increase the chance of detecting and describing a new or synergistic toxicologically important effect. While some reviewers may be critical of a study which does not determine an NOAEL, the interpretive value of an NOAEL for a ratio that is different than what will be used clinically is meaningless (See above arguments).

Safety Pharmacology

Section IV. C. (**lines 282-285**) strongly encourages the conduct of combination safety

pharmacology studies, but then the following lines (**285-288**) imply these studies are needed only when both drugs target the same organ system, a toxicity is associated with a class of compound…, or the intended patient population is compromised … This seems inconsistent and should be clarified. Lilly believes combination safety pharmacology studies are needed only when both agents target the same organ system or physiology. For example, if one or both agents produce sedation or affect seizure threshold, select CNS safety studies should be conducted. Similarly if both agents affect respiration but the molecular mechanism is unknown, combination respiratory pharmacology studies should be conducted. However, if the molecular target is known and is the same for both agents, the effect of the combination can be determined mathematically using simple Michalis/Menton kinetics, and no combination study is necessary (e.g. the potential for QT prolongation was attributable to inhibition of I_{Kr} conduction and was quantified by in vitro methods using hERG transfected cells).

Dose Selection

Determination of doses to employ in a combination study is highly dependent on prior knowledge from single agent repeat-dose studies. While in principle, exposures should be equal to or exceed those for maximal efficacy in patients, this may not always be tolerated in animals as suggested in **lines 268-273**. Therefore, we believe the doses of each agent should produce adverse effects which the animal model can tolerate and not be dependent on human therapeutic doses. Differences in target sensitivity or metabolism are common reasons for deviations from allometric linearity. Doses of each agent should be such that they produce some degree of toxicity but that the highest selected dose not be an MTD nor an NOEL. The doses should be selected such that if additive toxicity is observed (an expected response), the effect is not lethality. Thus, the additivity can be observed and studied. If synergy or an unexpected exaggerated response is observed, the effect should be characterized sufficiently to support further product development decisions. Negative interaction may also be seen, but while interesting, poses no additional risk for clinical development and is, therefore, less important.

Dose selection may require conduct of pilot studies if drug interaction is suspected and pharmacokinetic or pharmacodynamic interaction information is inadequate. Ideally, exposure of each agent in the high-dose combination group will match that in the concurrent single-agent control group. This provides the best comparison for determining a clinically meaningful interaction.

Lilly does not believe, as alluded to in the FDA document (**line 265**) or suggested by the CHMP, that exposure ratios or doses be similar in the combination animal repeat-dose study as those anticipated in human therapy unless these doses represent the MTD in animals. This approach reduces the sensitivity of the animal study in detecting potential adverse events, and their use in predicting potential human outcomes. Determination of the optimal dosing ratio will be most often determined after extensive clinical

evaluations. Thus it is unlikely that the final clinical ratio will be available at the time nonclinical studies need to be conducted.

Use of Nonclinical Combination Studies in Support of a Single Agent With a Drug Class

Although not addressed in the draft guidance, clarification is needed on the selection of molecules for toxicological evaluation when adjunctive therapy is intended with a drug class (i.e., atypical antipsychotics, statins, etc.). Performing combination toxicity and embryofetal studies on every possible combination of a drug class with an NME or marketed compound would likely not provide additional new information on the safety of the adjunctive therapy. Lilly recommends that toxicity studies with one molecule from a drug class could be used to evaluate the risk associated with the class in combination with the add-on therapeutic. The molecule selected from the class should have the greatest potential for interaction based on the safety considerations listed in Section II. A of the draft guidance. Generally, the molecule selected for study would have the broadest receptor interaction profile of the group and/or the greatest potential for metabolic interaction with the NME or marketed compound used in the adjunctive therapy. There may be some instances where more than one compound from the class may meet the above criteria and evaluation of more than one compound in toxicity studies may be justified based on diverse pharmacodynamics and/or pharmacokinetics. However, Lilly recommends that independent studies are not needed for every possible drug from a particular class.

Considerations for Pharmacokinetic Interactions

The potential for pharmacokinetic interactions resulting from administration of combinations has been raised in the Draft Guidance. Specifically, the question of a "potential metabolic interaction" is discussed on **lines 150-151,** and boxes 4 and 5 of Figure A recommend the conduct of in vitro metabolism studies to elucidate these potential interactions. However, the in vitro metabolism package (including calculated K_i values for multiple CYP isozymes) should be available for each individual drug or NME at this point in development, and this information would be sufficient to predict the potential for a metabolic interaction between the two drugs when given in combination. Thus, the individual in vitro metabolism data packages should provide sufficient knowledge to guide the design of clinical drug-drug interaction studies, and early clinical studies of the combination will fully characterize the pharmacokinetics of the combination.

The Draft Guidance also suggests that sponsors evaluate serum protein binding and monitor plasma concentrations of each drug in the toxicology studies **(lines 296-297**). However, if the sponsor conducts a toxicokinetic analysis and is familiar with the pharmacokinetic characteristics of the combination, it would seem that additional assessment of protein binding adds negligible value. This perspective is supported by a

recent publication by Benet and Hoener (*Clin. Pharmacol. Ther.* [2002], **71**:115-121), which concluded that when "changes in protein binding [are] caused either by drug interactions or by disease states…no adjustments in dosing regimens will be necessary except in rare cases of a drug with a high extraction ratio and narrow therapeutic index that is given parenterally…or, even rarer, a drug with a narrow therapeutic index that is given orally and has a very rapid pharmacokinetic-pharmacodynamic equilibration time." Therefore, since very few drugs or NMEs are expected to fall within these exceptional cases, additional assessments of in vitro protein binding interactions in human (or animal) plasma should not be considered necessary to support the drug development program of the combination.