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Bristol-Myers Squibb Company

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The Division of Drug Information (HFD–240)
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane, Rockville, MD 20857

Re: Docket No. 2006D-0344; Draft Guidance for Industry on Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling *(Federal Register, Vol. 71, No. 176, September 12, 2006)*

Dear Sir or Madam:

Bristol-Myers Squibb (BMS), a diversified global health care company, is pleased to have the opportunity to offer comments on the Draft Guidance for Industry on Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling. Our company's mission is to extend and enhance human life by providing the highest-quality pharmaceutical and related health care products. For this reason, we are interested in commenting on the Draft Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling. Our comments are set forth below.

We commend the FDA for their commitment to providing a comprehensive guidance to industry concerning drug interaction studies. The draft guidance makes recommendations on metabolism- and transporter- based drug interaction studies. The draft guidance recommends that an integrated in vitro and in vivo approach should be employed to address drug interaction concerns, which is scientifically sound and consistent with the FDA's Critical Path Initiative. The following are specific comments on points in the guidance.

1 Page 11, in the paragraph beginning on line 448, there is a recommendation indicating that it may be informative to evaluate the effect of multiple CYP inhibitors under certain conditions. While we understand the spirit of the recommendation, currently there is little information on "acceptable" inhibitor cocktails to use for this purpose (especially in a healthy volunteer setting). We recommend removing this paragraph until specific guidance, based on clinical experience, can be given to justify this approach.

2. Page 19, table 2: Examples of in vivo substrate, inhibitor, and inducer for specific CYP enzymes recommended for study (oral administration). Efavirenz is the only example listed as an in vivo probe for CYP2B6. Since bupriopion has been demonstrated and used as an appropriate probe for assessing human CYP2B6 activity in vivo, we recommend that bupriopion be included in the table in addition to efavirenz.

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3. Page 29, line 916. It is stated that "Noncompetitive and mechanism-based inhibitors are not dependent on the drug (substrate) concentration." While this statement is true for noncompetitive inhibitors, it is not true for mechanism-based inhibitors. Mechanism-based inhibition is time- and concentration-dependent. Since this paragraph deals with pre-incubation condition for mechanism-based inhibitors, we recommend that the original statement is replaced with the following statement: Mechanism-based inhibitors are time- and concentration-dependent.

4. Page 28, table 2: Chemical inhibitors for in vitro experiments. We have three comments for this table as follows:

   (1) Footnote (2): It is stated that "Furafylline and methoxsalen are mechanism-based inhibitors and should be pre-incubated before adding substrate." We recommend that other mechanism-based or time-dependent inhibitors such as phencycline, ticlopidine and troleandomycin, which are listed in the table as acceptable inhibitors, should be indicated by the same footnote(2).

   (2) Gemfibrozil is listed as an in vitro inhibitor for CYP2C8. Although gemfiborzil is a potent inhibitor for CYP2C8 in vivo, it is not a potent and selective inhibitor in human liver microsomes. The in vivo inhibition appears to be primarily mediated by the glucronide conjugate of gemfibrozil. Therefore, we recommend that gemfibrozil be excluded from the list of acceptable inhibitors for CYP2C8.

   (3) There is no preferred inhibitor for CYP2C19. Recent studies have shown that (+)*N*-3-benzylnirvanol is a potent and selective inhibitor for CYP2C19 in human liver microsomes. We suggest that (+)*N*-3-benzylnirvanol be included in the table as the preferred inhibitor for CYP2C19.

5 Page 32, Table 3: Preferred and acceptable chemical substrates for in vitro experiments. Testosterone 6β-hydroxylation is listed as one of the two preferred substrates for CYP3A4/5. We recognize that testosterone 6β-hydroxylation has been widely used as an in vitro research assay for CYP3A4/5 activity, and testosterone represents a distinctive binding site from other CYP3A4/5 substrates including midazolam, nifedipine and erythromycin. However, there is no relevant in vivo probe that can be used in human to test testosterone 6β-hydroxylase activity. Therefore, we recommend that testosterone 6β-hydroxylation be replaced with an assay such as nifedipine oxidation which represents not only a distinctive binding site, but also a class of clinically relevant substrates of CYP3A4/5. In the same regard, it would be very helpful to have a recommendation on which in vivo substrate is appropriate for human testing when only testosterone 6β-hydroxylation is inhibited by the test article in vitro.

6. Page 34, lines 1065 -1074. We have three comments for this section as follows:

   (1) Line 1070-1072: It is stated that "Any time-dependent and concentration-dependent loss of initial product formation rate indicates mechanism-based inhibition." The time- and concentration-dependency can only suggest metabolism-based inhibition. Other criteria for irreversible or quasi-irreversible inhibition are required to determine mechanism-based inhibition, for example, dilution studies to determine K_I and k_{inact}, covalent binding to the enzyme, and quasi-irreversible complex formation.

(2) Line 1072 -1074: It is stated that "Detection of time-dependent inhibition kinetics in vitro indicates follow-up with in vivo studies in humans." It is unclear what type (or to what extent) of time-dependent inhibition kinetics in vitro, such as K_I and k_{inact}, indicates that in vivo studies in humans should follow. Since the prediction of in vivo drug interaction for mechanism-based inhibitors using in vitro data is poor, we recommend that the statement is modified as follows: For mechanism-based inhibitors, if it is justified scientifically based on in vitro investigation, in vivo study in humans should be conducted.

(3) The terms "mechanism-based inhibition" and "time-dependent inhibition" are used interchangeably. Some time-dependent inhibitors are not mechanism-based inhibitors. There are stricter criteria for mechanism-based inhibitors than for time-dependent inhibitors. If the guidance is only for mechanism-based inhibitors, more clarification should be made in this section. For example, the following criteria should be included to determine whether or not the test article is a mechanism-based inhibitor, dilution studies to determine K_I and k_{inact}, covalent binding to the enzyme, and quasi-irreversible complex formation.

7. Page 35, APPENDIX C-3. There is no recommendation for categorization (strong-moderate-weak) of enzyme induction-related interactions. The categorization of strong, moderate, and weak for enzyme inhibition (based on AUC changes, page 9) is helpful, and a similar ranking of AUC changes due to enzyme induction with possible outcomes would be equally helpful to standardize the language across this field. As an example: AUC decreases greater than 50% would represent strong inducers; AUC decreases between 25-50% would represent moderate inducers; AUC decreases <25% would represent weak or insignificant inducers.

8. Page 35, lines 1091-1093. Chemical Inducers as a Positive Control. It is stated that "The positive controls should be potent inducers (>2-fold increase in enzyme activity of probe substrates at inducer concentrations <500 μM)". This is contradictory to the information contained in Table 5 that refers to phenobarbital and pyrazole at concentrations greater than 500 μM.

9. Page 36, lines 1112-1113: Design of In Vitro Drug Induction Studies. It is stated that ".....immortalized liver cells are acceptable if it can be demonstrated with positive controls that CYP3A4 and CYP1A2 are inducible". We have two comments on this statement: (1) it is not clear whether this refers to enzyme activity or other measurements such as mRNA and western blot; (2) immortalized cell lines do not appear to retain all of the functional mechanisms of enzyme induction as do primary hepatocytes. For example, the Fa2N-4 cell line responds appropriately to PXR- and AhR-mediated enzyme induction, but does not appropriately respond to CAR-mediated enzyme induction. In addition, some inducers may involve multiple mechanisms such as PXR- and CAR activation. To clarify those concerns, we recommend the following modifications to this statement: ".....immortalized liver cells are acceptable if it can be demonstrated with positive controls that CYP3A4, CYP1A2 and CYP2B6 enzyme activities are inducible"

10. Page 36, line 1115. It is stated that "Test drug concentrations should be based on the expected human plasma drug concentration (to) be used" The word "to" is missing. More importantly, it would be helpful to clarify whether the expected human plasma drug concentration refers to free or total drug concentration. To be consistent with the inhibition section, this should be total concentration.

11. Page 36, lines 1121-1125. There should be some mention that enzyme inhibition data (of the test article) is necessary when interpreting enzyme activity results from probe substrates, as inhibition can confound such results.

12. Page 36, lines 1127-1128. It is stated that "When conducting experiments to determine enzyme activity, the experimental conditions listed in section Appendix C-2 are relevant". In Appendix C-2, there are discussions about how probe substrates at K_m concentrations should be employed for enzyme inhibition study, however, this is not true for induction studies where concentrations approaching V_{max} are employed. We recommend that appropriate language such as "probe substrate concentration at 3-5-times K_m, or maximum solubility of the probe" should be added in this section.

13. Page 36, lines 1105-1135. In the section about design of in vitro drug induction studies, there is lack of criteria about acceptable cell viability. To strengthen the validity of cell-based enzyme induction models, appropriate measures of biochemical cellular toxicity would be beneficial. We suggest a minimum cell viability of >70% when initially plating cells. In addition, it is highly recommended that cell viability be measured at the end of the experiment on control and drug treated cells. Significant cytotoxicity caused by the test articles can result in false negative interpretations. We suggest that any test article (and its associated concentration) that causes >50% cytotoxicity in a biochemical toxicity assay should not be used in the final interpretation of results.

14. Pages 38-50, APPENDEX D. The terms "P-glycoprotein" and "efflux" are used inter-changeably though this section. Since there is lack of specific substrates and inhibitors for P-gp, it is difficult to assess the contribution of other efflux transporters such as BCRP and MRP-2 to the net flux ratio of the cell culture systems. Therefore, caution should be taken when concluding whether or not the test article is a P-gp substrate or inhibitor.

15. Page 42, section (c): Tissue culture considerations to ensure functionally polarized cells. No guidance is provided regarding the appropriate pH value for bi-directional studies. We recommend that pH 7.4 for both the donor and receiver chambers should be considered appropriate.

16. Page 43, line 1356. It is stated that "Optimal experiments should determine recovery of substrate, to allow estimation of metabolism and non specific binding." Howerver, there is no acceptance criteria for mass balance. We recommend that appropriate acceptance criteria for mass balance, such as >60%, should be provided in the guidance.

17. Page 45, lines 1428. It is stated that "where (REi/REa) represents the efflux ratio of the probe P-gp substrate in the presence of inhibitor concentration (I) relative to that for the control without inhibitor." There is another approach which has been widely used to calculate the inhibition potency (Kim et al, Pharm Res 1999, 16, 408-414). We recommend that this approach is included as an alternative.

18. Page 45, lines 1412-1414. It is said that "After incubation of the cells for 0.5-1 hour at 37°C, the medium is removed from the apical or basolateral side of the monolayer and replaced with the appropriate concentration of the selected probe P-gp substrate (see Table 2). As a preferred

practice, the test article as inhibitor should be maintained in the media on both sides for the entire duration of incubation to avoid false negatives.

19. Page 46, line 1149. It is stated that "A net flux ratio over 2 is considered a positive result." The net flux ratio over 2 as the cut-off criteria is too sensitive. Too many false positive results will be generated due to experimental variability. Another concern is that, if the net flux is just over 2, there is no enough dynamic range for the follow-up inhibition studies as suggested by the guidance (page 47, Figure 1). We recommend that a net flux ratio over 3 be considered a positive result.

20. Page 47, line 1474. As discussed in comment #19, we recommend that the following criteria be used in the decision tree (Figure 1): Net flux ratio ≥ 3 as a positive result, net flux ratio < 3 as a negative result.

21. Page 47, lines 1499-1501. It is stated that "A sufficient system produces net flux ratios of the probe substrates similar to values reported in the literature (a minimum net flux ratio of 2 is recommended)". Considering the dynamic range for inhibition studies and experimental variability, we suggest a minimum net flux ratio of 3.

22. Page 48, lines 1520-1525. The I/IC50 (or Ki) ratio has been recommended as an index to determine whether an in vivo drug interaction study is needed or not. It is helpful to have this guidance on how to use in vitro results to guide in vivo human studies. However, unlike the case for CYP inhibition, there are not many examples that can validate this recommendation.

23. Page 48, line1521. It is stated that "An in vivo drug interaction study with a P-gp substrate such as digoxin should be conducted." Since fexofenadine has been demonstrated and used as an appropriate P-gp substrate for clinical studies, we recommend that fexofenadine also be included as an alternative to digoxin.

24. Page 49, line 1527. It is stated that "Figure 1. ....." This should be Figure 2.

BMS appreciates the opportunity to provide comment and respectfully requests that the FDA give consideration to our recommendations. We would be pleased to provide additional pertinent information as may be requested.

Sincerely,

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