Society of Toxicologic Pathology

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August 6, 2001

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Re: (G:/3010DFT.DOC, 4/10/01

Dear Sir/Madam:

This communication contains comments and suggestions from the Society of Toxicologic Pathology regarding the draft Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs. Please forward to the appropriate parties.

Thank you for the opportunity to comment.

Sincerely,

RR Maronpot

Robert R. Maronpot, D.V.M., Diplomate ACVP, Diplomate ABT
President

/lcw
Enclosure

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SOCIETY OF TOXICOLOGIC PATHOLOGY
Scientific and Regulatory Policy Committee
Subcommittee on Immunotoxicity Testing

COMMENTS REGARDING
Guidance for Industry:
Immunotoxicology Evaluation of Investigational New Drugs
(G:/3010DFT.DOC, 04/10/01)

General comment: Given the relatively immature status of the field of immunotoxicology, we understand that interpretation of study findings with respect to immunodysfunction is quite difficult. We therefore agree with the use of terminology in this document that allows individual decision-making about the need for additional testing and the selection of appropriate tests for evaluation of immune function for each drug. At this time it is difficult to establish firm rules about the need for testing and the specific tests to be conducted. As the science of immunotoxicology matures and more definitive evaluations are available, this guidance will need to be updated.

Line 37: *"5. Adverse immunostimulation: Non-antigen specific activation of the immune system"*
Comment: Non-antigen specific activation of the immune system does not, of itself, constitute an adverse consequence; problems arise when such immune activation is uncontrolled.
Recommendation: Change the bullet to read "Non-antigen specific uncontrolled activation of the immune system".

Line 52: *"Changes in some parameters might not be cause for concern when the changes are small but statistically significant. For example, any decrease of more than 40 percent in total lymphocytes (ref) or 75 percent in granulocyte counts (ref) could be significant, while changes less than 40 percent and 75 percent may be only suggestive of the immunotoxicity."* **Comment:** Although only an example, the reference to percentage changes – suggesting a threshold for interpretation – may lead to incorrect interpretations of study data. This is true for all clinical pathology parameters, but especially for the leukocyte differential. Differences in species, study

population, husbandry, study procedures, etc., greatly affect the magnitude of changes and normal variability that can occur. Moreover, the measurement of one or a few parameters does not adequately describe the immunologic status of the host. The document needs to underscore that multiple endpoints are needed to corroborate the identification of immunosuppression/immunodysfunction. The example statement does not effectively clarify the previous two sentences, which are tenets of data interpretation in toxicological studies. **Recommendation:** Delete the sentence beginning with "For example" on line 53.

Line 75: *"A comparison of observed effects with vehicle-treated controls might be useful to determine whether there are toxicological effects of the drug that are stress inducing."* **Comment:** The second sentence of this paragraph describes procedural effects (not test article effects) that may cause "stress". On the other hand, the sentence beginning on line 75 describes drug toxicity that may be "stress inducing". The recommended additional sentence (below) clarifies that stress-inducing toxicity is not necessarily immunotoxic. **Recommendation: Add the following sentence to the end of this paragraph:** "While the toxicological effects of the drug must be considered, these secondary, stress-inducing effects of drug toxicity do not indicate direct effects on the immune system and should not trigger additional testing for immunotoxicity."

Line 86 – 90: Comment: This paragraph overstates the risk of drug concentration in reticuloendothelial tissues (i.e. monocyte/macrophage system) and its effect on the function of phagocytes. The function of the monocyte/macrophage system is to phagocytize and process foreign material, pigments, etc and remove them from the blood. Therefore it is not unusual to identify material within the monocyte/macrophage system and it would be inappropriate to initiate immunotoxicity studies on such evidence. If drug accumulates within the monocyte/macrophage system resulting in an adverse effect on the function of this system, it is very likely that there will be ample histologic evidence that such accumulation has a significant biological effect. Moreover, materials can accumulate in macrophages following drug exposure that are not drug specific, such as phospholipidosis secondary to cationic amphophilic drugs. Such responses are

well characterized and their full toxicologic impact well understood; immunotoxicity studies would be of academic interest only.

Line 109: *"• Decreased serum immunoglobulin levels"* **Comment:** The concentration of serum globulins is routinely determined "in standard non clinical toxicology studies". Quantification of immunoglobulin levels requires special techniques and this should not be required in standard studies. **Recommendation:** Remove this bullet line or change "immunoglobulin" to "globulin".

Paragraph ending on Line 129: Comment: The first sentence of the **paragraph beginning on line 131** describes detailed evaluation of cortical and medullary areas of lymphoid tissue. Further, it seems more appropriate to attach this thought to the end of the previous paragraph. **Recommendation:** Delete the sentence beginning on line 131 and add the following sentence to the end of the paragraph ending on line 129: "If routine histopathologic evaluation of these immune system-related tissues reveals possible effects, additional, detailed histopathologic examination of lymphoid tissues should be conducted to detect potential immunotoxic changes." Pertinent references for this detailed histopathologic examination are: 1.) The ICICIS Group Investigators. Report of validation study of assessment of direct immunotoxicity in the rat. Toxicology 125: 183-201, 1998. 2.) Kuper CF, Harleman JH, Richter-Reichelm HB, Vos JG. Histopathologic approaches to detect changes indicative of immunotoxicity. Toxicol. Pathol. 28: 454-466, 2000.

Line 132: *"Other indicators of immunosuppression in nonclinical toxicology studies include treatment-related infections and lymphoproliferative type tumors."* **Comment:** This is an overstatement, at least the reference to lymphoproliferative tumors. As written, this statement suggests that the finding of treatment-related lymphoproliferative tumors is indicative of immunosuppression. There are other, more likely, mechanisms of carcinogenicity besides immunosuppression. In addition, this statement will cause problems in the paradigm of drug development. Since the end of the 2-year rat study is near the end of the critical path for drug development, drug companies cannot wait until the end of the 2-year study to consider immune

function testing. The risk of delay in drug development may force "prophylactic" immune function testing to protect against the possibility that the 2-year study might reveal tumors, and there is insufficient justification for the additional use of animals that would result. **Recommendation: Change this as follows:** "Other possible indicators of immunosuppression in nonclinical toxicology studies include treatment-related infections and lymphoproliferative type tumors. If treatment-related infections or lymphoproliferative tumors are observed in nonclinical toxicology studies, a thorough retrospective analysis of clinical and histomorphologic findings should be undertaken to evaluate for possible immunosuppressive effects. This analysis should include material from the 2-year bioassay and from previous studies."

Line 134: *"Although decreases in serum immunoglobulin might be considered a relatively insensitive indicator of immunosuppression, this measurement is useful because it can be readily incorporated into the standard battery of clinical pathology tests."* **Comment:** The concentration of serum globulins is routinely determined "in standard non clinical toxicology studies". Quantification of immunoglobulin levels requires special techniques and this should not be required in standard studies. **Recommendation:** Change "immunoglobulin" to "globulin".

Paragraph beginning on Line 138: Comment: This paragraph illustrates inconsistencies and inaccuracies in the development of immunologic concepts. The paragraph is included in a section describing "Detection of Immunosuppression". Some parts of the paragraph are relevant to immunosuppression, specifically "direct bone marrow toxicity". However, reference to "drug-mediated intravascular hemolysis" and "immune-mediated cytolysis in immunosuppression" should be deleted or the paragraph moved. **Recommendation:** Move this paragraph to the section on "Autoimmunity" and modify it to correct for the inaccuracies described below (lines 141 and 148).

Line 141: Comment: The phrase *"...drug-mediated hemolysis from immune-mediated cytolysis in immunosuppression..."* is an incorrect statement. Hemolysis under such conditions represents

immunostimulation. **Recommendation:** See the recommendation for this paragraph beginning on Line 138, above.

Line 148: *"Detection of cell-bound antibodies can determine if the immunosuppressive effect has an autoimmune or anti-drug antibody component."* **Comment:** Again, this discussion is directed at hemolysis of RBCs and would be appropriate here only if the antibody response was directed at a lymphocyte subset. **Recommendation:** See the recommendation for this paragraph beginning on Line 138, above.

Section beginning on Line 160: "*Immune Cell Phenotyping*" Comment: This section appears to place undue emphasis on immune cell phenotyping. The ILSI subcommittee on flow cytometry has indicated that immunophenotyping is not a good indicator of immune status. Moreover, there are significant issues with source and consistency of immunologic reagents for cell markers across species, i.e., mouse, rat and dog. In addition, it has not been well established that splenocytes from rodents are a good surrogate for circulating white cell populations in the peripheral blood of other species, especially man.

Section beginning on Line 188: *"C. Immune Function Studies"* **Comment:** There is no mention of nonspecific immunity in this section. Neutrophil, natural killer (NK) and macrophage function are important and should be included in this section.

Line 199: *"However, there is a version in which the assay is integrated into standard nonclinical toxicology studies."* **Comment:** Immunization of the main study animals (no satellite animals used) has been suggested at times. However, it is not known if antigenic stimulation of a lymph node along with activation of the immune system in general will alter the PK and therefore the TK of a drug. If immunization of an animal with SRBC occurred at the same time that an antigenically active compound was administered, what would be the outcome? Will protein binding be altered? Will they interact in ways to change the overall toxicologic picture? Alternatively would immunostimulation by a T-dependent antigen counteract, hide, or blunt the appearance of

minimal-to-mild immunosuppression of a drug given at the same time? **Recommendation:** Delete the sentence. It is not additive to the discussion. If the SRBC assay is to be done, the laboratory should know enough about the assay to understand that integration into a standard toxicology study is a possibility.

Line 200: *"Animals in the study are immunized with an antigen (e.g., SRBC, tetanus toxoid) and....."* **Recommendation:** Change the sentence to read "Animals in the study are immunized with a T-dependent antigen (e.g., ...)" This change is recommended to emphasize that this test evaluates the T-cell-dependent antibody response.

Line 202: *"Although the ELISA variation is not a true test of immune function,it has demonstrated...."* **Comment:** The statement that the ELISA method is "not a true test of immune function" is incorrect. **Recommendation:** Delete the phrase and start the sentence with "This ELISA variation has demonstrated...."

Line 224: *"If a drug is intended for treatment of HIV infection or a related immune disease, immune function studies should be considered part of the safety assessment, even when no signs of immunotoxicity have been observed in the standard toxicology studies."* **Comment:** It is recognized that HIV patients are a susceptible population with respect to immune function. However, the mandatory inclusion of immunotoxicity testing for ALL drugs used to treat HIV appears unwise. Many drugs used for treatment of secondary HIV complications (i.e., antibiotics, antifungals, nutritional support products, etc.) do not target the immune system. For these products, evaluation of all data from standard non-clinical studies is most appropriate for evaluating potential immunotoxicity. Furthermore, evaluation of immune function for all drugs used in HIV patients will not likely protect this susceptible population as intended. It is stated that this is a susceptible population with "impaired immune function". Routine immune function testing in immunologically normal animals will not improve the safety assessment for HIV drugs in the patient population with "impaired immune function". Appropriate, immunologically impaired

animal models for evaluation of HIV drugs have not been established. Inclusion of this mandate in the guidance (and particularly in the flowchart depicted in Attachment 1) will likely confound the development of supportive therapeutics for the HIV population. Nevertheless, for HIV drugs that have a direct effect on the immune system, immune function studies are appropriate for safety assessment.

Line 234: *"True antigens are digestible by antigen-presenting cells (APC)."* **Recommendation:** Change the word "digestible" to "processed".

Section beginning on Line 272: ***"VI. HYPERSENSITIVITY":*** **General comment:** There are no useful models for predicting Types I, II and III hypersensitivity. Furthermore, testing for these types of hypersensitivity is neither validated nor predictive, yet the flow chart (Attachment 2) refers to specific tests to be "considered".

Line 457: *"Adverse immunostimulation refers to any antigen-nonspecific, inappropriate, or unintended activation of some component of the immune system."* **Comment:** Definition of "adverse" needs improvement. It should indicate an uncontrolled response or a response that targets inappropriate tissue. "Unintended" does not necessarily imply adverse.

Line 458: *"Chronic inflammation can be considered to result from adverse immunostimulation,..."* **Comment:** As stated, every incidence of chronic inflammation in a toxicology study would be incorrectly ascribed to perturbations of the immune system. **Recommendation:** Change the sentence to read: "Chronic inflammation may result from adverse immunostimulation...".

Line: 469: *"A relatively common manifestation of immunostimulation is leukocyte infiltration of tissue."* **Comment:** Leukocyte infiltration of tissues in rodents is a common background finding and may be increased in general organ toxicity as a response to direct chemical damage to a tissue. It should not be considered evidence of direct effects on the immune system as presented

in this document, unless there is additional supporting evidence. **Recommendation:** Delete the sentence.

Line 479: "*As the flowchart in Attachment 1 indicates, additional immunotoxicology studies to complement the standard repeat-dose toxicology studies are expected when the drug is administered by inhalational or topical routes.*" **Comment:** Route of exposure is not a good rationale for triggering additional testing. It is the compound, not the route that should trigger additional testing. For example, a respiratory sensitizer will cause sensitization if administered orally. **Recommendation:** Change the sentence to read: "As the flowchart in Attachment 1 indicates, additional immunotoxicology studies to complement the standard repeat-dose toxicology studies are expected when the drug is expected to have sensitizing potential." Also, change the flowchart in Attachment 1 to reflect this wording.

Line 484: *"...or mouse IgE test (MIGET)"* **Recommendation:** Remove "mouse IgE test (MIGET) from the "such as" list and from the flow chart (Attachment 1). MIGET is not a validated test. In fact, an attempt at validation of MIGET has failed.

Line 492: *"Ideally, the effect of maternal drug exposure on lymphoid system histopathology and hematology in the F_1 generation offspring should be included in the terminal examination."* (Also mentioned in **Line 221 and Attachment 1**) **Comment:** Standardized developmental immunotoxicology approach and methods are not defined. The recent ILSI sponsored Developmental Immunotoxicity Workshop (June 12 & 13, 2001, Washington, D.C.) confirmed this lack of defined methodology for developmental immunotoxicity testing. **Recommendation:** Since developmental immunotoxicity testing is an emerging science the wording here should indicate that the approach to testing will be handled on a "case-by-case basis".

Line 514: *"For further evaluation of immunosuppressive effects, two assays in particular should be considered: (1) immune cell phenotyping (by flow cytometry) and (2) the anti-sheep red blood cell plaque assay."* **Recommendation:** Add the following phrase to the end of the sentence: "or other tests that evaluate T-cell-dependent antibody response." **Comment:** As indicated above

(Line 160), item (1) appears to place undue emphasis on immune cell phenotyping. The ILSI subcommittee on flow cytometry has indicated that immunophenotyping is not a good indicator of immune status. Moreover, there are significant issues with source and consistency of immunologic reagents for cell markers across species, i.e., mouse, rat and dog. In addition, it has not been well established that splenocytes from rodents are a good surrogate for circulating white cell populations in the peripheral blood of other species, especially man.

Line 521: *"For example, when anemia is present, a Coombs test could indicate whether immune-mediated hemolytic anemia is the cause."* **Comment:** As written, this sentence suggests that any anemia would evoke the performance of Coombs test. **Recommendation:** Change the sentence as follows: *"For example, when anemia is present* ***and other findings are consistent with an immune-mediated hemolytic anemia****, a Coombs test could indicate whether immune-mediated hemolytic anemia is the cause."* Also, add the following sentence: "Findings consistent with an immune-mediated hemolytic anemia include histopathologic evidence of increased destruction of red blood cells in the spleen and/or bone marrow, hyperbilirubinemia, hemoglobinuria, regenerative response (reticulocytosis or erythroid hyperplasia and/or extramedullary hematopoiesis) without evidence of hemorrhage, and/or spherocytosis."

Line 528: *"Drug-induced autoimmunity suspected in toxicology studies is difficult to confirm with current methods. Nonetheless,...."* **Comment:** There is general acknowledgement that the predictability of animal models for immuno-allergic phenomena is unreliable (Choquet-Kastylevsky and Descotes, 1998) or nonexistent (Griem et al., 1998). Elucidation of the mechanism(s) of immuno-allergic reactions is confounded by the idiosyncratic and unpredictable nature of the reaction. **Recommendation:** Change the sentence beginning with "Nonetheless" as follows: "Nevertheless, consideration should be given to conducting some additional methods to further elucidate the potential for autoimmunity. Currently, most methods (such as the popliteal lymph node assay and specific biomarker assays) are experimental and not validated. Therefore,

the selection of appropriate methods should be based on the drug and the nature of the suspected autoimmune findings."

Line 532: *"If chronic toxicology studies or rodent bioassays indicate carcinogenic potential, the contribution of unintended immunosuppression to the findings should be evaluated."* **Comment:** Since there are other, more likely, mechanisms of carcinogenicity besides immunosuppression, it seems inappropriate and problematic to trigger immunotoxicity testing solely on the basis of carcinogenicity in a chronic bioassay. **Recommendation:** Change the sentence to read: "If chronic toxicology studies or rodent bioassays indicate carcinogenic potential, a thorough retrospective analysis of clinical and histomorphologic findings should be undertaken to evaluate for possible immunosuppressive effects. This analysis should include material from the 2-year bioassay and from previous studies. If this evaluation indicates that immunosuppression may have been a factor in the carcinogenicity, then appropriate evaluation for immunosuppression, such as a tumor host resistance model, should be considered.

Line 535: *"Tumor host resistance models are appropriate for determining carcinogenic immunosuppressive potential."* **Comment:** This statement is incorrect. Tumor host resistance models evaluate for potential immunosuppression. They do not evaluate for carcinogenic potential. In this context the direct linkage between carcinogenicity and immunosuppression is problematic. There are other mechanisms of carcinogenicity besides immunosuppression. Further, a finding of immunosuppression does not necessarily indicate that an observed carcinogenic finding was due to immunosuppression. Immunosuppression is just one of several potential causes of cancer. **Recommendation:** Delete the sentence.

End of text: Comment: Page 15 appears to be missing. The last page of the text is page 14 and the first page of the references is page 16.

Attachment 1: Based on the comments listed above, the following steps in the flowchart are inappropriate (the line number for the comment is indicated after the flowchart category):

1. *"Inhalational or topical administration?"* see comment for **line 479**
2. *"Likely to be used in pregnant women?"* see comment for **line 492**
3. *"Accumulation or retention in reticuloendothelial tissues?"* see comment for **line 86 – 90**
4. *"Treatment of HIV or related immune disease?"* see comment for **line 224**

Attachment 2: The annotation in the right margin opposite *"Evidence of hypersensitivity (4)?"* is *"IV.B"*. It should be VI.B.

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