Sara Radcliffe
Research Director
Preclinical Affairs

August 9, 2001

Dockets Management Branch
Food and Drug Administration, HFA-305
5630 Fishers Lane, Room 1061
Rockville, MD 20857

Re: Docket No. 01D-0177; Draft Guidance on Immunotoxicology Evaluation of Investigational New Drugs, *Reference to 65 Federal Register 175 (April 10, 2001)*

Dear Sir or Madam:

The Pharmaceutical Research and Manufacturers of America (PhRMA) represents the country's leading research-based pharmaceutical and biotechnology companies, which are devoted to inventing medicines that allow patients to lead longer, happier and more productive lives. Investing an estimated $30 billion in 2001 in discovering and developing new medicines, PhRMA companies are leading the way in the search for cures.

PhRMA has reviewed the Draft Guidance on Immunotoxicology Evaluation of Investigational New Drugs, *Reference to 65 Federal Register 175,* which was released for comments on 10 April 2001. We agree with the overall principles of this well written guidance, but would like to take this opportunity to offer the following comments.

General Comments

This document in general provides a good perspective on the state of immunotoxicology testing for pharmaceuticals. With this guidance, the Food and Drug Administration (FDA) is proposing that all investigational new drugs be evaluated for effects on the immune system with further provisions where additional nonclinical testing may be necessary. PhRMA agrees that the evaluation criteria presently included in standard repeat-dose toxicology studies are generally sufficient to assess potential effects on the immune system. Based on past experience, this practice has been adequate because drugs that have produced unintended immunosuppression in humans have not been found. The design of these studies can often accommodate inclusion of additional specific immunologic tests on a case-by-case basis driven by scientific need or risk/benefit for the class of drug. If immune system-related findings are noted, the guidance document indicates that follow-up studies are needed using a case-by-case approach in order to better understand the potential safety implications of those findings.

1100 Fifteenth Street, NW, Washington, DC 20005 • Tel: 202-835-3440• FAX: 202-835-3597 • E-Mail: sradclif@phrma.org

Nevertheless, PhRMA is concerned that the proposed guidance does not factor in 1) the inability of the assays to be able to distinguish a biologically significant effect and 2) the lack of validation of the assays with respect to human prediction. Overall, we are concerned that the development of important pharmaceuticals may be curtailed on the basis of immunologic changes observed in nonclinical testing that either can not be interpreted in the species tested or have no relevance to humans.

PhRMA agrees that in most situations a dramatic decrease in the antibody response to a T-cell-dependent antigen or other parameter should be a concern for possible human immunotoxicity. However, this may not always be the case and we give as an example Videx ®(didanosine). This drug has been shown to clearly improve the immunologic function and survivability of patients infected with HIV. Immunologic testing in normal mice showed a 90% reduction in the ability of the animals to respond to a T-cell dependent antigen, which the authors interpreted as a profound effect (Phillips et al., 1997). Nevertheless, this effect did not prove relevant to the intended human population. In fact, it was not even predictive of any significant adverse effect in the species tested. In a 2-year carcinogenicity study in rats, female animals given the same dose that produced the "profound" effect actually had a significant increase in their lifespan (SBA Videx, 1991).

The guidance also evaluates the concerns and complexities around drug hypersensitivity or autoimmune reactions and indicates that in most instances there are no standard preclinical tests available for reliably determining the potential for drugs to cause these adverse effects in humans. However, the flow chart presented in attachment 2 gives the impression that there are defined assays to test for these adverse reactions by listing non-validated assays for hypersensitivity or autoimmune testing together with validated assays for determining immunosuppression. PhRMA recommends that an explanation be given with this chart to clearly distinguish validated from non-validated assays along with scientific rationale for further evaluating hypersensitivity or autoimmunity, if there is evidence for these reactions.

Specific Comments

Lines 50-60: PhRMA strongly agrees with the FDA that changes in immune parameters need to be biologically significant and not just statistically significant to trigger follow-up studies, and that a dose response relationship is important. However, with our present state of knowledge, it is often not possible to discern the degree of change that would be biologically significant. As you are aware, the immune system has great reserve and redundancy and can often compensate when only one component is affected. Thus, a very large change in a parameter may be required to affect host resistance. We therefore recommend that the uncertainty of what may be considered biologically significant be emphasized in the guidance.

Lines 70-77: PhRMA agrees that environmental factors may cause stress-induced immunologic effects, and that in carefully designed studies these effects should be reflected in non-drug-treated control animals. However, it is inherent to the purpose of toxicology studies to produce significant toxicity, which itself can be a source of stress to the animal, and it is often

difficult or impossible to distinguish a direct immunologic effect of the drug at doses that might induce stress-related immunologic changes. Thus, PhRMA believes the agency should discuss the importance of studying the potential for immunotoxic effects at doses that do not produce overt toxicity.

Lines 86-90: The term "reticuloendothelial tissues" should be replaced with "phagocytic cells" or "lymphoreticular tissues." Most often a compound that is retained in tissues, and especially within phagocytic cells, is either benign or behaves as an adjuvant in stimulating immune cell responses. Yet, standard testing of macrophage function (as recommended in this guidance) generally evaluates for suppression of non-specific phagocytosis and killing. There is little in the literature to support that localized retention of a pharmaceutical (non-metal or non-metalloid) compound in lymphoreticular tissues alters these general innate immune responses *in vitro*. In addition, it is unclear whether enhancement, as well as suppression, of phagocytic or killing activity would be considered "adverse." FDA should add a justification to explain why additional testing should be performed if drug accumulates in macrophages even when no signs of immunotoxicity are apparent (such as with the accumulation of certain antibiotics).

Lines 109 and 134-136: Because total serum immunoglobulin is known to be an insensitive indicator of immunosuppression, we recommend that it not be incorporated into a standard testing battery for immunotoxicity. Histopathologic changes in lymphoid organs are more sensitive indicators.

Lines 138-150: This paragraph intermingles a description of myelosuppression with autoimmune-mediated effects on erythrocytes. Antibody mediated drug-induced hemolysis can occur, but this is not in itself an immunosuppressive effect. The only autoimmune phenomenon potentially relevant to this section might be immune-mediated neutropenia, an exceedingly rare event that is poorly documented. We recommend that this section focus on myelosuppression as being a cause of immunosuppression; the reference to autoimmune phenomena should be moved to the appropriate section of the guidance.

Line 107: "Increased incidence of tumors" should be changed to "Increased incidence of certain tumor types (e.g. lymphoma/leukemia)." Data obtained with known immunosuppresive agents do not demonstrate an increased incidence of most tumor types, suggesting that the relationship between tumorigenesis and immunosuppression is not generalizable.

Lines 143-150: We recommend that the lack of full Good Laboratory Practice (GLP) compliance for follow-up studies should not limit the value of these data to support clinical studies or registration. Many of these studies are investigative in nature, particularly when incorporated into an ongoing study to elucidate a possible immunologic mechanism. In these situations, methods used must often be defined in a very short period of time, or GLP-validated methods may not exist, particularly in non-rodent studies. PhRMA recommends a statement that the work be done "in the spirit of GLPs."

Lines 160-186: The guidance recommends the characterization of immune cell phenotype in follow up studies if immunosuppression is observed in nonclinical toxicology studies.

Phenotyping studies were recommended since immune cell phenotype changes have been demonstrated by the National Toxicology Program (NTP) (Luster et al., 1992) to be one of the best single correlations with host resistance against pathogens or tumors. However, since the NTP examined the spleen cells of B6C3F1 mouse for these studies, it is not known if the same correlation exists for rats, dogs and monkeys or with peripheral blood lymphocytes (PBLs). With non-rodent species, the analysis of PBLs will be much more practical. Moreover, since PBLs would be used to monitor adverse effects in clinical trials, it makes more sense that PBLs be used for the preclinical studies. This difference between validation in mouse spleen and practical use of rat, dog, monkey and human PBLs needs to be reconciled before stating that immune cell phenotyping in preclinical studies is a validated approach. This is further supported by recommendations made at a recent workshop on the application of flow cytometry to immunotoxicity testing (International Life Sciences Institute Immunotoxicology Technical Committee, 2001). The workshop panel strongly emphasized that, for regulatory purposes, the application of flow cytometry data is problematic if statistically significant changes are highlighted without evidence for any corresponding biological significance.

Line 173-174: The proportion of NK cells in spleen or in peripheral blood of rodents is very low, and there is little in the literature to suggest that determination of absolute numbers of NK cells in these tissues is apt to uncover important immunotoxic effects. Thus, the added time, expense and other resources required to include these cells in phenotype analysis seems unwarranted, and PhRMA requests that the routine use of this assay be reconsidered.

Lines 183-184: "Both percentage and absolute cell counts can be determined by a single method ...". It may be useful to add here that absolute cell counts for spleen are preferably based on gram weight rather than whole spleen.

Lines 199-204: PhRMA does not agree that the assessment of antibody titer by ELISA is not a true test of immune function, and we recommend that this definition be modified. The ELISA measures a different endpoint of the same immune function. The plaque assay measures the number of antibody-forming cells in the spleen; the ELISA quantitates the amount of antibody produced from all immune organs, not just the spleen. We do agree that either assay should be acceptable. In fact, the ELISA method adds a number of advantages because time course can be followed and recovery assessed within the same animals by using a different antigen. SRBCs are used as a T-cell dependent antigen only for historic reasons, and the assays used were developed prior to the advent of ELISA methodologies. Indeed, sponsors should be encouraged to develop other T-dependent antigens (KLH, OVA, DNP-OVA, etc) to evaluate "immune function," which are likely to provide a more robust and consistent response and/or assay. We therefore recommend that either assay be considered acceptable.

Lines 217-222 and 491-494: It is unclear whether the draft guidance requires that the sponsor consider a study of the F1 offspring every time a drug could be used in pregnant women, or only specifically for drugs that would be prescribed for a condition linked to the pregnancy. It is also not clear at what age the draft guidance requires that the F1 generation be evaluated. PhRMA recommends that these sections be clarified. Furthermore, although this paragraph is under the section of "Immune Function Studies," the parameters the agency recommends to be

evaluated are not immune function parameters. We suggest moving this under a different heading to avoid confusion.

Lines 224-228: We agree that in some situations, such as for drugs intended for use in an immunocompromised population, more thorough testing of immune function should be conducted. However, it is important that data generated from immune function studies do not trigger termination of the drug's development, but rather serve as guidance for parameters/biomarkers that could be monitored in human trials. PhRMA urges that wording to this effect be added to the guidance.

Line 257: "Under certain circumstances, attempts should be made to determine the potential antigenicity of large molecular weight drugs." Should that read "small" rather than "large"? In our experience, large molecular weight drugs are usually antigenic. Thus, large molecular weight drugs should be routinely monitored for antigenicity. This section is unclear and PhRMA urges that it be clarified.

Lines 257-259 and 488-490: The potential of a drug to be haptenic does not on its own warrant the need for the evaluation of anti-drug antibody responses in standard toxicology assays. There are a number of drugs on the market that are known to produce reactive intermediates that bind to macromolecules but are associated only with a very low incidence of clinical hypersensitivity reactions. The decision to evaluate an anti-drug antibody response should be based on findings observed in the study or earlier studies suggesting that an antibody response to the drug may have occurred. In contrast to lines 257-259 and 488-490, lines 407-412 indicate that anti-drug antibody responses should be conducted if the test compound belongs to a class known to produce hypersensitivity reactions through covalent binding. However, studies have demonstrated that these compounds (e.g., sulfonamides, penicillins) do not produce an anti-drug antibody response when administered via a clinically relevant route in rats (without adjuvant or immunizing with drug-protein conjugates; Kitteringham et al., 1987: Gill et al., 1997). Reasons for the lack of an anti-drug antibody response may be attributed to the amount of reactive intermediate generated, how quickly it is inactivated, and the immunogenicity of the hapten-protein conjugate. Thus, evaluating anti-drug antibody responses in routine toxicity studies will not be helpful and should be recommended only if warranted by specific findings suggesting an anti-drug antibody response might have occurred. Studies that have examined the relationship between the amount of covalent binding and immunogenicity of the hapten have not been reported. Thus, PhRMA recommends that FDA no recommend covalent binding studies to determine potential antigenicity at this time.

Lines 265-267: "Assays to identify anti-drug immune responses should be considered part of nonclinical assessment, because peptides, polymer, and protein drugs and classes are known to be potentially haptenic." This sentence is confusing and needs to be reworded. Although it might be useful to develop an ELISA or blastogenic assay for the purpose of evaluating hypersensitivity responses to a compound-derived hapten, determination of the precise hapten-protein complex(es) which initiate the specific response can be extremely difficult. In addition, grading an in vitro reaction to derived antigenic moiety(ies) based on lymphocyte proliferative responses or antibody production generally tends to have very limited sensitivity and specificity,

and to be poorly predictive of *in vivo* responses. Thus, consideration of this testing methodology seems only rarely plausible and appropriate, and then only for very selective compounds. Thus, we recommend that the usefulness of these assays be clarified.

Lines 282-283: Small molecular weight compounds can be antigenic -- not allergenic -- if they bind directly to proteins, either as the parent or via metabolites. They are allergenic if they produce an exaggerated or pathologic reaction. We suggest changing "allergenic" to "antigenic" in line 282.

Lines 283-287: We suggest that FDA add that the type of hypersensitivity reaction can also depend on genetic background.

Lines 293-300: PhRMA agrees that the active systemic anaphylaxis and passive cutaneous anaphylaxis assays add little predictive or mechanistic value and should not be conducted on a routine basis.

Lines 313-314: Only a few laboratories have investigated cytokine gene or protein expression patterns in local draining lymph nodes of mice exposed cutaneously to well-established potent respiratory sensitizers (e.g., trimellitic anhydride). Results of these studies have been mixed, with expression analysis of only a single cytokine (IL-4) showing potential to distinguish possible respiratory sensitizers (Dearman et al., 1999; Vandebriel et al., 2000). It is also well documented that the cytokine patterns elicited in an induced hypersensitivity response can vary significantly with mouse strain. Thus, PhRMA believes that it is premature to suggest evaluation of cytokine expression patterns as a means of testing for compounds of unknown potential as respiratory sensitizers and that this should not be recommended.

Lines 367-398 and 481-484: We agree that all dermal drugs should be routinely tested for the potential for dermal sensitization, since validated and predictive assays are available.

Lines 311-326, 478-484, and 562-566: We do not believe that sufficient data are available to justify using contact sensitization assays to screen for respiratory sensitization potential of inhaled drugs. These data should be referenced, if available. Scientific rationale argues against testing the potential of a compound to act as a Th-2-promoting respiratory sensitizer by evaluating the compound's capacity to induce a Th-1-like contact hypersensitivity reaction. Thus far, cutaneous application for induction in testing for respiratory sensitizers has been used to differentiate compounds that can induce both skin (Th-1-like) and respiratory (Th-2-like) hypersensitivity reactions from those that induce only contact hypersensitivity. Compounds with strictly respiratory sensitizing potential have not been adequately evaluated by the LLNA assay methodology. Thus, although testing inhalants for cutaneous hypersensitivity may be warranted, the method should not be considered valid for determining respiratory sensitizing potential. In addition, the unmodified murine LLNA does not discriminate between skin sensitizers and irritants. Although a variety of reported modifications for this latter purpose have been reported, these modifications have not yet been standardized or validated. PhRMA therefore urges that FDA not recommend this approach.

Lines 312-313 and 484: The mouse IgE test has not been adequately validated for the detection of respiratory sensitizers and FDA should not recommend it at this time.

Lines 321-326: This guidance recommends that the guinea pig method of Karol (1995), which involves dermal or inhalation induction followed by inhalation challenge, be used for inhalation drugs. Since the model of Karol is very time consuming, expensive, and difficult to conduct, perhaps other alternatives such as the tiered approach for evaluating respiratory sensitizers of low molecular weight chemicals described by Sarlo and Clark (1992) should be considered, although they should be validated for pharmaceutics prior to more routine use.

Lines 528-530, Attachment 2: This section states that the PLNA and specific biomarker assays might provide insight into potential autoimmune mechanisms. However, lines 444-446 state that the PLNA may have promise, but no extensive evaluation has been reported that would support any recommendation for its use in drug development. We agree that this assay has not been validated and therefore believe that it should not be recommended for detecting autoimmunity-inducing potential. In addition, guidance needs to explain the term "specific biomarker assays." If this is meant to be markers of T-cell activation and effects of a drug on markers of TH2 cell induction (line 448-449), FDA needs to include more information and justification for these markers. Since the PLNA and biomarkers of T-cell activation are not validated methods to assess for potential autoimmunity induction, PhRMA urges that FDA not recommend these assays.

Lines 532-536: The guidance recommends that, if a compound is found to be tumorigenic in rodent bioassays and is suspected of being immunosuppressive (unintended), follow-up tumor host-resistance assays should be considered. It further states that these host-resistance assays are appropriate for determining carcinogenic immunosuppressive potential. PhRMA is unaware of studies that demonstrate the usefulness of host-resistance assays to determine if immunosuppression results in increased tumorigenesis, and we therefore question the value this may provide to the risk assessment process. PhRMA recommends that FDA provide additional support for this recommendation.

PhRMA appreciates the opportunity to comment on this guidance, and we would be happy to provide any additional needed information.

Sincerely,

Sara Radcliffe

Sara Radcliffe

References

Dearman RJ, Hilton J, Basketter DA, and Kimber I (1999). Cytokine endpoints for the local lymph node assay: considerations of interferon-gamma and interleukin-12. *J Appl Toxicol* 19:149-55.

Gill HJ, Hough SJ, Naisbitt DJ, Maggs JL, Kitteringham NR, Pirmohamed M and Park BK (1997). The relationship between the disposition and immunogenicity of sulfamethoxazole in the rat. *J Pharmacol Ex. Thera* 282:795-801.

ILSI Immunotoxicology Technical Committee (2001). Application of flow cytometry to immunotoxicity testing: summary of a workshop. *Toxicology* 163:39-48.

Luster MI, Portier C, Pait DG, White KL Jr., Gennings C, Munson AE and Rosenthal GJ (1992). Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam Appl Toxicol* 18:200-210.

Kitteringham NR, Christie G, Coleman JW, Yeung JH and Park BK (1987). Drug-protein conjugates-XII. A study of the disposition, irreversible binding and immunogenicity of penicillin in the rat. *Biochem Pharmacol* 36:601-608.

Phillips KE, McCay JA, Brown RD, Musgrove DL, Meade BJ, Butterworth LF, Wilson S, White KL Jr, Munson AE (1997). Immunotoxicity of 2',3'-dideoxyinosine in female B6C3F1 mice. *Drug Chem Toxicol* 20(3):189-228.

Sarlo K and Clark ED (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. *Fundam Appl Toxicol* 18:107-114.

Summary Basis of Approval for Videx (didanosine), New Drug Application (NDA), October 9, 1991.

Vandebriel RJ, De Jong WH, Spiekstra SW, Van Dijk M, Fluitman A, Garssen J, VanLoveren H (2000). Assessment of preferential T-helper 1 or T-helper 2 induction by low molecular weight compounds using the local lymph node assay in conjunction with RT-PCR and ELISA for interferon-gamma and interleukin 4. *Toxicol Appl Pharmacol* 162:77-85.