

ILSI HEALTH AND ENVIRONMENTAL SCIENCES INSTITUTE
International Life Sciences Institute (HESI)

April 11, 2005

Food and Drug Administration,
Division of Dockets Management [HFA-305],
5630 Fishers Lane, rm. 1061,
Rockville,
MD, 20852

Docket Number 2005D-0022

RE: Comments on the Food and Drug Administration, International Conference on Harmonisation: 2005 Draft Guidance on S8 Immunotoxicity Studies for Human Pharmaceuticals; Availability.

To Whom It May Concern:

The ILSI Health and Environmental Sciences Institute (ILSI HESI) appreciates the opportunity to provide comments to the FDA on the Draft Guidelines for International Conference on Harmonisation: Draft Guidance on S8 Immunotoxicity Studies for Human Pharmaceuticals; Availability (*Federal Register vol. 70, No. 25* February 8, 2005). The comments are collated under the auspices of HESI and were generated from members of the Immunotoxicology Technical Committee (ITC). The comments are submitted in two parts: 1) a two page summary indicating general comments; and 2) specific comments and recommendations.

ILSI HESI is a global branch of the International Life Sciences Institute, a public, non-profit scientific foundation with branches throughout the world. The mission of ILSI HESI is to stimulate and support scientific research and educational programs that contribute to the identification and resolution of health and environmental issues of concern to the public, scientific community, government agencies, and industry. ILSI HESI provides an international forum to advance the understanding and application of scientific issues related to human health, toxicology, risk assessment and the environment.

ILSI HESI is widely recognized among scientists from government, industry and academia as an objective, science-based organization within which important issues of mutual concern can be discussed and resolved in the interest of improving public health. As part of its public benefit mandate, ILSI HESI's activities are carried out in the public domain, generating data and other information for broad scientific use and application, and include participation from government, industry, and academic scientists. ILSI HESI's programs are supported primarily by its industry membership. ILSI HESI also receives support from a variety of US and international government agencies.

Please contact Dr. Ciaran Faherty at 202-659-3306 or cfaherty@ilsa.org with any questions regarding these comments and/or the HESI Immunotoxicology Technical Committee. We look forward to working cooperatively with the FDA on scientific improvements to the 2005 draft Guidelines.

Sincerely,



Enclosures:

Section I. General Comments (p. 1-2)
Section II Specific Comments (pp. 3-19)

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ILSI HEALTH AND ENVIRONMENTAL SCIENCES INSTITUTE
International Life Sciences Institute (HESI)

Comments by
HESI Immunotoxicology Technical Committee

to
Food and Drug Administration
on the
**International Conference on Harmonisation: 2005 Draft Guidance on S8
Immunotoxicity Studies for Human Pharmaceuticals; Availability**
(*Federal Register vol. 70, No. 25* February 8, 2005)

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Section I- General Comments

Overall, the guidance is very well written and supports a scientifically driven, case-by-case approach driven by what is known about the compound, its target, and findings observed in standard toxicity studies. This document represents a well designed summary of the current position but may however require further preparation to provide a clear and descriptive guidance for these issues. The document ends somewhat prematurely without any clear recommendation for a testing strategy. In addition, it is strongly recommended to include references in support of statements throughout the document.

The primary areas of greatest concern to HESI ITC scientists are as follows:

- While conducting the weight of evidence review to determine the need for additional nonclinical immunotoxicity assessment, it was suggested that discussions with clinical physicians could be advantageous. The early collaboration between nonclinical and clinical professionals would provide an opportunity for better interpretation of the immunotoxicity risk assessment to humans and allow the inclusion of biomarkers of potential immunotoxicity earlier in clinical trials, if warranted.
- It was suggested that the current guidance document should consider including biotechnology compounds to provide a more thorough guideline to companies developing these products. However, there were concerns that inclusion of biotechnology compounds would require more extensive considerations that may necessitate an additional section to the guidance or may in turn merit separate guidance.
- It was suggested that a notation be included in the present ICH document stating that existing regulatory guidance on immune-related effects other than immunosuppression (e.g., contact, respiratory, developmental) will remain in force after ICH S8 is adopted.
- There was concern regarding the host resistance studies section. In particular, it was suggested that discussion on the limitations of host resistance assays should be included in the guidance. Furthermore, it was commented that the need to conduct host resistance assays should in turn encourage sponsors to discuss these protocols with the relevant agency(ies). Please see section II for discussion on host resistance studies, pp. 16-17.
- There were a number of serious concerns regarding the interpretation of stress in the guidance document. In particular, the guidance should consider a well supported discussion on the definition of ‘stress’ and the sagacity of recommending specific physiologic indices of ‘stress’ to provide a clear strategy for obtaining compelling evidence of ‘stress’. Please see section II for discussion on the interpretation of stress, pp. 13-14.

Section II Specific Comments

1. Introduction

Comment- It is recommended that the scope of the guidance be stated at the start of the document.

L63-64- It is recommended to add hypersensitivity (drug allergy) to L63 "these include suppression or enhancement of the immune response *and hypersensitivity (drug allergy)*" to make it consistent with L68 & 71.

L67- In reference to "Drug or drug-protein....." It is recommended to include references to support this statement.

1.1 Objectives of the Guideline

Comment- It is suggested that the content of section 1. & 1.1 is redundant. Is it possible to have only one section?

L65-66- It is not evident that a generally enhanced immune response should contribute to autoimmunity. It is suggested that this sentence be removed, rephrased or supported.

L75- It is recommended to change "approaches to identify compounds (non-biologics) ~~which~~ that have the potential". It is very important to clarify if this guidance is for compounds with expected or unexpected immunotoxicity as well as unintended or intended immunotoxicity.

1.2 Background

L82-89 In discussing the value of performing immune function studies on anti-proliferative compounds, for clarity, it is suggested to add "*anti-proliferative*" to precede "drug-risk assessment" in L86.

L91-97- It is recommended to simplify/shorten this sentence.

L96- It is recommended to insert "...induced necrosis *or* apoptosis...".

L97- Examples of drugs that give unintended immunosuppression should be given as have been done for "intended" immunosuppression.

1.3 Scope of the Guideline

Comment- The title of the guidance indicates "Human pharmaceuticals", supported by "All new investigational drugs" in the top box of Figure 1. However, L103 recommendations pertain only to "low molecular weight drugs (non-biologicals)"? In addition, it is recommended to include a section to define the difference between testing strategies for drugs with intended vs unintended immunosuppression. That is, some

additional clarification is needed to determine when the pharmacologic effect becomes immunotoxic ?

L103- It seems that the term, "low molecular weight drugs", does not make sense in this case. It is recommended that "low molecular weight drugs (non-biologicals)" should be changed to "non-biological drugs". Alternatively, if this term is to be used, then a definition for the low molecular weight should be given.

L103- It is suggested that 'biologic compounds' be included in this document.

L107- This sentence states that the guideline can apply to "immune" observations observed in clinical studies. This is not consistent with earlier statements that the guidelines are for preclinical testing. This required clarification.

L107- It is recommended that "toxicologic issues" be replaced with "*immunotoxicity issues*".

L109-111- It is recommended that this sentence "The term immunotoxicity in this guideline will primarily refer to immunosuppression, i.e. a state of increased susceptibility to infections or the development of tumors" should be included at the end of the Introduction section (i.e. L71-72) to add clarity to the document.

L111- Increased tumor development is listed as an indicator of immunotoxicity. It is recognized that immune surveillance plays a role in tumor development for some tumors. However, an increase in tumor development alone does not necessarily indicate immunosuppression and of itself should not mandate functional immunotoxicity testing.

L112- It is recommended to add a section here dealing with how the data to be generated from this guidance will be used. For example, will this data be used to guide clinical monitoring or risk assessment or risk characterization or what?

L113-114-. It is recommended to insert, "It is beyond the scope of this guideline to provide specific guidance on how each immunotoxicity study should be performed. General guidance is provided in Appendix 1. *Furthermore, drugs intended to induce immunomodulation are not within the scope of this guideline.*", to provide clarity regarding the exclusion of drugs for which immunomodulation is the pharmacological effect.

1.4 Overview

L118- This section states that the guideline is for all new investigational drugs but earlier it is stated that it is not for biologics. Additional clarification should be given in Section 1.4.

L118-119- It is recommended to insert "All new investigational drugs should be evaluated for the potential to produce *unintended* immunosuppression."

L120- - It is recommended to insert “Methods include standard toxicity study (STS) *endpoints* and...”.

2. GUIDELINE

2.1 Assessment of potential Immunotoxicity

L138- In reference to “known drug class effects”. It is recommended that the guidance document should include a statement regarding drug classes with known effects that have been demonstrated to have no cause for concern in humans.

L139- In reference to “disposition of the drug”. Because there are many drugs that distribute into organs and tissues where no toxic response is observed (e.g., liver, muscle, adrenal gland) it is recommended that this statement requires additional discussion and clarification.

L130-. It is recommended to delete “...involves the standard ...”.

2.1.1 Standard toxicity studies

L146-. It is suggested that the sentence should end with “...following:”.

L148- The listed changes are all reflective of a direct effect on immune cells. However, it should be considered that neutrophilia or lymphocytosis may also be observed secondary to infection and could be suggestive of an indirect effect on the immune system.

L149-150- It is recommended to either omit, "pancytopenia or other blood dyscrasias", to be consistent with appendix 1, section 1., or include RBC and platelet parameters in appendix 1, section 1.

L151 It is recommended to insert “Alterations in immune system organ weights *and/or* histology”.

L153- It is known that serum Ig levels are a very insensitive parameter. Therefore, it is suggested that serum Ig should not be considered as a separate point in this document if no good examples of drugs affecting this parameter can be provided.

L153- It is recommended to delete "immuno globulins" as immunoglobulins are not routinely measured in standard toxicity studies.

L153-. It is recommended to acknowledge that serum globulin measurements are not only insensitive, they are nonspecific since other globulins (predominantly made by the liver) are present in that component--acute phase proteins, for example. Increases may occur with inflammation, dehydration, etc. and decreases may occur with liver failure, malnutrition, hemorrhage, malabsorption, etc.

L155-. It is recommended that the use of immunoglobulins/globulins be kept consistent throughout the document.

L158- It is suggested that carcinogenicity in the absence of genotoxicity should not mandate functional immunotoxicity testing. In addition, these carcinogenicity studies are not performed for all drugs, and if conducted, they may be conducted during phase III, after significant human exposure. Further, it is suggested to insert the statement, “(5) Evidence of carcinogenicity, especially in the absence of genotoxicity. *Under most circumstances, when increased incidence of tumors is observed in standard rodent bioassays, this effect is likely related to genotoxicity, hormonal effects, liver enzyme induction, hyperplasia, or other relatively well understood mechanisms. However, for some investigational drugs the cause of tumor findings in nonclinical studies might not be apparent. In cases where a potential role of immunosuppression is plausible based on a weight-of-evidence review of the relevant data, functional assays should be considered.*”, as referenced in FDA Guidance (2002).

L160-. If findings are observed that are suggestive of a suppressive effect on immune function, the objectives of additional immunotoxicity testing need to be clear. Specifically what needs to be understood about the effect on the immune system so that risk/benefit can be assessed? It is suggested that this point needs to be reemphasized in section 2.2.

L160 – It is recommended to insert a statement to the effect that “...*findings from STS or other causes for concern...the decision to conduct...*”.

L165- It is recommended to replace the word ‘and’ with either ‘or’, ‘and/or’ since statistical significance does not necessarily equal biological significance or adversity.

L167- It is recommended to insert “*dose/exposure dependency or relationship*”.

L167-. In reference to “dose dependency”, it is recommended that this point requires further comments. From an immunological perspective, it is known that immunotoxic effects may lack dose dependency. However, from a toxicological view, one may think that a dose response effect is necessary for the effect to be regarded as “true”.

L169- It is recommended to change "study duration" to "*treatment duration*".

L173- "produces" should be "*produce*"

L160 -162-. The draft guideline lacks clarity regarding the decision to perform additional studies based on an overall review of the relevant data rather than on a single observation. To clarify the decision to perform additional studies, based on one or more observations of immunotoxic potential, it is recommended to modify the statement, “If the findings from the STS indicate that there are signs of immunotoxicity, the decision to conduct

additional immunotoxicity testing should be considered in a weight-of-evidence review of the data *and not based only on a single sign from the list above.*”.

2.1.2 Other Causes for Concern in the Weight-of-Evidence Review

L181-191- It is recommended to include guidance for compounds known to modulate adaptive immune response.

L182- In reference to “significant immunosuppression”, it is suggested that the term ‘significant’ needs to be defined. Does this mean a certain degree (%) of effect on an antigen or pathogen challenge response? It is also suggested that ‘Immunosuppression’ needs to be defined with respect to intended, unintended, or both.

L183- It is recommended that examples of the anti-inflammatory drugs to which this sentence refers be included.

L185-. The statement, “their ability to suppress adaptive and/or innate immune responses is not clear”, needs additional clarification. Is this true? The answer would be “yes” for steroids, methotrexate, and anti-TNFs, but what about for anti-IgE? Does this only relate to non-biologicals?

L186- The statement, “Information on the ability of the compound to affect the immune system can be gathered as part of the pharmacological studies conducted during the discovery or early development phases”, needs additional clarification. If these studies demonstrate an effect on function, will they be acceptable or will a GLP study be necessary? In addition, does this statement mean that all anti-inflammatory drugs should be tested in assays covering all the different parts of the immune system?

L186-190; Many non-GLP pharmacology studies conducted during discovery or early development phases do not focus on safety, and thus often do not have adequate animal numbers or valid control groups to make decisions on safety. In reference to “deciding if additional immunotoxicity studies are required”, the decision to conduct additional immunotoxicity testing should be based on the findings in early stage toxicology studies rather than pharmacology/efficacy studies. However, this is not to say that nonGLP studies should not be considered in the decision to conduct additional immunotoxicity testing. The use of nonGLP pharmacology studies should be further clarified.

L188-. Does this term refer to studies prior to Phase I? It is suggested that the statement, “...early development phases”, requires further definition of ‘early development’.

L189-191-. It is recommended to delete the sentence, “The decision to..... approach” as it does not provide any further information.

L193-. It is suggested that the statement, “(2) The targeted patient population...”, is too broad and requires a more precise definition. For example, as written this statement could include elderly patients simply by age criteria, which would mean almost all drugs

that are developed, would need to be tested. The extent of this statement should be more specifically stated/defined. One consideration would be if the specific disease state's hallmark is immunosuppression. In addition, do RA patients receiving DMARDs and asthma patients on steroids represent an immunocompromised target population?

L197- In reference to "Compounds structurally similar...immunotoxicity testing", it is important to note that there is no established database for structure search or comparison among immunosuppressive drugs. Thus this approach may happen in a random manner but not as a systematic evaluation.

L200-202- It is recommended to revise the sentence, "If the compound and/or its metabolites...be considered.", as there are many drugs that distribute into organs and tissues where no toxic response is observed. Additionally, innate immunity can extend to any cell type having natural defenses against infection, blocking portal of entry, etc., e.g., GI epithelial barrier, mucous producing cells, cells lining the tear ducts, salivary gland, etc. This point needs additional discussion and clarification.

L201-202- It is recommended to explain the definition for 'high concentrations' and the method of assessment.

L204- It is not clear what would be expected for drugs that are intended to suppress immune function versus those that are not. What would be the appropriate approach for drugs with known and expected or intended immunosuppressive activity like immunomodulators?

L204-206-. It is recommended to modify the statement, "If signs of immunotoxicity are observed in STS *only or in conjunction with one of the above four factors*, it is recommended that the sponsor conduct studies of drug effect on immune function or provide justification for not performing the evaluations."

Comment- Literature data on alterations in immune function observed in knockout mice or in association with human polymorphisms could be included as 'other causes for concern' in paragraph 2.1.2 in determining the need for additional testing and/or determining which tests should be conducted.

2.2 Selection and Design of Additional Immunotoxicity Studies

2.2.1 Selection of assays

L217- This paragraph needs clarification. It is suggested to insert "If specific cell types are affected in STS, assays that measure function of that specific cell type might be conducted, *which could include a TDAR*".

L217: It is suggested to revise this statement by replacing/adding the following "*If additional immunotoxicity testing is warranted*, it is recommended that an immune function study be conducted."

L217- It is suggested to start this paragraph with “Where a specific target is not identified, ...”.

L222- In the statement "Immunophenotyping of...can be conducted...", it is recommended that “can” be replaced with “*may*”.

L222-. The Immunophenotyping statement is not clear. Is the guidance that only if immunophenotyping is observed in preclinical studies should it then be used in clinical trials?

L223-. It is suggested to insert "*may provide*" before "useful". Depending on the source of the cells and the relevancy of that source to human circulating lymphocyte populations, there may or may not be a useful clinical biomarker identified by immunophenotyping leukocytes. For example, if a peripheral lymph node draining a site of absorption or inflammation has a depletion or increase in a certain cell type identifiable via immunophenotyping, it doesn't necessarily follow that there will be an effect on circulating lymphocytes in that or another species.

2.2.2 Study Design

L227-228- It is recommended that the first sentence in this section should indicate "*28 consecutive daily doses in mice or rats*", and the word “oral” be deleted as some compounds may be administered via a different route.

L229-. It is recommended that “route of animal exposure” replace “route of administration”.

L232-. It is suggested that additional perspective on what is considered “stress” would be helpful and as this is dealt with in more detail a reference to the appendix, section 1.4 should be included.

L234-236-. It is recommended that these two sentences be clarified and that a number of references be added. In addition, it is recommended to change L235 from "Under most" to "Under *some* circumstances”.

2.2.3 Evaluation of Need for Follow-up testing

3. Follow-up immunotoxicity studies

L245-247 and corresponding decision point in flow chart (Figure 1)- It is not clear what is meant by the phrase, "if changes are observed with immunotoxicity testing". The previous section, 2.2.3 states, "if the overall risk benefit ...is acceptable, then no follow up testing...". Is it the intent that the recommendation in L245-247 refers to changes that occur at clinically relevant exposures or under conditions where no NOAEL was established? This point needs to be clarified. As written, it appears that this paragraph is

meant to cover strategies for follow up if the risk assessment is unacceptable, not if any changes are observed.

L253-. In reference to "that specific component or associated function could be monitored". It is recommended that this be discussed further to reduce ambiguity.

Comment- The host resistance assay gives information regarding the biological impact/relevance of the immunotoxic event, not necessarily the specific cell type affected or the mechanism of action.

L252-255-. Further clarification is required regarding the value of additional animal studies when follow-up immunotoxicity studies are included in the design of the clinical program. It is suggested to modify the statement, "In situations where the development candidate might have a pharmacological effect on the immune system, that specific component or associated function could be monitored. *However, if immunotoxicity testing is included in the design of the clinical program (e.g., due to the targeted patient population and/or known effects of the investigational drug), follow-up preclinical immunotoxicity testing may not be required regardless of findings in the STS.* Additional guidance is beyond the scope of this guideline."

4. Timing of immunotoxicity testing in relation to clinical studies

L259-261-. The document is vague with regard to the timing of immunotoxicity testing. It is suggested that "additional immunotoxicity studies" should be completed before exposure of a large population of patients. Is this Phase III? It is suggested that *follow-up assays* (NK cell/M functional assays and host defense assays) rather than *additional immunotoxicity assays* (e.g. TDAR assay) be completed before exposure to a large population of patients.

L260- It is suggested that the term, "large population", be defined. A possible distinction could be made to clarify that the "large patient population exposure" as a multi-dose study. Furthermore, it would be helpful if guidance could be provided on whether this means Phase II, Phase III or Phase IV development. If the intent is to allow for incorporation of appropriate immunotoxicology endpoints in clinical studies, then one might infer that the intent is to perform nonclinical immunotoxicity assessments concurrent with, or prior to Phase II.

L265-267- It is recommended that the last sentence in this section be reworded to increase the impact, for example "If the target patient population is immunocompromised, immunotoxicity testing *should* be initiated at an earlier time point in the development of the drug." This revised language allows for flexibility while eliminating a large degree of ambiguity encapsulated by the use of "can be initiated".

Flow Diagram Comments

Figure 1- The brief list of recommended considerations listed in one of the Figure boxes should be discussed in the body of the text. For example, reversibility and dose comparison are not discussed previously. This is also true for some of statements in the follow-up box at the bottom of Figure 1 (e.g., biomarker development).

Appendix 1

1. Standard Toxicity Studies

Comment-. Nowhere in this document is there anything about cytologic evaluation of bone marrow. There is a statement referring to documents from "professional toxicological pathology societies"; STP has recently produced such a document, which was endorsed by the ICH expert working group, but only gives bone marrow evaluation a superficial acknowledgement. It may be appropriate for this document, then, to either provide some guidance on when it is appropriate to evaluate cytology, or in general to expand on the bone marrow discussion. It could be discussed in this section, 1.1. A statement such as "*unexplained decreases in two or more peripheral blood cell lines or histopathologic findings may suggest the need for cytologic evaluation of the bone marrow.*" would be a good starting point.

1.1 Hematology and Clinical Chemistry

L286- It is recommended to include, "e.g. nephrotoxicity, *hepatotoxicity, malabsorption/malnutrition, and acute phase responses*".

L288-. It is suggested to insert, "Although serum immunoglobulins, *without antigenic challenge*, are an ...".

1.2 Gross Pathology and Organ Weights

L293-. It is suggested that a more specific list of tissues to be examined be provided here. In addition, the ability to examine 'all' lymphoid tissues is questionable.

L293- To remain consistent with the STP Best Practice Guideline for the Routine Pathology Evaluation of the Immune System document, it is suggested to add the following to the end of the first sentence: "and any gross lesions of a lymphoid organ should also be collected for microscopic evaluation."

L294-. It is recommended to delete, "more", from this sentence.

L296- In reference to canine spleen weight, it is highly variable depending upon completeness of exsanguination. Practical experience suggests that complete exsanguination is difficult to achieve in a reproducible fashion. It is recommended that because the value of spleen weights for canine in toxicology testing is questionable, this point may not be mandated. In addition, it is suggested that the primate spleen does not

respond well, if at all, to hypovolemia by contracting. Bleeding out may therefore, only be important for dogs.

1.3 Histopathological Examination

Comment- Histology of a draining lymph node and at least one additional lymph node is recommended. Should the additional lymph node be a draining node or a distant lymph node? The STP Immunotoxicology Working Group does not recommend histopathologic examination of peripheral non-draining lymph nodes. It is suggested that from the STP WG this recommendation be considered.

Comment- Should histology of the Peyer's patch be recommended even in cases when the test material is administered through a non-oral route?

L303- It is suggested that the term "GALT" be used, rather than the more narrow "Peyer's patches" be used.

L305- In reference to the use of histopathological changes in the NALT. The feasibility of this approach is questionable due to the small size of the NALT in rodents.

L312- It is recommended to insert "*a detailed*" rather than a "semi-quantitative", as the "semi-quantitative" compartment approach is not universally accepted.

L313- It is recommended that this guidance should refer the reader to the STP Best Practice document for further information regarding use of a unified terminology description for histopathologic evaluation. Three primary points are emphasized in the evaluation: 1) each lymphoid organ has separate compartments that support specific immune functions, 2) these compartments can and should be evaluated individually for changes, and 3) descriptive, rather than interpretative terminology, should be used to characterize changes within these compartments. In order to achieve an accurate, consistent and useful "semi-quantitative description" it is necessary to develop consensus on terminology used in characterization of lymphoid tissue changes. Whenever possible, semi-quantitative/descriptive terms (i.e. reduced numbers of lymphocytes) rather than interpretative terms (i.e. lymphoid atrophy) for registering lymphoid tissue abnormalities is recommended. To illustrate this point further, consider potential stress-induced changes of the thymus; a semi-quantitative description such as: "thymus, cortex, decreased lymphocytes, marked" would be preferable to "thymic involution".

L320- It is recommended to list the lymphocytes first, since effects on lymphocytes are more consistent findings across species and time points than the neutrophil effects.

L322- It is recommended to insert "weight *and/or* histologic evidence of adrenal cortical hyperplasia can be...".

L323- It is recommended to change, "decrease", to, "*decreased*".

L324-. It is suggested to insert a statement to the effect "...decreased activity, *toxicities in other organ systems severe enough to compromise well-being*), ...

1.4 Interpretation of stress related changes

Comment (L316-326). There is the perception, which is reflected in this section, that stress-related changes in immune parameters observed in toxicology studies should automatically be dismissed. In contrast, it is believed that significant effects on immune parameters should be reported (regardless of the mechanism). Without benefit of mechanistic studies, it becomes a judgment call, made without proof, that the stress response (defined as neuroendocrine-immune effects) is not drug-related or will be absent in humans. The reporting of significant changes in immune parameters may also help alert clinicians to the immunomodulatory properties of therapeutic drugs that may have otherwise been overlooked. Additional support for limiting the stress exclusion is discussed below.

Discussion- In standard toxicity screening, doses sufficient to produce overt toxicity and significant body weight loss (such as MTD levels) are expected to induce a stress response with associated increase in corticosterone levels (Pruett, et al., 1993; Pruett 2001). However, as pointed out by the same authors, there is less evidence regarding a stress effect on the immune system at lower doses of toxicity, and only in cases where a drug substantially elevates glucocorticoid levels, should the possibility of immunosuppression by this mechanism be considered. There is also recent evidence that animals can accommodate to chemical-related increases in serum corticosterone over time and that Sprague-Dawley rats (a strain frequently used in toxicology studies) are relatively insensitive to ethanol induced corticosterone effects. Additionally, these animals actually accommodated to the corticosterone release over 30 days but still presented with reduced thymus weights and depressed immune function (Hebert et al., 2005).

Routinely ascribing changes in immune parameters as stress related in standard toxicology studies has led to the general misconception that any weight loss will indirectly affect immune parameters such as thymus weight. In fact, very significant weight loss must occur before most immune parameters are affected. For example, in a 2-week food restriction study in Sprague-Dawley rats, immune cell changes were manifested only after the degree of weight loss reached moderate to severe levels defined as terminal body weights that were reduced to 40-60% of control (Levin et al., 1993). WBC counts were least sensitive to body weight loss with bone marrow cellularity being the most sensitive. Decreased thymus and liver weights occurred only in animals with body weight loss greater than 30%. Further evidence that the immune system is relatively insensitive to weight loss per se is supported by several studies (Sharer, 1977; Oishi et al, 1979; Pickering and Pickering 1984, Ogawa et al., 1985).

Relative to thymus weight decreases and stress, it is recognized that not all chemically induced reductions in thymus weights leads to immunotoxicity in the test species (Comment et al., 1992). However, because this relationship can only be determined by

functional testing of the immune response, decreases in thymus weights provide a sensitive first tier indicator, or biomarker, of potential consequences of chemical or drug treatment on the immune system and therefore should not be ignored.

Therefore, evidence of stress should be compelling and reliable physiologic indices defining this effect should be measured. If stress-related effects on immune parameters at doses other than the MTD are diagnosed in a standard toxicology study, they should be reported (regardless of whether it is a direct or indirect mechanism) because the overall effect is potentially detrimental to immune responsiveness and should be identified. The only advantage to understanding the mechanism is if it is believed that humans treated with the drug will not have the same stress response, however, proving this is beyond the current scope of routine toxicity screening studies.

L321- It is recommended that the use of, (“starry sky” appearance)”, be omitted as this is only one of the many changes that can be observed.

2. Additional Immunotoxicity Studies

Comment-. The assays listed in this section represent only some of the assays available to assess immune responses. It is recommended that other tests be considered that could be useful e.g. mitogenic and allogenic reactions, complement activation. In addition, it may be worth mentioning the use of BrdU and TUNEL labeling as a useful indicator of cell turnover.

2.1 Assay Characterization and Validation

Comment-. While there is a general approach to many sections within the guidance document, allowing flexibility towards the science, there is little to no specific information regarding the functional assays and immunophenotyping sections. The degree of detail is not sufficient to conduct these assays. It is recommended that the assay details be explained to sufficiently describe the assays to non immunotoxicologists.

2.2 T-cell Dependent Antibody Response (TDAR)

L352- What is considered the most appropriate endpoint for KLH response?

L353-. There are a number of ways that TDAR data can be expressed, how the assays can be conducted, what immunogens are used and whether they should or should not be conducted with adjuvant. While these are all very important questions that need to be addressed, it is not the scope of this document to discuss specific assays and how they should or should not be conducted or evaluated. If these statements are to be included, they should be supported by references.

L355-356-. It is recommended that the literature within this section clarify the justification for use of outbred versus inbred rats.

L360-. It is recommended that, “*and both IgM and IgG antibodies can be evaluated during the study*”, be inserted. Recent publications indicate that primary antibodies of IgG class may be a more sensitive endpoint than IgM, and these references should be included.

L369- It is recommended not to specifically select the rat in reference to the TDAR as this should apply to any species.

2.3 Immunophenotyping

L379-385: This section appears to be out of place and may confuse the reader. If the intent is to present that immunohistochemistry is an alternative method to flow cytometry but has limitations, it is recommended that this paragraph be reworded and a conclusion drawn as to when it is appropriate to use either technology platform. In addition, although immunohistochemistry may be useful in some circumstances this technique may not be used routinely.

L389-. It is recommended to insert, “*toxicity studies and when peripheral blood is used as the leukocyte source,...*”.

L390-. It is suggested that additional perspectives (i.e. examples) on how flow cytometry can be used for antigen specific responses of lymphocytes would be useful to the reader.

L392 The statement, “However, flow cytometry can be used to measure antigen-specific immune responses of lymphocytes”, should either be deleted or a reference found to support this statement.

L393-. It is recommended to change "can" to "may".

L394-. It is recommended that absolute counts are preferred when assessing peripheral blood (PB). If studies are conducted in mice, then splenocytes will need to be evaluated and percentages used to express the data. There also may be times when splenocytes along with PB phenotyping may be appropriate for a specific drug.

L394-396-. It is recommended to delete the last sentence "It is recommended that.....changes" as this point is already addressed in L376.-378. In addition, if only one is to be used (absolute vs percentages), absolutes are preferred. But sometimes it's useful to look at both. It is suggested to modify this to read "*both absolute numbers and percentages be used...*".

Comment-. It is suggested that evaluating ratios (e.g. T/B, CD4/CD8) can also help in assessing drug-related changes.

2.4 Natural Killer Cell Activity Assays

L402-. It is recommended to pluralize the word “assay” to “assays”.

L404-. It is suggested to indicate that the chromium in this document is 51Cr so that the wider readership can fully understand the reference to “radioactive”. In addition, it is recommended to delete “of”.

L406-. It is suggested to insert "cytotoxicity *and generate a curve*".

2.5 Host Resistance Studies

L411-. It is recommended to insert “pathogen (*bacterial, fungal, ...*)”.

L414-. It is recommended to insert “*Candida albicans*” as this model has been established and published.

L425-. It is recommended to pluralize “assay” to “assays”.

Comment-. It is strongly recommended that a statement be included to highlight the limitations of host resistance assays thus encouraging sponsors to discuss host resistance assays with the agency(ies) prior to conducting them.

Discussion-Host resistance assays have a number of limitations, and should not be undertaken lightly. The most common rationale for conducting host resistance assays is to obtain information which will be relevant to human risk assessment, i.e. provide an assessment of overall immune status and ability to fight infectious disease, particularly if a functional deficit has been identified using an assay which evaluates only a part of the immune system. However, because of species differences in receptors, cytokines, innate immunity, and susceptibility to specific organisms, animal host resistance assays are not always translatable to human risk. Virulence, dose, and other factors related to the organism as well as the immune competency of the subject all contribute to the outcome of the experiment. It is difficult to mimic a clinical situation in terms of organism, exposure, specific intended patient population, and other factors. For example, the assays cannot be used for quantitative risk assessment, because it would be difficult to determine a NOAEL which is translatable to clinical significance. To illustrate this point, doses of test compound which produce slight immunosuppression may result in a significant decrease in host resistance with high challenge levels. While the test compound dose is controllable and exposure to test compound in humans relative to animals is reasonably predictable, it is difficult to determine the challenge level which would be appropriate in a clinical setting. There is a risk of over- and under-interpretation in that a positive finding in a manipulated system may not have any relevance to humans, and conversely a negative finding in a controlled system may underestimate the risk to humans with pre-existing disease.

Selection of the organism can be problematic, especially if the compound has a very specific target, and results with one infectious agent may not translate to resistance to another infectious agent. Selection of the species can confound results, as well. Host resistance assays in monkeys, dogs, or rabbits are extremely problematic from a humane perspective and because of the unavoidable background incidence of disease in these animal models. In addition, host resistance assays in these species are not routine assays with adequate experimental experience, validation, and/or historical data; in contrast with a few specific rodent assays with a limited number of different types of bacteria or tumor cells.

For humane and cost reasons, the number of animals per group is generally kept small in these species (e.g. monkeys, dogs and rabbits). Assessing highly variable readouts in highly variable species with a small group size is not an ideal experimental design. The best understood and least variable species for host resistance assays are rodents, but, in this era of increasingly specific targets, they are less likely than the “large animal” species to have the receptors, cytokines, or immune mechanisms appropriate to the drug being tested. Because of the potential animal rights issues and for valid scientific, humane and ethical reasons, sponsors should discuss the preclinical program with regulatory authorities prior to conducting host resistance assays. If there is a legitimate need to conduct host resistance assays for a given program, guidance should be sought beforehand in order to design the most meaningful experiments and avoid the need to repeat them. The assays should be designed to ask specific, directed questions.

2.6 Macrophage/Neutrophil Function

L433-. It is suggested to insert “...(phagocytosis, oxidative burst, *cytolytic activity*)...”. In addition, it is suggested that under macrophage / neutrophil function it may be worth adding mast cell functionality as a further parameter to measure where appropriate.

L436-. It is suggested that the sentence “In vitro exposure to test compound can also be investigated” is redundant when used here.

L441-. This section lacks any suggestion that non-radioactive methods and/or alternative measures from chromium (51Cr)-labeled SRBC might be useful for assessing macrophage/neutrophil function in vivo (eg, in vivo chemotactic responses). It is recommended that some mention and/or discussion would be useful to the reader.

L440-. It is recommended to change this sentence to “...removed. *The radioactivity of the tissues is then counted.*”.

2.7 Assay to measure Cell-Mediated Immunity

Comment-. There are publications and quite a bit of work conducted previously with the rat DTH using footpad injections. These publications are not mentioned. It is recommended that the rat DTH be mentioned in this paragraph with the references; Exon et al., 1990, 1986.

L448-. It is recommended to change “cells” to “cell”.

Comment-. There is no discussion for the need to assess the developing immune system during reproductive toxicity testing. It is recommended that the inclusion of some mention and/or discussion of this topic would be of value. In addition, it is recommended to use references where possible throughout this document.

Suggested References

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