INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

IMMUNOTOXICITY STUDIES FOR HUMAN PHARMACEUTICALS

S8

Current *Step 4* version
dated 15 September 2005

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

S8
Document History

First Codification	History	Date	New Codification **November 2005**
S8	Approval by the Steering Committee under *Step 2* and release for public consultation.	18 November 2004	S8

Current *Step 4* version

S8	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	15 September 2005	S8

IMMUNOTOXICITY STUDIES FOR HUMAN PHARMACEUTICALS

ICH Harmonised Tripartite Guideline

Having reached *Step 4* of the ICH Process at the ICH Steering Committee meeting on 15 September 2005, this guideline is recommended for adoption to the three regulatory parties to ICH

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IMMUNOTOXICITY STUDIES FOR HUMAN PHARMACEUTICALS

1. INTRODUCTION

1.1 Objectives of the Guideline

The objectives of this guideline are to provide (1) recommendations on nonclinical testing approaches to identify compounds which have the potential to be immunotoxic, and (2) guidance on a weight-of-evidence decision making approach for immunotoxicity testing. Immunotoxicity is, for the purpose of this guideline, defined as unintended immunosuppression or enhancement. Drug-induced hypersensitivity and autoimmunity are excluded.

1.2 Background

Evaluation of potential adverse effects of human pharmaceuticals on the immune system should be incorporated into standard drug development. Toxicity to the immune system encompasses a variety of adverse effects. These include suppression or enhancement of the immune response. Suppression of the immune response can lead to decreased host resistance to infectious agents or tumor cells. Enhancing the immune response can exaggerate autoimmune diseases or hypersensitivity. Drug or drug-protein adducts might also be recognized as foreign and stimulate an anti-drug response. Subsequent exposures to the drug can lead to hypersensitivity (allergic) reactions. Much of the science and method development and validation efforts in the past have been focused on evaluating drug development candidates for their potential for either immunosuppression or contact sensitization. No standard approaches for human pharmaceuticals are currently available for testing for respiratory or systemic allergenicity (antigenicity) or drug-specific autoimmunity; testing for these endpoints is not currently required in any region. There are no regional differences in testing approaches of skin sensitization.

Immunosuppression or enhancement can be associated with two distinct groups:

1) Drugs intended to modulate immune function for therapeutic purposes (e.g., to prevent organ transplant rejection) where adverse immunosuppression can be considered exaggerated pharmacodynamics;

2) Drugs not intended to affect immune function but cause immunotoxicity due, for instance, to necrosis or apoptosis of immune cells or interaction with cellular receptors shared by both target tissues and non-target immune system cells.

Anti-proliferative agents used to treat cancer are an example of drugs that produce unintended immunosuppression. In such instances, adverse findings in nonclinical studies are predictive of human immunotoxicity in a rather straightforward manner. That is, specific assays to determine immunotoxicity are probably not valuable in drug risk assessment since the target tissues are usually rapidly dividing cell types, such as bone marrow-derived immune system progenitor cells. Hence, the adverse effects on immune function can be predicted based on pharmacologic activity and can usually be reliably evaluated in non-clinical studies. For other types of compounds not intended to suppress the immune response, distinction between exaggerated pharmacodynamics and non-target effects can be less obvious. As an example, some anti-inflammatory compounds have an effect on certain innate immune functions but do not necessarily affect the adaptive immune response.

1.3 Scope of the Guideline

This guideline is focused on providing recommendations on nonclinical testing for immunotoxicity induced by human pharmaceuticals. It is restricted to unintended immunosuppression and immunoenhancement, excluding allergenicity or drug-specific autoimmunity.

This guideline applies to new pharmaceuticals intended for use in humans, as well as to marketed pharmaceuticals proposed for different indications or other variations on the current product label in which the change could result in unaddressed and relevant immunotoxicity issues. In addition, the guideline might also apply to drugs for which clinical signs of immunotoxicity are observed during clinical trials and following approval to market. The guideline does not apply to biotechnology-derived pharmaceutical products covered by ICH S6 Guideline[1] and other biologicals.

Existing guidance documents on sensitization or hypersensitivity remain in force and are not affected by this document. It is beyond the scope of this guideline to provide specific guidance on how each immunotoxicity study should be performed. General methodology guidance is provided in the Appendix.

1.4 Overview

The general principles that apply to this guideline are:

1) All new human pharmaceuticals should be evaluated for the potential to produce immunotoxicity;

2) Methods include standard toxicity studies (STS) and additional immunotoxicity studies conducted as appropriate. Whether additional immunotoxicity studies are appropriate should be determined by a weight of evidence review of factor(s) in section 2.1.

The description of the guideline below will follow the recommended decision process in immunotoxicity evaluation as shown in the flow diagram (Figure 1). More detailed descriptions of the testing methods are in the Appendix.

2. GUIDELINE

2.1 Factors to Consider in the Evaluation of Potential Immunotoxicity

Factors to consider that might prompt additional immunotoxicity studies can be identified in the following areas: (1) findings from STS; (2) the pharmacological properties of the drug; (3) the intended patient population; (4) structural similarities to known immunomodulators; (5) the disposition of the drug; and (6) clinical information.

The initial screen for potential immunotoxicity involves standard toxicity studies. Data from rodent and non-rodent studies from early short term to more chronic repeat-dose studies should be taken into consideration. Additional details on the parameters that should be evaluated and the reporting of histopathology findings are provided in the Appendix.

2.1.1 Standard Toxicity Studies

Data from STS should be evaluated for signs of immunotoxic potential. Signs that should be taken into consideration are the following:

1) Hematological changes such as leukocytopenia/leukocytosis, granulocytopenia/granulocytosis, or lymphopenia/lymphocytosis;
2) Alterations in immune system organ weights and/or histology (e.g., changes in thymus, spleen, lymph nodes, and/or bone marrow);
3) Changes in serum globulins that occur without a plausible explanation, such as effects on the liver or kidney, can be an indication that there are changes in serum immunoglobulins;
4) Increased incidence of infections;
5) Increased occurrence of tumors can be viewed as a sign of immunosuppression in the absence of other plausible causes such as genotoxicity, hormonal effects, or liver enzyme induction.

Changes in these parameters could reflect immunosuppression or enhanced activation of the immune system. Immunosuppression is usually reflected by reduced values of immune parameters, whereas immunoenhancement is usually reflected by increased values. However, these relationships are not absolute and can be inverted in some cases.

Similar to the assessment of risk with toxicities in other organ systems, the assessment of immunotoxicity should include the following:

- Statistical and biological significance of the changes;
- Severity of the effects;
- Dose/exposure relationship;
- Safety factor above the expected clinical dose;
- Treatment duration;
- Number of species and endpoints affected;
- Changes that may occur secondarily to other factors (e.g., stress, see the Appendix, section 1.4);
- Possible cellular targets and/or mechanism of action;
- Doses which produce these changes in relation to doses which produce other toxicities; and
- Reversibility of effect(s).

2.1.2 Pharmacological Properties

If the pharmacological properties of a test compound indicate it has the potential to affect immune function (e.g., anti-inflammatory drugs), additional immunotoxicity testing should be considered. Information obtained from the nonclinical pharmacology studies on the ability of the compound to affect the immune system could be used in a weight of evidence approach to decide if additional immunotoxicity studies are needed.

2.1.3 Intended Patient Population

Additional immunotoxicity studies might be warranted if the majority of the patient population for whom the drug is intended is immunocompromised by a disease state or concurrent therapy.

2.1.4 Structural Similarity

Compounds structurally similar to compounds with known immunosuppressive properties should also be considered for additional immunotoxicity testing.

2.1.5 Disposition of the Drug

If the compound and/or its metabolites are retained at high concentrations in cells of the immune system, additional immunotoxicity testing should be considered.

2.1.6 Signs Observed in Clinical Trials or Clinical Use

Clinical findings suggestive of immunotoxicity in patients exposed to the drug could call for additional nonclinical immunotoxicity testing.

2.2 Weight of Evidence Review

A weight of evidence review should be performed on information from all the factors outlined above to determine whether a cause for concern exists. A finding of sufficient magnitude in a single area should trigger additional immunotoxicity studies. Findings from two or more factors, each one of which would not be sufficient on its own, could trigger additional studies. If additional immunotoxicity studies are not performed, the sponsor should provide justification.

3. SELECTION AND DESIGN OF ADDITIONAL IMMUNOTOXICITY STUDIES

3.1 Objectives

If a cause for concern is identified, additional immunotoxicity studies should be performed to verify the immunotoxic potential of the compound. These studies can also help determine the cell type affected reversibility, and the mechanism of action. This type of information can also provide more insight into potential risk and possibly lead to biomarker selection for clinical studies.

3.2 Selection of assays

If the weight-of-evidence review indicates that additional immunotoxicity studies are called for, there are a number of assays which can be used. If there are changes in standard toxicity testing data suggesting immunotoxicity, the type of additional immunotoxicity testing that is considered appropriate will depend on the nature of the immunological changes observed and the concerns raised by the class of compound. It is recommended that an immune function study be conducted, such as a T-cell dependent antibody response (TDAR). If specific cell types that are affected in STS are not known to participate in a TDAR, assays that measure function of that specific affected cell type might be conducted (see the Appendix). Where a specific target is not identified, an immune function study such as the TDAR is recommended.

In addition, immunophenotyping of leukocyte populations, a non-functional assay, can be conducted to identify the specific cell populations affected and might provide useful clinical biomarkers.

3.3 Study Design

To assess drug-induced immunotoxicity, a generally accepted study design in rodents is a 28 day study with consecutive daily dosing. Adaptations of immunotoxicity assays have been described using non-rodent species. The species, strain, dose, duration, and route of administration used in additional immunotoxicity studies should be consistent, where possible, with the standard toxicity study in which an adverse immune effect was observed. Usually both sexes should be used in these studies, excluding nonhuman primates. Rationale should be given when one sex is used in other species. The high dose should be above the no observed adverse effect level (NOAEL) but below a level inducing changes secondary to stress (see Appendix, section 1.4). Multiple dose levels are recommended in order to determine dose-response relationships and the dose at which no immunotoxicity is observed.

3.4 Evaluation of Additional Immunotoxicity Studies and Need for Further Studies

Results from additional immunotoxicity studies should be evaluated as to whether sufficient data are available to reasonably determine the risk of immunotoxicity:

1. Additional studies might show that no risk of immunotoxicity can be detected and no further testing is called for;
2. Additional studies might demonstrate a risk of immunotoxicity but fail to provide sufficient data to make a reasonable risk-benefit decision. In this case further testing might help provide sufficient information for the risk-benefit decision;
3. If the overall risk-benefit analysis suggests that the risk of immunotoxicity is considered acceptable and/or can be addressed in a risk management plan (see ICH E2E Guideline[2]), then no further testing in animals might be called for.

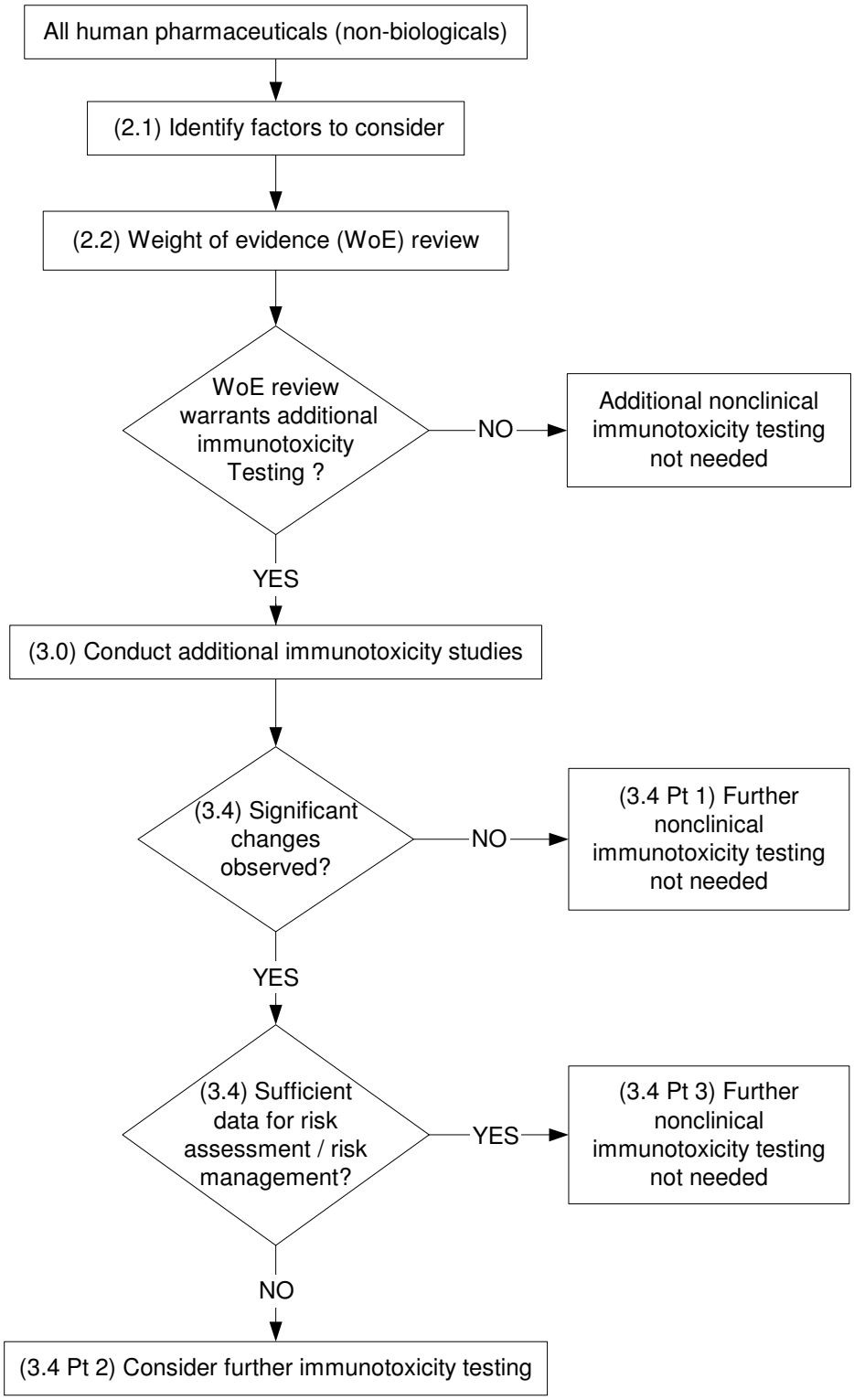
4. TIMING OF IMMUNOTOXICITY TESTING IN RELATION TO CLINICAL STUDIES

If the weight-of-evidence review indicates that additional immunotoxicity studies are appropriate, these should be completed before exposure of a large population of patients, usually Phase III. This will allow for the incorporation of monitoring immune system parameters in the clinical studies if appropriate. The timing of the additional immunotoxicity testing might be determined by the nature of the effect by the test compound and the type of clinical testing that would be called for if a positive finding is observed with the additional immunotoxicity testing. If the target patient population is immunocompromised, immunotoxicity testing can be initiated at an earlier time point in the development of the drug.

5. REFERENCES

1. ICH Harmonised Tripartite Guideline (S6) “Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals”
2. ICH Harmonised Tripartite Guideline (E2E) “Pharmacovigilance Planning”

Figure 1: Flow Diagram for Recommended Immunotoxicity Evaluation



APPENDIX: Methods to Evaluate Immunotoxicity

1. Standard Toxicity Studies

The following table lists the parameters that should be evaluated in standard toxicity studies for signs of immunotoxicity. These parameters (excluding hematology and clinical chemistry) and methods for obtaining samples and evaluating tissue sections are described in more detail in documents from professional toxicological pathology societies.

Parameter	**Specific Component**
Hematology	Total and absolute differential leukocyte counts
Clinical Chemistry	Globulin levels[1] and A/G ratios
Gross pathology	Lymphoid organs / tissues
Organ weights	Thymus, spleen (optional: lymph nodes)
Histology	Thymus, spleen, draining lymph node and at least one additional lymph node, bone marrow[2], Peyer's patch[3], BALT[4], NALT[4]

[1] Unexplained alterations in globulin levels could call for measurement of immunoglobulins.

[2] Unexplained alterations in peripheral blood cell lines or histopathologic findings might suggest that cytologic evaluation of the bone marrow would be appropriate.

[3] Oral administration only.

[4] For inhalation or nasal route only. BALT: bronchus-associated lymphoid tissues. NALT: nasal-associated lymphoid tissues

1.1 Hematology and Clinical Chemistry

Total leukocyte counts and absolute differential leukocyte counts are recommended to assess immunotoxicity. When evaluating changes in globulin levels, other factors should be taken into account (e.g., liver toxicity, nephrotoxicity). Changes in serum globulins can be an indication that there are changes in serum immunoglobulins. Although serum immunoglobulins are an insensitive indicator of immunosuppression, changes in immunoglobulins levels can be useful in certain situations in order to better understand target cell populations or mechanism of action.

1.2 Gross Pathology and Organ Weights

All lymphoid tissues should be evaluated for gross changes at necropsy. However, this can be more difficult for the Peyer's patches of rodents due to the small size. Spleen and thymus weights should be recorded. To minimize variability of spleen weights in dogs and monkeys, bleeding the animals thoroughly at necropsy is recommended. Atrophy of the thymus with aging can preclude obtaining accurate thymus weight.

1.3 Histopathological Examination

Histopathological changes of the spleen and thymus should be evaluated as an indicator of systemic immunotoxicity. The lymphoid tissue that drains or contacts the

site of drug administration (and therefore is exposed to the highest concentration of the drug) should be examined. These sites include the Peyer's patches and mesenteric lymph nodes for orally administered drugs, bronchus-associated lymphoid tissues (BALT) for drugs administered by the inhalation route, nasal-associated lymphoid tissues (NALT) for drugs administered by the inhalation or nasal route (if possible), and the most proximal regional draining lymph nodes for drugs administered by the dermal, intramuscular, intradermal, intrathecal, or subcutaneous routes. The specific node selected and the additional lymph node should be at the discretion of the sponsor based on the sponsor's experience. For intravenously administered drugs, the spleen can be considered the draining lymphoid tissue.

It is recommended that a "semi-quantitative" description of changes in compartments of lymphoid tissues be used in recording changes and reporting treatment-related changes in lymphoid tissues.

1.4 Interpretation of Stress Related Changes

With standard toxicity studies, doses near or at the maximum tolerated dose can result in changes to the immune system related to stress (e.g., by exaggerated pharmacodynamic action). These effects on the immune system might be mediated by increased corticosterone or cortisol release or other mediators. Commonly observed stress-related immune changes include increases in circulating neutrophils, decreases in circulating lymphocytes, decreases in thymus weight, decreases in thymic cortical cellularity and associated histopathologic changes, and changes in spleen and lymph node cellularity. Increases in adrenal gland weight and/or histologic evidence of adrenal cortical hyperplasia can also be observed. Thymic weight decreases in the presence of clinical signs, such as decreased body weight and physical activity, are too often attributed to stress. These findings on their own should not be considered sufficient evidence of stress-related immunotoxicity. The evidence of stress should be compelling in order to justify not conducting additional immunotoxicity studies.

2. Additional Immunotoxicity Studies

2.1 Assay Characterization and Validation

In general, the immunotoxicity test selected should be widely used and have been demonstrated to be adequately sensitive and specific for known immunosuppressive agents. However, in certain situations, extensive validation might have not been completed and/or the assay might not be widely used. In these situations, a scientific/mechanistic basis for use of the assay is called for and, if feasible, appropriate positive controls should be incorporated.

There can be variations of response for each type of immunotoxicity test used by different labs. In most situations, these changes do not affect the ability of the assay to assess immunotoxicity. However, to ensure proper assay performance and lab proficiency, several standard technical validation parameters should be observed. These parameters can include determining intra- and inter-assay precision, technician-to-technician precision, limit of quantitation, linear region of quantitation and test sample stability. In addition, assay sensitivity to known immunosuppressive agents should be established. It is recommended that each laboratory test a positive control concomitantly with an investigational compound or periodically in order to demonstrate proficiency of performance, except for studies with non-human primates.

For immunophenotyping, if properly validated technically, the addition of positive controls for each study might not be needed.

Immunotoxicity studies are expected to be performed in compliance with Good Laboratory Practice (GLP). It is recognized that some specialized assays, such as those described below, might not comply fully with GLP.

2.2 T-cell Dependent Antibody Response (TDAR)

The TDAR should be performed using a recognized T-cell dependent antigen (e.g., sheep red blood cells (SRBC) or keyhole limpet hemocyanin (KLH)) that results in a robust antibody response. The endpoint selected should be justified as the most appropriate for the chosen assay and the selected species.

Antigens for immunization should not be used with adjuvants without justification. Alum might be considered acceptable for use only in non-human primate studies. The relative TDAR response can be strain-dependent, especially in mice. With outbred rats, there can be significant variability among rats within the same group. Inbred rat strains could be used with provision of sufficient exposure data to bridge to the strain used in the STS.

Antibody can be measured by using an ELISA or other immunoassay methods. One advantage of this method over the antibody forming cell response is that samples can be collected serially during the study. In monkeys, serial blood collection can be important due to the high inter-animal variability in the kinetics of the response. For these studies, data can be expressed as the sum of the antibody response over several collection dates (e.g., area under the curve).

When SRBC antigens are used for an ELISA, the preparation of the capture antigen that is coated on the plates is considered critical. Whole fixed erythrocytes or membrane preparations can be used as the SRBC capture antigen. ELISA results should be expressed either as concentration or as titer, but expression as optical densities is not recommended.

2.3 Immunophenotyping

Immunophenotyping is the identification and/or enumeration of leukocyte subsets using antibodies. Immunophenotyping is usually conducted by flow cytometric analysis or by immunohistochemistry.

Flow cytometry, when employed to enumerate specific cell populations, is not a functional assay. However, flow cytometry can be used to measure antigen-specific immune responses of lymphocytes. Data obtained from peripheral blood can be useful as a bridge for clinical studies in which peripheral blood leukocytes are also evaluated. It is recommended that absolute numbers of lymphocyte subsets as well as percentages be used in evaluating treatment-related changes.

One of the advantages of immunohistochemistry over flow cytometry is that tissues from standard toxicity studies can be analyzed retrospectively if signs of immunotoxicity are observed. In addition, changes in cell types within a specific compartment within the lymphoid tissue can be observed. Some of the lymphocyte markers for certain species are sensitive to formalin fixation and can only be localized in tissue that are either fixed with certain fixatives or flash frozen. Quantitation of leukocytes and intensity of staining is much more difficult with immunohistochemistry.

When immunophenotyping studies are used to characterize or identify alterations in specific leukocyte populations, the choice of the lymphoid organs and/or peripheral blood to be evaluated should be based on changes observed. Immunophenotyping can be easily added to standard repeat dose toxicity studies and changes can be followed during the dosing phase and periods without drug exposure (reversal period).

2.4 Natural Killer Cell Activity Assays

Natural killer (NK) cell activity assays can be conducted if immunophenotyping studies demonstrate a change in number, or if STS studies demonstrate increased viral infection rates, or in response to other factors. In general, all NK cell assays are *ex vivo* assays in which tissues (e.g., spleen) or blood are obtained from animals that have been treated with the test compound. Cell preparations are co-incubated with target cells that have been labeled with ^{51}Cr. New methods that involve non-radioactive labels can be used if adequately validated. Different effector to target cell ratios should be evaluated for each assay to obtain a sufficient level of cytotoxicity and generate a curve.

2.5 Host Resistance Studies

Host resistance studies involve challenging groups of mice or rats treated with the different doses of test compound with varying concentrations of a pathogen (bacteria, fungal, viral, parasitic) or tumor cells. Infectivity of the pathogens or tumor burden observed in vehicle versus test compound treated animals is used to determine if the test compound is able to alter host resistance. Models have been developed to evaluate a wide range of pathogens such as *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Candida albicans*, influenza virus, cytomegalovirus, *Plasmodium yoelii* and *Trichinella spiralis*. Tumor host resistance models in mice have used the B16F10 melanoma and PYB6 sarcoma tumor cell lines.

Host resistance assays can provide information on the susceptibility to particular classes of infectious agents or tumor cells and can have an impact on the risk management plan. In addition, they can have an important role in identifying or confirming the cell type affected by a test compound. Moreover, host resistance assays involve innate immune mechanisms for which specific immune function assays have not been developed. In conducting host resistance studies, the investigator should carefully consider the direct or indirect (non-immune mediated) effects of the test compound on the growth and pathogenicity of the organism or tumor cell. For instance, compounds that inhibit the proliferation of certain tumor cells can seem to increase host resistance. An *in vitro* assay to test direct effects on the organism is recommended.

2.6 Macrophage/Neutrophil Function

In vitro macrophage and neutrophil function assays (phagocytosis, oxidative burst, chemotaxis, and cytolytic activity) have been published for several species. These assays assess macrophage/neutrophil function of cells exposed to the test compound *in vitro* or obtained from animals treated with the test compound (*ex vivo* assay). *In vitro* exposure to test compound can also be investigated. An *in vivo* assay can also be used to assess the effects on the reticuloendothelial cell to phagocytize radioactively or fluorescently labeled targets.

2.7 Assays to Measure Cell-Mediated Immunity

Assays to measure cell-mediated immunity have not been as well established as those used for the antibody response. These are *in vivo* assays where antigens are used for sensitization. The endpoint is the ability of drugs to modulate the response to challenge. Delayed-type hypersensitivity (DTH) reactions with protein immunization and challenge have been reported for mice and rats. Models in which contact sensitizers are used have been explored in mice but have not been well validated or extensively used. Cytotoxic T cell response can be generated in mice using a virus, tumor cell line, or allograft as the antigenic challenge. Monkey DTH reactions have also been reported. However, these reactions in monkeys are very difficult to consistently reproduce. In addition, one should make sure that the DTH response is not mistaken for an antibody and complement mediated Arthus reaction.