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Guidance for Industry

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION

Points to Consider in the Preclinical Development of Immunomodulatory Drugs for the Treatment of HIV Infection and Associated Disorders

This document addresses basic scientific issues that should be considered when evaluating the activity of immunomodulatory drug products in a preclinical setting. The guidance provided is specifically targeted to the development of immunomodulators intended for use in the treatment of HIV infection and associated disorders. All preclinical studies that define activity, as described below, should be submitted to the IND under the heading: MICROBIOLOGY. For general information regarding the chemistry, manufacturing and controls, preclinical pharmacology and toxicology, and clinical considerations pertaining to all drugs reviewed by the Division of Antiviral Drug Products, please refer to the document entitled "Points to Consider in the Preparation of IND Applications for New Drugs Intended for the Treatment of HIV-Infected Individuals" (document #P1). Specific guidance pertaining to the preclinical assessment of activity for antiretroviral drugs is provided in the Pre-IND document #P2, "Points to Consider in the Preclinical Development of Antiviral Drugs", which is available upon request.

General Comments

Adequate and well controlled preclinical studies to define activity play an important role in the drug development process for potential immunotherapeutic agents. Sufficient information should be obtained during the course of these studies to establish a rationale for efficacy, to allow for the generation of a risk/benefit profile and to facilitate the design of appropriate clinical trials. In most cases, Phase I human studies can begin when sufficient preclinical data are available to demonstrate a favorable risk/benefit profile for the drug in the intended patient population. Additional preclinical information pertaining to relevant therapeutic variables and the mechanisms of drug action are needed prior to the initiation of Phase II (efficacy) trials.

During the preclinical phase of drug development a potential immunomodulator should be thoroughly evaluated for activity in at least 2 independent assay systems. Generally, when an immune-based therapy is under development for treatment of an infectious disease, drug activity can best be determined in an appropriate infected animal model. *In vitro* systems are often used to supplement or support these studies and may be necessary to generate more detailed information about specific molecular and cellular mechanisms of drug action. When a compound cannot be

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adequately assessed in animals, *in vitro* studies may serve as the primary basis for drug activity evaluation. In general, a comprehensive drug development program should utilize both animal models and *in vitro* systems to maximize the value of the preclinical data obtained. However, the best approach for study of any specific immunotherapy should be determined on a case-by-case basis, taking into account the unique characteristics of the drug and its proposed clinical application.

The following information should be included in the Microbiology/Immunology portion of an Investigational New Drug (IND) submission:

NON-CLINICAL ACTIVITY STUDIES

Evidence of immunomodulatory activity sufficient to provide a rationale for human use should be submitted prior to the initiation of clinical studies. An appropriate rationale is essential in that it provides the context in which the safety risks are assessed. While both clinical and nonclinical experience from other disease applications may serve as supporting evidence of drug action, this experience does not preclude the need to define relevant activity parameters in an appropriate virus-infected system.

The preclinical evaluation of activity should include the identification and characterization of specific immunological changes associated with drug activity, including potentially adverse immunologic events. In addition to overt immunosuppression, the potential for activation of virus replication (see section 3, below), up-regulation of inappropriate immune responses, or disturbance of essential regulatory mechanisms are examples of deleterious effects that should be considered as part of the evaluation of activity.

While this document focuses on HIV indications, most of the considerations outlined here also apply to the evaluation of immunomodulators for use in other viral diseases. Specific examples involving HIV targets or models will not apply and specific issues such as viral activation may not be an issue.

Studies submitted to an IND as evidence of drug activity must be presented in sufficient detail to allow for a thorough and independent review of the data. Abstracts and summary tables are <u>not</u> adequate. It is important that all appropriate controls be included with each data set and that quantitative studies be subjected to an appropriate statistical evaluation for significance.

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1. Defining Immunomodulatory Activity *In Vitro*

In vitro studies can contribute valuable information to the drug development process. The ability to isolate cells and to closely control testing conditions makes cell culture systems particularly useful for evaluating mechanism(s) of drug action, including characterization of specific effector cell populations or responses that may contribute to drug activity *in vivo*. Data from *in vitro* studies can also be used to guide the selection of dosage regimens for animal models, to identify potential markers for *in vivo* activity and for preliminary assessment of virus activation potential (discussed in section 3).

If data obtained from *in vitro* studies are to be used to provide a rationale for Phase 1 clinical trials, the items listed below should be addressed in the IND submission.

a. Identify appropriate cell type(s) to carry out *in vitro* characterization of the immunomodulator under development. The choice of experimental systems and/or methods for analysis will be dictated to a significant degree by the nature of the drug substance. However, use of animal cells for drug activity evaluation should be restricted to *ex vivo* studies performed in support of animal model experiments. If *in vitro* studies are used to provide the primary evidence of drug activity, these data should be obtained using a variety of different <u>human</u> immune cell types, including primary and established cell cultures.

b. Define the basic parameters of the chosen system, including cell source, immunological activities (if any) mediated by the cells under normal conditions, dependence on specific cytokines, and the presence of endogenous viruses.

c. Identify the specific functions/markers that will be followed to evaluate drug activity in the chosen cell system. Endpoints should be clearly defined. Where appropriate, specific criteria for classification of responses as positive, negative or no change should be provided, along with the standards used to establish rating criteria. For all *in vitro* studies, the number of replicates per experiment should be sufficient to allow for the determination of statistical significance.

   Examples of drug activities that may be followed include cellular proliferation or cytokine production following exposure to antigenic, mitogenic or allogeneic stimuli; cytotoxic responses directed against virus-infected cells or tumor targets; chemotaxis, phagocytosis and/or killing of ingested organisms.

d. Define the dose-response curve for the drug in relation to the activities or marker(s) defined above. Immunomodulators often have a biphasic or bell-shaped activity curve. Consequently, it is important to record both up- and down- regulation of a given function and the dose levels at which these effects occur. Care should be taken to define activity as thoroughly as possible over the full range of biologically relevant drug concentrations. Also, sufficient information from cytotoxicity testing should be provided to demonstrate that the concentrations required to achieve the desired effect(s) do not overlap with cytotoxic drug levels.

e. If drug activity directly involves cell types that are targets for HIV infection *in vivo*, repeat the studies outlined in section 1 a-d to compare the activity of the compound in the presence and absence of virus. Determine what impact (if any) the virus infection has on the dose-response curve and the cytotoxicity of the drug.

Drug impact on virus replication should also be evaluated using an appropriate measure of virus titer. Data from these experiments may overlap or complement studies specifically designed to evaluate antiviral activity (discussed in section 1 f, below) or virus activation potential (see section 3 a-d).

f. Immunomodulators may exhibit direct antiviral effects in addition to their immunologic activity. However, immunomodulation and antiviral effects may occur at substantially different drug concentrations. Hence, there is a need to carefully define and compare dose/response relationships for both types of activities.

If a significant degree of direct antiviral activity is observed in the experiments described above:

Determine the drug concentration that inhibits cell growth by 50% (ID_{50}). An effort should also be made to establish the level of drug associated with 50% cell death (TC_{50}).

Calculate the *in vitro* therapeutic index (TI) of the drug (i.e., the ratio of cytotoxic to antiviral activity as a function of drug concentration). If the therapeutic index exceeds 1 and the difference is statistically significant, then the compound should be evaluated further in a variety of different human cell lines and primary cell cultures. If the antiviral activity is significant and reproducible, the compound may be classified as an antiviral agent with secondary

immunomodulatory properties. The preclinical development of antiretroviral agents is discussed in a separate "Points to Consider" document (#P2).

2. *In vivo* Animal Protection Studies

Animal models of retrovirus infection are an important resource for the preclinical evaluation of potential HIV immunotherapeutics. None of the models currently available provides a perfect replicate of HIV infection in humans. However, at the present time they offer the best nonclinical approach for demonstrating or defining possible correlations between immunomodulatory activity and a positive clinical outcome. Dose response curves generated in animals can provide an activity profile that takes into account drug effects on the immune system as a complete, interactive unit. This is particularly important for potential immunomodulators because these compounds often affect more than one cell type or function, with a total spectrum of activity that reflects both direct and/or indirect drug effects. Animal models can further provide the means for defining the effects of changes in dose, schedule and route of administration on drug activity. The presence of an appropriate virus infection in the "whole animal" model makes it possible to assess the impact of cumulative changes in immune function on an infectious disease process. Drug evaluation carried out in a retrovirus-infected animal model may allow for the detection of drug effects that may not be present, or are not accurately reproduced in an uninfected host.

As part of the nonclinical evaluation of activity the following information pertaining to animal models should be submitted:

a. Identify and characterize an appropriate animal retrovirus model in which drug activity can be adequately evaluated. A model system should be selected that most closely approximates the human disease manifestations that the drug is intended to treat. A list of examples of retrovirus animal models that have been used, or have been proposed for use in HIV drug studies is provided in Appendix 1.

b. Provide a complete description of the model. This should include relevant information about the virus, inoculum size, route of infection, time course of the resulting disease and characteristic pathology in the untreated host.

c. Identify the specific endpoint(s) to be used for evaluation of drug activity. In addition to defining general outcome parameters (i.e., effects on morbidity and mortality), an effort should be made to identify and characterize specific drug-associated changes that

contribute to activity. A reliable measure of virus load should be included as an outcome parameter for all studies carried out in an infected host.

d. Define the dose-response curve for the drug in the presence and absence of virus.

e. Determine how changes in the following parameters effect drug activity in the model system;

- multiplicity of infection (M.O.I.)
- time of initiation of therapy
- dosing schedule
- route of administration

3. Virus Activation Studies

Some immunomodulatory compounds have been shown to enhance HIV replication or alter the state of infection *in vitro*. In at least one instance, virus activation, as measured by an increase in serum p24 antigen levels, was also observed *in vivo* in human clinical trials. Clearly, the potential for drug-associated up-regulation of virus production is an important factor to consider in the analysis of risk/benefit. Consequently, the following studies should be included in the preclinical evaluation of immunologically active drugs being considered for use in an HIV-infected patient population:

a. Using primary and established cell lines of <u>human</u> origin determine what effects, if any, the compound has on HIV replication *in vitro*. These studies should include an assessment of drug impact on acute and chronic (i.e., low level persistent) infections.

At a minimum, activation potential should be evaluated in the following human cell types: freshly isolated PBMC's, at least one established T cell line and one cell line representative of the monocyte/ macrophage lineage. Studies employing a variety of different human cell cultures and HIV strains are encouraged.

Please note: The evaluation of virus activation potential requires a model that utilizes HIV as the infectious agent and also allows for quantitation of virus load. Data on virus activation obtained from animal models are acceptable only if these criteria are met.

b. Up-regulation of virus production may result from a direct effect of the compound on infected cells, or indirectly through the induction of cytokines or other endogenous factors. It is conceivable that additional

mechanisms exist through which the infectivity or replication competency of HIV may be enhanced by the action of an immunomodulatory agent. Where evidence exists to suggest that virus production is up-regulated by the drug under study an effort should be made to identify the stage(s) of virus replication that are affected by the compound and to explore the probable mechanism(s) by which this activation takes place (e.g., up-regulation of virus receptors leading to enhanced binding or entry, enhancement of viral transcription/ translation, facilitation of cell-to-cell spread, etc.).

c. Immunomodulatory agents that activate virus replication in preclinical studies should be evaluated in combination with a suitable antiviral agent. These studies are outlined below in section 4.

The studies listed in section 4 a-c are important even if the immunomodulator is intended to enhance immunity to a specific opportunistic pathogen or neoplasia associated with HIV infection. For these compounds, the sponsor should also be alert for possible adverse effects of immunomodulation on the non-HIV target organism or tumor cell.

4. Concomitant Drug Therapy Involving an Antiretroviral Agent and an Immunomodulator

The tendency to promote virus replication does not necessarily preclude the use of an immunomodulator in an HIV+ patient population. It does, however, indicate a need for concomitant administration of an effective antiretroviral agent. Proposed combinations should be evaluated in a preclinical setting to determine how the drugs interact at biologically relevant concentrations.

Concomitant drug therapy involving immunomodulators and antiretrovirals may be proposed and tested for a variety of reasons other than the tendency for an immunomodulator to activate virus. In each case, appropriate models for testing will be determined by the nature of the compounds involved and their respective therapeutic targets.

If an immunomodulator will be administered concomitantly with an antiviral drug the following experiments should be performed:

a. Identify primary human cell cultures and/or established cell lines representative of *in vivo* virus-infected tissues. Proposed combinations

should be tested in these cell lines in the presence and absence of HIV, as described below in b through d.

b. Establish the range of effective concentrations for the antiviral agent in the virus-infected system. A dose/response curve should also be generated for the immunomodulator if it mediates direct antiviral activity at biologically relevant drug concentrations (e.g., drug levels in the range of achievable serum concentrations). Similarly, dose/response curve(s) for cytotoxicity should be obtained in the same model system.

c. On the basis of the dose-response curve(s) defined in section 4b and the nature of the 2 drugs under study, select an appropriate mathematical method to determine if the proposed combination is synergistic, antagonistic or additive with respect to antiviral activity. Examples of potentially useful methods include, but are not limited to the Median Dose Effect Equation [1-4], the "COMBO" approach [5,6], "3D" or "Synergy" [7], fractional inhibition concentration method [8-10], the universal response surface approach[11] and other variations or extensions of the isobologram approach (reviewed in reference 7). Irrespective of the model employed, the determination of interaction profiles should include an assessment of the statistical significance of the result [4,12,13]. References are provided in Appendix II.

d. Evaluate the antiviral effects of the drug combination over the full range of activity defined by the dose/response curves of the individual agents. Specific concentrations and/or drug ratios to be tested will be dictated, at least in part, by the method selected for data analysis. However, special attention should be paid to drug concentrations that involve clinically relevant levels of the individual compounds.

e. Determine what effect, if any, the immunomodulator has on the cytotoxicity of the antiviral agent.

The studies described in section 4 a-e can contribute valuable information to facilitate the design of subsequent clinical trials (e.g., may help to identify optimal drug ratios and/or concentrations to maximize beneficial drug effects). These data should be available prior to the initiation of Phase II (efficacy) studies involving the concomitant therapy regimen.

The following studies are recommended to support a clinical trial involving concomitant therapy with an antiretroviral and an immunomodulator in HIV+ patients:

f. Repeat 4 a-e to evaluate the effects of the antiviral agent on the activity/cytotoxicity of the immunomodulator. It is likely that the *in vitro* model system employed for the antiviral studies will not be appropriate for these experiments. In most instances, one or more of the systems identified in section 1 or 2 can be used to address this issue.

5. Concomitant Drug Therapy not involving an antiretroviral agent

If the primary treatment regimen under evaluation involves an immunomodulatory drug administered concomitantly with another immunomodulator or a therapeutic agent other than an antiretroviral (e.g., antifungal, antiparasitic, antibacterial, antiviral or antineoplastic drug), the following studies should be performed;

a. Determine if the drug combination under study is synergistic, antagonistic, or additive using the procedure outlined in section 4 a-e (above), substituting the appropriate assay system in place of that described for the antiretroviral agent.

b. Examine the effects of the drug combination on HIV replication, as described in section 3.

6. Miscellaneous Studies

a. If concerns exist about the potential antigenicity of a drug compound which may adversely impact activity, this potential should be thoroughly evaluated in appropriate animal models.

b. General requirements concerning validation of composition, lot-to-lot consistency, and potency are discussed in the chemistry section of the P1 preIND document. However, special care should be taken to ensure that the immunomodulatory activities associated with a specific drug product are due, in fact, to the drug substance and not to low level contaminants such as double-stranded RNA from endogenous viruses in plant extracts or endotoxin.

7. Clinical Microbiology Laboratory Test Methods

A description of the proposed clinical trial should include the following information:

a. The immunological and virological parameters that will be followed during the course of the clinical study should be specified in the protocol. A brief statement of rationale to support the selection of these parameters (as it relates to the immunologic/virologic activity profile of the compound established during preclinical development) should also be provided.

b. The proposed schedule for testing of each of these parameters should be clearly specified, including the number and timing of baseline measurements.

c. The endpoints that will be employed in the evaluation of each parameter measured must be specified. Where appropriate, specific prospective criteria for classification of responses as positive, negative or no change should be proposed. Standards used to establish rating criteria should be clearly defined.

d. A description of the protocols and reagents to be employed in the measurement of the immunological and virological markers/functions should be included in an appendix accompanying the protocol.

Appendix I: Animal Models

1. Infected Animal Models: Examples of possible model systems for testing anti-HIV drugs are shown below. It is important to note that some of these models are still under development, and have not been validated. Also, this list is not all-inclusive and will be subject to revision as new (or refined) models become available.

Murine (for HIV)
- LP-BM5
- Cas-BrM
- Friend Leukemia virus
- Rauscher Leukemia virus (Ra-SFFV, Ra-MuLV)
- Reconstituted SCIDs, GVH models, transgenic animals or xenotransplantation models (HIV)

Feline (FeLV, FIV)
Monkeys, macaque (SIV)
Chimpanzee (HIV)
Rabbits (HIV, BIV)
Sheep (Lentiviruses)
Chickens (ALV)

2. Genetically Engineered/modified animals (e.g., inbred animal strains with known susceptibility to specific transmissible tumors, genetically-defined immunodeficient animals, or inbred animal strains with inducible immunodeficiency states).

The animal models listed in this group can be used to evaluate specific effects mediated by an immunomodulator on the basis of protection against, or recovery from the predetermined immune dysfunction. These models may allow for the generation of dose-response curves, or some other form of quantitation, and may also be useful in the examination of cell subset interactions. However, these systems should <u>not</u> used in place of an appropriate animal model of retrovirus infection.

3. Immunologically normal, uninfected animals.

In general, immunologically competent animals are not considered to be appropriate models for characterization of immunomodulators as a drug class. They do, however, play a necessary role as "normal" controls in the evaluation of activity in retrovirus-infected models.

Appendix II: References

1. Chow, T.C. & Talalay, P. (1983) Analysis of combined drug effects: A new look at a very old problem. *Trends Pharmacol. Sci.* 4, 450-454.

2. Chow, T.C. & Talalay, P. (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* 22, 27-55.

3. Rideout, D.C. & Chow, T.C. (1991) Synergism, Antagonism, and Potentiation in Chemotherapy: An Overview. In: Synergism and Antagonsim in Chemotherapy, pp. 3-60. Academic Press, Inc.

4. Spector, T. & Hajian, G. (1981) Statistical methods to distinguish competitive, noncompetitive and uncompetitive enzyme inhibitors. *Analytical Biochem.* 115, 403-409.

5. Weinstein, J.N., Bunow, B., Weislow, O.S., Schinazi, R.F., Wahl, S. M., Wahl, L.M, & Szebeni, J. (1990) Synergistic drug combinations in AIDS therapy. In AIDS: Anti-HIV Agents, Therapies, and Vaccines. *Ann. N. Y. Acad. Sci.* 616, 367-384.

6. Bunow, B. & Weinstein, J.N. (1990) COMBO: A new approach to the analysis of drug combinations *in vitro.* In AIDS: Anti-HIV Agents, Therapies, and Vaccines. *Ann. N.Y. Acad. Sci.* 616, 490-494.

7. Prichard, M.N. & Shipman, C. (1990) A three dimensional model to analyze drug-drug interactions. *Antiviral Res.* 14, 181-206.

8. Bauer, D.J. (1955) The antiviral and synergistic actions of isatin thiosemicarbazole and certain phenoxypyrimidines in vaccinia infection in mice. *Br. J. Exp. Pathol.* 36, 105-114.

9. Jackson, R.C. (1984) A kinetic model of regulation of the deoxyribonucleoside triphosphate pool composition. *Pharmac. Ther* 24, 279-301.

10. Johnson, J.C. & Attanasio, R. (1987) Synergistic inhibition of herpesvirus replication by acyclovir and phosphonocompounds. *Intervirology* 28, 89-99.

11. Greco, W.R., Park, H.S. & Rustum, Y.M. (1990) Application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-β-D-Arabinofuranosylcytosine. *Cancer Res.* 50: 5318-5327.

12. Berenbaum, M.C. (1988) Isobolographic, algebraic, and search methods in the analysis of multiagent synergy. *J. Am. Coll. Toxicol.* 7, 927-938.

13. Gessner, P.K. (1988) A straightforward method for the study of drug interactions: an isobolographic analysis primer. *J. Am. Coll. Toxicol.* 7, 987-1012.