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REPORT OF A WHO INFORMAL CONSULTATION  
ON PRECLINICAL AND CLINICAL ASPECTS OF THE  
USE OF IMMUNOMODULATORS IN HIV INFECTION

GENEVA  
3-5 APRIL 1989



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# Report of a WHO informal consultation on preclinical and clinical aspects of the use of immunomodulators in HIV infection

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## Summary

A wide spectrum of immunomodulatory strategies offer promise for treating people with HIV infection; however, numerous gaps still exist in our understanding of the normal regulation of the immune system during the progression of HIV infection. Preclinical development of immunomodulators must include a strong rationale for the use of the immunomodulating substance substantiated by appropriate laboratory studies, *in vitro* as well as *in vivo*. Preclinical studies must demonstrate the safety of the proposed therapeutic agent, including an assessment of the potential for adverse effects on immune function and virus replication. Combined therapeutic modalities should also be appropriately evaluated at the preclinical level.

Clinical evaluations should be instituted only after a strong rationale is substantiated and safety concerns are fully considered. Initial studies should be conducted with patients at an intermediate stage of HIV-disease progression. Immunomodulators may exhibit unusual dose-response patterns, and this should be considered when designing the trials.

The consultation recommended that WHO continue to provide a forum for the timely exchange and validation of information related to the development and clinical evaluation of immunomodulators for the treatment of individuals infected with HIV.

## 1. Introduction

The acquired immunodeficiency syndrome (AIDS) is characterized by severe alterations of the immune system that result in characteristic opportunistic infections (OIs) and often death. Most of the currently available therapies, as well as the majority of therapies undergoing clinical evaluation in the United States of America and Europe, were discovered by virtue of their antiviral activity, e.g., their ability to inhibit replication of the human immunodeficiency virus (HIV) which causes AIDS, in cell culture systems when added at concentrations that were not toxic to the cells. Therapies approved to date in the United States include zidovudine, or AZT, for individuals with AIDS, and interferon alpha for those with Kaposi's sarcoma (KS). Zidovudine inhibits the virus encoded reverse transcriptase, an enzyme essential to virus replication. The mechanism of action of interferon alpha against KS remains unclear, but it may be through its antiproliferative action.

Since alterations in the immune system are the primary characteristic of HIV infection, therapies targeted toward modulation of the immune response have been under intense evaluation. Immunomodulators could prove therapeutically beneficial by restoring the damaged immune system, decreasing the incidence of opportunistic infections, reducing virus load, or preventing other manifestations of disease. Combination therapies may also prove beneficial in cases where the immunomodulator counteracts toxic effects of the drug that prevent its utilization at recommended doses or enhances the therapeutic effect of either therapy alone.

Since there are growing numbers of infected individuals, particularly in developing countries, who may not have easy access to available therapies, it is critical that additional antiviral and immunomodulatory therapies be found and evaluated as expeditiously as possible.

Preclinical and clinical evaluations of immunomodulators for use as single agents or in combination with other immunomodulators or antiviral drugs offer unique opportunities as well as special challenges. To devise a consensus regarding the approaches required to achieve these evaluations in an expedient manner, the WHO Global Programme on AIDS organized an informal consultation to review the state of the art of immunomodulator therapies for the treatment of those infected with HIV, and to formulate recommendations to the scientific community and WHO. The informal consultation was held in Geneva on 3-5 April 1989 and was attended by 13 participants from 10 countries (Annex). The meeting was

opened by Dr J. Esparza, Acting Chief of the Biomedical Research Unit (BMR/GPA), and Dr A. Jurado (BMR/GPA) explained the method of work. Professor A. Pompidou (France) was elected chairman, Dr M. Johnston (USA) was co-chairman and rapporteur, and Dr E.C. Cooper (USA) was co-rapporteur.

## 2. General concepts

### 2.1 Definition and classification of immunomodulators

Immunomodulators are substances of diverse origin that modulate the response of immune competent cells through a signalling event. They differ from other immune-based therapies, such as cytotoxic T-cells, bifunctional antibodies, and immunoadhesins, that have direct killing effects on virus-infected cells. Immunomodulators may exert their beneficial effects by a variety of means, including reduction of virus load either indirectly or directly by killing virus-infected cells.

Immunomodulators include substances that augment, modulate, or restore the immune response and agents that modulate the sensitivity to endogenous or exogenous agents. Since the pleiotropic activity and the multi-target action of most immunomodulators complicate a functional categorization, a classification for the immunomodulating agents based on their origin - homologous and heterologous - is proposed (Table 1). Homologous immunomodulators are those that the body is capable of producing; examples are cytokines, such as the interleukins and interferons, which are described in more detail below. It is proposed that the homologous immunomodulators be further characterized by two subgroups: those generated by the immune system (intra-system) such as interleukins, and those produced outside the immune system (extra-system) such as neurohormones. The second major category (heterologous) comprises agents from any other biological sources and synthetic materials such as polynucleotides and small organic molecules.

### 2.2 Cytokines as immunomodulators

Cytokines are a group of proteins that have potent activity in a number of physiological systems. They are especially important in the regulation and development of the immune response but are also involved in hemopoiesis and the neuroendocrine system. Cytokines also control the interactions of the immune system with other physiological processes.

Cytokines are secreted by and act on a number of different cell types, in particular lymphocytes and monocytes. They are usually produced in very small amounts and have extremely potent biological activities. Cytokines identified to date number over 20, and many appear to have multiple overlapping activities.

Cytokines are usually divided into the interleukins, the colony stimulating factors, tumor necrosis factors/interferons, and other cytokines that do not fit clearly into these categories. Cytokines that have been characterized, cloned, and purified are listed in Tables 2 through 5, along with their most commonly known actions.

Cytokines act on target cells through interaction with specific receptors that are either normally present on cell surfaces or are induced by stimuli, e.g., interaction with antigen. It should be emphasized that many of the data supporting the secretion and effector function of cytokines is based on *in vitro* studies using transformed cell lines. Such experimental systems may not reflect *in vivo* physiological processes involved in normal immune regulation or immunomodulation by exogenous agents.

The *in vivo* administration of some cytokines may result in the induction of severe adverse reactions that require discontinuation of therapy. For example, interleukin-2 (IL-2) can cause severe fluid retention and volume overload. In contrast, other cytokines such as granulocyte CSF (G-CSF) do not seem to be associated with any serious side effects.

A significant body of literature describing the activities and potential uses of cytokines and other endogenous immunomodulators has been published. One rationale for the use of these agents to treat individuals infected with HIV is to administer small doses to restore disease-associated deficiencies in the immune system. Alternatively, they may be given in larger amounts to achieve a magnified immunomodulatory effect expected to result in reduction of virus load or prevention of opportunistic infections. Cytokines are also undergoing evaluation for their ability to alleviate the toxic effects of certain antiviral therapies. Cytokines being evaluated as potential therapies for AIDS include the interferons, G-CSF, GM-CSF, IL-2, IL-3, and erythropoietin (Table 6).

### 2.3 Synthetic immunomodulators

Synthetic immunomodulators offer several advantages over recombinant cytokines. They can often be produced inexpensively and reproducibly. They are more often active following oral administration and, like cytokines, usually induce pleiotropic effects, sometimes through the induction of single or multiple cytokines. Because of the ease of administration and low cost, such agents may prove to be extremely valuable in developing countries. Numerous immunomodulators are undergoing preclinical and/or clinical evaluation as potential therapies (Table 6).

### 2.4 Goals of immunomodulator therapies

Since the first reported AIDS case in 1981, much has been learned about the immunologic abnormalities associated with HIV infection at various stages of the disease. Many of these abnormalities were observed during *ex vivo* studies of immune competent cells and may not reflect the activity of those cells in the body.

For example, peripheral blood mononuclear cells (PBMC) from HIV-1-infected patients with AIDS or AIDS-related complex (ARC) clearly have no or at least decreased capacity to secrete interferon-alpha in *ex vivo* assays. This cytokine is usually produced as a host defense mechanism in response to a virus infection. Since the decrease in interferon-alpha production occurs before changes in other clinical parameters, such as T4/T8 ratio, or elevated neopterin level, the amount of interferon-alpha produced by PBMC *in vitro* is proposed to serve as an early prognostic parameter of the progression of the disease from Walter Reed classification stage 2 (WR2) to stage 3 (WR3).

In contrast, the capacity of PBMC to produce interferon-gamma *in vitro* (T lymphocyte-mediated) declines only late in infection at WR5 to WR6. Additional parameters such as the change of natural killer (NK) cell activity (peak at WR2 and a subsequent drop at WR4 to WR6 almost to 0%) during the progression of the disease. Similarly, the secretion of tumor necrosis factor (TNF)-alpha by macrophages (increase during stages WR3 to WR5) may contribute to precise staging and understanding of the mechanisms of the disease.

Further evaluation of these and other immune parameters is paramount to understanding the pathogenesis of the virus, the body's response to the virus, and the progression of disease. Current knowledge of the defects that result from infection with HIV has led to several rationales for the administration of immunomodulators to AIDS patients. In general, these include:

- improvement or restoration of effector cell function through increasing the number of effector cells or augmenting effector cell activity;
- replacement of natural biological effectors or mediators;
- augmentation of sensitivity to endogenous or exogenous antiviral mechanisms; or
- increasing tolerance to antiviral agents.

Although numerous immunomodulators have been described, it is not possible at this time to recommend that specific classes of immunomodulators be employed at specific stages of disease. As more is learned regarding the nature of disease

progression and the exact immune mechanisms that can successfully keep the virus in check, it may prove beneficial to consider evaluating specific types of immunomodulators (e.g., stimulating agents, inhibitory agents, regulatory agents) at certain stages of the disease.

Investigators proposing to use immunomodulators for HIV infection should be aware that there is now evidence to suggest that the immune dysfunction observed during the course of HIV-related disease (perhaps including the destruction of CD4+ cells) may, in part, result from autoimmune reactions. The origin of these responses is unclear, although one explanation may be that HIV infection of macrophages induces the secretion of numerous cytokines such as IL-1, IL-6 (BSF-2), and tumor necrosis factor (TNF) that have powerful immunoactivating and inflammatory properties. The resulting activation of the immune system could lead to aberrant humoral and cellular autoimmune reactions that may be associated with the destruction of the CD4+ lymphocyte subpopulation as well as a diverse range of clinical manifestations including thrombocytopenias, neuropathies, and arthritides. Thus, considerable care should be taken in preclinical and clinical trials to ensure that these pathologic sequelae are not amplified.

### 3. Preclinical evaluation of immunomodulation

Because of the urgent need to evaluate all therapies as expeditiously as possible, concerted worldwide effort must be directed at appropriate preclinical evaluations. These evaluations must precede entry of any immunomodulator into clinical trial. Poorly designed or executed evaluations may delay the development of potentially critical therapies and may not identify the most effective therapies from the growing array of possible choices.

Preclinical evaluation of an immunomodulator should consist essentially of two components. First, a solid scientific rationale for use of the agent as an immunomodulator for treating HIV infection and/or its sequelae must be developed. This may be accomplished through *in vitro* and/or animal model studies. Second, the agent must be shown to be safe to administer to humans. Mechanisms of toxicity and the pharmacological and toxicological properties of the agent should be determined, in addition to the potential effects of the agent on the replication of HIV in T-cells and macrophages.

#### 3.1 Developing a scientific rationale

Preclinical research is required to provide a solid rationale for the proposed use of the immunomodulator and to assist clinicians in optimizing the clinical protocol through identification of an appropriate laboratory marker of immunomodulation. Although even the most carefully designed and controlled preclinical research may not be predictive of the outcome of therapeutic trials in humans, valuable information can be obtained regarding the ability of immunomodulators to reduce virus load, or to protect the host from the toxic effects of one of the agents.

##### 3.1.1 Effects on immune parameters *in vitro*

One of the critical goals of preclinical research is to provide a scientific rationale for use of the immunomodulator as a potential therapy in HIV-infected individuals. Such aims should be accomplished by demonstrating the effectiveness of the immunomodulator in at least two systems consisting of *in vitro* or animal studies.

*In vitro* assays assist in the identification of the effector cell(s) that may be responsible for the proposed beneficial effect of an immunomodulator. In turn, this information is helpful in optimizing the clinical protocol. The more routine *in vitro* assays include evaluating the activity of NK cells, lymphokine activated killer (LAK) cells, monocyte/macrophages, T-cells, and B-cells in the presence and absence of the immunomodulator. Proliferation assays measure the ability of a cell population to divide upon exposure to an appropriate stimulus such as antigen or mitogen. Proliferation in response to specific antigens such as HIV antigens, tetanus toxoid, and *Candida* are strongly recommended over mitogen responsiveness. Killing/cytotoxicity assays usually measure killing of chromium-labelled tumor or virus-infected target cells. In most cases it would be recommended to demonstrate

HIV-specific responses, e.g., that enhanced killing results from activation by HIV antigen or in killing of HIV-infected cells or cells expressing HIV antigens. Recombinant vaccinia virus constructs that express viral antigens have proven very useful in these assays, making measurement of HIV-specific responses possible without working with infectious HIV.

Monocyte function may be evaluated through migration assays, phagocytosis assays, the release of oxygen radicals upon stimulation, and/or the production of IL-1 in culture. In addition, the ability of immunomodulators to elicit a response through production of naturally occurring cytokines such as interferons and interleukins can be evaluated using assays for these cytokines.

Certain standard and quantitative *in vitro* assays may prove useful in screening compounds or mixtures for immunomodulatory activity. The Jerne hemolytic plaque assay detects stimulation of helper T-cell activity in the B-cell response that leads to antibody production, which can be easily measured.

PBMC from patients in WR4 - WR5 or normal PBMC treated with nonviable HIV preparations or purified gp120 respond *ex vivo* to virus with a reduced production of IFN alpha. These cells can be exploited as an *in vitro* model for immunocompromised PBMC and used to screen compounds for immunomodulating activity using restoration of the ability to produce IFN alpha as a marker. Cell suspensions are incubated with a suitable virus preparation (e.g., vesicular stomatitis virus) in the presence or absence of a potential immunomodulator. Inducibility is measured by <sup>3</sup>H-dThd incorporation rates, the amount of IFN produced (antiviral assay), or production of IFN mRNA (dot blot hybridization).

In addition to evaluating potential immunomodulators in *in vitro* assays of immune function, it is necessary to determine their direct effects on HIV and their effects on the antiviral activity of other agents. New immunomodulators should be tested for potential stimulation of viral replication, which could occur through direct interaction with the virus or indirectly through immune modulation. In addition, they should be evaluated in combination with an antiviral agent, such as Zidovudine. Data from these assays will indicate if the combination is synergistic, antagonistic, or additive. Similarly, it is advisable to evaluate the effect of the antiviral agent on the activity of the immunomodulator. Information on the *in vitro* activities of combinations is critical since most immunomodulators will, after initial clinical evaluation, also be evaluated in individuals receiving antiviral therapy.

### **3.1.2 *In vivo* evaluations in retroviral models**

In certain cases, *in vitro* evaluations may provide sufficient data for an immunomodulator to be used in HIV-infected individuals. In most cases, however, establishment of a sound scientific foundation can be facilitated through utilization of appropriate animal models. This is particularly critical for new immunomodulators that have not undergone clinical evaluation for other indications. Ideally, in small animal models, the agent should demonstrate (1) an immunomodulatory activity that would benefit an individual infected with HIV; and/or (2) efficacy against an appropriate pathogen, as measured by survival and/or reduction in pathogen load. However, because of the limitations of currently available animal models, it may not always be possible to demonstrate the latter, even for agents with therapeutic potential.

Use of small animal models can facilitate identification of the effector cell population, e.g., the target cell or cells responsible for the beneficial effect of the immunomodulator. The effector cell will be the activity or activities that most closely parallel efficacy. One method of verifying the effector cell(s) would be to determine what purified cell population(s) derived from a treated/infected/protected donor protects naive animals from infection.

Identifying the effector cell greatly facilitates the design and optimization of the numerous parameters of a preclinical protocol, including dose, route, schedule, and timing of administration of the agent or agents. Optimizing the protocol is of

particular importance in the administration of immunomodulators, where efficacy may not be directly proportional to dose. The optimal immunomodulatory dose (OID) is the dose that provides the maximum degree of effector cell activity and the greatest therapeutic effect to the individual. The OID is sometimes lower than the maximum tolerated dose. In some cases, doses in excess of the OID may have no therapeutic immunomodulatory effect and may even have adverse immune effects. For example, interferon gamma and TNF have been demonstrated to have bell-shaped dose-response curves in mice. Not all cells in the body may be affected equally by equal doses. Biphasic response curves have been observed after administration of IL-2 to mice; higher doses effectively stimulated NK and LAK cells, whereas lower doses were more effective in stimulating T-cells. Careful optimization of the preclinical protocol greatly facilitates clinical evaluation of the immunomodulator. Physicians more easily optimize a clinical protocol using the optimized preclinical protocol as a starting point and following an appropriate laboratory marker indicative of the proposed effector cell activity.

In developing a rationale for the use of an immunomodulator to treat HIV-infected individuals, researchers are encouraged to demonstrate immunomodulation and a positive clinical outcome in an animal model. There is, at present, no single ideal model for such purposes. However, there are numerous retroviral small animal models that serve as useful tools of the preclinical researcher. Though not identical to HIV in humans, these experimental retroviral infections provide rapid answers with clearly defined endpoints and are quantitative and cost-effective. Further, there is rarely a problem with patient compliance.

Unfortunately, in developing a model that can be utilized over a short time course, investigators often administer very high levels of virus. This approach may not be most indicative of the HIV load that an immunomodulator would be expected to combat in humans.

The immune defects present in these small animal retroviral models bear numerous similarities to the defects found in AIDS patients, thus making them the models of choice, at present, for immunomodulator evaluation. For example, infection of mice with the LP-BM5 virus results in several functional abnormalities in T- and B-cells that are similar to those observed in AIDS patients. Suppression of IL-2 production and LAK cell production occurs in experimental and natural infection of cats with feline leukemia virus (FeLV) (W. Tompkins, personal communication). Thus, if the immune defect or effector cell targeted by the immunomodulator is shared by the HIV/human and retrovirus/animal systems, the animal model may be useful in establishing a rationale for using the immunomodulator in HIV-infected individuals.

One potential limitation is that most of these small animal models have as a feature a lymphoproliferative disorder, which is believed to be a primary cause of the observed immune dysfunction. In most cases, lymphoproliferation parallels infection over a certain range. Researchers often utilize inhibition of splenomegaly as a measure of antiviral effect. It is conceivable that an immunomodulator or other agent may appear to alleviate infection by blocking lymphoproliferation directly with no change in virus load. Direct measurement of virus load is, therefore, an important component of the overall evaluation. Another difficulty in evaluating immunomodulators in small animal models is that many cytokines, such as the interferons, are relatively or absolutely species specific. With rare exceptions, murine and feline cytokines have either not been cloned or are not available in the quantities needed for animal studies.

Even though existing small animal retroviral models share many common features with HIV infection, it is not yet known if any model will be predictive of efficacy of immunomodulators in HIV-infected individuals. Until better models become available, as many immunomodulators as possible should be evaluated in small animal retroviral models so that sufficient data can be accumulated over the next several years to determine which models are most predictive of the outcome in HIV-infected individuals. Until then, the models will continue to provide valuable information on mechanisms of action of immunomodulators and on approaches to

protocol optimization. It is clear from the number of therapies currently under development that additional discriminating factors for entry into phase I trials must be developed. Small animal models offer promise for use as this discriminating factor for the future.

Because HIV infection of chimpanzees does not produce the immunosuppressive effects observed in HIV-infected humans, the use of chimpanzees for evaluating immunomodulators is generally inappropriate and unwarranted. Simian immunodeficiency virus infection of monkeys appears at present to be the animal model most similar to HIV infection of humans.

However, because of limitations in the number of animals available for therapeutic evaluations, the demand for evaluation of antivirals, cost, and other concerns, evaluation of immunomodulators in SIV-infected monkeys could be justified only in very unusual cases, such as evaluating a species-specific immunomodulator in combination with an antiviral agent. Even then, the numbers of animals and expense would be limiting and unlikely to yield statistically significant results.

Transgenic animals that express all or a portion of the HIV genome and severe combined immunodeficient mice reconstituted with human cells (SCID/hu) that can be infected with HIV offer new and very exciting directions for developing small animal model systems to study HIV infections. These systems will permit analysis of antivirals directed against genes and gene products not present in small animal retroviruses. Given evidence that certain cytokines as well as other types of immune stimulation activate HIV expression *in vitro*, transgenic models that permit quantitation and dissection of this process *in vivo* would be highly valued. SCID/hu mice will also permit evaluation of immunotherapies against HIV on human cells in an *in vivo* situation and thereby strengthen our ability to establish the scientific rationale for administration of an immunomodulator to HIV-infected individuals. Problems associated with species barriers may also be minimized since this model utilizes human cells. Development of the SCID/hu and similar models that employ human cells infected with HIV are critical to the successful evaluation of immunomodulators as therapies and offer the highest hopes at present for a fully predictive small animal model for HIV infection. The rapid development and utilization of the SCID/hu model is imperative.

One of the best uses of small animal retroviral models is to evaluate combination therapies provided the virus molecular target and the immune target are both present in the model system. It is likely that immunomodulators will commonly be used in combination with an antiviral agent. Preliminary information on the synergy, antagonism, or additive nature of two agents given together can be evaluated *in vitro*, but cell culture systems may not be predictive of *in vivo* situations due to variables such as biodistribution, pharmacokinetics, etc. Therefore, combination therapies proposed for the clinic should receive appropriate preclinical evaluation in small animal models to determine how the immunomodulator may affect the activity of the antiviral agent, and how the antiviral agent may affect the activity of the immunomodulator *in vivo*.

Several immunomodulators, alone and in combination with antivirals, are currently undergoing preclinical evaluation (Table 6). Many of these agents are currently undergoing clinical evaluation, but evaluation in animal models is continuing to provide additional information on protocol optimization and effector cell activities.

### 3.1.3 Interplay of clinical and preclinical research

There are many difficulties in optimizing clinical protocols designed to determine efficacy of an immunomodulator in patients infected with HIV. First, even if the effector cell is identified through careful preclinical research, the sites of effector cell activity (e.g., spleen, bone marrow, neuronal cells) may not be easily accessible for routine measurements during clinical trial, or the number of cells obtained from these sites may be insufficient to allow assessment of drug activity. The assays available for clinical evaluation may be different from those employed in preclinical studies. While preclinical researchers often employ sophisticated

analysis of macrophage, T-cell, and NK cell activity, and/or measurement of mRNA levels, clinicians usually employ less sophisticated techniques, such as mitogen responsiveness, to determine immunomodulator activity. Lastly, individuals infected with HIV display a range of immune defects that may vary with the stage of the disease. These differences could result in therapeutic responses that differ quantitatively and perhaps qualitatively with the stage of the disease.

Many of these difficulties can be approached through careful preclinical identification of laboratory markers of efficacy. Laboratory markers are factors or activities that are related to therapeutic efficacy and are easily quantified in available clinical samples. For example, the serum concentration of the HIV protein p24 is commonly believed to be related to virus load. A commonly employed immunological marker is mitogen responsiveness of a patient's isolated peripheral lymphocytes, although this parameter is probably not directly related to effector cell function.

Preclinical researchers need to establish better laboratory immunological markers that reflect the degree of effector cell activity and that can be employed in the clinic to help optimize the clinical protocol for each immunomodulator. In turn, research clinicians need to employ more sophisticated techniques in monitoring patient responses to immunomodulator therapies. For example, assays that measure effector cell activity against HIV-infected cells would constitute a more specific indicator of immunomodulation than the delayed type hypersensitivity measurements commonly employed in the clinic as a measure of immune competence. Similarly, lymphocyte proliferative responsiveness to specific antigens such as HIV, *Candida*, or tetanus are recommended over mitogen responsiveness. Whatever the effector cell activity or laboratory marker measured, precise determination of the baseline value is required to evaluate the significance of changes that result with therapy.

In summary, preclinical research must first provide a solid rationale for use of the immunomodulator in HIV-infected individuals. This can be accomplished through evaluations *in vitro* or in small animal models. Further, preclinical research can lead to definition of the effector cell population(s), establishment of valid laboratory markers, as well as provide clinicians with valuable information concerning the optimal protocol design and the variables that might best be measured to establish activity in humans. Ideally, these approaches may someday make possible employment of a wide range of doses and schedules, each tailored to the individual and established through monitoring an effector cell activity or laboratory marker known to predict efficacy while avoiding toxicity.

### 3.2 Determining safety

The potential pitfalls of immunomodulator therapy must be considered along with the scientific rationale. The pleiotropic effects of some cytokines imply that cells and systems other than the desired target(s) may be affected. As an example, IL-2 induces cytotoxic T-cells, promotes proliferation of NK cells, stimulates JAK-cell activity, and also acts as a B-cell growth factor. Similarly IL-4 and IL-7 act on both B- and T-cells (at least *in vitro*). Cytokines such as IL-6 and especially IL-1 possess activity affecting a diverse array of target cells. Therapy involving such multi-active agents must therefore be used with care.

Production of additional targets for HIV infection or other undesirable effects may occur in addition to the desired treatment effect, e.g., adjuvant, hemopoietic, or cell stimulatory activity. Further, stimulating cells to replicate may increase their susceptibility to infection or may enhance virus replication. For example, stimulation of peripheral T-cells with IL-2 has been shown to increase infectivity by HIV *in vitro* and stimulation of monocytes/macrophages with recombinant GM-CSF increases HIV production. In contrast, immunomodulators may have benefits that outweigh their possible negative effects and warrant continued investigation at both the preclinical and clinical levels.

Following establishment of a solid rationale, the second critical component of preclinical development of immunomodulators for the treatment of AIDS is

evaluating the product's safety. In addition to traditional animal toxicity studies, the safety evaluation should involve screening for adverse effects on immune function and virus replication.

### 3.2.1 Effects on HIV production and opportunistic infection systems

An important component of the preclinical evaluation of immunomodulators with potential for treating those infected with HIV consists of evaluating the effect of the immunomodulator on HIV production in T-cells and in monocytes/macrophages, in the presence and absence of an antiviral agent such as zidovudine. In many cases, investigators do not perform anti-HIV testing, under the presumption that immunomodulators would not be expected to affect virus production. However, immunomodulators are usually pleiotropic in their effects and may lead to activation of HIV production from infected T-cells and/or macrophages, or production of suitable targets for HIV infection.

One documented example is the activation of HIV production in fresh peripheral monocytes/macrophages by granulocyte-macrophage colony stimulating factor (GM-CSF). GM-CSF was first proposed for use in HIV-infected patients to counteract the toxic effects of Zidovudine. However, when monocytes/macrophages were cultured and infected in the presence of GM-CSF, HIV production was increased over 10-fold. However, GM-CSF and Zidovudine have been found to be synergistic *in vitro*. When cells were placed in the presence of the combination prior to or at the time of HIV infection, HIV production was lower than that observed in the presence of Zidovudine alone. Although the toxic effects of the combination *in vivo* have not yet been explored, this example verifies the need to fully evaluate immunomodulators for their effects on HIV production *in vitro* prior to clinical trial.

Similarly, development of *in vitro* screening assays to evaluate immunomodulatory activity against the opportunistic infections (OIs) should be more fully developed. When these are available, immunomodulators should also be evaluated for their potential adverse effects in promoting replication of opportunistic pathogens in *in vitro* systems.

### 3.2.2 Pharmacology, toxicology, and formulation

Preclinical development of drugs to IND status in the United States of America requires that certain pharmacologic and toxicologic studies be performed. In most cases, the route of administration that is proposed for clinical trials should be employed in preclinical studies.

The types of tests normally required include determining the pharmacokinetics of the substance. Distribution and kinetic data will help guide development of a clinical protocol in terms of the dose and frequency required to achieve the proposed therapeutic level of the agent. The planned clinical protocol, in turn, will help in the design of appropriate preclinical toxicology experiments. Acute toxicity studies in one or two species should be done if the immunomodulator is not species-specific. If the immunomodulator is species specific, e.g., demonstrates activity in primates but not lower animals such as rodents or canines, toxicity studies in monkeys are strongly recommended. The doses and animal model chosen depend to a great extent on the activity of the immunomodulator expected in the animal compared with that expected in humans. For example, if an immunomodulator is 10-fold less active on rodent cells than human cells because of a 10-fold lower binding affinity to the effector cell, higher concentrations will be required in toxicity studies. Doses sufficient to result in some observable toxicity should be used. Null effect dose or no observed effect level at which no side effects are observed should also be determined.

Determination of the effects of repeated dosing on the toxicity profile is highly recommended. A subchronic (30-day) study with repeated dosing that mimics the schedule for the proposed phase I trial should be performed. Assays to detect and monitor levels of antibody specific for the immunomodulator must be established to evaluate the immune response to the immunomodulator during phase I trials in

cases where the agent has antigenic potential because of its size or demonstrated antigenicity in animals.

Appropriate quality control measures are required to assure lot-to-lot standardization of immunomodulatory activity as well as assuring safety. Immunomodulators should be evaluated for contaminants, including substances such as viruses and nucleic acids, that could prove quite harmful even when present in very small amounts. In addition, sensitization assays should be performed to provide assurance that administration of the agent will not sensitize the recipient to *other* stimulants or therapies.

### 3.3 Other indications

#### 3.3.1 Early stages of disease

Evaluation of immunomodulators in HIV-infected individuals prior to their progression to frank AIDS has been suggested as preferable to evaluation in patients with more advanced disease and more severely depressed immune systems. The rationale for this argument is that a therapy that works by immune-system stimulation may have a greater chance for success in a more immune-competent individual.

Use of anti-HIV drugs for post-exposure chemoprophylaxis has already begun in two populations: those accidentally exposed to HIV and children born to HIV-infected mothers. Special toxicology studies for these applications are needed during the preclinical development in order to assure safe use of the agent in these populations. These special toxicology studies should also be done for any immunomodulator under consideration for use in these patient populations and should include immune function studies in uninfected and developing animals, placental toxicity of the agent, developmental toxicology (teratology, neurologic, behavioural), toxicology in preweaning animals, *in vitro* genetic toxicology screening, target organ toxicity (hematopoietic, neurotoxicity), and standard/nonstandard target organ responses in adults and neonates. These studies should be coordinated with efficacy studies to prioritize drugs for testing and allow continued development of drugs while phase I and phase II trials are under way and before phase III trials are begun.

#### 3.3.2 Opportunistic infections

An area of research that has received little attention is the potential utilization of immunomodulators for the treatment of opportunistic infections associated with AIDS. Inhibition of macrophage function is believed to be responsible for *Mycobacterium avium-intracellulare* complex infection leading to disseminated disease in AIDS patients. TNF alone or with IL-2 has been shown to be associated with macrophage killing of *M. avium*. *Toxoplasma gondii*, an opportunistic pathogen of the central nervous system of AIDS patients, also evades phagocytic killing by suppressing oxidative burst and acidification of endosomes. Interferon gamma has been shown to stimulate macrophages to block *T. gondii* replication *in vitro*.

Although the promise of using immunomodulators for treating OIs has been established through *in vitro* assays, animal models for evaluating immunomodulator therapies should be utilized to more firmly establish the scientific rationale for clinical studies of immunomodulators to treat specific opportunistic pathogens associated with HIV infection. Since some cytokines may act on cells (e.g., macrophages) that can be co-infected with an opportunistic pathogen and HIV, or with more than one opportunistic pathogen, *in vitro* and animal model co-infection systems must be developed and employed to enable investigators to evaluate immunomodulator effects in co-infection systems. In conclusion, animal models should be utilized to establish the scientific rationale for immunomodulator therapies for the OIs and to guide development of clinical protocols for treating OIs in the face of an underlying HIV infection. This may be an ambitious goal since animal models for some OIs may be difficult to optimize and implement on a routine basis.

In this regard, delivery and formulation of immunomodulators should be addressed. In most instances, recombinant cytokines have been administered intravenously (i.v.) or subcutaneously (s.c.) with the goal of attaining therapeutic levels in the serum. If the goal of the immunomodulator therapy is to block or prevent infection by an opportunistic pathogen that resides primarily in the lung, aerosolized delivery of cytokines should be thoroughly explored. Similarly, encapsulation of cytokines in liposomes may result in selective delivery of the cytokine to phagocytic cells and enhance activity of the cytokine against parasites, while decreasing the overall dosage required and minimizing side effects. Thus, formulation and route of delivery are related to both the scientific rationale and safety of the proposed therapy and must be carefully considered.

### 3.3.3 Cytokines as adjuvants

Increased information on the biology of cytokines suggests they may offer new alternatives to previous formulations referred to as 'adjuvants'. The general purpose of using an adjuvant in conjunction with an immunogen is to provide an optimal and long-lasting state of immunity, classically measured through antibody production. However, induction and/or improvement of cell-mediated responses is also critical in the host's response to viral infection. A number of cytokines, namely, IL-2, IL-4, IL-6, IFN, and BMFF, have been found to enhance the process of immunization at various levels. For example, IL-2 augments the primary expression of IgM; this effect may result from a two-step mechanism where IL-2 induces production of lymphokines by helper T-cells. In addition, immunization with genetically engineered constructs containing relevant viral genes together with the gene encoding IL-2 have shown promising results.

*In vivo* and *in vitro* experiments have demonstrated that IL-6 has both IgM and IgG enhancing capacities that make it a potential candidate for boosting the immune response.

The broad range of pleiotropic activities of IFN-gamma includes modulation of Ig-isotype expression in mice. Additional work has found that the administration of IFN-gamma together with antigens (malaria) enhances immune responses. IFN-gamma may mediate these effects through its macrophage activating activity, thereby increasing the efficiency of antigen presentation.

Finally, specific reconstitution of immunosuppressed *in vivo* antigen specific secondary antibody response has been achieved through administration of partially purified lymphokine preparations (Con-A stimulated rat spleen, EL-4 mouse, and Jurkat human T-cell clones).

These results suggest that cytokines and perhaps other immunomodulators deserve further exploration for their potential utility as adjuvants to boost response to vaccines designed to prevent primary infection with HIV.

## 4. Clinical aspects of the use of immunomodulators in HIV infections

In developing new drugs for the treatment of HIV infection, clinical development time must be compressed because of the extraordinary public concern regarding this devastating disease and the resultant need to make safe and effective new therapies available as rapidly as possible. Therefore, a more abbreviated approach to clinical development has evolved, which may be substituted for the traditional progression of phase I trials in normal volunteers, followed by phase II trials in patients with the disease of interest, followed by larger phase III trials to further characterize safety and confirm efficacy.

Rather than beginning clinical studies of a new AIDS drug in uninfected normal volunteers, phase I studies are designed to detect signs of drug activity in humans, i.e., preliminary evidence of efficacy. Such trials are sometimes referred to as 'phase I/II trials,' to denote their expanded scope. In turn, the initial phase II efficacy trials which follow phase I are randomized, controlled, and of sufficient size to allow adequate evaluation of the potential efficacy of the drug based on definitive clinical

endpoints appropriate to the stage of disease under study. In the setting of HIV infection, designing the initial controlled trials as definitive efficacy trials rather than limited exploratory trials is critical, to prevent the generation of supportive but non-definitive data that would make justification of further controlled testing difficult while failing to provide convincing demonstration of efficacy. Therefore, initial phase II studies should be structured to serve the multiple purposes of confirming safety and activity data from phase I trials, providing data on intermediate variables, such as immune parameters, and allowing adequate evaluation of definitive clinical endpoints. Again, because such trials incorporate the goals traditionally associated with phase III trials, they are sometimes referred to as 'phase II/III trials'.

Following completion of such definitive phase II trials, additional important information can be obtained through the conduct of phase III/IV open or dose comparison studies during the pre-approval or post-marketing phases.

#### 4.1 Prerequisites for initial clinical trials

Since the limited state of our knowledge concerning the mechanism of immune suppression in AIDS and the lack of a validated animal model for HIV infection, immunomodulatory drugs must be considered for clinical trials solely on the basis of demonstration of some potentially useful biological activity in preclinical models. In some cases, the available models may be limited to *in vitro* systems or to animal models of unclear relevance to HIV infection in humans. In order to justify testing in humans, any observed influence on immune function should be thoroughly characterized in at least two independent *in vitro* and/or animal assay systems, using proper controls and the most appropriate techniques. Wherever possible, new immunomodulators should be evaluated in animal models to assess *in vivo* effects prior to administration to humans, although in some cases, suitable animal models for demonstrating the biologic activity of interest will not be available. When appropriate HIV-infected animal models are available, the immunomodulator should be tested in these systems before administration to humans.

The goal of preclinical development of a new immunomodulator should be to characterize the full range of its immunomodulatory effects as comprehensively as possible. If a new drug exhibits some biological activity of potential therapeutic benefit, the failure of other assay systems to demonstrate additional effects should not discourage clinical development, but should provide important guidance in the selection of patient population, study design, and parameters to be monitored.

Regardless of the specific nature of the drug under study or the models used in preclinical investigation, there should exist a sound theoretical rationale for the use of any new immunomodulatory agent in humans, i.e., the demonstrated biological activity should be one that can be expected to be of benefit at some stage of HIV-related disease.

One or more representative biological immune parameters, which are believed to reflect the immunomodulatory effect of potential therapeutic benefit and which are quantifiable, should be defined during the preclinical phase of development. Such parameters, which for simplicity's sake are termed 'immune markers', should be representative of the immunomodulatory effects of clinical interest, reproducible, and practical for clinical application and should be followed throughout the clinical programme.

In addition, a potential immunomodulator should be evaluated *in vitro* for its possible ability to affect virus replication before it is introduced into humans. Demonstration of enhancement of virus replication will allow incorporation of appropriate safety precautions in the clinical study design; whereas identification of any direct anti-HIV activity will be essential to the interpretation of results of clinical trials. In view of the limited predictive value of HIV culture systems, stimulation of virus replication *in vitro* would not necessarily prohibit clinical testing of an otherwise promising new drug. However, such an observation would

warrant careful monitoring for evidence of unexpected increases in virus burden or rate of clinical progression during human trials.

In addition to these unique aspects of the preclinical development of immunomodulators, all standard preclinical safety requirements including chemical identity, purity, pharmacology, toxicology, mutagenicity, and quality control standards of the product and production process must be provided prior to administration of a new agent to humans. Furthermore, drugs which might be expected to have antigenic potential, either by virtue of their size or because of demonstrated antibody formation in animals, require a suitable assay to detect human antibody formation against the drug substance.

#### **4.2 Selection of patient population**

Currently, the population of patients that in most circumstances appears to be most appropriate for initial study of a new immunomodulator are patients at an intermediate stage of HIV disease progression, i.e., asymptomatic or minimally symptomatic patients with CD4+ cell counts in the range of 300 to 500/mm<sup>3</sup>. Use of this patient group allows the investigator to avoid a number of potential problems associated with the study of immunomodulators in patient populations with very early or advanced disease. Less advanced (more immunocompetent) patients may be less suited to providing clinical evidence of improved immune function as they require more time to reach definitive clinical endpoints and are less likely to have immunologic abnormalities at baseline that will respond to pharmacologic intervention in an objectively measurable fashion.

Furthermore, the relatively good short-to-moderate-term prognosis in immunocompetent seropositives makes it more difficult to justify exposing them to a new agent that could have deleterious effects that may be difficult or impossible to detect preclinically or in phase I trials. In the case of a new immunomodulator, there is the particularly disturbing potential for unanticipated adverse immune effects that could precipitate acceleration of disease progression.

More advanced patients, i.e., those with AIDS and advanced ARC, introduce different problems. The toxicity of a new agent is more difficult to evaluate in more symptomatic patients than in a population of asymptomatic or minimally symptomatic patients. In addition, since these patients are eligible for zidovudine therapy, concurrent administration with the investigational immunomodulator will make it more difficult to interpret any observed treatment effects. Patients who cannot tolerate zidovudine could be studied with a new immunomodulator alone. However, these patients are not optimal candidates for early investigation of a new drug because they are more likely to be at an advanced stage of infection with a higher sensitivity to toxicity, as well as apt to display a reduced level of responsiveness to immunomodulatory therapy because of the severe degree of immunosuppression.

While these considerations highlight the advantages 'moderate risk' patients hold for the early study of new immunomodulatory drugs, it is important that studies of other patient populations be planned to follow quickly after the initial phase I studies. The effects of immunomodulators, both therapeutic and deleterious, may vary substantially with the stage of HIV-related disease. In addition, the efficacy of some immunomodulators may be dependent on, or greatly enhanced by, concurrent antiviral therapy. It is important that the impact of disease stage and concurrent antiviral therapy be characterized early in the course of drug development, to optimize planning for controlled studies.

#### **4.3 Design of phase I studies**

The goals of phase I investigation of immunomodulators intended for the treatment of HIV infection go far beyond the considerations of safety, tolerance, and pharmacokinetics traditionally associated with phase I clinical studies. The foremost goal of phase I trials using immunomodulators should be *in vivo* confirmation of biological activity in humans, as measured by an effect on the immune marker identified in preclinical studies. Pharmacokinetic information

should be obtained, if possible, and its correlation, if any, with treatment-associated effects on the immune marker should be assessed. Extensive immune function testing should be monitored regularly in order to detect any unanticipated immunomodulatory effects, whether potentially therapeutic or detrimental. In addition, broad immune function testing during human trials may serve to identify an immune marker for biological activity in humans that is superior to those derived from preclinical studies.

Safety data to be collected should, of course, include monitoring of standard safety parameters such as vital functions and hematologic and chemical laboratory parameters. In addition, a general screen of immune function should be included in safety monitoring, to assess the potential for adverse immunomodulatory effects, and a marker of virus burden, such as p24 antigen level, should be followed to allow detection of any treatment-related enhancement (or reduction) of virus replication. Finally, in the interests of obtaining maximal information as quickly as possible, evidence of clinical activity, such as improvement in weight, symptoms, or CD4+ cell count, should be sought, although failure to affect these parameters should not disqualify a new agent from further testing in controlled trials.

If a phase I trial with a new immunomodulator is to provide useful characterization of its safety, tolerance, pharmacokinetics, and biological and clinical activity, it is imperative that careful attention be given to dose levels and frequency of administration. In some cases, the optimal dosing frequency may differ substantially from traditional schedules of administration, since relatively infrequent exposure to an immunomodulator may result in sustained alterations in immune function. Similarly, the optimal immunomodulatory dose (OID) may bear little relation to the maximum tolerated dose (MTD) on which traditional phase I studies focus. Doses higher than the OID may have no immunomodulatory effect or may even have adverse effects.

Animal studies should be designed to identify such atypical dose-response patterns, and the planning of clinical trials should include a careful analysis of the animal data, to allow selection of the most appropriate dose levels and schedules for clinical study.

As discussed previously, in most circumstances, the first phase I trial of a new immunomodulatory agent should be conducted in HIV-infected individuals with moderate immune suppression, e.g., CD4+ counts in the 300 - 500/mm<sup>3</sup> range.

Cohorts of approximately five patients each should be entered at increasing doses with the goals of defining the active range and toxicity of the new agent, the OID, and the MTD and characterizing the biological effect. As the information to be obtained is so much more extensive than simple acute organ toxicity and pharmacokinetics, such a clinical trial program may last considerably longer than conventional phase I studies. Trials should be planned for at least 3 months, to allow sufficient time for demonstration of a stable influence on immune function and detection of any clinical activity.

#### **4.4 Prerequisites for phase II/III studies**

In order to justify proceeding from phase I/II into larger phase II/III randomized trials, the clinical data obtained in the early studies must continue to support a favorable risk-benefit assessment. At a minimum, the immune marker established preclinically should change in a favorable direction as a result of administration of the experimental agent. Additional evidence of benefit may be derived from data regarding other measures of immune function, as well as weight, clinical symptoms, p24 antigen levels, and CD4+ cell counts.

It is recommended that phase II/III studies be performed simultaneously in two or more relatively homogeneous populations of HIV-infected patients. As stated above, phase I/II tolerance/activity studies should be performed in each population prior to initiating larger controlled studies.

## 4.5 Design of phase II/III trials

### 4.5.1 Patients with moderate disease

The design of phase II/III studies in the group of moderately immunocompromised patients should be randomized, blinded, and placebo-controlled. The major clinical endpoint should be progression to an objective and prospectively defined degree of immunologic and clinical compromise sufficient to meet the criteria for zidovudine eligibility. An example of an appropriate primary endpoint would be a sustained decline in CD4+ cell count from greater than 300 to less than 200, associated with the development of an objectively defined clinical sign or symptom indicative of disease progression. Patients should continue to be followed for development of opportunistic infections, other serious complications of HIV infection, and death, even after zidovudine eligibility is reached, at least for the duration of the trial period.

Controlled studies of immunomodulators should be planned to last at least one year, preferably longer. Periodic interim analyses should be planned prospectively, to provide for detection of any early occurrence of a predefined highly significant difference in rate of progression between treatment groups. The number of patients to be studied should be determined in advance based on the expected progression rates in the placebo group and a predefined expected improvement in rate of progression in the experimental group. A 50% decrease in rate of progression over a year would be a 'good' response, although a lesser but statistically significant response might also be acceptable.

### 4.5.2 Advanced patients

Randomized studies in more advanced zidovudine-eligible patients should be designed separately for newly diagnosed, i.e., zidovudine-naive patients, and those who have previously been treated with zidovudine. As noted, randomized trials should be preceded by phase I studies demonstrating acceptable toxicity and reasonable evidence of activity in these patient groups.

#### 4.5.2.1 Zidovudine-naive patients

Newly diagnosed zidovudine-eligible patients, having either advanced ARC or early AIDS/OI, who have *not* received zidovudine before can be randomized to zidovudine plus placebo or to zidovudine plus the new immunomodulator. Patients should be followed for at least one year (unless a planned interim analysis shows a remarkable difference in progression of the two treatment groups). The primary endpoint in these more advanced groups should be a difference in survival or the incidence of AIDS-defining opportunistic infections or life-threatening neoplasms, although "lesser" clinical parameters and laboratory parameters (measures of virus burden, CD4+ cell counts, and the immune marker) should be closely followed. Using frequency of opportunistic infections as a definitive endpoint is problematic, particularly with the advent of effective prophylaxis against *Pneumocystis carinii* pneumonia and the anticipated development of other regimens that will prevent or delay the occurrence of many opportunistic infections. The ability to interpret any controlled trial of a new agent for HIV infection will be dependent on the uniform use of such prophylactic agents in all treatment groups. Since longer periods of time may be required to reach the endpoints to be used in these trials, planning of a longer treatment period and extended follow-up is desirable.

#### 4.5.2.2 Patients already receiving zidovudine

Zidovudine-eligible patients who have already taken on zidovudine for some minimum period of time can be randomized to the new immunomodulator or placebo. Although this group is more advanced clinically and probably less homogeneous than the newly zidovudine-eligible/zidovudine-naive group of patients, it allows the introduction of a single new agent in patients who are already "tolerating" zidovudine, making interpretation of changes in clinical status somewhat easier. In view of the considerable heterogeneity that can be introduced by variations in dose and duration of prior zidovudine therapy, studies in this population should incorporate careful definition of entry criteria for prior zidovudine exposure and/or stratification.

#### 4.6 Additional investigations

One concern is that it may be difficult under some circumstances to determine a definite endpoint for clinical efficacy study. Survival, as initially used for zidovudine may be difficult to use today as the current one-year survival of 80% under zidovudine requires a large sample size and extended observation periods. This type of study could be done in a multicenter approach, but these would have the additional problem of standardizing differences in the occurrence and management of OIs. Laboratory surrogate markers of HIV infection, or a combination of them, and quality-of-life parameters, should be studied as alternative endpoints for early clinical trials.

If initial phase II/III studies show efficacy sufficient to support registration of the drug (i.e., approval for marketing), the sponsor should study the agent in other populations, such as zidovudine-intolerant patients, patients with Kaposi's sarcoma, patients with HIV-induced neurologic diseases, pediatric patients, and earlier asymptomatic patients (e.g., CD4 counts greater than 500), provided such studies are justified by the rationale and safety profile. If possible, these phase III/IV trials should be comparative and randomized; otherwise, the sponsor should study the drug in open-label or dose-comparison trials to obtain as much useful information as possible on the safety and efficacy of the drug in these different groups of HIV-infected patients. In most instances, larger phase III/IV trials in new populations should be preceded by small pilot studies demonstrating the safety of the new drug in the population in question.

The use of immunomodulators may raise special concerns for some patient populations. For example, additional considerations related to planning clinical trials of immunomodulators in HIV-infected patients with Kaposi's sarcoma (KS) include the following:

- a) The assumption is made that immunomodulation may be a mechanism of anti-KS activity.
- b) An adequate rationale must be proposed to support the potential efficacy of the drug against KS.
- c) An appropriate KS population for initial phase I testing would be patients having CD4 counts greater than 200/m<sup>3</sup> and no prior history of opportunistic infections. KS lesions should be documented to be progressive in the 2 months prior to treatment with the experimental immunomodulator.
- d) Phase II studies may be open and noncomparative, with the objectives of defining clinical response rate and duration of response, as well as further defining toxicity. WHO oncology criteria should be employed in defining responses. The new drug should be compared with the current treatment of choice in a randomized phase III trial, with special emphasis on recording duration of response, other beneficial effects with respect to AIDS, and toxicity in both treatment groups. Alternatively, a more accelerated program of phase I/II and phase II/III studies may be planned.

#### 5. General conclusions

Preclinical evaluation represents an important step in bringing immunotherapies to clinical trials with persons infected with HIV. These evaluations must provide a solid scientific rationale for using the immunomodulator in HIV-infected individuals and also provide the required safety information. In addition to traditional manufacturing controls and pharmacologic, and toxicologic data, preclinical studies of a new immunomodulator should provide information regarding any potential for enhancement of virus replication or anti-HIV activity and should fully characterize its immunomodulatory effects. Furthermore, the studies should identify biological activities that can serve as immune markers during clinical trials. A thorough evaluation of the effects of immunotherapies in small animal models will contribute to the scientific rationale and complement *in vitro* results. Animal model studies will also provide information critical to the optimization of the clinical protocol. This is of particular importance in designing a combination protocol, where the variables are seemingly endless and simply

cannot be fully evaluated in clinical trials. Administration of three agents further increases the possible protocol variations. The appropriate utilization of small animal model systems will facilitate entry of immunotherapies into clinical trial and assure that each potential therapy is given the best chance for success. Development of improved small animal models that involve HIV infection of human cells, such as the SCID/hu model, is imperative and should be of the highest priority.

Because of the need to make safe and effective new therapies available as rapidly as possible, clinical development time for new immunomodulators for the treatment of HIV infection must be compressed, and hence the scope of trials conducted at each phase of study will be considerably broader than in traditional models of drug development. Phase I/II studies should be planned to provide confirmation of *in vivo* biological activity in humans as well as preliminary evidence of clinical activity, in addition to generating safety, tolerance, and pharmacokinetic data. Phase II/III studies should be designed to allow evaluation of definitive clinical endpoints sufficient to support approval for marketing, in addition to confirming safety and activity data from phase I/II and providing data on intermediate variables such as measures of immune function. Following definitive phase II/III trials, additional data can be obtained during phase III/IV studies. In designing clinical trials, careful attention must be given to selecting the patient population, dose level, and frequency of administration, as these factors may have considerable impact on the ability to detect the activity of drugs of this class.

The following summarizes the major points of this report:

- a) The wide spectrum of immunomodulatory strategies offers promise of the potential utility of immunomodulators in the treatment of individuals infected with HIV.
- b) Numerous gaps still exist in our understanding of the normal regulation of the immune response and alterations in the immune system during the progression of HIV infection; therefore, vigorous research in both areas is vital.

These research efforts should include a search for appropriate laboratory markers of immune function that are predictive of the state and progression of disease and are therefore helpful in monitoring the efficacy of therapeutic regimens. Development and utilization of innovative small animal models that utilize HIV infection of human cells, such as the SCID/hu mouse, are strongly encouraged, as those models offer great promise for expediting evaluation of anti-HIV and immunomodulator therapies.

- c) Preclinical development of immunomodulators must include development of a strong rationale for the use of the immunomodulating substance to treat HIV-infected individuals. The rationale must be substantiated by appropriate laboratory studies in two systems, which may include *in vitro* as well as *in vivo* studies. Small animal models are extremely useful for evaluating immunomodulator therapies and should continue to be employed to determine which may be most predictive of potential clinical benefit. Reliable laboratory assays that reflect the immunomodulator activity should be developed to facilitate comprehensive monitoring of the immunomodulator during the clinical evaluation phase.
- d) Preclinical studies must demonstrate the safety of the proposed therapeutic agent. Pharmacokinetic and toxicity studies in animals should provide relevant information to satisfy these requirements. Safety evaluation should include assessment of the potential for adverse effects on immune function and virus replication, in addition to providing more traditional toxicity data.
- e) Combined therapeutic modalities should be appropriately evaluated at the preclinical level to find more efficacious schemes or to prevent toxic effects sometimes present when single agents are employed at effective doses.
- f) Clinical evaluations beginning with phase I trials should be instituted only after a strong rationale is substantiated and safety concerns are answered.

- g) After entry of immunomodulators into phase I, preclinical or nonclinical investigation should continue to improve information pertaining to the mechanism of action, effector cell activities, and protocol optimization.
- h) Initial clinical studies of a new immunomodulator should be conducted with patients at an intermediate stage of HIV disease progression, i.e., asymptomatic or minimally symptomatic patients with CD4+ cell counts in the range of 300 - 500/mm<sup>3</sup>.
- i) Immunomodulators may exhibit unusual dose-response patterns, and hence the design of initial clinical trials should involve careful consideration of the available animal data regarding the effects of various dose levels and treatment schedules on immune function.
- j) In order to justify further clinical testing, phase I studies of a new immunomodulator should provide *in vivo* confirmation of biological activity in humans, as measured by an effect on the immune marker identified in preclinical studies.
- k) Safety monitoring of phase I studies employing immunomodulators should include immune function testing and measures of virus burden, in order to detect potential adverse effects related to immunomodulatory activity.
- l) The initial phase II/III efficacy trials of a new immunomodulator should be randomized, controlled, blinded, and of sufficient size to allow adequate evaluation of the potential efficacy of the drug based on definitive clinical endpoints.
- m) During or following definitive phase II/III trials, additional studies should be initiated in other populations of patients with HIV infection. In general, each phase III/IV study should be preceded by a small pilot study, in order to demonstrate the safety of the drug for the population in question.

## 6. Recommendations to WHO

- a. WHO should continue to provide a forum for the timely and expeditious exchange and validation of information related to the development and clinical evaluation of immunomodulators for the treatment of individuals infected with HIV.
- b. WHO should continue to promote and facilitate the exchange of reference reagents for standardization and research purposes.

Table 1  
**Classification of immunomodulating compounds**

**Homologous origin**

- Intra-system
  - Interleukins
  - Interferons
  - Tumour necrosis factors
  - Antibodies
  - Colony stimulating factors
- Extra-system regulators
  - Neurohormones
  - Endocrine hormones
  - Diverse, physiologic regulators

**Heterologous origin**

- Viruses
- Bacteria
- Fungi
- Plants
- Animals
- Synthetic
  - Oligonucleotides, polynucleotides
  - Sulphated compounds
  - Bioengineered molecules

Table 2  
**Cloned and purified cytokines and their properties**

**Interleukins**

<b>Factor</b>	<b>Actions</b>
Interleukin-1 alpha and beta	Multiple: inflammatory, fever, cytotoxicity, interleukin-2 production, haemopoietic activity
Interleukin-2	T- and B-cell proliferation and differentiation macrophage activation, NK cell activation
Interleukin-3	Progenitor growth and differentiation, mast cell growth
Interleukin-4	T- and B-cell proliferation and differentiation, IgE and IgE receptor induction
Interleukin-5	Eosinophil differentiation (B-cell growth)
Interleukin-6	B-cell differentiation, acute phase response. T-cell growth
Interleukin-7	Pre-B-cell growth. T- and B-cell stimulation
Interleukin-8	Neutrophil activation and attraction

Table 3  
Cloned and purified cytokines and their properties

**Interferons/Tumour Necrosis Factors**

<b>Factor</b>	<b>Actions</b>
Interferon (IFN) alfa and beta	Antiviral, growth inhibition, differentiation, NK activation, MHC antigen expression
Interferon (IFN) gamma	Antiviral, macrophage activation, MHC antigen expression, growth inhibition
Tumour necrosis factor (TNF) alfa and beta	Multiple, cytotoxicity, cachectia, haemolytic necrosis, bone resorption, fever
Transforming growth factor (TGF) beta	T- and B-cell proliferation and differentiation, IgE and IgE receptor induction

Table 4  
Cloned and purified cytokines and their properties

**Colony stimulating factors**

<b>Factor</b>	<b>Actions</b>
Granulocyte CSF (G-CSF)	Growth and differentiation of granulocytes
Macrophage CSF (M-CSF)	Growth and differentiation of monocytes/macrophages
Granulocyte macrophage CSF (GM-CSF)	Growth and differentiation of granulocytes/monocytes
Interleukin-3 (multi-CSF) (IL-3)	Growth and differentiation of progenitor cells, mast cells

Table 5  
Cloned and purified cytokines and their properties

**Others**

<b>Factor</b>	<b>Actions</b>
Low MW B-cell growth factor	B-cell proliferation
Erythropoietin	Erythropoiesis
Leukemia inhibitory factor	Macrophage differentiation

Table 6  
**Immunomodulatory compounds under evaluation for potential treatment of HIV**

<b>Agent</b>	<b>Development</b>	<b>Indication</b>
<b>Biologics</b>		
IL-2	Clinical	ARC/AIDS/KS
IFN alpha	Clinical	ARC/AIDS/KS
IFN beta	Clinical	ARC/AIDS/KS
IFN gamma	Clinical	ARC/AIDS/KS
TNF	Clinical	ARC/KS
G-CSF	Clinical	ARC/AIDS
GM-CSF	Clinical	ARC/AIDS/KS
Erythropoietin	Clinical	AIDS
Peptide T	Clinical	ARC/AIDS
IMREG-1	Clinical	AIDS/ARC/KS
IMREG-2	Clinical	AIDS/ARC/KS
Anti IFN Ig	Clinical	AIDS
Polio vaccine	Clinical	AIDS
Ampligen	Clinical	ARC/AIDS
MTP-PE	Clinical	KS
CD4 protein (Soluble)	Clinical	ARC/AIDS
Anti-leu (3A)(Idiotype-based)	Preclinical	
Poly IC:LC	Preclinical	
<b>Drugs</b>		
THF gamma 2	Clinical	Asym/ARC/AIDS
Thymopentin (TP-5)	Clinical	Asym/ARC
Thymostimulin (TP-1)	Clinical	ARC
Myelopeptides	Clinical	AIDS/ARC
Methionine enkephalin	Clinical	AIDS/ARC
Naltrexone (Commercial)	Clinical	ARC/AIDS
DHEA (dihydroepiandrosterone)	Clinical	ARC/AIDS
CL 246,738	Clinical	ARC/AIDS/KS
AS-101	Clinical	AIDS/ARC
Bropirimine	Clinical	KS
AL-721	Clinical	ARC/AIDS
Disulfiram	Clinical	ARC/AIDS
DNCB	Clinical	KS
Imuthiol (DTC)	Clinical	ARC/AIDS
Isoprinosine	Clinical	ARC/AIDS
Carrisyn	Clinical	ARC/AIDS
Indomethicin (Commercial)	Clinical	ARC/KS
Lithium Carbonate (Commercial)	Clinical	AIDS
D-Penicillamine (Commercial)	Clinical	ARC
Lentinan	Clinical	ARC
Glycyrrhizin	Clinical	Asym/ARC
Coumarin	Preclinical	
Etiocholanedione	Preclinical	
MVE-2	Preclinical	
Oxamizole	Preclinical	
Imexon	Preclinical	
Phenytoin (commercial)	Preclinical	
Diethylcarbamazine (DEC)	Preclinical	

## **Annex**

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