The Forum for Collaborative HIV Research

Immune-based Therapies and HIV Disease

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Scientific Chair: Alan Landay, Ph.D.

Facilitator: June Bray, Ph.D.

The Forum for Collaborative HIV Research, a project of the Center for Health Services Research and Policy at the George Washington University School of Public Health and Health Services, was founded in 1997. The goal of the Forum is to facilitate discussion regarding emerging issues in HIV clinical research and the transfer of research results into care.

The Forum is a coalition of government agencies, clinical researchers, health care providers, pharmaceutical companies, and patient advocates. An Executive Committee made up of representatives from each of the above named constituency groups governs the Forum. The Executive Committee determines the subject and scope of the Forum projects. The Forum brings these constituencies together to identify gaps and impediments in the understanding of the medical management of HIV disease and develops recommendations to fill those gaps. The Forum is a public / private partnership, which receives financial support from its governmental and industry members and with in-kind support from its membership within the academic research, patient care, and advocacy communities.

For more information about the Forum or to download reports from this meeting or prior ones, visit the Website at

www.hivforum.org

Table of Contents

Introduction 1

Recommendations for IBT Research 2

Presentation Summaries 6

Clinical and laboratory outcomes in cancer immunotherapy 6

Presented by Nora Disis, M.D., University of Washington 6

HIV immune-based therapies: A research-clinician perspective 11

Presented by Roy Gulick, M.D., MPH, Cornell University 11

Defining the bar: A community perspective 15

Presented by Brenda Lein, Project Inform 15

Report from the Forum's "Immune-based Therapies and HIV Disease: European and Australian Perspectives" meeting, Scotland, October 26, 2000 20

Presented by Michael Lederman, MD, Case Western Reserve University 20

Importance of innate immunity in HIV infection 22

Presented by Jay A. Levy, M.D., University of California at San Francisco 22

Progress with structured treatment interruptions and IL-2 26

Presented by Kendall Smith, M.D., Weill Medical College of Cornell University 26

Patterns of immunodominance in CTL responses directed against HIV-1 and the potential role of therapeutic immunization 30

Presented by Spyros Kalams, M.D., Partners AIDS Research Center,Massachusetts General Hospital and Harvard Medical School 30

In search of the Holy Grail 35

Presented by Michael Lederman, M.D., Case Western Reserve University/University Hospitals of Cleveland 35

Quantitating human T-cell responses to cancer vaccines 40

Presented by Kim Lyerly, M.D, Duke University 40

Design and analysis issues for studies of immune-based therapy 43

Presented by Victor DeGruttola, Sc.D. and Ronald Bosch, Ph.D., Harvard School of Public Health..... 43

FDA standards and perceptions for the development of IBTs for the treatment of HIV Disease.....47

Presented by William Schwieterman, M.D., U.S. Food and Drug Administration 47

Agenda *53*

Participant List……...…….57

Introduction

The use of highly active antiretroviral therapy (HAART) has led to decreased mortality and morbidity but not to HIV eradication. HAART regimes allow immune reconstitution but not normalization of the immune response. Therefore, additional therapeutic approaches need to be developed that include the use of immune based therapy. New approaches in immune-based therapy (IBT) must be designed to capture endpoints that will allow approval of these agents by regulatory agencies. The challenge is to identify, develop, and validate biomarkers that can serve as surrogate endpoints in evaluating the efficacy of immunologic agents in human immunodeficiency virus (HIV) disease.

Reflecting the heightened interest in this area of research is the recent focus on surrogate markers of IBT efficacy. The Antiviral Drugs Advisory Committee of the U.S. Food and Drug Administration's Center for Drug Evaluation and Research held a meeting on October 16, 2000, to discuss the use of surrogate markers of bioactivity in the early development of immunomodulatory agents for the treatment of patients with HIV. In conjunction with annual meeting of the International Congress on Antimicrobial Agents and Chemotherapy in Glasgow, Scotland, the Forum for Collaborative HIV Research held a meeting on "Immune-based Therapies and HIV Disease: European and Australian Perspectives" on October 26, 2000. The quest for such markers was also the topic of the workshop reviewed here, which was sponsored by the Forum on December 7–8, 2000, in Washington, DC. The workshop, entitled "Immune-Based Therapies and HIV Disease," centered on several discussion points:

- What are the appropriate indices to measure immune competence?
- What are the appropriate research designs and endpoints for trials evaluating IBTs?
- What can we learn from the research on IBTs in diseases other than HIV?
- What are the requirements of regulatory agencies for the approval of IBTs?

As background information for participants attending the Forum's workshop, a report *Immune-based therapy for HIV treatment and prevention: A review of clinical endpoints for trials of immunologic agents* was prepared to address issues related to these discussion questions.

The report reviewed study designs and endpoints used in phase II and phase III trials of 13 IBTs, eight of which have been approved by the U.S. Food and Drug Administration (FDA) for use in disease conditions other than HIV and five of which are under study for use in HIV-infected individuals but not yet approved for this indication. The background paper is available on the Forum's Website at www.hivforum.org.

The workshop participants included representatives from academia, government, advocacy groups, and pharmaceutical companies. The Forum encouraged a multidisciplinary approach, looking across a number of areas outside the area of HIV research for endpoints, outcomes, surrogates, and assessments that may apply in clinical trials of IBTs in HIV disease. When a repertoire of appropriate surrogate markers of bioactivity is available to correlate with increased resistance to infections, reduced disease progression, and survival, additional protocols for clinical trials of IBTs can be developed and implemented to rapidly and accurately assess how effectively these therapies prolong survival, protect against infection, and improve quality of life.

Recommendations for IBT Research

Research in the field of IBT for treating HIV disease continues to be hampered by several barriers. Perhaps the biggest barrier is that the highly effective treatment now available to people living with HIV/AIDS is a two-edged sword. Highly active antiretroviral therapy effectively suppresses viral loads, allowing the immune system to come back to a level that enables the infected individuals to live longer with a high quality of life. The challenge arises because these therapies make it difficult to show additional benefits attributable to adjunctive therapies.

The group was challenged to identify markers to serve as surrogates for clinical endpoints in trials of IBTs for HIV disease, suggest study designs for implementing studies of IBTs, and develop recommendations for agents to investigate. To achieve these goals, the Forum convened investigators from cancer and immune diseases to allow for cross-fertilization and generation of new areas of investigation. Other participants included representatives from the community of people living with HIV/AIDS, academia, government and the pharmaceutical companies to help bring IBT therapies from the laboratory bench to the bedside.

During the course of the workshop, breakout groups met to discuss questions posed by the Forum. Each breakout group subsequently presented its findings to the reassembled larger group. A review of the responses and ensuing discussions revealed that the recommendations generated fall into three main action items, which are discussed below in more detail:

- Action Item 1: Advance our understanding of immune pathogenesis of HIV disease.
- Action Item 2: Identify and validate appropriate markers and determine how they can be used in research of trials of immune-based therapies.
- Action Item 3: Design clinical trials of IBTs based on surrogate markers of bioactivity, keeping in mind some general considerations.

The recommendations advanced by the breakout groups and ideas suggested by the presenters and by participants during the question-and-answer sessions are discussed below within the framework of these three action items.

Action Item 1: Advance our current understanding of immune pathogenesis.

- We need appropriate animal studies with IBTs for the examination and evaluation of new cytokines such as IL7.
- Investigators should explore the immune pathogenesis of HIV using the current tools to define functional deficits in the immune system.
  (1) Apply tetramer ELISPOT and intracellular cytokine assays to evaluation defects in HIV specific immune responses;
  (2) Apply assays of T cell development to evaluate mechanism of CD4 depletion (e.g. TREC);

Action Item 2: Identify and validate appropriate markers of immune-based function

- Viral load, in absence of validated markers of immune competence, is one of the strongest candidates for serving as a surrogate for clinical endpoints. In addition, the development of viral resistance to antiviral drugs must be monitored. Possible related study endpoints include:
  (1) Time to suppression of viral load;
  (2) Further reductions in viral load beyond that achieved with HAART;
  (3) More durable response to HAART;

(4) Endpoints based on percentage of patients who remain virus-free after six months of antiviral withdrawal (analogous to clinical trials of agents for treating hepatitis C).

- Biomarkers used as surrogate endpoints must be useful, reproducible, suitable for use in multicenter environments, stable during freezing, shipment, and storage of biologic specimens, and, perhaps, appropriate for kit testing.
- The participants proposed several markers of bioactivity that could serve as immune-based surrogates for clinical endpoints:
  (1) CD4+ cell counts could be monitored to see if IBT can preserve CD4+ cell counts;
  (2) Immune responses to "vaccine" antigens could serve as in vivo measures of adaptive immune competence.
  (3) Related markers of bioactivity include time to onset of opportunistic infection, and the amount and duration of OI prophylaxis necessary to maintain patient's health.

Action Item 3: Design clinical trials of IBTs based on surrogate markers of bioactivity, keeping in mind some general considerations.

There is a need for validated surrogate markers of bioactivity for trials of HIV-specific agents based mainly on viral load response and for trials of therapies that seek to boost the immune system in a more general way.

Develop a definition of immunologic success and failure

- Develop a definition of immunologic success and failure to use across trials based upon current data to apply in preliminary evaluation of IBT activity. One of outcome of this workshop should be to task a working group to develop such a definition.

Design of trials around viral load markers.

- Structured treatment interruptions present an excellent opportunity for investigating IBTs, however, we lack basic knowledge regarding structured treatment interruptions. We need to identify a clinical indicator—a "trigger"—for reinitiating HAART, but we must always bear in mind patient safety especially in regard to STIs

and possible development of viral resistance. Studies conducted in the context of structured treatment interruptions can look for:

(1) No viral rebound;

(2) Viral rebound to a lower setpoint;

(3) Rank-based comparisons of viral relapse looking at whole curve to give more power to the analysis.

- Specific anti-HIV therapies that are of interest to the workshop participants include DNA vaccines, dendritic cell immunization, local administration of some materials that are toxic systemically, and leukopheresis for ex vivo treatment. The participants mentioned several agents—anti-CD40 ligand, monoclonals to B7, and anti CD-20 (rituximab) — that show activity in other diseases, but it was believed that there is inadequate rationale to try these drugs in an HIV setting.

Design of studies around general immune enhancement.

Measuring adaptive immune responses to immunization is a final, common-pathway "readout," reflecting the host's ability to maintain health in the face of opportunistic infections and other microbial challenges. Recommendations to measure general immune response include:

- Examine antibody levels as a measure of B-cell response or use delayed-type hypersensitivity testing to measure induction of CD4+ cell response.
- Evaluate a general immune-boosting therapy by studying patients who have relatively high CD4+ cell counts and are ART naï ve to see how long they can maintain their CD4+ cell counts in absence of ART but while receiving a general immune-enhancing treatment.
- Target individuals with lower CD4+ counts since they would experience more clinical events. These patients are more immunocompromised and it may be more difficult to stimulate their immune systems.
- Enroll patients who are "mildly failing" antiviral therapy and have viral loads of 500–1000 copies of HIV RNA. Try to stimulate immune response, perhaps with an immunogen, to see if the viral load will become undetectable.

General considerations for IBT clinical trials.

- Clinical trials should rely upon a multifaceted approach to determine bioactivity of IBT based upon virologic, immunologic, and clinical endpoints.
- Investigators should employ complementary trial designs (e.g., different patient populations, induction versus preservation effects, etc.). The different patient populations for such studies should include those who are naï ve to ART and/or IBT, those who are virally suppressed on ART, and "salvage" patients.
- When designing studies, keep in mind that how the registration studies are conducted affects how the product label will read ultimately. Consider whether the label should say, "for acute HIV infection" or "for treatment of HIV."
- Set up a central laboratory to handle testing for the individual study sites, similar to the system put in place for the ACTG trials. That way, testing could be done under reproducible conditions using standard operating procedures. Different centers specialize in different assays or a single center could do all assays under one roof.
- Store everything, including peripheral blood mononuclear cells, DNA, urine, and so forth. Develop a "blanket" consent form to allow future genetic testing of blood, cells, and DNA that may be used for future genetic testing. We must assure patients that their data will be protected now and into perpetuity.

Presentation Summaries

Clinical and laboratory outcomes in cancer immunotherapy

Presented by Nora Disis, M.D., University of Washington

Immune-based therapies have been exploited in several other fields, including cancer treatment. In fact, many researchers in the HIV field previously worked in other areas of research. To enhance the usefulness of this meeting, several experts in fields other than HIV have been invited to participate. One of them is Dr. Disis who discussed some clinical applications of cancer immunotherapies but focused primarily on biological surrogates and laboratory measurements.

Development of cancer immunotherapies. The application of cancer immunotherapies developed along parallel lines to the application of cancer chemotherapies. Early studies involved administering therapies to patients with advanced disease to try to induce tumor shrinkage by stimulating the immune system. Intuitively, such studies seem a bit ludicrous because patients with advanced disease have little intact immune response to stimulate. Now, we are working from the theoretical to the clinical. Studies have advanced over the past several years to more advanced, rational clinical applications to measure the kind of immune response that theoretically could be stimulated with a particular agent. The goal now is to develop a method for monitoring immune responses so that the generation of a particular immune response can be correlated with a clinical outcome.

How is immunotherapy applied in treatment of cancer?

- Vaccines are used in early or minimal disease states.
- Immunotherapy can be targeted to specific disease burdens.
- Reproducible immunologic measurements can be developed.
- The most robust techniques can be applied universally.
- Phase II studies can be designed to validate a marker as well as determine clinical effect.

The role of tumor-specific antigens. Human cancer is immunogenic. The biggest boon in cancer research has been the identification of more than 200 tumor antigens, among them MAGE 1,2,3; gp100; prostate specific antigen (PSA); prostatic acid phosphatase (PAP); mucin, carcinoembryonic antigen (CEA); HER-2; and MART. They have been defined by virtue of the fact that people with cancer mount immune responses against these antigens. Defining tumor antigens has led to the development of highly quantitative immune assays to try to discern which proteins are expressed in malignancy and get a handle on magnitude of immune responses in cancer patients.

It is not true that the immune systems of cancer patients are entirely compromised. When, for example, cancer patients are vaccinated against tetanus toxoid, they mount the same level of immunogenic response as normal blood donors do. One of the problems, at least with solid tumors, in terms of preexisting immune responses, is that these antibodies are present only at very low levels. Such factors limit the types of immune therapies that might be appropriate for these patient populations.

Treating according to disease burden. Another big issue in cancer immunotherapy is better application of the types of available therapies, whether it is adaptive immunotherapy, cytokine therapy, or cancer vaccines, or infusion of competent T-cells, etc., according to the level of disease burden. Analogous to measuring viral load in HIV patients to discern their disease status is staging of cancer. Studies can focus on patients with no evidence of disease, slight disease, localized disease, or advanced disease. The level of disease in stage III and IV patients can be reduced by using some combination of chemotherapy and surgery. Clinical trials are starting to look at patient populations with less advanced disease.

Then, IBT can be studied in a variety of ways: in patients with micrometastatic disease, localized disease, or undetectable disease. Among the questions that can be addressed are:

- Are there different types of IBTs that can be applied to reduced disease burden?
- Can treatment prevent or delay recurrence or metastasis?
- What level of antigen-specific T-cells is needed to prevent, eradicate, or limit disease?
- Which biologic surrogates are indicative of clinical outcomes?

Boosting immune response to tumor-specific antigens. An important facet of cancer research is the search for an agent to augment the immune response against tumor-specific antigens. Several groups have published data based on delayed-type hypersensitivity (DTH) testing and other means that show that cancer patients can be vaccinated and that they can mount an immune response against cancer antigens. (Peptide-based vaccines work in rodent models.) The issue is to circumvent immune systems so that the vaccines are not foreign proteins. Generation of cytotoxic T-lymphocytes (CTLs) and stimulation of cell-mediated immunity are the goals of cancer vaccines.

A vaccine study described. Dr. Disis and her colleagues are looking into a HER-2/neu peptide vaccine against the HER-2 oncogenic protein. HER-2/neu protein, a growth factor receptor, is

- overexpressed in 20% of adenocarcinomas
- composed of intracellular and extracellular components

- immunogenic in patients with HER-2/neu overexpressing breast, ovarian, and colon cancer, resulting in low levels of immunity with antibody responses and CTL precursor frequencies at the barely detectable range.

She discussed an arm of their study that investigated stimulation of immunity using putative T-helper epitopes, each 15—18 amino acids long, derived from the HER-2/neu protein. Within the natural sequence of the 15- to 18-mers were HLA-A2 epitopes specific for the proteins. It was a concept of using peptides to generate both helper and CD8+ CTL immunity within the same epitope with the hope of supplying CD4+ T-cell help and gathering evidence of long-lasting immunity that persisted after active immunization was over. This highlights another aspect of cancer vaccine work, which is not only trying to generate a certain magnitude of immunity but also discerning which T-helper arms are involved in the immune response.

The study population for phase I study of HER-2/neu peptide vaccine had the following characteristics:

- stage III/IV breast, ovarian, lung cancers
- HER2 antigen overexpressed
- already treated to maximal response using surgery, chemotherapy, hormones, or localized X-ray therapy
- must not be anergic as evidenced by DTH to recall antigens.

Twenty patients were enrolled. The question in phase II trials, looking at time to progression, would not be: "Does this vaccine correlate with time to progression?" but rather, "Does the *immune response* to HER-2/neu protein correlate with time to progression?" They looked at the T-cell response and precursor activity after peptide immunization, as well as HLA-2 binding motifs. Patients developed significant immune responses to two of the better-defined HLA-A2 epitopes to which they had been exposed. Some developed very significant precursor populations.

Building clinical tools from laboratory surrogates. The next step is to validate these responses measured in the laboratory with a therapeutic response. For a laboratory assay to be a useful clinical tool, it should

- require a minimal amount of clinical material

- have a short turnaround time
- be highly quantitative and reproducible
- be sensitive to a broad range of responses
- uses cryopreserved cells (especially important for T-cell assays)
- *measure a critical immune effector function.*

A critical immune effector function is a laboratory surrogate that correlates to the clinical response of interest. They are very important for phase II trial design in cancer vaccinology because these critical immune effector functions must correlate with protection or tumor shrinkage.

Table 1 lists some attributes of quantitative T-cell assays that are under consideration by the Immunologic Monitoring Consortium for possible application to many different antigen systems in cancer vaccinology (figure 1). Dr. Disis has been working on these assays with the overall goal for phase II trial design being the validation of these biologic surrogates.

Feature	Cytokine flow cytometry	ELIspot	MHC tetramer
Limit of detection	1:5000	1:1000–1:100000	1:10000–1:50000
Assay time	8 hr	24–48 hr	2 hr
Functional readout	Yes	Yes	No
Non-MHC restricted	Yes	Yes	No
Easily automated	Yes	No	Yes

Table 1. Comparison of quantitative T-cell assays being used in cancer studies and infectious disease research. Source: Holden Maeker, Ph.D., Becton Dickenson, San Diego, 2000.

The Consortium, which was established under a collaborative agreement with the National Institutes of Health, is monitoring these assays for clinical use; investigators in the cancer research community can apply to the consortium to have their clinical trials analyzed using some of the techniques. The Consortium is concentrating on the accuracy, precision, specificity, limits of detection, and robustness of these assays to turn these assays into clinical tools.

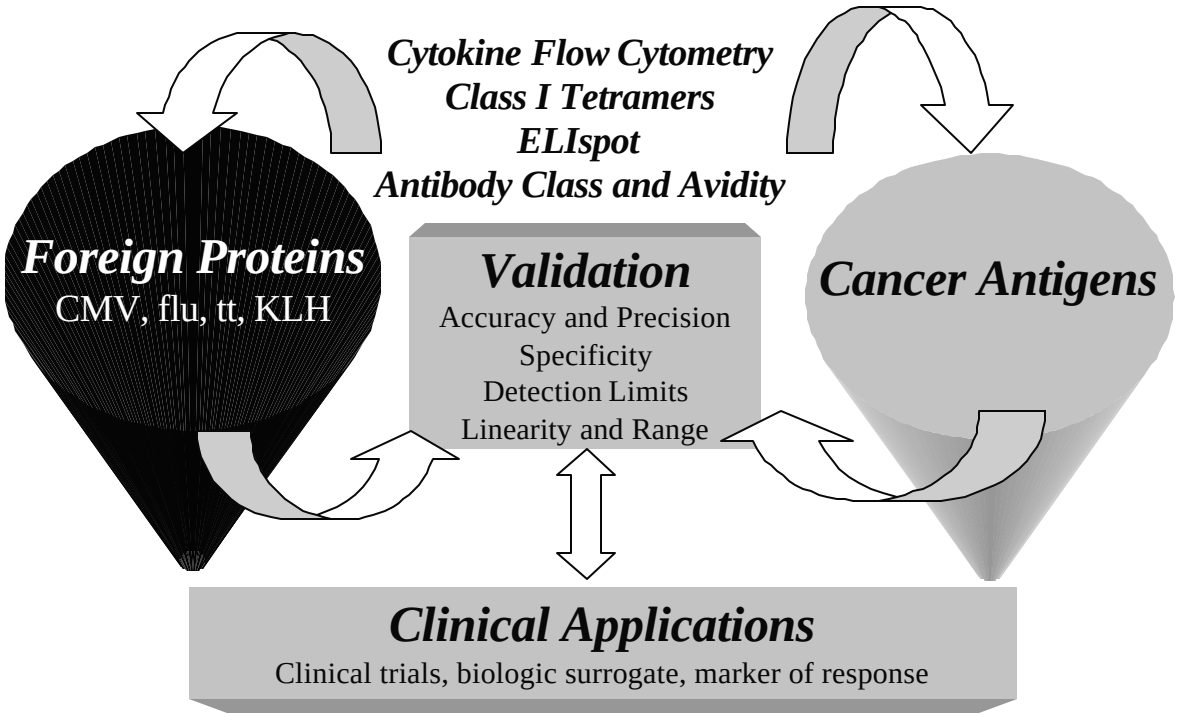

Figure 1. Activities of the Immunologic Monitoring Consortium. Source: Nora Disis, M.D., University of Washington. 2000.

HIV immune-based therapies: A research-clinician perspective

Presented by Roy Gulick, M.D., MPH, Cornell University

Dr. Gulick's presentation touched on some of the history of HIV therapy, current lines of research, and an overview of how IBT might fit into the arena of antiretroviral therapy (ART). Perhaps progress in treating HIV/AIDS can be measured against these poignant benchmarks:

- 1996: "It's the virus, stupid." This phrase came to be heard during the advent of viral load testing and triple drug treatment regimens that dramatically suppressed viral replication.
- 1998: "It's the immune system, stupid." Over the next few years the clinical realities of HAART regimens—toxic side effects and development of viral resistance—set in along with the realization that virus eradication was not just around the corner.

- 2000: “Stop calling me stupid! Can’t we just work together on this?” We have come a long way, but it is clear that we have miles to go. All approved therapies as of 2000 are antiviral treatments, but these are not enough.

A short history of antiretroviral therapy. In 1987, the first antiretroviral was approved by the FDA. With single-agent treatment, viral loads decreased but rebounded as the virus mutated

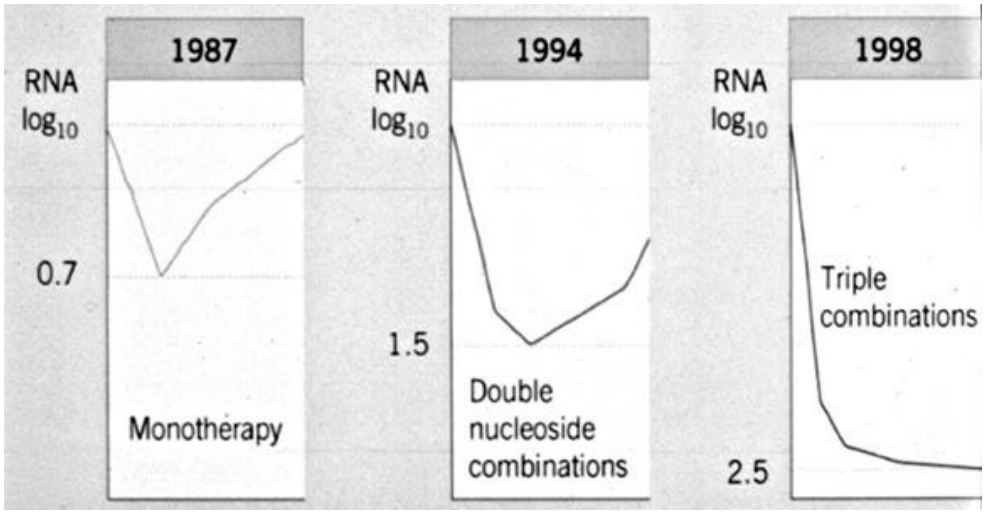

Figure 2. Evolution of changes in HIV RNA levels with different antiretroviral regimens. Diagram courtesy of Joseph Cervia, MD, Long Island Jewish Hospital,

and became resistant (figure 2). Double and triple combination therapies maintained viral suppression and delayed clinical progression. Clinical studies have documented that these regimens lead to virologic suppression and enhancement of CD4+ counts. Furthermore, these results correlate with the clinical picture (death rate). The death rate was cut by two-thirds between late 1995 and early 1996 when the use of protease inhibitors (PIs) became widespread.

These results have been replicated throughout the United States and the developed world. The goals of therapy today are

- To suppress HIV RNA (viral load) to as low a level as possible and for as long as possible
- To preserve or enhance immune function
- To delay clinical progression of HIV disease.

Currently 15 approved antiretroviral drugs are available. Dr. Gullick also discussed the treatment guidelines of the Department of Health and Human Services (www.hivatis.org/upguidaa.html). Clinical trials support the use of all the combinations listed in the guidelines. The arsenal of antiretrovirals includes one investigational and 15 approved drugs: 6 nucleoside reverse transcriptase inhibitors (NRTIs), 3 non-nucleoside reverse transcriptase inhibitors (NNRTIs), 1 investigational nucleotide reverse transcriptase inhibitor (tenofovir), available by an expanded access program, and 6 PIs. Clearly, ART reduces viral loads and leads to reconstitution of the immune system.

The evidence for immune reconstitution includes decreased mortality and decreased morbidity (fewer OIs), the ability to discontinue OI prophylaxis, the resolution of chronic OIs and other, "untreatable diseases" (e.g., Kaposi's sarcoma, cryptosporidiosis, CNS lymphoma). This improved immune status has been characterized using several parameters, namely expansion of CD4+ cell populations, improved lymph node structure, and enhanced immune function (e.g., DTH responses). Despite these positive clinical indicators, ART does fail patients. Clinical cohort studies show that rates of virologic failure range from 20% in a Swiss study of ART-naïve individuals to 63% in a Baltimore cohort (table 2).

Clinical cohort	***N***	**Virologic failure** (% Above limit of detection, time)
Amsterdam	271	40%, 48 wk
Cleveland	310	53%, 1 yr
Johns Hopkins	273	63%, 1 yr
Swiss	1517 experienced 1157 naïve	38%, 2 yr 20%, 2 yr
UCSF	337	50%, 48 wk

Table 2. Treatment failure: cohort studies. Source: Roy Gulick, M.D., MPH, Cornell University, 2000.

Dr. Gulick postulated several reasons for such failures:

- Insufficient virologic activity
- Incomplete immunologic recovery
- Adherence
- Side effects—acute and longer term
- Baseline resistance or cross-resistance
- Drug levels and drug interactions
- Tissue reservoir penetration
- Other unknown reasons.

Future of antiretroviral therapy. To offer further clinical benefits, treatment regimens continue to evolve. Antiretroviral regimens now include quadruple drug combinations. Pharmokinetic parameters have been enhanced so that higher, more stable plasma drug levels are achievable. New ARTs are being developed. And, people living with HIV now have access to simplified dosing regimens, combination pills, and new drug formulations.

Measures are being taken to reduce the toxicity of ART and minimize side effects. Such measures include a PI-sparing approach, the PI- and NNRTI-sparing approach, and structured treatment interruptions (STIs). These measures create a niche for IBT in HIV treatment, as they may be able to help maintain or enhance overall immune function, maintain or enhance HIV-specific immunity, increase the proportion of patients responding to ART therapy, and/or improve the durability of response to ART. Studies have shown that patients with the highest CD4+ cell counts do better on the same triple drug regimen than others with more advanced immunodeficiency. This is less true of viral load levels themselves.

Such results seem to indicate that a complementary approach—a combination of ART and IBTs—may be the most effective. Complementary use of IBTs may minimize ART-associated toxicity and improve quality of life for those living with HIV by supporting the immune system even with lower doses of ART or periodic treatment interruptions.

Structured treatment interruptions (STIs) in acute, suppressed HIV infection may serve to enhance HIV-specific immunity, providing not an immune-based therapy, but rather an immune-based strategy. In chronic, suppressed HIV infection, STIs may enhance immunity or provide a break from ART and its side effects. Furthermore, in chronic, nonsuppressed HIV infection, STIs

may allow resistant HIV strains to revert to wild-type virus and improve response to subsequent ART. These hypotheses remain to be proven.

The search for IBTs. The quest for IBTs has focused on identifying agents that are potent, effective, easy to administer, well tolerated, readily available, and inexpensive. Among the issues that must be considered for IBT clinical trials are the following:

- Develop IBT within the framework of the current standard of care using antiretroviral therapy (i.e., ARV +/- IBT).
- Consider a variety of study populations (naïve to ART and/or IBT, virally suppressed on antiretrovirals, "salvage" patients).
- Consider virologic, immunologic, and clinical endpoints.
- Evaluate possibility of conducting trials of IBTs during STIs.
- Assess benefits and risks.

Dr. Gulick observed that antiretroviral therapy in 2000 effectively suppresses viral load levels, enhancing the immune system and decreasing HIV-related morbidity and mortality. Despite these gains, however, 10% to 20% of subjects in clinical trials and up to 60% of patients experience treatment failure. The role of IBT in HIV therapy may be to improve the magnitude and durability of responses to ARV therapy, or IBT may allow discontinuation or interruption of ART to decrease toxic effects and improve quality of life for people living with HIV. Certainly, it will be challenging to design, conduct, and analyze studies with concomitant ART and IBT but that will be the likely setting for any clinical studies of IBT.

Defining the bar: A community perspective

Presented by Brenda Lein, Project Inform

One outcome of the recent FDA meeting on IBT was the identification of the need to "define the bar" for licensure of an IBT for treating HIV disease. The bar certainly should not be set higher than it is for antiretrovirals, nor should it be so low that it is not meeting clinical and community needs. It was this community perspective that Ms. Lein brought to the discussion of research goals for studies of IBTs for treatment of HIV disease.

The goals of HIV therapies. The primary goal of such studies should be to develop therapies that improve longevity and quality of life for people living with HIV/AIDS. The phrase, quality of life, includes minimizing reliance upon treatments that are toxic or involve complicated, burdensome dosing schedules that negatively affect day-to-day life. Another goal of IBT research should be to further clinical research by elucidating the pathogenesis of HIV disease, providing future directions for therapeutic research, and developing new research tools. Ultimately, these goals must meld into one overarching objective: to develop a cure for HIV/AIDS.

If IBTs are to be part of the solution, we need reliable and useful new tools to enhance management and monitoring of people living with HIV/AIDS. Such new tools, in the form of surrogate endpoints, will not only enhance research into IBT, but may expedite evaluations of efficacy of new compounds. Such endpoints, to be useful, must be reproducible, suitable for use in multicenter environments, stable during freezing/shipping/storing of biologic specimens, and, perhaps, appropriate for kit testing. We need to be able to validate these indices, as well.

Validity of surrogate markers. Ms. Lein described an assessment framework for evaluating surrogate endpoints that was developed by Mildvan, Landay, DeGruttola, Machado, and Kagan (Clin Infect Dis 1997;24:764-74):

- *Type 0—Measure of prognosis (natural history).*
- *Type 1—Measure of drug activity.* Type 1 marker that has commonly been used in anti-HIV drug evaluation and is seemingly sufficient for "accelerated licensing" applications. (Note: Mellor's data are Type 0 data).
- *Type 2—Measure of drug effect.* A type 2 indicator is almost interchangeable with a clinical endpoint. If such an indicator is *perfectly* validated (unlikely) to clinical outcome, demonstration of an effect on marker/indices would be a clinical endpoint.

Type 1 markers have generally been the basis for licensure of drugs for treating HIV disease, not type 2. In fact, type 0 data have supported the approval of drugs for HIV disease. When we define "the bar" for IBT approval and new assays in support thereof, we must keep in mind the precedents set during the approval of antiretrovirals and not become discouraged by the lack of type 2 markers. Which indicators served as the bases for approval of existing HIV treatments?

Basis for antiretroviral approvals. To give some perspective to these questions, Ms. Lein reviewed some of the history of drug approvals for HIV. In the era before viral load could be measured reliably:

- ddI was approved on basis of 24-week data, which showed a mean difference of 13 CD4+ cells compared to the control group (AZT). (In fact, both groups showed declines in CD4+ cell counts, but the decline was less pronounced in the ddI-treated group.) The main clinical trial was supported by a large safety data set from an expanded access program. (ACTG116b/117).
- ddC was approved on the basis of 28-week data in 111 people. The ddC-treated group demonstrated a mean increase of 21 CD4+ cells compared to the control group (AZT). This study was supported by a relatively small safety data set from expanded access program (ACTG 119). A second study did not support approval.
- 3TC was approved on the basis of 52-week data, which showed a mean difference of about 100 CD4+ cells in the 3TC-treated group compared to controls who received a combination of AZT+3TC versus an AZT-monotherapy control. Viral load data at week 24 (1.2 log versus 0.3 log) supported approval, though viral load was merely at type 0 level. Supported by a relatively large safety data set from an expanded access program.

The FDA then moved beyond looking merely at log decrease in viral load to a more important assessment: sustained response as indicated by percentage of subjects achieving undetectable HIV RNA.

- Indinavir (IDV) was licensed on the basis of a pivotal study, which consisted of 24-week data in 97 people. The IDV-containing triple-therapy regimen induced ~1.8 log reduction in viral load, AZT+3TC induced ~0.8 log reduction, and IDV alone ~1 log reduction. Percent of subjects achieving undetectable HIV RNA with AZT+3TC was 0, with IDV alone, 40, and for those on triple therapy, 85. CD4+ cell count increases were 25 on dual therapy, 120 on indinavir monotherapy, and 125 on triple therapy (Merck 035).
- Efavirenz (EFZ) was approved on the basis of a 24-week study in 450 people. About 75% of individuals receiving AZT+3TC+EFV had fewer than 400 copies HIV RNA

versus 65% on IDV+EFV versus 57% on AZT+3TC+IDV. No difference in CD4+ cell count was observed among the treatment arms; the overall increase was about 143.

- Lopinavir was approved on the basis of a 24-week study in 653 people. Seventy-nine percent of subjects receiving lopinavir attained HIV RNA <400 copies versus 70% of those on nelfinavir. Mean increase in CD4+ cell count was 154 versus 150 for the nelfinavir group.

Endpoints for immune-based therapies. When it comes to the landscape of IBT and research into immune indices research, there are more questions than answers. The issue of which immune indices are deemed ripe for development, to a large degree, must be separated from discussions of specific IBTs in development. It is not useful to discuss "IBT endpoints" because the endpoints will be different for interleukin 12 than they will be for IL-7 or HIV immunogen, for example. Each immune-based therapy has a different mechanism of action. The effects of each IBT will be manifested as different markers. To achieve breakthroughs in IBT treatment of HIV disease, we need collaborative product- and technology-specific discussions that include representatives of government, academia, industry, regulatory agencies, and the community. We need to know more about regulatory requirements and the decision-making process so that we can understand how these requirements do or do not promote answers to questions of import to community and science. It practically goes without saying, that resources are needed as well as cooperation and data sharing.

Success through collaboration. Great strides have been made in other areas, for example, therapeutic drug monitoring. Pharmacologists and industry worked collaboratively to develop these tools. Another example is the development of viral resistance testing—a cumbersome process back in the 1990s—for which a number of groups worked together using the ACTG Department of Defense consensus assay. Now genotypic and phenotypic assays are widely available and making therapeutic differences in people's lives. Ms. Lein also extended kudos to endocrinologists and virologists who developed a definition of lipodystrophy. As we now face similar challenges with IBT, perhaps we can apply the same models to develop a definition of immune deficiency in HIV based on laboratory abnormalities.

Next steps. Ms. Lein proposed the following as next steps:

1. Conduct small, product- or technology-specific meetings to determine what can be done short of clinical endpoints and identify alternatives to clinical endpoints. Decide what level of uncertainty is acceptable.
2. Examine obstacles to IBT development including lack of structure for bench-to-bed, pathogenesis-driven research, study section education, new mechanisms for R01-like research, incentives for industry, and so forth. Propose ways to surmount these obstacles.
3. Convene a selection committee to identify IBTs and assays ripe for development, develop timelines, and commit resources.
4. Select assay(s) for further development, refinement, and evaluation.
5. Draft development plans and timelines with "stop" and "go" parameters clearly defined.
6. Establish validation committee(s).

In closing, Ms. Lein issued a challenge: No one but the people in this room can and will define the "bar." The FDA is not mandating this definition; it expects an answer to come from you, as a collaborative group. We have more power in this discussion than what we are exhibiting or wielding; we must overcome the largest barrier: the lack of committed focus and follow-through from us as a group.

During the discussion subsequent to Ms. Lein's presentation, Dr. Schweiterman of FDA's Center for Biologics Evaluation and Research made three points. First, he echoed Ms. Lein's call for an open discussion of all participants including the FDA, academia, the pharmaceutical industry, and the community. The Agency, in his opinion, has an obligation to be frank about what the requirements are, for example, for the SILCAAT and ESPRIT trials. The FDA does not have the answers; there is a need for creative thought when it comes to the problems around demonstrating the efficacy of immune-based therapies. This is the forum for such creativity to be expressed.

Second, he challenged the group to compare the concepts of *bioactivity* and *efficacy* and to use the terms in deliberate and appropriate ways. This contrast is a useful concept for distinguishing the de facto chemical study from earlier kinds of studies that measure

cooperatively and complementarily the scientific hypothesis underlying phase I and phase II studies, which look at many endpoints. For phase III pivotal studies, one should have a firm idea of the characterization of that drug and know what to look at in terms of effect. It is such a daunting task to come up with a product development plan that the task should be broken down into chunks. He suggested that for this workshop that *bioactivity* is probably the most appropriate term.

Third, he emphasized that the outcome of this meeting should be solid results to push the academic side into action.

Another point of discussion centered on identifying target populations who may benefit from IBT therapy. One group may see IBT as a way to reduce use of ARTs that are causing undesirable side effects (e.g., lipodystrophy). A second group may be those who are failed by ART. An additional target population may be individuals who are biologic successes but immunologic failures (low viral load, but low CD4+ cell count). These people do better clinically if they are doing better immunologically.

Another participant suggested that the immune response itself has profound antiviral effects. We know how to measure antiviral effects and have the tools to do so. How do we design studies to look at the antiretroviral response?

Report from the Forum's "Immune-based Therapies and HIV Disease: European and Australian Perspectives" meeting, Scotland, October 26, 2000

Presented by Michael Lederman, MD, Case Western Reserve University

Dr. Lederman reviewed the discussions that occurred in the Forum meeting in Glasgow. Ron Mitsuyasu reviewed the agenda of the AIDS clinical trials dealing with IBTs, including the ones for interleukin-2, GM-CSF, interleukin-12, therapeutic vaccine trials, and some treatment withdrawal studies. Other discussions focused on vaccine candidates; the ones we have are simply not sufficiently active. Dr. Lederman highlighted several ongoing studies:

- For the QUEST study of ALVAC vaccine in patients identified during acute infection, patients will receive HAART alone, HAART + ALVAC, or HAART + ALVAC + Remune.

- David Cooper talked about the studies in Australia looking at fowlpox vector. The advantage of the particular vector he is using is that it can be grown to high titers and it has plenty of room to insert genes for adjuvant molecules. The ones they are looking at now are interleukin-12 and interferon-gamma.
- Much effort in Australia has been dedicated to the standardization of assays to use on cryopreserved clinical specimens.
- Mago Clerici talked about some of his group's studies of treatment interruptions in the setting of antiviral resistance and failures as a consequence of viral resistance. Those studies primarily rely upon laboratory endpoints.
- Another speaker from a research group in Barcelona highlighted their IL-2 trials.
- Another speaker discussed trials of hydroxyurea.
- Another spoke of a study demonstrating CD4+ enhancement in patients receiving cyclosporin A concurrently with the initiation of HAART. Results suggest that the administration of cyclosporin A enhances the redistribution or survival of cells after viral suppression is initiated.

What came out of the meeting were two approaches in terms of IBTs. The first approach involves using an immunologic agent or immunization, treatment, or strategy to enhance HIV-specific immunity. In this regard, the readouts are fairly straightforward and all we need is some imaginative ways of designing studies so as to optimize our ability to use those readouts. The second approach uses agents that enhance general immunologic competence. This is where we have failed. There are many of these very exciting molecules, but not one such agent has been approved or developed sufficiently to use as therapy.

What is really needed, according to Dr. Lederman, is a general measure of immunologic competence, not just the CD4+ cell count alone. We need to measure host's ability to maintain health in the face of OIs and other microbial challenges. Laboratory endpoints may help us for some agents, but ultimately, because so many different mechanisms of actions are involved with the myriad immunologic agents, we need a measure of immunologic competence that can be generalized for use across many different trials of many different agents.

Importance of innate immunity in HIV infection

Presented by Jay A. Levy, M.D., University of California at San Francisco

In discussions of HIV, one area that has not received sufficient attention is innate immunity. Most of what has been reported and written on immune response and immune testing has focused on the adaptive immune response. Researchers have been working on the problem of HIV infection for some 18 years now (primarily on aspects of the adaptive immune system) and have not yet solved it. We have not yet begun to approach what is critical from an immunologic perspective: namely the immune response that is present before HIV infection—the innate immune system. Two cell types appear important in the innate immune response in HIV infection: the interferon producing cell (IPC) and CD8+ cells.

Unexplored territory. In looking at CD8+ cell antiviral responses and immune function, Dr. Levy and his colleagues realized the importance of dendritic cells and their precursors one of which is the interferon-producing cell. These are part of the innate immune system. Table 3 summarizes his comparison of the innate and adaptive immune systems.

Characteristic	Innate immune system	Adaptive immune system
Quick response (min to days)	+	-
Delayed response (days to weeks)	-	+
Antigen-specific	-	+
Memory responses	-	+
Gene rearrangement	-	+
Conserved throughout evolution	++	+

Table 3. Comparison of innate and adaptive immune systems. Source: Jay Levy, M.D., University of California at San Francisco. 2000.

Very few HIV/AIDS investigators are looking at components of the innate system, including complement and the defensins. The innate immune system consists of cellular and soluble components:

- dendritic cells
- neutrophils
- macrophages
- NK cells
- gamma-delta T-cells
- cytokines (e.g., interferon)
- interferon-producing cells (IPCs)
- B-1 cells
- chemokines
- defensins
- complement
- lectin-binding proteins (collectins), among them, mannose-binding lectins; mutations in the expression of this innate ligand are associated with greater ability to be infected and lowered capability to resist infections. Mannose-binding lectins have been shown to bind to the envelope protein of HIV.
- fever (acute phase reactants, cathelicidins, pentraxins)

In brief, the innate system offers the earliest response to infection and may, in fact, ward off certain bacteria and other microbes that enter through mucosal lining cells. This protective system is active even in HIV-infected individuals. The innate immune system is located in mucosal lining cells of the skin—right where it is most needed. If the innate system fails, there is no time for the adaptive immune response to kick in. By taking a long perspective, one can see that were it not for the innate immune system, the adaptive immune system could never have evolved.

Role of interferon-producing cells. Dr. Levy emphasized the role of the interferon-producing cell (IPC), which is a precursor to the dendritic cell (DC2), the dendritic cell that enhances Th-2 type responses. The monocyte-derived type of dendritic cell enhances the Th-1 response. Interferon-producing cells play a role via interferon production in warding off initial HIV infection, subsequent opportunistic infections, and HIV-associated cancers.

In 1986 Frederick Siegal showed that type I interferons were very important in HIV infection. If an individual showed high interferon-alpha production in stimulated peripheral blood mononuclear cells and/or if the individual had a high CD4+ cell count, the clinical picture was fairly stable with little susceptibility to opportunistic infections. The people who lacked these parameters progress to AIDS.

The principal cell for making interferons are plasmacytoid T-cells. In the 1970's Lennert and colleagues described these cells in lymph nodes. Subsequent studies by Siegal and Yong-

Jun Liu have shown that this elusive cell was the IPC that makes thousands of units of interferon-alpha and -beta. The characteristics of the IPC can be summarized as follows:

- It is CD4+/lin-/CD11c-.
- It is a dendritic cell precursor (DC2).
- It has plasmacytoid morphology.
- It represents 0.2% to 0.9% of the peripheral blood and is also found in bone marrow.
- Loss of IPCs usually mirrors CD4+ cell reductions in HIV infection.
- It secretes interferon with exposure to herpes simplex virus and other pathogens.
- Its numbers are reduced in HIV infection.
- The cells are well represented in HIV long-term survivors; present in reduced numbers in progressors; and few, if any, can be found in AIDS patients (figure 3).

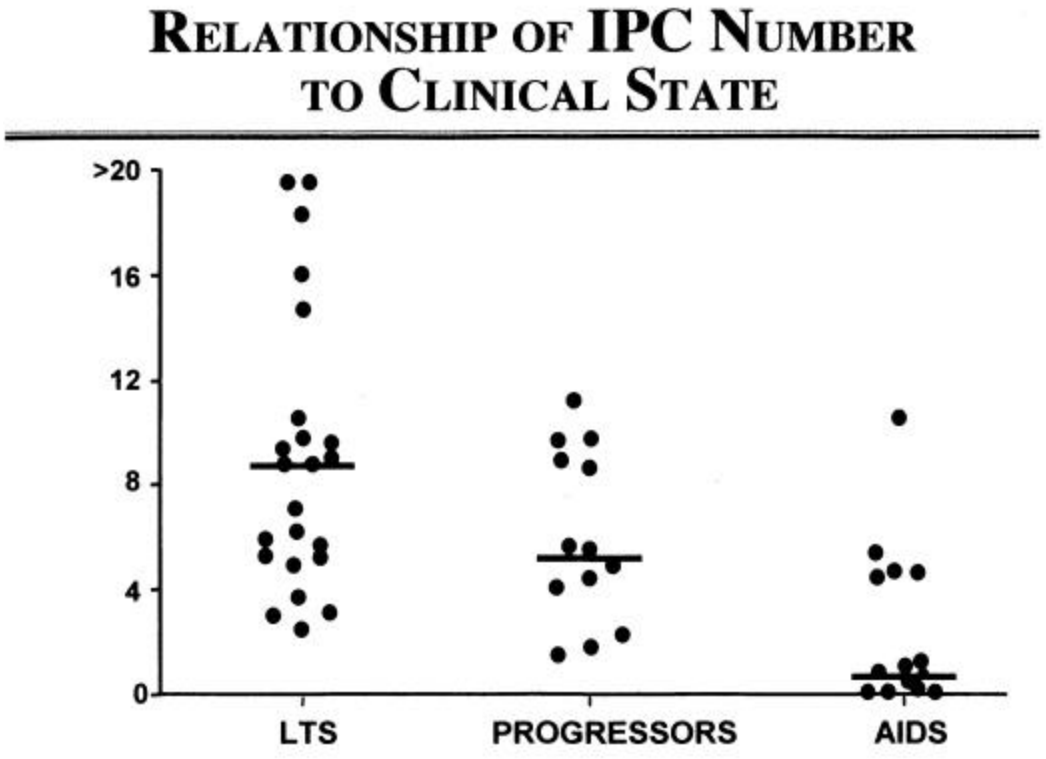

Figure 3. Relationship of IPC number to clinical state. Source: Jay Levy, M.D., University of California at San Francisco. 2000

The known roles of interferon-alpha seem to explain the parallels between IPCs and HIV disease. For example, it is know that interferon-alpha has antiviral, anti-tumor, and

immunomodulatory functions. Some of the latter are activation of NK cells, enhancement of MHC expression, and boosting of cellular responses. Recent studies show that interferon-alpha plays a role in directing the system toward the Th-1 response. Yet, Dr. Levy says, one of the most useful exercises in understanding the potential roles of different cells in this disease is studying the exceptions.

For example, in general, the CD4+ counts in Kaposi's sarcoma (KS) patients were not that low; they were well over 20% of total lymphocytes. Some patients had low IPCs, and they were the ones who had recurrent lesions. Other KS patients had normal levels of IPCs and no recurrent lesions. These findings suggested to Dr. Levy and his colleagues that IPCs were warding off the onset of new Kaposi's sarcoma lesions. The most dramatic findings were in a very few individuals who had very low CD4+ cell counts and sometimes high viral loads who had refused all prophylaxis and anti-retroviral therapy but remain healthy for 6 to 10 years. Their IPC counts were in the normal range. The IPCs, part of the innate immune system, are apparently able to ward off the types of infections that lead to AIDS. In addition, Siegal has shown that with initiation of HAART the IPC counts can be restored in patients who had lost most of these cells.

The noncytotoxic antiviral response of CD8+ cells. Dr. Levy's group believes that the CD8+ cell noncytotoxic antiviral response is also part of the innate immune system. When CD8+ cells are added in very low numbers to infected CD4+ cells, they suppress viral replication at transcription. There is no killing of the CD4+ cells, which continue to replicate and maintain function. He summarized this noncytotoxic activity as shown in the following:

- It is observed with activated CD8+CD28+ cells.
- It is active against HIV-1, HIV-2, and SIV.
- It is a nonlytic mechanism.
- It blocks transcription.
- It does not block activation or proliferation of CD4+ cells.
- It is mediated by a novel factor, called CD8+ antiviral factor, which, like cell contact, suppresses transcription.
- It is a very rapid response, initiated before antibody response in people who are acutely infected.

This activity exists in exposed uninfected individuals and does not last more than one year post-exposure. Thus this lack of memory for this response resembles a feature of the innate immune. Perhaps some approaches could be employed to enhance the characteristic responses associated with the innate immune system.

The two-edged sword. One participant pointed out that like the adaptive system, the innate system is a two-edged sword. NK-T cells can inhibit tumor immune surveillance through a mechanism that involves production of IL-13. By inhibiting that mechanism, one can prevent tumor recurrence. This is an example of how an innate response orchestrated through NK-T cells and cytokines can inhibit an adaptive immune response. Gamma-delta cells can also be increased in HIV infection, but they may be harmful because they eliminate cells that are beneficial to the host.

Next steps. Reports have appeared in the literature documenting that Flt-3 ligand, M-CSF, and G-CSF can bring back IPCs in individuals who formerly had reduced numbers of these cells. Dr. Levy's goal is to enhance mature IPCs from stem cells to provide immune therapy based on the innate immune system.

Progress with structured treatment interruptions and IL-2

Presented by Kendall Smith, M.D., Weill Medical College of Cornell University

Dr. Smith asked that the workshop participants keep in mind one central tenet: ***that the body's immune response itself is antiviral***. Therefore, to determine efficacy of IBTs, monitoring viral loads seems to be the most direct and rational choice for indicating bioactivity. To this end, we should consider administering IBTs while patient viral load is maximally suppressed with effective antivirals, then testing efficacy (IBTs bioactivity) by observing their effects on suppressing or controlling relapse of plasma viral load when the antiviral treatment is withdrawn.

The example of hepatitis C. This is exactly the approach being applied to the study of pegylated interferon and other antivirals in the treatment of hepatitis C. The investigators treat the patients for 48 weeks and then they withdraw the treatment. Six months later, they assess viral loads in the patients to determine the sustained viral response rate (percentage of patients who remained virus free 6 months after cessation of antiviral therapy). With pegylated interferon

and ribavirin, approximately half of patients remain virus free at the 6-month point. This is the type of test that investigators should be employing with HIV.

A model for T-cell responses and proliferation. Dr. Smith then went on to describe a study that his group conducted in 1999. The hypothesis was that a combination of antigen plus IL-2 should maximally stimulate immunity to HIV itself. The study was based upon a model for T-cell responses and proliferation. The model has been ongoing in his laboratory for some 20 years. When antigen is introduced with good antigen-presenting cells, CD4+ and CD8+ cells are initially quiescent. Once the CD4+ cells are stimulated via T-cell receptors, they change. The change becomes manifest in the CD4+ compartment through the production of large quantities of IL-2. With polyclonal activation, as many as 60% of the CD4+ T cells express the IL-2 gene and produce detectable IL2 within 4-6 hours of stimulation. They also express IL-2 receptors on their surfaces so they can respond to the IL-2 that they produce. The CD8+ compartment is different; these cells also produce IL-2 and express IL-2 receptors but only about one-third of them produce IL-2. Because CD-8+ cells are only half as numerous as CD4+ cells, some 80% of the elaborated IL-2 comes from CD4+ compartment in a normal immune reaction.

This finding truly supports the rationale of using IL-2 as a therapy because if you have anything that limits the production of IL-2, not only will there be deficiencies in the CD4 compartment, but also there will be deficiencies in the CD8+ compartment because CD8+ cells largely rely upon the CD4+ cells for help. The help comes in the form of IL-2. Tetramer studies indicate that there is a huge clonal expansion after an experimental viral infection. In the LCMV (mouse) model system, there is a 5-log increase in the number of CD8+ cells within a week. In an IL-2 knockout mouse, this proliferative expansion of the CD8+ is reduced by 90%. These knock-out mice have all the other cytokines, which are potential T-cell growth factors. Therefore, there is something very unique about IL-2, particularly for the expansion of CD8+ cells.

Interleukin-2 also activates NK cells, which differ from the T-cell compartment because they constitutively produce IL-2 receptors. If IL-2 is used therapeutically, not only is the adaptive immune system affected but also the innate, non-antigen-specific aspect of the immune system. Because 90% of the NK cells only express the beta-gamma dimer of the IL2 receptor and lack the alpha chain, very high doses of IL-2 are necessary to activate huge numbers of NK cells. Therefore only high-dose IL-2 administration leads to the cascade effect of

proinflammatory cytokine production, which is described as a "cytokine storm" with all its adverse side effects.

Exploiting immune responses to HIV re-exposure via STIs. Dr. Smith's group initiated a study at Cornell to address two questions:

- Can discontinuing HAART promote HIV-specific immunity?
- Can IL-2 treatment augment HIV immunity?

To answer these questions, they treated HIV patients with low-dose IL-2 therapy in daily, continuous fashion to saturate the high-affinity IL-2 receptors. On average, the patients had been HIV+ for 2 years. They started in January 1999 with the idea that perhaps HAART could be discontinued, and, if the virus returned, it would serve as an antigen source, giving specificity to the resultant immune reaction. The daily, continuous infusion of IL-2 would circumvent any potential deficiency of IL-2 and maximally expand the CD8+ compartment. They initiated a protocol to discontinue HAART in patients who had undetectable viral loads, normal numbers of CD4+ cells, and elevated levels of CD8+ and NK cells as a result of daily low dose IL-2 treatment. The IL-2 treatment continued throughout the study, even after the discontinuation of HAART. They monitored HIV levels and lymphocyte subsets after discontinuation of HAART, and the patients had received HAART for at least 3 months and IL-2 for at least 3 months. So far they have followed 16 patients through this protocol.

When they discontinued HAART, there was a rapid increase in viral load with a doubling time of about a day and a half, reaching peak concentrations at about the 2-week mark. Coincident with these rises in viral load were increases in CD8+ cell counts. The rises in CD8+ cells lagged behind increases in viral load, indicating that antigen was necessary to stimulate CD8+ cell production. There was a transient decrease in CD4+ cells, and no change in NK cell counts. After the CD8+ T cells increased, they found then that the virus concentration decreased subsequently over the next 2 weeks and settled at a plateau at about 10-fold below the peak value. Interestingly, the patients fell into two groups—one with rapid viral load declines (half-life of 1.5 days) and the other with slow viral load declines (half-life of 5 days). Also, the patients whose viral loads dropped most quickly after peak (greatest slopes) were the ones who achieved plateaus at the lowest levels of viremia, which were ~ 100-fold lower than the peak levels. By comparison, those subjects whose viral loads dropped more slowly did not go down as

far, only ~ 50% decrease. These findings correlated with the CD8+ cell counts; the patients with fast viral decay experienced the greatest increases in CD8+ cell counts.

They have followed 4 of these individuals for a year, and then they reinstituted HAART followed by another STI. They found:

- Viremic relapse in all subjects with a similar latent interval (time to detection, ~ 14 days)
- A similar rate of virus increase, i.e. t1/2 = 1.5 days.
- > Tenfold lower peak viral load in 3 of 4 subjects
- CD8+ cell count remained elevated and at a higher level than first STI
- No change in CD4+ or NK cell counts.

Dr. Smith concluded from this study of STIs and IL-2 that:

- Cessation of HAART leads to viral relapse in 100% of HIV-infected individuals in contrast to hepatitis C picture, where there is only a 50% relapse rate.
- In relapse, host CD8+ cells appear to control viremia, reducing the peak viral load ~ 10-fold.
- Immunity is possible in chronically infected individuals; the dogma of treating early with antiretrovirals may not be the best approach.

These results have just been published in the November/December 2000 issue of *HIV Clinical Trials*.

Dr. Smith believes that the future of immune stimulation is bright because of the availability of the new study endpoints of viral and lymphocyte dynamics that occur after cessation of therapy. Antigen-specific T-cells can also be monitored. Investigators can test different agents—therapeutic vaccines and cytokines, for example—while patients are maximally suppressed, then determine the effectiveness of the IBTs by a short-term interruption of antiviral therapy. In addition, this approach for immunotherapy can be extended to other infectious diseases and cancer.

A new study. Dr. Smith went on to describe a new randomized, controlled, 2-step phase II trial of an HIV vaccine and IL-2 that he is doing in collaboration with Frederick Siegal's group at St. Vincent's, David Warren at SUNY-Brooklyn and Israel Lowy at Mt Sinai in New York.

The study, which is powered to detect a difference in trough viral load of one-half log, consists of:

- Step 1—While patients receive HAART they are randomized to receive (1) a vaccine placebo, (2) an HIV vaccine (ALVAC vcp1452), (3) vaccine placebo + daily IL-2, or (4) an HIV vaccine plus daily IL-2 for 12 weeks.
- Step II—discontinuation of HAART for a minimum of 12 weeks, during which time viral load, lymphocyte concentrations, and numbers of HIV-specific cells will be monitored.

This clinical trial design has the advantage of requiring only a 24-week (6 month) commitment on the part of the volunteer, and includes a treatment interruption for all subjects. The alternative, of testing the efficacy of IBTs while continuing to administer HAART, and then following the progression to AIDS-defining illnesses, requires many more volunteers and several years of commitment on the part of each individual, as demonstrated by the recent Remune trial.

Patterns of immunodominance in CTL responses directed against HIV-1 and the potential role of therapeutic immunization

Presented by Spyros Kalams, M.D., Partners AIDS Research Center,Massachusetts General Hospital and Harvard Medical School

Now that we have the ability to suppress viremia with anit-retroviral medications, we can start thinking about boosting HIV-specific immunity. Some combination of the known therapeutic approaches will likely provide the answer. Dr. Kalams discussed some of his findings from the acute seroconverter cohort.

Models of viral latency. Other speakers addressed immune protection in chronic viral infections, and perhaps we can apply that model to HIV infection. For example, with herpesviruses, the initial infection is contained, and the virus remains latent in the host but is controlled by a healthy immune system.

The lymphocytic choriomeningitis virus (LCMV) model in mice has been helpful for dissecting out mechanisms of immune control. After infection, there is rapid induction of the CD8+ response. With tetramer and intracellular cytokine technology, it has been demonstrated

that up to 70% of CD8+ cells are specific for the virus within 8 days postinfection. One patient with acute HIV infection they looked at years ago had some 8% of his whole peripheral blood T-cell response dominated by three clones that were all specific for the same epitope (Data submitted for publication). In the case of HIV, however, the CD4+ helper response is destroyed around the same time. That may be one of the reasons that control of viremia is not attained after HIV infection For example:

- In CD4+ knock-out mice, CD8+ cells do not expand to quite the same levels after acute infection, they are not as functional, and they do not persist.
- Neutralizing antibodies may also play a role in ultimate control of LCMV infection. Such antibodies may also play a role in control of HIV viremia as evidenced by data from one of the acute seroconverters studied by Dr. Kalams's group during a treatment interruption.

Viral setpoints. What are some of the factors that may be influencing the viral setpoint? Some people who are nonprogressors or who are able to control their viremia may have been infected with an attenuated virus. Several host genetic factors have been described (CCR5 mutations, certain HLA alleles) that have been associated with long-term control of viremia. Finally, there are the variables associated with the host's immune response. It is the latter factor that may be augmented with specific therapies.

Cytotoxic T-lymphocytes probably control viremia by soluble factors as well as direct killing. Some of the studies in macaques have made great advances in the field, with the Letvin group doing CD8+ T cell depletions and showing dramatic increases in viremia. Dr. Kalams's group showed an association between helper responses and control of viremia in chronically infected subjects in a cross-sectional study. Helper response seems to be most powerful predictor of control of replication in subjects not already on HAART. In fact, people with high levels of help tend to have high levels of CTLs. There are some people with no help but with detectable levels of CTLs by precursor frequency, yet these people do not control viremia. It appears, then, that the helper response is the crux of the matter in HIV.

What happens during immune reconstitution with HAART?

- Increases in CD4+ counts, first the memory cells and then increases in the numbers of naï ve cells at a much slower rate.
- Recovery of proliferative responses to mitogens and recall antigens.
- Rare recovery of HIV-specific proliferative responses.
- Decreased incidence of OIs on HAART, sometimes allowing prophylaxis to be safely discontinued.

Dr. Kalams suggested some IBT approaches that have may offer clinical benefits: cytokines, adoptive cell transfers, therapeutic vaccination, treatment interruptions, and gene therapy.

Cytokines. With IL-2, certainly CD4+ numbers increase, although when HAART is discontinued even if IL-2 is continued, the viremia rebounds. The question is whether we somehow train these cells that are coming back to become HIV-specific? Studies are ongoing with IL-12, which appears to augment CTL activity and NK activity in vitro.

Adoptive cell transfer. Some of these studies are quite interesting. The idea early in this field was to expand HIV-specific CTL isolated from infected subjects and reinfuse them. Work by Koenig et al. showed little success as the virus was able to mutate and escape recognition by the infused clone. Phil Greenberg's group infused marked T-cells and showed that the infused cells did home to the lymph nodes, but there was no change in viral load. Another study in Oxford by Tan et al. showed that the infused cells underwent rapid apoptosis.

Otto Yang and Margo Roberts have been doing some work in the area of universal T-cell-receptor-modified cells. They took cytotoxic T-cells and genetically modified them to have a CD4+ molecule directly linked to the cytolytic machinery. In theory, these modified cells would be able to recognize HIV-infected cells. In vitro, they seemed to kill HIV-infected cells at the same rate as epitope-specific cytotoxic CD8+ T-cells. At least the early trials using large infusions of these cells have not had an anti-viral effect. The re-infusion of large numbers of antigen-specific cells has not enjoyed the success in HIV that, say, the reinfusion of CMV-specific cells has attained.

A very promising approach with dendritic cells has been applied by Dhodapkar and Bhardwaj. These cells can be grown up quite easily and do not require multiple infusions via subcutaneous injections. Augmented influenza-specific memory CTL responses have been

documented, as well as new helper responses. This is a nice approach to put to the test in clinical trials.

Several assays have been proposed to measure responses in chronically infected subjects, but which epitopes are the appropriate ones to target? Dr. Kalams has evaluated dominant CTL responses in HIV-infected subjects. He showed a slide revealing frequencies of five HLA-A2 epitopes. These epitopes are listed in the HIV molecular immunology knowledge database on the Web. Seventy percent of A2 people who are chronically infected tend to recognize a p17 epitope (p17/77-85, SLYNTVATL). There are varying degrees of recognition of this epitope. Either individual epitope responses could be targeted to fill in the repertoire of HIV-specific responses, or general immunotherapeutic regimen whether it is canarypox, DNA vaccine, etc. could be used. By tracking individual T-cell responses, investigators could discern how immunogenic the vaccines are.

Another notable finding is that it takes a long time and a great deal of exposure to develop a response to the p17/77-85 epitope. Although seventy percent of chronically infected individuals recognize this epitope. Dr. Kalams and colleagues found that acute seroconverters do not recognize this epitope, even after they develop other CTL responses. This fact would be important to keep in mind during vaccine development, because vaccines that do not have high-level antigen expression may not elicit these responses in sero-negative subjects. Also, the difference in the degrees of recognition of particular epitopes is a pitfall of using one tetramer as a surrogate for the entire immune response.

Therapeutic vaccines. Results to date have been difficult to interpret. gp160, for example, has been shown to be safe and to induce helper responses. No therapeutic benefit has been demonstrated in clinical trials, although these trials occurred during the pre-HAART era was and it may be difficult to show a benefit during ongoing viral replication.

Remune, the center of much controversy, is a safe product and a good inducer of helper responses. When one examines correlates of control of viremia, responses to HIV *gag* protein are strongly associated with control of replication. If one looks at acute seroconverters treated early, nearly all of them mount vigorous helper responses. Efficacy, however, has been difficult to evaluate in clinical trials where patients were immunized initially in the absence of anti-retroviral therapy. When subjects began therapy with anti-retroviral drugs, viral load measurements were not done frequently enough to determine whether the relapse rate of viremia was decreased in the

Remune group. Another difficulty has been that in a retrospective analysis of a large trial, therapy failures due to changes in anti-retroviral regimens because of drug side-effects were not distinguished from failures due to relapse of viremia (ACTG 816). Trials designed to augment HIV-specific helper responses in chronically infected individuals are still warranted.

Canarypox vectors have been shown to be safe in seronegative subjects, and have elicited CTL responses. Few studies have been done in HIV-positive subjects although several are ongoing now. Although these constructs produce "pseudovirions", it is not yet known how robust a helper response is generated.

Treatment interruption. The rationale behind treatment interruption is that it uses the subject's own virus for stimulation (also a disadvantage). Some evidence of efficacy has been shown in small numbers of subjects treated with HAART early after infection (before seroconversion). Data on acute seroconverters suggest that STIs can be a useful adjunct to other approaches. Little evidence exists to show efficacy in chronic infection. Of 8 individuals who interrupted therapy, 3 did not require reinitiation of therapy; they remained below 500 copies and have sustained low viral loads. The others rebounded within 50 days to viral loads above 100,000 without a repeat of their acute seroconversion syndrome. A second interruption was initiated in these 5 patients after they reached undetectable levels for at least 8 weeks, and 2 more had a sustained control of viremia below 500 copies/ml. Ongoing studies are attempting to determine the immune correlates of control of viremia in these individuals.

The future of IBTs in HIV. Ongoing ACTG clinical trials include the following:

- A5024—IL-2, ALVAC (vCP1452) or both
- A5049—IL-12 and vaccine with a B7 epitope
- A5051—HAART +/- recombinant IL-2 with long-term follow-up
- A5058s—Remune and ALVAC(vCP1452) to see if a combination of agents designed to induce helper responses and CTL responses is more effective than either one alone.
- A5068—STI and canarypox(vCP1452).

These trials will likely point to some promising agents. The ideal immunotherapeutic regimen in an HIV setting, according to Dr. Kalams, would suppress viremia, generate naï ve CD4+ cells that are "trained" to recognize HIV, and augment CTL responses.

In search of the Holy Grail

Presented by Michael Lederman, M.D., Case Western Reserve University/University Hospitals of Cleveland

Dr. Lederman opened with a question: Do we need IBTs for treating HIV disease? The answer is yes. Treatment with HAART does not restore immune function or the numbers of immune cells to normal. To bolster his position, he showed some data from ACTG 315, which provided some evidence of immune restoration as gauged by delayed-type hypersensitivity (figure 4). The study involved individuals with moderately advanced disease. After a year of a potent antiviral regimen, the median CD4+ cell count went from a mean of 180 or so to about 350. Despite this clear evidence of immune restoration, 60% of patients remained anergic; their functional abnormalities persisted. (Only about 10% of the general population is anergic.) CD4+ cell rises were even more modest during years 2 and 3 of HAART. Unfortunately, we do not yet know how much immune capability is necessary to need have a normal life expectancy. Nevertheless, the numbers of immune cells achieved in this study appeared to be sufficient to protect against OIs at least in the short term even though immune function is still compromised.

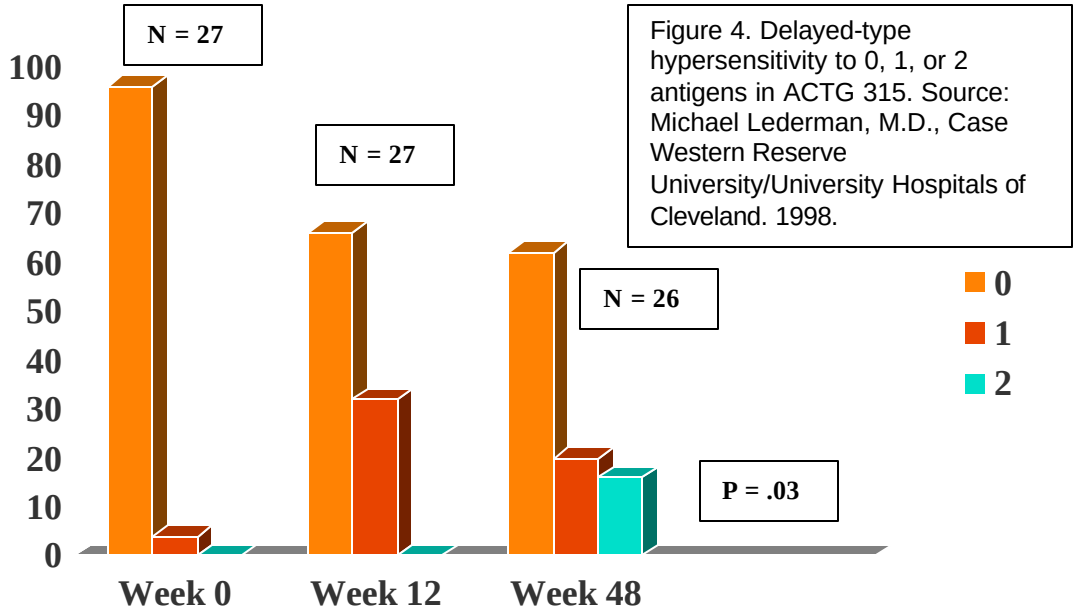

Figure 4. Delayed-type hypersensitivity to 0, 1, or 2 antigens in ACTG 315. Source: Michael Lederman, M.D., Case Western Reserve University/University Hospitals of Cleveland. 1998.

What about the patients who are virologic successes but immunologic failures; that is, with HAART they do not achieve increases in circulating CD4+ cell counts although their viral loads go to undetectable levels? This situation occurs in 5% to 20% of HAART virologic successes. This is a population at risk of succumbing to OIs. The need to enhance immune capability is real.

Role of IBT in HIV disease. These scenarios suggest two possible and distinct roles for IBT in HIV disease: to improve HIV-specific immune response and to enhance general immune competence. The latter role is the one that Dr. Lederman focused on in his presentation. As he sees it, developing agents to enhance immune competence presents a number of challenges:

- The need to demonstrate clinical benefit
- Paucity of surrogate markers to predict clinical benefit after IBTs
- The conundrum of IBTs
- The cost for development is prohibitive.

The conundrum of IBTs arises because they generally work best in the people who need them least—those who have relatively well-preserved immune function. Some, for example IL-2 may be most active when HIV replication is already suppressed via antiretroviral therapy. Furthermore, studying patients with suppressed HIV-1 replication and preserved immune function makes clinical endpoint trials costly and impracticable because of the need for a large number of study subjects.

Surrogates for clinical endpoints. So far, the only laboratory markers that have clearly been shown to predict clinical benefit in ART trials are plasma HIV RNA and circulating CD4+ T-cell counts. CD4+ cells counts are a reflection of general immune competence. The strengths of the CD4+ marker as a surrogate are:

- Circulating CD4+ cell counts predict outcome in natural history studies.
- CD4+ cell counts rise with ART, and the increase predicts clinical course.
- CD4+ cell counts can be used as a guide for OI prophylaxis to help determine when it should be instituted and when it can safely be withdrawn.

So, what happens to CD4+ cells when an agent such as IL-2 is administered? Dr. Lederman presented some data from the ANRS 048 study to help answer this question. This study, among others, has shown that with intermittent administration of IL-2 that CD4+ cells

counts rise significantly compared to controls who do not receive IL-2. The increases are polyclonal, and the cells are apparently functional ex vivo.

Is a simple increase in the numbers of circulating CD4 cells likely to provide clinical benefit? In the first phase of HAART (the first 8-12 weeks), when the cells are likely being redistributed, the increase in CD4+ cells is temporally associated with clinical benefit. At the same time, viral replication is also decreased, and if viral replication itself is associated with immune competence, this may confound analyses of such data. At least the data are suggesting that just making enough of these cells available in the peripheral circulation is conferring a clinical benefit.

The ESPRIT and SILCAAT trials are addressing this very question of whether IL-2 by increasing the number of CD4+ cells in the peripheral circulation improves outcomes in HIV infection. Dr. Lederman then proposed a hypothetical: Suppose these trials show that IL-2 confers clinical benefits for people with HIV disease and that this benefit is entirely attributable to increased CD4+ cells in circulation. Would this information help us develop other IBTs, say, IL-12, IL-15, or GM-CSF? The answer obviously is no, this information would not help us at all because all these agents work all by different mechanisms.

A final common pathway readout. Even though they are useful for prospective and retrospective studies and they are great tools for exploring pathogenesis, in vitro laboratory markers really have limited utility for developing new IBTs. What is needed is a final, common pathway readout. Presently we use clinical endpoint trials as a final common pathway readout.

Participants are randomized to treatment assignment, and then they are observed for OIs, other AIDS-defining complications, or death. An OI really is a final common pathway of immune failure. An OI can be seen as the clinical consequence of an inadequate immune response to microbial challenge. The immune response to OI antigens is a reflection of the adaptive immune response to OI antigens. Merely counting OIs as a clinical readout of immune competence is a bad idea for a couple of reasons, according to Dr. Lederman. For one, there is no control over the timing or the population frequency of challenge. Because only a fraction of the study population will contribute meaningful endpoints to the statistical analysis, the sample size requirement is enormous. Even worse, reaching endpoints means that the patients in the study are at risk of morbidity and death.

Another approach is to examine immune responses to "vaccine" antigens as an in vivo measure of adaptive immune competence. Table 4 compares the adaptive immune response to an OI to the response to "vaccine" antigens. Immunization is a form of microbial challenge. In HIV disease, response to immunization is associated with disease stage (Schooley, ACTG 209/214) and is predictive of subsequent disease course (Redfield, N Engl J Med 95). Furthermore, enhanced immune response to recall and OI antigens is associated with better control of HIV replication and decreased immune activation (Valdez, AIDS 2000).

Immune response to opportunistic infection	**Immune response to "vaccine" antigen**
Failure of adaptive immune response to antigen challenge	Measure adaptive immune response to antigenic challenge
May be CD4+, CD8+, B-cell dependent	May be CD4+, CD8+, B cell dependent (responses measured by assays of CD4+, CD8+, B-cell function)
May require both afferent and efferent arms of immune system	Requires both afferent and efferent arms of the immune system

Table 4. Comparison of adaptive immune responses to opportunistic infections and "vaccine antigens." Source: Michael Lederman, M.D., Case Western University/University Hospitals of Cleveland. 2000.

By using immunization as a microbial challenge, one can use

- Complex or simple antigens to measure CD4+ T-cell or B-cell responses
- Methods of intracellular gene expression to induce CD8+ T-cell responses employing DNA or RNA, or virus or viral vectors
- Tests of both the afferent limb and effector limbs of the immune response
- Serum antibody levels to measure B-cell responses
- Lymphocyte proliferation, cytokine expression or delayed-type hypersensitivity to measure induction of CD4+ T-cell responses
- CTL assays, Elispot, and tetramer staining to measure induction of CD8+ cell responses
- Intracellular cytokine expression to provide detailed, cell-specific characterization of responses.

Using response to immunization as a final common pathway for testing adaptive immune responses to microbial challenge offers a number of advantages. Patients can be randomized to treatment. Endpoints are responses to immunization (discrete or continuous responses), and, as such, are measures of adaptive immune competence. Endpoint timing (vaccination challenges) and immunologic monitoring are determined by trial design and not by chance. *All* trial participants contribute to the number of endpoints. This strategy avoids putting trial participants at risk for morbidity or mortality of clinical endpoints based on OIs. And, finally, trials can be streamlined based upon a predictable ending date.

What is needed to put this concept into practice? To put this strategy to best use, we need consensus methods and reagents for immunization. We need good recall antigens and neoantigens. We need antigens and vectors to test B cell responses as well as CD4+ and CD8+ T-cell responses. We need consensus methods for measuring immune responses to microbial challenge.

To validate immunization responses as predictors of OI protection, we must conduct cross-sectional studies, and we need to measure responses to immunization in individuals who have had clinical responses to antiviral therapies. Likewise, we must try to examine immunization responses as a consequence of IBTs.

What are the barriers to this strategy? We lack the tools to elucidate the role of the nonadaptive "intrinsic" immune defenses in host defenses against HIV. But, given the current paradigm, will we be able to develop IBTs for treatment of HIV disease? The answer given by Dr. Lederman was, "probably not." Despite the plethora of exciting and active molecules that have been identified and produced in the last 10–15 years, not one immune-enhancing agent has been approved for use in HIV infection. We must explore different approaches to the development of safe clinically useful approaches to explore immune function in HIV disease.

The workshop participants generally were very supportive of this approach. Using antigens from opportunistic pathogens may have therapeutic implications as well. Monitoring in vivo immune competence is certainly a powerful, surefooted approach. Among the issues raised by the assembled group were

- From the perspective of immune restoration, there is a real and different response to HIV than there is to *Mycobacterium avium*, cytomegalovirus, or other opportunistic pathogens.

- Once a treatment group has been exposed to a set of neoantigens, then the group will no longer be naï ve to those antigens and any further testing would require a whole new set of "neoantigens."
- This method may still require validation against clinical endpoints

Quantitating human T-cell responses to cancer vaccines

Presented by Kim Lyerly, M.D, Duke University

Parallels have become more obvious between the cancer and HIV fields. Studies of cancer therapeutics also rely on clinical endpoints, and there are surrogate markers of those clinical benefits. Clinical endpoints are survival (may take years, large numbers of patients), tumor response/shrinkage (not always lined to survival benefit), delay in progression/recurrence (not always linked to survival benefit), quality of life (based not on survival benefit, but rather enhancement of life quality—a worthwhile goal). Some surrogate markers (reduction of tumor burden) do not necessarily correlate with clinical benefit. For example, if a patient has bilateral pulmonary metastases, removal of one lung reduces tumor burden by 50% but does not confer clinical benefit.

How cancer vaccines are tested for safety and feasibility. The vaccine studies that Dr. Lyerly's group has focused on very early clinical trials of novel therapeutic agents in cancer, allowing the introduction of relatively unproven agents in people with near-terminal disease. This presents special challenges because these people are not likely to mount robust responses to vaccines in light of their immunocompromised state. The main objectives of such studies, as mandated by the FDA, are safety and feasibility. Although these studies are powered for those goals, they do also look at secondary endpoints for hints of biological activity, namely

- tumor response/shrinkage
- tumor marker response—for example, decline or rise in prostate specific antigen or the rate of said rise or fall—are not 100% correlated with clinical benefit
- other clinical markers—induction of vitiligo, for example, which is an indicator of immune response indicates melanoma vaccine efficacy
- delay in time to progression or delay in time to recurrence

- increase in overall survival
- adverse effects.

Getting to the true response rate. The problem collapses to one of statistics. In oncology trials, the goal is to explain clinical results and why oftentimes results reported by one group cannot cannot be replicated by other groups. Dr. Lyerly and his colleagues use a computer model for a given cancer therapeutic agent into which they enter the variables of interest, run the experiments electronically, and show the data. Using the model, they can enter a response rate of 5%, for example, a level that would not be of much interest clinically in the oncology field. With a sample size of 10—a very common number in safety/feasibility studies—they apply a random number generator using the model and the first trial shows a response rate of 10%. The next trial shows no responders, and so forth. What happens is that the data showing responders are published and the negative data are not. The reality is that we cannot continue to do this because these data sets are equivalent. One strategy to overcome this dilemma is to increase sample size, thereby reducing the confidence intervals and yielding a more reliable descriptor of the true response rate. As cancer investigators seek resources to do such studies, they get powering sufficient for the safety/feasibility issues for the sample size of 10–15 patients, but if these samples sizes are extended to a more robust level, the studies can give a much truer picture.

What is the point of immunologic monitoring?

- It may be predictive of clinical benefit.
- It is an objective measure of biologic activity that may allow investigators to prioritize the myriad available approaches. (One could test a vaccine that resulted in a broad, strong immune response before testing similar vaccines that generated only weak immune responses.)
- It underpins an understanding of the mechanism of action for optimization.

Anti-myeloma vaccine. Dr. Lyerly reported on progress made in his laboratory in collaboration with Larry Kwak. They immunized in vitro peripheral blood mononuclear cells from patients with malignant myeloma and were able to generate an idiotype (Id)-specific T-cell response (Li et al. Blood 2000;96(8):2828-33). The CD8+ response was lytic for the autologous

malignant myeloma cells of the patients. First example of a T-cell response that was lytic for the malignant plasma cells.

Dendritic cell (DC) vaccines are more complicated than people realize. Consider these characteristics of DCs:

- Maturation of DCs. They derive from a variety of lineages and achieve a phenotype that allows them to be effective antigen loaders. They are highly phagocytic. CD83 antigen is a marker of maturation; treatment with TNF-alpha causes the DCs to mature and produce the CD83 marker. If one matures DCs by adding TNF-alpha and then loads them with antigen, the T-cell response is less than if they were first loaded with antigen and then matured with TNF-alpha.
- DCs matured with TNF-alpha will produce large amounts of IL-12 when stimulated with combinations of cytokines; when treated with individual cytokines, levels of IL-12 were much lower.
- Route of administration is very important with DC-based vaccines. In studies with immature In^{111-}labeled DCs administered intravenously, Dr. Lyerly observed early trafficking to the lungs with radiography but no trafficking at all to areas of metastatic tumor. Later, they moved to the spleen and liver. The expectation was that DCs could be injected into the skin and they would migrate to the draining lymph nodes, but the reality was that they migrated only slowly and rarely. Fewer than 1% of intradermally injected DCs migrate.

Flt-3 ligand. In a recent article in the *Journal of Clinical Oncology*, it was reported that in metastatic colon cancer patients were treated with Flt-3 ligand as a subcutaneous injection every day for a week. The result was a very significant rise in their monocyte populations that drops off when the Flt-3 ligand is withdrawn. There is a population of myeloid-type DCs that is characterized by CD11c positivity and low levels of CD123. There is a rapid and significant expansion of this subset with Flt-3 administration. It may be these cells that provide important immunologic function, perhaps an antiviral function. Flt-3 ligand may be a well-tolerated agent that can lead to high circulating levels of these cells.

Functional analyses. Insofar as functional analyses are concerned, many in the cancer vaccine field now consider Elispot analysis to be the gold standard. It is also very important to do

direct assessment of peripheral blood mononuclear cell (PBMC) immune function because it minimizes in vitro artifact. It is shocking to many that if a peptide is used to expand the PBMC population that after 12 days, the majority of expanded T-cells are now peptide specific. If after 12-18 days' stimulation, the number of tetramer-positive T-cells is less than a fraction of a percent, that it probably not a robust immune response although others may say otherwise.

Dr. Lyerly's group often relies upon intracellular cytokine staining of antigen-specific T-cells. They look at class I and class II responses. When Dr. Lyerly's group examined T-cell responses to tetanus vaccine as a recall antigen using these assays, they found an unexpected CD8+ response in addition to CD4+ response. With multiple immunizations, they found a plateau of the CD4+ response, but they found very significant levels of CD8+ responses occurring 2-3 weeks following tetanus immunization. In fact, 1.5% of the circulating CD8+ cells were responding to the tetanus toxoid.

Defining immune function. Dr. Lyerly summed up by saying that we now have the tools to thoughtfully and carefully do the analyses that will have important roles in defining the immune function in cancer patients. This is an amazing opportunity to converge technologies and use shared antigens and reagents to understand T-cell biology in cancer, HIV disease, and in normal individuals.

Design and analysis issues for studies of immune-based therapy

Presented by Victor DeGruttola, Sc.D. and Ronald Bosch, Ph.D., Harvard School of Public Health

How can we improve efficiency of IBT studies? How can we identify patterns of immunologic markers that predict viral control or clinical outcomes? These were the questions addressed by Dr. DeGruttola.

Selecting an appropriate trial design. Trial design is determined by the scientific question and the population under study. For example, if the question is: "For treatment-naïve patients, could IBT used with HAART to reduce the virologic setpoint?" an appropriate study may be to follow naïve patients for 3 months before treatment to establish their viral setpoint. Initiate HAART until viral suppression is achieved and maintained for some period of time, and

then randomize the patients to IBT or placebo. Use as an endpoint whether the virologic marker level following an STI is above or below the pretreatment setpoint. Statistical analysis in this case would be fairly straightforward.

For other trials, however, statistical innovation can improve efficiency. For treatment-experienced patients, the question may be whether an IBT can slow virologic rebound following HAART. Once again, patients can be randomized to an IBT or placebo after virologic suppression is achieved. In this case, though, rather than just looking at viral setpoint or time to viral rebound, by using nonparametric, rank-based comparisons of the entire rebound curves, the investigator can make use of *all* data that can be collected following the STI.

Innovative statistical approaches. The presenters pointed out ways in which innovative statistical methods can play a role in clinical trials. On such method relies upon efficient comparisons at each time point. Virologic curves tend to be complex, peaking and then falling off. For this type of analysis, compare *each* individual from one group to *each* person from the other group at *each* time point. This way, all information is used by performing a rank-based analysis on summary scores. The advantages of this approach are:

- It allows use of all data, even if the patients were unable to have an STI because viral load was never fully suppressed.
- Analyses are unaffected by missing observations so long as they occur randomly. If missing data points occur nonrandomly (if the patient is not feeling well and so misses several appointments), additional statistical assumptions would be necessary to accommodate information from such subjects.
- This approach allows a natural way to accommodate information from studies in which subjects restart therapy when needed. .

Trial design can be made more efficient using other statistical innovations, such as factorial designs using simultaneous randomizations to two or more different therapies. For example, the ACTG 5058 study is looking simultaneously at the Remune HIV immunogen and ALVAC vaccine.

Another approach is the use of stratifying factors/entry criteria to identify homogeneous patient groups for discerning early indicators of benefit. The more homogeneous the patient group within a stratum, the greater the ability to detect subtle, early effects of an IBT. This

approach can improve efficiency in early proof-of-concept studies but is not applicable to phase III efficacy trials in which the goal is to have a very diverse patient population. One question is how can a homogeneous patient population be identified? The investigator must establish a measure of similarity for patients according to laboratory markers of interest (e.g., multiple immunologic markers). Once scores are assigned across a broad range of immunologic markers, they can serve not only as stratification factors but also as endpoints.

The problem of high-dimensional data. To identify patterns of immunologic markers investigators in the HIV field have been collecting information on a broad range of immunologic markers on a relatively small patient population, giving rise to a problem known in statistics as *high-dimensional data*. We need to accommodate the increasing number of markers. With many immunologic markers to be measured, it is generally not efficient to investigate the prognostic value of each marker individually particularly when the patient population is small. Many approaches can be applied to high-dimensional data; investigators can cluster the data to determine clusters of patients with similar marker values across the full spectrum of measurements. This approach works when certain markers tend to correlate with other markers. Then we can ask, "Does membership to a cluster predict some outcome of interest, for example, virologic or immunologic response?"

One way to investigate this question is to use prediction-based classification via a recursive partitioning splitting algorithm. The basic idea is to start with many observations and an outcome of interest. By employing a splitting algorithm, the association of certain observations with certain outcomes becomes apparent. This type of analysis, when applied to data on viral resistance, reveals that membership in these clusters, as defined by genotype, is highly predictive of the phenotype (resistance). Those relationships can then be further investigated.

How the clustering approach can be used. Dr. Bosch then presented some analyses using this type of approach for immunologic data developed by the AIDS Clinical Trials Group. His group is doing some exploratory studies to look for interesting patterns and perhaps to identify subsets of patients or specimens that may be ripe for further investigation.

As an example, he discussed ACTG 5026S, an immunology substudy of ACTG 5025. There were 38 evaluable subjects who had data at baseline and at follow-up. The full study had involved individuals who had <200 copies of HIV RNA copies/mL. This was a rollover of

ACTG 343, in which subjects were treated with indinavir+3TC+ZDV. The patients were then randomized in ACTG 5025 to one of three arms: (1) continue the triple antiretroviral therapy, (2) switch from NRTI component (3TC+ZDV) to ddI +d4T, or (3) continue ART regimen and add hydroxyurea (HU).

Applying a clustering approach to the resultant high-dimensional data, Dr. Bosch's group clustered subjects based on changes in 10 immunologic vectors from baseline to follow-up (mean of weeks 16 and 24). These methods can also be applied to 100s or 1000s of dimensions. The clustering method relies upon several iterative steps:

- Each subject begins as his or her own cluster (38 clusters initially).
- The two closest clusters are combined using a high-dimensional distance measure based upon a Euclidean distance in a 10-dimensional space (37 clusters result).
- Continue combining clusters according to their closeness in Euclidean space; each iteration reduces the number of clusters by one.
- Repeat process until two clusters remain.

They then generated box plots of flow cytometry T-cell subset data by clusters. Two clusters fell out of the analysis: cluster 1 (13% had received HU) and cluster 2 (53% had received HU). Cluster 1 had significantly higher numbers of CD4+, CD8+, naï ve CD4+ subset, and naï ve CD8+ subset. In cluster 1, 13% experienced virologic failure versus 40% in the cluster 2, although this difference was not statistically significant probably due to small sample size (P = .12 by Fisher's exact test). It is important to remember that clustering can be driven by many different things. In this example, all these data could be explained by decreased T-cells generally.

The power of these approaches. These results show that high-dimensional statistical techniques can reveal patterns in immunologic responses useful for exploratory data analysis and future research. The real power of this method is its efficiency, as demonstrated with studies involving many more vectors because it allow exploration of many relationships simultaneously. In summary, by applying this technique, investigators seeking IBTs that will be effective in HIV disease can

- Answer questions more quickly and with fewer patients
- Identify subtle patterns that may be indication of clinical benefit

- Guide selection of specimens and assays for further, in-depth analyses
- See what patterns appear—separate and apart from outcome predictors
- Identify the major contributors—which variables have no effect and which ones may have an effect—to clustering and outcome predictors
- Analyze many types of data, including categorical or classification data.

FDA standards and perceptions for the development of IBTs for the treatment of HIV Disease

Presented by William Schwieterman, M.D., U.S. Food and Drug Administration

Dr. Schwieterman opened by identifying some challenges and issues facing investigators who engage in researching IBTs for preventing and treating HIV disease. Among these challenges are postmarketing adverse reactions, limitations of current ART, lack of agency clarity about requirements, the compelling need for new therapies, and the absence of validated surrogates for IBTs. Myriad biomarkers are available for study, but we lack standards and even normal values for IBT surrogate markers, nor is there an impetus for collecting data on these markers. Finally, we face the enormous difficulty of validating surrogates, and even when such surrogates can be validated, we cannot infer validation of surrogates across classes of therapies because of the different mechanisms of action.

Dr. Schwieterman's remarks centered on 10 questions, which are covered in the sections that follow.

#1. What is the current agency standard needed for the approval of IBTs?

The U.S. Food and Drug Administration (FDA) has relied upon clinical outcomes—mortality, number of opportunistic infections (OIs), time to OI—for conventional approval of antiretroviral drugs. Accelerated approval has been granted based upon reductions in viral load.

For IBTs, no validated surrogate markers for clinical outcomes exist, although reductions in serum viral load are viewed by many clinicians as likely to be indicative of a beneficial effect. Dr. Schwieterman suggested that investigators use a complement of study designs to look at different effects (clinical, virologic, and immunologic) and different patient populations. Studies

designed to ascertain the benefit of IBTs on clinical or virologic outcomes should be central to any IBT product development plan. Ultimately what is needed for approval is a compelling and consistent description of efficacy and an overall favorable benefit to risk assessment.

#2 Because IBTs are not necessarily antiretroviral, isn't using viral load illogical as an endpoint for testing these therapies?

Viral load should not necessarily be viewed solely as a measure of mechanism of action but rather should be viewed with IBT therapies as a measurement of an HIV-specific immunocompetence. However, it's important to keep in mind that changes in viral load following IBT therapy is unvalidated as a, measures of protection against microbial infection, just as are other more direct measures of immune function.

In the absence of any validated surrogates, no definitive conclusions can be drawn about the utility of changes in viral load or immune function to predict benefit for IBTs. Dr. Schwieterman stated that it is difficult to interpret clinical relevance of treatment-induced changes on unvalidated markers of disease states, or, (stated otherwise), association of disease effects with clinical outcomes is not proof that therapeutic-induced changes in those effects affects clinical outcomes. Surrogates rarely capture the totality of clinical benefit of a product, nor do they capture unknown effects of treatment. Great care, therefore, must be used when inferring clinical benefit of an IBT therapy when unvalidated surrogate markers are used as outcome measures for treatment success.

#3 What role do immune markers play in the development of IBTs?

Many immune markers can serve as corroborative measures of bioactivity. They can be applied for management, dosing, selection of patient populations for study, elucidation of pathogenesis, understanding of mechanism of action, hypothesis generation for phase II trials, and so forth. Standardized collection of immune biomarker data is important to include on the product label as evidence of efficacy. And, although beyond the mandate of the FDA, tissue storage is vital for future studies.

#4 Isn't the question of developing and validating immune biomarkers the same as developing standards for efficacy?

In a word, no. These are really two different issues. Biomarkers:

- Are *critical* to the development of many areas of HIV therapeutic research
- *Could be* validated and expedite future product approval
- Beg the question of clinical validation, i.e., predictability of clinical benefit
- Can be used in clinical studies, but clinical outcomes or strong supportive measures (changes in viral load) are necessary for approval.

#5 What advice does the agency have for sponsors initiating development of IBTs?

Dr. Schwieterman reminded the group to keep in mind the ultimate goal, which is to bring a product to market that benefits patients. That said, investigators should generate a hypothesis about the product and the medical need that it meets and even give some early consideration to the product label so that agency reviewers can help investigators not only with individual studies but also with the overall product development plan.

Use phase I and II trials to characterize product safety and bioactivity based upon a spectrum of many different outcome measures. Phases II and III should be complementary. Phase II data should be used to study and potentially optimize various clinical trial parameters and generate a hypothesis. Phase III trials should address and test the hypothesis generated in phase II, i.e., characterize the product's medical niche and the potential benefits that the product confers.

The agency often suggests to sponsors that they capitalize upon the power of complementary trial designs by utilizing different patient populations, looking at induction versus preservation effects, and so forth. The focus should remain on different aspects of viral load as a central measure of immune competence, including partial reductions, viral setpoints, development of resistance, viral peaks, or maintenance of an antiretroviral effect after a structured treatment interruption (STI).

#6 Which trial designs are acceptable or preferable for IBTs?

Many acceptable trial designs exist. Dr. Schwieterman suggested a few designs that may be helpful for clinical trials for IBTs. For example, for patients and physicians already using

STIs as a therapeutic strategy, they should consider using STIs as an investigative tool in IBT research. Some 40% to 60% of patients end up discontinuing HAART because of harsh side effects or lack of efficacy. These limitations present an opportunity for evaluating IBT as an adjunctive treatment, for inducing a maintenance effect following induction, and for developing HAART-sparing regimens (preserving antiviral effect while on or after HAART. Trials of STIs need to be developed with great attention to patient safety parameters, and to ensuring equipoise and patient informed consent The key issue is that STIs are, in some settings, potentially valuable opportunities for use in phase I and II studies. Investigators can observe the effects of IBTs during the time when patients are immediately off therapy and then later? It is important to remember that phase III trials must show *clinical benefit* to gain approval of the drug Small trials, although not often powered to determine whether a product confers beneficial clinical outcomes, can nevertheless be used to gather critical information on the biologic effects of an agent. Of note, as products are developed that address clinical problems in any given field, other outcome measures can be developed and considered as measures of clinical benefit. Consider the example of drugs and biologics used in kidney transplantation and the evolution of endpoints in this field. Initially, the endpoint used by investigators was effects of the IBT on graft rejection, then, with the advent of better treatment, the endpoint came to be flares, and then, with further progress, other endpoints have developed. Perhaps this analogy can apply to IBTs.

#7 Does the agency consider any particular class or type of biomarkers preferable or "ripe" for use in clinical studies?

The Agency has no particular opinion about any the primacy or utility of any particular biomarker or set of biomarkers. Rather agency reviewers usually defer these assessments to experts in the field. Furthermore, the Agency supports attempts to build consensus about biomarkers but recognizes that it is difficult to standardize or generalize because of the plethora of candidate IBTs, their specific mechanisms of action, and the specific nature of most assays. Nevertheless, Dr. Schwieterman re-emphasized the need for data collection and tissue storage in conjunction with any clinical trials based upon surrogate biomarkers.

#8 Does the Agency consider any patient populations preferable for initial study?

The Agency, according to Dr. Schwieterman, has no particular preference about which patient populations might be studied first or are most likely to benefit from IBT therapy. Certainly IBTs are potentially useful in all patient populations. For example, studies could look at early disease to see if IBT could preserve immunocompetence or in middle-to-late disease to see if IBT could boost the immune response. Dr. Schwieterman did offer that IBTs may have utility in patients who are neither totally immunocompromised but neither totally immunocompetent either. Ultimately the decision about which patient population to address in a given trial must be based on risk-benefit analysis; this is the currency for decisions about many factors, including state of disease, prior treatment, animal data, anticipated toxicity, and so forth.

#9 How can the HIV community learn from other fields of IBTs?

One example that the HIV community may be able to study, and perhaps emulate, is the design of clinical trials for treatment of chronic hepatitis C with interferon-alfa, an IBT. No validated surrogates were used for initial approval of this agent, but HCV viral load was thought to be the best indicator of clinical benefit. The endpoint was percentage of patients with sustained suppression of viral load 6 months after discontinuation of treatment.

Another example is the basis for approving anti-tumor necrosis factor agents for treatment of rheumatoid arthritis. Here, the parameters measured were signs and symptoms of rheumatoid arthritis, X-ray evaluation of joints, and assessments of function. Clinical outcomes were used to assess benefits, and unvalidated surrogates (X-ray evaluation of joints) were used to corroborate the outcomes.

#10 How can the community, academia, industry, and the agency work together to develop IBTs?

The role of the Agency is to clarify meaningful endpoints and help design successful strategies for industry so that new safe and effective products are made available to patients. Appropriate forums for agency participation to this end are many, and include academic meetings, meetings with sponsors, guidance, advisory committee meetings, FDA representation on committees, liaisons with National Institutes of Health, and liaisons with the community.

The Agency relies on professional societies and experts in the field for guidance and advice on standards of care, efficacy standards, data and tissue collection, and the like. In addition, the Agency makes use of its databases in post hoc meta-analysis.

In conclusion, Dr. Schwieterman stated that although no validated surrogates are yet available for IBTs, viral load appears to be a strong measure of immune competence. He again emphasized that HAART's shortcomings may afford opportunities for evaluating IBTs during STIs. The need is great for new therapies, organization, coordination, and continued dialogue between the Agency and all parties.

AGENDA

Immune-Based Therapies and HIV Disease Meeting
Washington, DC
December 7-8, 2000

December 7, 2000

10-10:30 A.M. **Welcome & Introduction**

Facilitator: June Bray, Ph.D.

Forum Director: David Barr

Scientific Chair: Alan Landay, Ph.D.

Goals of Immune-Based Therapies

10:30- 11 A.M.	Clinical and Laboratory Outcomes in Cancer Immunotherapy	Nora Disis, MD
11-11:30 A.M.	HIV Immune-based Therapies: Research/Clinical Perspective	Roy Gulick, MD, MPH
11:30 A.M.-12:00 P.M.	Defining the Bar: A Community Perspective	Brenda Lein
12:00 P.M.-12:10 P.M.	Report from the Forum's "Immune-based Therapies and HIV Disease: European & Australian Perspectives" Meeting, Scotland, October 26, 2000	Michael Lederman, MD
12:10 –12:15 P.M	***The Importance of Innate Immunity in HIV Infection***	Jay A. Levy. MD.
12:15-12:30 P.M.	***Discussion***	
12:30 – 1:30 PM	***Lunch***	
1:30 – 2:00 PM	Progress with structured treatment interruptions and IL-2	Kendall A. Smith, MD
2:00-2:30 P.M.	Patterns of immunodominance in CTL responses directed against HIV-1 and the potential role of therapeutic immunization	Spyro Kalams, MD
2:30-3:00 P.M.	*Break*	
3:00 –5:00 PM	**Breakout sessions**	
	A. Obstacles in designing clinical trials for IBTs	Ronald Bosch, PH.D.
	B. Obstacles in implementation of IBT clinical trials	Larry Fox, MD
	C. Barriers for industry in developing an IBT	Mike Robertson, MD
	D. Immune modulators in other diseases and applications to HIV	Brian Kotzin, MD
5:00 –6:00 PM	Wrap-up (Large Group)	

December 8, 2000

8:30 –9:00 A.M.	In Search of the Holy Grail	Michael Lederman, MD
9:00 – 9:30 A.M.	Quantitating Human T Cells Responses to Cancer Vaccines	Kim Lyerly, MD
9:30-10:20 A.M.	Design and Analysis Issues for Studies of Immune-based Therapy	Victor DeGruttola, ScD Ronald Bosch, PhD
10:20-10:50 A.M.	FDA Standards and Perceptions for the Development of IBTs for the Treatment of HIV	William Schwieterman, MD
10:50-11:05 A.M.	Break	
11:05 A.M.–1:00 P.M.	***Breakout Sessions***	
	A. Identification of new therapies and potential markers (virologic, immunologic, clinical) of immune competence	Nava Sarver PhD/ Elizabeth Adams. MD
	B. Research designs for evaluating IBTs	Victor DeGruttola , ScD
	C. Validation plan for IBT	Richard Pollard, MD
	D. FDA perspective on validation of IBT markers	Sherri Lard, MD
1:00 – 2:00 P.M.	Lunch	
2:00 –4:00 P.M.	Summary Reports (Large Group)	

Immune-Based Therapies & HIV Disease Meeting
Participants List
Washington D.C.
December 7& 8, 2000

Elizabeth Adam, M.D.
DAIDS/NIAID/NIH
6700 Rockledge Dr
Bethesda, MD 20895-7620
eadams@niaid.nih.gov
W: 301-496-0700
F: 301-435-9282

Andrew Badley, M.D.
Ottawa Hospital, General Campus
501 Smyth Road
Ottawa, Ontario K1H8L6, Canada
abadley@ogh.on.ca
W: 613-737-8998
F: 613-737-8682

Jay Berzofsky, M.D., Ph.D.
NIH
9000 Rockville Pike
Building 10--Room 6B-12
Bethesda, MD 20892-1928
berzofsk@helix.nih.gov
W: 301- 496-6874

Ronald Bosch. Ph.D.
Harvard School of Public Health
651 Huntington Avenue, FXB#603
Boston, MA 02115-6017
ronbosch@sdac.harvard.edu
W: 617-432-3024
F: 617-432-2843

Barry Bredt
UCSF/sfgh Core Immunology Laboratory
Box 1353
San Francisco, CA 94143-1353
barryb@itsa.ucsf.edu
W: 415-206-5210
F: 415-206-8200

Sandra Bridges, Ph.D.
DAIDS/NIAID/NIH
Rm. 4128
6700-B Rockledge – MSC 7626
Bethesda, MD 20892-7626
sb33j@nih.gov
W: 301-496-8198
F: 301-402-3211

Judy Britz, M.D.
Cylex Inc.
8980-I Old Annapolis Road
Columbia, MD 21045
jbritz@cylex.net
W: 410-964-0236
F: 410-964-0367

Pat Bucy, M.D., Ph.D.
University of Alabama at Birmingham
Spain Wallace Building- W286
619- 19th Street South
Birmingham, Al 35233-1924
bucy@uab.edu
W: 205-934-6246
F: 205-975-7074

Scott Cairns, Ph.D.
NIH/NIAID
6700-B Rockledge St
Bethesda, MD 20892
scairns@niaid.nih.gov
W: 301-402-4239
F: 301-402-3211

Richard T. Davey, M.D.
NIAID/NIH
Building 10, Rm. 11C-103
Bethesda, MD 20892-1880
rd11q@nih.gov
W: 301-496-8029
F: 301-402-4097

Victor De Gruttola, Ph.D.
Harvard School of Public Health
677 Huntington Ave.- Kresg Building –Room 626A
Boston, MA 02115
Victor@sdac.harvard.edu
W: 617-432-2820
F: 617-432-2832

Yvette Delph
TAG
14907 Running Ridge Lane
Silver Spring, MD 20306
Ydelph@aol.com
W: 301-438-9751
F: 617-438-9753

James F. Demarest, Ph.D.
Glaxo Wellcome Research and Development
Five Moore Drive Research Triangle Park
North Carolina 27709-3398
jfd53641@glaxowellcome.com
W: 919-483-9972
F: 919-315-5243

Mary L. (Nora) Disis, M.D.
University of Washington, Oncology
Box 356527
Seattle, Washington 98195-6527
ndisis@u.washington.edu
W: 206-616-1823
F: 206-543-8557

William Duncan, Ph.D.
Division of AIDS-NIAID-NIH
6003 Executive Blvd, Solar Bldg.- room 2C19
Rockville, MD 20852-7620
Wd6u@nih.gov
W: 301-402-0130
F: 301-402-3171

Raphaelle El Habib
Aventis Pasteur
1541 Avenue Marcel Merieux
69280 Marcy L' Etoile, FRANCE
raphaelle.elhabib@aventis.com
W: 33-4-3737-3228
F: 33-4-3737-3639

Linda Forsyth, M.D.
Center for Biologics, FDA
HFI-40
Rockville, MD 20857
forsythL@cber.fda.gov
W: 301-827-5151
F: 301-827-5394

Lawrence Fox, M.D., Ph.D.
NIH, NIAID, DAIDS, TRP
6700-B Rockledge Dr room5104
Bethesda, MD 20892-7624
lfox@niaid.nih.gov
W: 301-496-0700
F: 301-435-9282

Genoveffa Franchini, M.D.
NCI/NIH
9000 Rockville Pike
41 Library Drive, 41/D804
Bethesda, MD 20892
veffa@helix.nih.gov
W: 301-496-2386 ext. 4840
F: 301-496-8394

William Freimuth, M.D., Ph.D.
Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20850
william_freimuth@hgsi.com
W: 240-314-1200
F: 301-294-1449

Raj Gandhi, M.D.
Mass General Hospital, GRJ504
55 Fruit Street
Boston, MA 02114
rgandhi@partners.org
W: 617-724-9670
F: 617-726-7653

Merril Gersten, M.D.
Agouron Pharmaceuticals
10350 North Torrey Pines Road
La Jolla, CA 92037-1020
merril.gerston@agouron.com
W: 619-622-8813
F: 619-678-8247

Richard Ginsburg, M.D.
Wyeth-Lederle Vaccine
Clinical Research
401 North Middletown BLD.140-4
Pearl River, NJ 10965
ginsber@war.wyeth.com
W: 914-732-42083
F: 914-732-5517

Jeffrey A. Gustavson
CCG representative for Immunology RAC, ACTG
1537 Jones #101
San Francisco, CA 94109
jeff_gustavson@tesseract.com
W: (415) 834-4056
F: (415) 981-4600

Bob Huff
American Foundation for AIDS Research
120 Wall Street, 13th Floor
New York, NY 10005
Bob.huff@amfar.org
W: 212-806-1761
F: 212-806-1608

Spyros A. Kalams, M.D.
Massachusetts General Hospital East
149- 13th Street, Room 5217
Charleston, MA 02129
Kalams@helix.mgh.harvard.edu
W: 617-724-4958
F: 617-726-4691

Barney Koszalka, Ph.D.
Glaxo Wellcome Research and Development
Five Moore Drive
Research Triangle Park
North Carolina 27709-3398
bk24976@glaxowellcome.com
W: 919-483-2313
F: 919-483-6147

Alan Landay, Ph.D.
Rush Presbyterian – St. Lukes Medical Center
Immunology /Microbiology Dept Rush Medical College
1653 W. Congress Parkway
Chicago, IL 60612
alanday@rush.edu
W: 312-942-6554 F: 312-942-2808

Roy Gulick, M.D., M.P.H
Cornell Clinical Trials Unit
Medical College of Cornell University
525 East 68th Street
New York, NY
Rgulick@med.cornell,edu
W: 212 746-4177
F: 212 746-8852

David W. Haas, M.D.
AIDS Clinical Trials Center
1211 21st Avenue South, Suite 539
Nashville, TN 37212-1302
david.w.haas@vanderbilt.edu
W: 615-936-1173
F: 615-936-1170

Jeffrey M. Jacobson, M.D.
Mount Sinai Medical Center I
One Gustave L. Levy Place. Box 1009
New York, NY, 10029
kalams@helix.mgh.harvard.edu
W: (617) 724-4958
F: (617) 726-4691

Richard S. Kornbluth, M.D., Ph.D.
UCSD
9500 Gilman Dr- Stein Clin Res Bldg, Rm 304
La Jolla, CA 92093-0679
rkornbluth@ucsd.edu
W: 858-552-8585, ext 2620
F: 858-552-7445

Brian L. Kotzin, M.D.
University of Colorado Health and Science Center
4200 East Ninth Avenue
Denver, CO 80206
Brian.kotzin@uchsc.edu
W: 303-315-6977
F: 919-483-6147

Sheryl Lard-Whiteford, Ph.D.
FDA/CBER/QAS/HFM-4
Rockville Pike
Rockville, MD 20852
lard@cber.fda.gov
W: 301-827-2389
F: 301-827-5867

Mike Lederman, M.D.
Case Western Reserve University/University Hospital
11100 Euclid Avenue, FOL5083
Cleveland, OH 44106-5083
lederman.michael@clevelandactu.org
W: 216-844-8786
F: 216-844-5523

Bruce Levine, Ph.D.
University of Pennsylvania/Molec. and Cell.
5th floor BRB II/III/6160
421 Curie Blvd.
levinebl@mail.med.upenn.edu
W: 215-573-6788
F: 215-573-8590

Jay A. Levy, M.D.
University of California, SF
Box 1270,S 1280
San Francisco, CA 94110
jalevy@itsa.ucsf.edu
W: 415-476-4071
F: 415-476-8365

Brenda Lein
Project Inform
205 13 Th Street – Suite 2001
San Francisco, CA 94103
blein@projectinform.org
W: 415-558-8669
F: 415-558-0684

Judy Lieberman, M.D., PhD.
Harvard Medical School
Center for Blood Research
800 Huntington Avenue
Boston, MA 02115
lieberman@cbr.med.harvard.edu
W: 617-731-6470
F: 617-278-3493

Katherine Ruiz Luzuriaga, M.D.
University of Mass. Med. School
Biotech II Suite. 318
373 Plantation St.
Worcester, MA 01605-2377
katherine.luzuriaga@umassmed.edu
W:508-856-6282
F:508-856-5500

Herbert Kim Lyerly, M.D.
Duke University
401 Med Sci Res Bldg
Box 2606 Med Ctr, Durham, NC 27710
k.lyerly@cgct.duke.edu
W: (919) 681-8350
F: 919-681-7970

Don A. Madren, RPh.
Agouron Pharmaceuticals, Inc
10777 Science Center Dr
San Diego, CA 92121-1111
don.madren@agouron.com
W: 858-622-8868
F: 858-622-8054

Donna Mildvan, M.D.
Beth-Israel Medical Center
Infectious Disease
First Avenue & 16th Street
New York, NY 10003
mildvan@ix.netcom.com
W: 212-420-4005
F: 212-420-4498

Luis Montaner
Wistar Institute
3601 Spruce St.
Philadelphia, PA 19104-4268
montaner@wistar.upenn.edu
W: 215-898-9143
F: 215-573-7008

Mike Norcross, Ph.D.
FDA
Center for Biologics HFI-40
Rockville, MD 20857
norcross@cber.fda.gov
W: 301-827-0793

Bob Munk, PhD.
New Mexico AIDS Infonet
34A Puma Way
Arroy Seco, NM 87514
Bobmunk@ix.netcom.com
W: 505-776-8032
F: 505-776-5324

Richard Pollard, M.D.
University of Texas
301 University Blvd. – Suite 518
Galveston, TX 77555-0835
rpollard@utmb.edu
W: 409-772-4979
F: 409-772-3461

Robert R. Redfield, M.D.
University of Maryland at Baltimore
Institute of Human Virology
725 W. Lombard St, 5th floor
Baltimore, MD 21201
redfield@umbi.umd.edu
W: 410-706-4613
F: 410-706-4619

Michael Robertson, M.D.
Merck & Co, Inc
10 Sentry Parkway
P.O. Box 4 BL3-4
West Point, PA 19486
michael_robertson@merck.com
W: 610-397-7051
F: 610-834-7555

Marjorie Robinson, PharmD.
Abbott Laboratories HIV Franchise
1160 N Federal Hwy #214
Fort Lauderdale, FL 33304
marjore.robinson@abbott.com
W: 954-525-8275
F: 954-523-7277

Mario Roederer
VRC/NIH
40 Convent DR, Rm. 5509
Bethesda, MD 20892-3005
marior@mail.nih.gov
W: 301-594-9491

Patricia Roth, Ph.D.
Becman Coulter
11800 SW 147th Ave, M/S 21-A06
Miami, FL 33116-9015
patricia.roth@coulter.com
W: 305-380-2548
F: 305-380-3131

Nava Sarver, Ph.D.
NIAID-NIH
Targeted Interventions Br. – Room 2C01
6003 Executive Blvd – Solar Building
Rockville, MD 20852-7620
ns18p@nih.gov
W: 301-496-2970
F: 301-402-3211

William Schwieterman, M.D.
FDA
HFI-40
Rockville, MD 20857
schwieterman@cber.fda.gov
W: 301-827-5094
F: 301-827-5394

Rafick–Pierre Sekaly, Ph.D.
Universite De Montreal
C.P. 6128, Succursale Centre-Ville
Monreal, Quebec
Canada
rafick-pierre.sekaly@umontreal.ca
W: 514-843-2961
F: 514-843-2962

Gene M. Shearer, Ph.D.
NCI/NIH
Experimental Immunology Branch
9000 Rockville Pike
Bldg. 10, Room 4B17
Bethesda, MD 20892
gs22y@nih.gov
W: 301-496-5464

Rebecca Sheets, Ph.D.
FDA/CBER/OVRR/DVRPA
1401 Rockville Pike HFM-475
Rockville, MD 20852
Sheetsb@cber.fda.gov
W: 301-827-2330 F: 301-827-2523

Gail Skowron, M.D.
Brown Univ. Rogers & Williams Hospital
825 Chalkstone Avenue
Providence, RI 02908-4735
gail_skowron@brown.edu
W:401-456-2437 F: 401-456-6839

Kendall Smith, M.D.
Weill Medical College of Cornell University
1300 York Avenue, Box 41
New York, NY 10021
kasmith@med.cornell.edu
W: 212-85+-4608
F: 212-746-8167

Peter R. Sottong, M.S.
Cylex Inc.
8970-D Rt. 108
Columbia, MD 21045
psottong@cylex.net
W: 410-964-0236
F: 410-964-0367

Jean-Marc Steens, M.D.
SmithKline Beecham Biologicals
Rue de I'Institut 89
B-1330 Rixensart
Belgium
jean-marc.steens@sbbio.be
W: 32 2 656 73 51
F: 32 2 656 81 33

Kim Struble, Pharm.D.
Center for Drugs, FDA
2517 Baltimore Rd. #4
Rockville, MD 20853
strublek@cder.fda.gov
W: 301-827-2483
F: 301-827-2510

Tracy Swan
ACTG CCG IRAC Alternate
175 Elm St
Cambridge, MA 02139
tracyswan1@cs.com
W: 617-591-6774
F: 617-591-6784

Scott Thaler. M.D.
Merck & Co., Inc
P.O. Box 4, UNC 151
West Point, PA 19486
scott_thaler@merck.com
W: 610-397-2625
F: 610-397-3621

Elaine Thomas, Ph.D.
Immunex Corp.
51 University St.
Seattle, WA 98101-2936
thomase@immunex.com
W: 206-389-4032
F: 206-682-9927

Joe Toerner, M.D.
FDA
5600 Fishers Lane, HFD-530
Rockville, MD 20857
toernerJ@cder.fda.gov
W: 301-827-2330
F: 301-827-2510

Fred Valentine, M.D.
New York University Medical Center
550 First Ave-CD515
New York, NY 10016
valenf01@gcrc.med.nyu.edu
W: 212-263-6565
F: 212-263-8264

Pierre Vandepapeliere, M.D.
Rue De L'Institut 89
1330 Rixensart
pierre.vandepapeliere@sbbio.be
W: 32 3 656 87 33
F: 32 2 656 90 72

Fulvia Veronese, Ph.D.
NIH - Office of AIDS Research
2 Center Drive Room 4E16 MSC 0255
Bethesda, MD 20892
fv10x@nih.gov
W: 301-496-3677
F: 301-496-4843

Carol Weiss
FDA/CBER Office of Vaccines HFM-466
NIH Bldg 29, Room 532
29 Lincoln Dr
Bethesda, MD 20892-4555
weissc@cber.fda.gov
W: 301-402-3190
F: 301-496-4684

Karen Weiss, M.D.
Office of Therapeutics Research and Review,
Center for Biologics, FDA
HFI-40
Rockville, MD 20857
weissk@cber.fda.gov
W: 301-8275093
F: 301-827-5394

Cara C. Wilson, M.D.
University of Colorado Health Sciences Center
Division of Clinical Immunology
Campus Box B-168
4200 E. Ninth Avenue
Denver, CO 80262
cara.wilson@uchsc.edu
W: 303-315-6659
F: 303-315-7642

Susan Wilson, Ph.D.
Chiron Corporation
4560 Horton St, mailstop 3
Emeryville, Ca 94608
susan.wilson@cc.chiron.com
W: 510-923-8142
F: 510-923-2586