Report from the 3rd International STI Workshop

Montréal, Quebec, Canada
March 23-24 2002

A collaborative project sponsored by:
Foundation for AIDS & Immune Research
The Forum for Collaborative HIV Research
Gay Men's Health Crisis
Project Inform
Treatment Action Group

Acknowledgments

The 3rd International STI Workshop was made possible through a generous grant from the Foundation for AIDS and Immune Research.

The STI steering committee (see back page) wishes to thank the following companies and organizations for their generous support of the meeting:

Abbott Laboratories
Agouron
Bristol Myers Squibb
Forum for Collaborative HIV Research
Gilead Pharmaceuticals
GlaxoSmithKline
Roche Pharmaceuticals
Treatment Action Group
ViroLogic

We gratefully acknowledge the dedication and commitment of Ben Collins and Stephen Pelton, who handled the meeting logistics with extraordinary skill and expertise. We are also indebted to the volunteers and staff of Project Inform for their assistance in putting the meeting materials together.

Richard Jefferys has written this report. He managed to capture all the essentials with accuracy and understanding. We are extremely grateful for this superb documentation of the Montreal workshop.

Linda Grinberg (1951 – 2002)
In Memoriam

Linda Grinberg, a fearless AIDS treatment activist, founder and president of the Foundation for AIDS and Immune Research (FAIR) and a long-time Board member of Project Inform died quietly at home on May 27, 2002 of complications of pulmonary hypertension and HIV infection.

Linda played a critical role in treatment activism and was a unifying force within the activist community. She energized people through her enormous intellectual curiosity and her ability to understand and explain complex science. She engaged activists, scientists and government leaders in creating the three international meetings on Structured Treatment Interruption. Without her drive and energy, it is unlikely that these meetings would have occurred.

Diagnosed with AIDS in 1991, doctors told Linda Grinberg that she had approximately one year to live. Not content to accept that, Linda educated herself about available treatments and became a resource to many other HIV positive people who sought out her counsel. Her work was by no means limited to the creation of the STI meetings. She was a leading force in the Coalition for Salvage Therapy, an activist coalition that worked with drug companies and government to hasten development of new therapies for people who had outlived their treatment options. In 1995, she founded FAIR, the Foundation for AIDS and Immune Research. FAIR makes grants to help support innovative, proof of concept studies that might otherwise go unfunded. FAIR's work is guided by a distinguished scientific advisory board.

Linda was an inspiration to every AIDS activist and scientist who knew her or her work. No task was too difficult –or too menial for her. She enthusiastically did whatever had to be done. We have all lost a wise, powerful activist and an incredibly dedicated person. She is deeply missed by all.

Martin Delaney
Founding Director
Project Inform

Introduction

Over a wintry, cold weekend this past March researchers and community activists gathered in Montreal for the 3rd International Structured Treatment Interruption (STI) Workshop. Since the workshop was inaugurated in 1999, STI research has diversified into a number of different sub-fields, reflecting the variety of settings in which treatment interruptions are being investigated.

STI Workshop Overview
Mark Dybul, National Institutes of Health (NIH)

The task of outlining a "conceptual framework" for the meeting fell to Mark Dybul from the NIH, who described the multiplicity of reasons why STIs are being studied, and raised the outstanding questions regarding potential risks and benefits of these strategies.

STI Strategy	Rationale	Potential Risks	Potential Benefits
Interruption for individuals with multi-drug resistance	Diminish resistant virus population	Decreased CD4 count, increased viral load, opportunistic infections	Improved response to salvage regimen
Interruption for individuals on effective treatment	Reduce time on HAART	Unknown impact on long term clinical outcome, increased viral load, decreased CD4 count, decreased quality of life, increased risk of transmission, drug resistance	Reduced toxicity, reduced treatment costs, improved quality of life
Interruption to allow auto-immunization	Improve HIV-specific immunity, allowing for host virus control	Drug resistance, immune escape, decreased CD4 count, increased viral load	Improved immune control of viral replication off HAART

Even within these overall strategies, a number of different research approaches are possible. Interruptions may be for fixed periods, or guided by CD4 and/or viral load thresholds. Additional treatments may be used to try and extend the period for which HAART can safely be withheld, such as therapeutic vaccines, immune suppressants or cytokines (e.g. IL-2, GM-CSF). In a research setting, interruption can be used as a "read out" period to assess any benefits of these additional treatments.

Reviewing current knowledge regarding auto-immunization, Dybul cited data from studies suggesting that a significant number of individuals with acute infection may manifest improved control of viremia off HAART after one or more treatment interruptions. However, a long-term clinical benefit has yet to be demonstrated. HIV's ability to mutate and escape from HIV-specific immune responses (discussed in more detail later in the workshop) may also make the benefits of this approach temporary. Based on study designs tried to date, auto-immunization appears to be an elusive goal in individuals with chronic HIV infection. Improved control of HIV replication after HAART withdrawal has only been demonstrated in 15-30% of individuals and the contribution of STI to this effect – as opposed to the results that would have been obtained with treatment cessation – remains unclear.

Dybul emphasized that the risks and benefits of each STI strategy remain largely unknown, and may not always conform to expectations. For example, adherence to intermittent regimens may be difficult and reduced drug costs might be offset by increased monitoring costs or increased overall treatment costs due to increased transmission. Citing the relationship between viral load and transmission risk described by Thomas Quinn and colleagues (Quinn 2000), Dybul presented a model of the possible effect of STI on HIV transmission. Based on a hypothetical cohort of young individuals averaging approximately 100 coital acts per year, Dybul's conclusion was a single 18 month STI – with an associated viral load increase to 38,000 copies or above – could double the number of new infections, thereby increasing overall treatment costs, if the couples did not use barrier methods. As Dybul emphasized, this example illustrates the potential for tension between individual and public health priorities. Dybul suggested that these tensions may play out differently in resource rich and resource poor settings, and presented a table to illustrate his point:

	Resource Poor Setting	Resource Rich Setting
Potential benefits	Cost, toxicity, quality of life	Toxicity, quality of life
Potential risks	Transmission, drug resistance, long term immune system damage	Drug resistance, long term immune system damage

Dybul also highlighted some the scientific issues that might come into play as STI strategies are explored in the global setting:

- Will viral load rebound kinetics differ based on the immune activation profile and/or the HIV subtype?
- Will the response to HAART differ based on these same factors?
- Will drug resistance patterns be influenced by HIV subtype?

In conclusion, Dybul stressed the need for randomized controlled trials that address questions about the relative risks and benefits of various STI strategies in diverse global settings.

Therapeutic Immunization and Immune Modulation

Overview
Richard Koup, Vaccine Research Center, NIH

Rick Koup provided the introductory overview of the first session of the STI workshop, which focused on the potential role of therapeutic immunization in STIs. The overall goal is to improve control of HIV replication when HAART is interrupted by enhancing HIV-specific immunity. Koup outlined the many unknowns that lie behind this appealing concept. Chief among them is whether HIV-specific immune responses can, in fact, be enhanced. As Koup pointed out, simply expanding pre-existing immune responses with a vaccine may confer little benefit given that these responses were incapable of controlling viral load before HAART was initiated. He suggested that it will be important to consider the quantity, quality, and location of the HIV-specific immune response.

With these caveats, Koup reviewed data demonstrating the importance of CD8 cytotoxic T-lymphocytes (CTL) in controlling HIV replication:

- Several studies have reported a negative correlation between HIV-specific CTL responses and viral load, although this is not a consistent finding and may be influenced by the methodology employed (Ogg 1998, Betts 2002)
- SIV-infected macaques depleted of CD8 cells by monoclonal antibody treatment experience a dramatic rise in SIV viral load (Schmitz 1999)
- There is an association between the appearance of HIV-specific CTL responses and reduction of viral load in primary HIV infection (Koup 1994)

However, in chronic infection there is a large and broadly targeted HIV-specific CTL response, which is typically incapable of controlling viral evolution and progression. . Koup listed some possible explanations for this apparent contradiction, including:

- Viral escape from the CTL response (Koup 1994a)
- Defects in the HIV-specific CD4 T cell response. In support of this hypothesis, Koup cited data from Tony Fauci's group showing that replacement of CD4 T cell help *in vitro* with a CD40 ligand trimer improves HIV-specific CTL function (Ostrowski 2000)
- Differentiation defects in the CTL response. Most HIV-specific CTL do not express the chemokine receptor CCR7, a marker for "central" memory T cells (Champagne 2001)
- Maturation defects in CTL. HIV-specific CTL appear to contain lower levels of perforin compared CMV-specific CTL (Appay 2000)

Koup stressed the difficulty of understanding the cause and effect relationship between these observations and chronic HIV replication, and posed the question: Is it defects in the HIV-specific immune response that lead to the failure to fully control viral replication, or does ongoing viral replication and consequent antigenic stimulation cause the defects in the HIV-specific immune response?

In terms of the potential for therapeutic vaccination to begin to address this question, there are a number of potentially encouraging findings. While HAART leads to a reduction in the numbers of HIV-specific CTL, they do not disappear entirely; there is a persistent memory response that could conceivably be boosted with therapeutic immunization. It has also been shown that not all possible HIV epitopes are targeted by CTL, suggesting that immunization might be employed to broaden the response. In support of the notion that such an approach might be beneficial, Koup cited the acute infection study by Eric Rosenberg demonstrating that an augmentation of the HIV-specific CD4 response and concomitant broadening and strengthening of HIV-specific CTL correlated with improved control of viral replication in the absence of HAART (Rosenberg 2000). Koup pointed out the need to ascertain whether broadening the CTL response benefits individuals whose virus has escaped extant HIV-specific CTL.

Koup ended his talk with a few theoretical considerations related to therapeutic immunization. One concern is that only pre-existing – and likely ineffective – memory T cells will respond to a vaccine. Creation of new memory T cells requires activation of naïve T cells by specialized antigen-presenting cells (APCs). Current models suggest that pre-existing memory cells might out-compete naïve T cells for access to APCs expressing vaccine antigens, and thus prevent induction of new memory cells. However, recent data from murine models demonstrated concomitant naïve and memory responses to a viral re-challenge (Turner 2001), supporting Koup's contention that the task may not be impossible. Koup explained the importance of inducing truly new memory T cell responses in order to increase the number of viral epitopes targeted and/or create better quality (i.e. more functional) HIV-specific memory T cells. Ending on a cautionary note, Koup pointed out that the development of an effective therapeutic vaccine is unlikely to be any easier than developing a preventive product.

Data Presentations

Results from ALVAC/gp160 Trial
Xia Jin, University of Rochester

Xia Jin presented the results of a therapeutic vaccine study conducted at the Aaron Diamond AIDS Research Center in New York (Jin 2002). The open-label trial employed Aventis-Pasteur's canarypox-based vaccine vector, ALVAC vCP1452 (which encodes several HIV protein sequences including *gag*, *env* and *pol* plus CTL epitopes from *nef* and *pol*) and a recombinant gp160 protein booster, in combination with HAART. Study participants began treatment with HAART within 90 days of HIV infection, and received at least four vaccinations. Sixteen participants later voluntarily elected to discontinue HAART, and Jin compared the kinetics of their viral load rebound and ultimate set point to historical controls that had not received any immunizations.

In terms of immunogenicity, the ALVAC vaccine performed poorly. CD4 T cell responses (as measured using the lymphoproliferative response assay) to *env* and/or *gag* were seen in 7/14 study participants but were not sustained. HIV-specific CTL targeting

at least one antigen (*env*, *gag*, *pol* or *nef*) were also detected in 7/14 individuals by intracellular staining for the cytokine interferon-gamma. After treatment cessation, control of viral load was not significantly different in vaccinees compared to the controls. Questioned about these disappointing results, Jin noted that the dose of ALVAC employed – 10^7 particle-forming units – is relatively low compared to other candidate vectors and suggested that higher doses might improve the response (a possibility supported by recent studies in HIV-negative volunteers – see Gupta 2002). Unfortunately, Aventis Pasteur claims that manufacturing problems have precluded widespread study of higher ALVAC doses.

Remune as a Therapeutic Vaccine
Gregory Robbins, Massachusetts General Hospital (MGH)

Gregory Robbins from Bruce Walker's group at Massachusetts General Hospital in Boston summarized data from a small 10-person study of Remune, a vaccine based on the traditional "whole-killed" approach (the construct contains an inactivated HIV recombinant A/G isolate from Zaire, missing only the gp120 and gp160 proteins). Designed by polio vaccine pioneer Jonas Salk and manufactured by the Immune Response Corporation (IRC), Remune has been dogged by controversy since it first entered human trials in the late 1980s. Robbins and colleagues felt that Remune's checkered history had precluded a rational, independent assessment of its immunogenicity in people on HAART and set out to fill this gap. Despite the small numbers the study was blinded and randomized to ensure maximum rigor, and all assessments of immunogenicity were conducted independently of IRC in Bruce Walker's laboratory.

The trial enrolled individuals with chronic HIV infection who had a CD4 count over 250 at the time of starting HAART. Participants were required to have had a viral load less than 500 copies for over six months and less than 50 copies at the time of study entry. Immunizations with Remune in incomplete Freund's adjuvant (IFA) or IFA alone were given at weeks 0, 12, 24, 36 and 48. HIV-specific CD4 T cell responses (to recombinant p24, native p24 or the entire vaccine) were assessed by a proliferation assay, with the results expressed either as a stimulation index or as counts-per-minute.

By both measures, the vaccine induced a statistically significant increase in HIV-specific CD4 T cell responses compared to IFA alone. These results echo those of a similar study conducted by Fred Valentine at New York University, presented four years ago at the International Conference on AIDS in Geneva. Robbins also looked at HIV-specific CTL responses using an ELISpot assay, but no clear changes in magnitude or breadth were detected. In answer to questions about whether Remune-induced responses would have any impact on viral load, Robbins stated that the MGH team had intended to conduct a larger study of Remune combined with STIs. However, this study was stopped during enrollment when the sponsor (Pfizer, Inc.) withdrew support.

Modeling Therapeutic Vaccine Strategies in Monkeys
Jeff Lifson, National Cancer Institute (NCI)

Jeff Lifson provided a review of his work in the rhesus macaque model. Macaques can be infected with a number of different simian immunodeficiency viruses (SIVs) derived from their natural host, sooty mangabeys. While these viruses are not pathogenic in the mangabey, a number of well-characterized isolates can cause simian AIDS in macaques, typically within 1 -2 years. Lifson has been employing these viruses (SIVsmE660, SIVmac239 and SIVmac251) to investigate questions about antiretroviral immune responses that cannot easily be addressed in humans.

The initial focus has been on modulating very early events in the course of SIV infection through the use of antiretroviral drugs. In a study that was recently published, Lifson's group administered the drug PMPA (now marketed as tenofovir) 24 hours after inoculating macaques with SIVsmE660 (Lifson 2001). Treatment was continued for 1-2 months and then stopped. Perhaps surprisingly, all animals were able to control viral replication after treatment cessation although some experienced a transient "blip" immediately after PMPA was withdrawn. The macaques displayed SIV-specific proliferative responses and CTL, but the magnitude of the response was not exceptional. Some of these animals have subsequently controlled re-challenges with the genetically divergent isolate SIVmac239. To formally demonstrate that the viruses were being controlled rather than cleared, Lifson used a monoclonal antibody to temporarily deplete CD8 T cells. This experiment led to a transient viral load rebound made up of both SIVsmE660 and SIVmac239 viruses, which were subsequently brought to undetectable levels as the effects of the antibody waned.

Lifson's group is now trying alternative regimens and schedules in order to further investigate the potential for immune control of SIV in macaques. In one study, a combination of PMPA and FTC was administered 30 days after infection with SIV. Treatment was then stopped eight weeks later. In this case, control of SIV was somewhat less robust: 3/4 animals maintained viral loads around 1,000 copies or less after a year of follow-up, while one animal resembled untreated controls with a viral load over 1 million copies.

In keeping with the theme of the workshop session, Lifson also previewed preliminary data on a very small therapeutic vaccine study performed in collaboration with Chris Miller from the University of California. Four macaques were infected with SIVmac251 and then twenty weeks later combination therapy with PMPA and FTC was initiated. A week after beginning treatment, two animals were immunized with a whole inactivated vaccine (derived from a different SIV isolate, mac239) together with adjuvants called CpG motifs. Three more immunizations followed at monthly intervals, and then antiretroviral treatment was stopped a month after the final shot. The remaining two animals received no vaccine but also had their treatment withdrawn at the same time in order to serve as controls. One vaccinated macaque displayed transient control of viral load off therapy, but subsequently experienced a rebound to pre-treatment levels. Viral load in the other vaccinated animal and both controls rapidly returned to a level

equivalent to that seen prior to initiation of PMPA and FTC. Lifson feels that the dose of both vaccine and adjuvant used in this experiment were suboptimal, and his group is continuing to pursue this line of research.

In conclusion, Lifson reminded workshop participants that monkeys are not humans, but nevertheless these models can provide useful "proof of concept" data prior to moving candidate approaches into human trials.

Open Group Discussion

Following the initial presentations, there was an open period when workshop participants could present additional data relevant to the topic of therapeutic immunization.

Genoveffa Franchini, NCI

Veffa Franchini from the National Cancer Institute described her therapeutic vaccination efforts, also in the SIV/rhesus macaque model. Franchini performed a study in monkeys infected with the highly pathogenic primary isolate SIVmac251 for six months, and then treated with a triple combination of PMPA, ddI and d4T. The vaccine employed was a weakened strain of vaccinia known as NYVAC encoding the *gag*, *pol*, *env*, *tat*, *rev* and *nef* genes of SIV. Animals were treated for eight months and immunized three times (at six week intervals) prior to an STI. Sixteen weeks after treatment cessation, there was statistically significant reduction in viral load in the vaccinated macaques compared to the controls ($p=0.024$), but this difference was subsequently lost due to a recrudescence of viremia in these animals.

Franchini then reviewed her ideas for potentially improving the response to vaccination, which include:

- Higher doses of vaccine
- Combination vaccines (e.g. DNA prime followed by NYVAC boost)
- Addition of cytokines such as IL-2, IL-7 and IL-15

In preliminary experiments, all of the above approaches have shown efficacy in terms of improving the magnitude of the SIV-specific CTL response compared to NYVAC alone, including the first ever study of a vaccine encoding recombinant human IL-7 in addition to SIV antigens. Concluding, , Franchini stated that her data offers no evidence that inducing new SIV-specific T cell responses adds "fuel to the fire" in terms of increasing virus replication during an STI, but suggests that therapeutic vaccination may be able to contribute to enhanced containment of viral load in the absence of drug therapy.

John Shiver, Merck Research Laboratories

The vaccine development efforts of Merck Research Labs have garnered a substantial amount of attention recently. Merck is pursuing an HIV vaccine strategy based on a DNA prime followed by a boost utilizing an adenovirus vector. While the head of the program,

Emilio Emini, has presented data from studies in HIV-negative volunteers (see www.retroconference.org for a webcast of his presentation at the 2002 Retrovirus Conference), the company also has an ongoing therapeutic program in HIV-infected individuals. Shiver reviewed the basics of the two vaccine constructs, which comprise naked DNA in either saline or one of two adjuvants (aluminum phosphate or a novel co-polymer called CRL-005) and an attenuated adenovirus (which in natural form causes colds). Both encode HIV *gag* but will eventually be modified to carry *gag*, *pol* and *nef*.

The aim of Merck's therapeutic program is to assess the immunogenicity of the vaccines in individuals on HAART with the hope of eventually evaluating the vaccine's ability to enhance control of viral load when drug therapy is interrupted. Shiver presented a complex slide containing data on *gag*-specific CD4 and CD8 T cell responses from participants in their ongoing phase I adenovirus vaccine study. The data is still blinded, but Shiver pointed to some individuals who appear to be developing responses of increased magnitude over the course of the study, and obviously he hopes that these represent active vaccine recipients. Noting that pre-existing responses to HIV can complicate these analyses, Shiver underscored the importance of collecting detailed baseline data on HIV-specific T cell responses prior to study commencement. Mark Dybul asked both John Shiver and Veffa Franchini their opinion on the role of "priming" in the setting of therapeutic immunization, suggesting that perhaps infection itself might be expected to perform this task. Franchini responded that DNA priming clearly improves the T cell response to a subsequent boost in her macaque experiments. The Merck program has yet to reach the stage of combining the DNA and adenovirus vaccines into a prime-boost schedule.

Kendall Smith, Cornell University

Kendall Smith has pioneered the study of low-dose interleukin-2 (IL-2) as a potential immune modulator in HIV infection. At the workshop, Smith outlined the role of IL-2 in promoting antigen-specific T cell expansion and described work that aims to delineate the role of this cytokine in enhancing HIV-specific T cell responses in the setting of STI. Smith reviewed a recent study (Smith 2000) that utilized what he describes as a "diagnostic treatment interruption" (DTI) to assess the ability of the HIV-specific immune response to control viral load without help from HAART. Based on an analysis of nine individuals with chronic HIV infection, the results demonstrated a fairly consistent pattern of virus rebound: viral load became detectable in a mean of 19 days (range 14 – 30) and peaked at around two and a half weeks. Over the next four to six weeks, viral load declined by about a log to a set point, concomitant with a 200% expansion in the CD8 T cell compartment. Using these data as a rough gauge of the antiretroviral potential of the immune response in chronic infection, Smith is now attempting to improve upon it. He is conducting an ongoing 92-person trial of the ALVAC HIV vaccine (or placebo) either with or without low dose subcutaneous IL-2 which will also feature a 12-week DTI after four immunizations have been administered. Fourteen participants have completed one DTI and Smith volunteered some preliminary observations regarding these individuals. The IL-2 arms are not blinded and Smith reported that CD4 counts increased by 25% in the IL-2 recipients during the DTI

compared to a gradual decrease in the non-IL-2 group, while CD8 counts increased by an average of 250 cells in the former group compared to 150 in the latter. Assessing HIV-specific CD8 T cell responses post–DTI using an intracellular cytokine staining assay, Smith noted that while cells producing both interferon-gamma and TNF-alpha could be detected, HIV-specific IL-2 production was absent, which he believes supports the notion of giving exogenous IL-2. Martin Delaney from Project Inform asked whether pre-HAART baseline viral loads were available for study participants; Smith replied that while these data were unavailable, the study was intended to be sufficiently powered to detect between-group differences in viral load setpoints.

Kathie Grovit-Ferbas, University of California at Los Angeles

The last discussant in the therapeutic immunization session was Kathie Grovit-Ferbas. In collaboration with her colleague Judith Currier, Dr. Grovit-Ferbas has developed a novel whole-killed HIV vaccine that retains its envelope proteins (traditional killing methods cause HIV to shed gp120 and gp160). Grovit-Ferbas described preliminary experiments in macaques comparing the ability of constructs with and without env to induce antibodies capable of binding viral isolates. Three macaques immunized with the env-containing vaccine (three times at 5 week intervals) in an adjuvant called QS21 displayed binding titers ranging from 1:64,000 to 1:250,000..

The next step for this candidate is a phase I dose-escalation trial in HIV-infected individuals on HAART whose viral load is suppressed to less than 50 copies. The primary goal of this study will be to demonstrate safety, but secondary endpoints will include antibody titers, vaccine-specific CD4 proliferative responses, vaccine-specific interferon-gamma production and the size of the HIV reservoir.

Immune Escape and Immune Control

Given that one strand of STI research is woven around the idea that immune control of HIV replication might conceivably be enhanced, it is important to consider whether the prodigious ability of the virus to mutate will limit the effectiveness of antiretroviral immune responses (a phenomenon loosely analogous to the development of drug resistance). The second session of the STI workshop focused on this emerging issue.

Immune Escape and STI
Marcus Altfeld, MGH

Marcus Altfeld from Bruce Walker's group at MGH provided an update on their acute infection STI study. The cohort of fourteen individuals were enrolled prior to full seroconversion and treated with HAART until viral load was suppressed to less than 50 copies for at least 8 months. Criteria for restarting HAART after treatment interruption was a single viral load measurement over 50,000 or viral load over 5,000 for three consecutive weeks (an additional criteria of a CD4 drop of greater than 25% was not met by any of the participants).

Altfeld reported data on the status of these individuals as of February 2, 2002. Twelve are currently off HAART, while two are on. Eight of the individuals currently off therapy have maintained viral loads less than 5,000 copies for at least six months – three years of follow up. An additional three have maintained viral loads less than 20,000 for two to four years of follow up and have chosen to remain off therapy. Control of viral load was achieved after a single interruption in some participants; others required two or three interruptions. In all these cases, an increase in the magnitude and breadth of HIV-specific CD8 T cell responses was associated with improved containment of HIV viremia. Of the two individuals still receiving HAART, one is scheduled to undergo further STIs while the other has not demonstrated any evidence of improved viral load despite repeated interruptions and is considered to have failed to benefit from the STI approach utilized in this study. All participants have maintained their CD4 counts and only one case of drug resistance has been documented (reported on by Cecile Tremblay later in the workshop).

Overall, Altfeld felt that these data supported the hypothesis that STIs could provide an auto-immunization effect, at least in the setting of acute infection. He contrasted these results with those from the Swiss-Spanish Intermittent Treatment Trial (SSITT), wherein only 23 out of 133 chronically infected participants reduced their viral load to less than 5,000 copies after undergoing a series of two week STIs (seven of whom had viral loads this low prior to ever starting HAART). Speculating as to the reasons why outcomes might be different in chronic infection, Altfeld cited the preservation of HIV-specific CD4 T cell responses and the relative homogeneity (sameness) of the viral population in treated acute infection (Altfeld 2001), as well as the fact that STIs in chronic infection may redistribute and expand pre-existing CD8 T cell responses from the lymph node rather than induce new virus-specific CD8 T cell responses (Altfeld 2002).

STI Immune Escape in the SIV Model System
Genoveffa Franchini, NCI

Veffa Franchini has studied viral escape from cellular immune responses in SIV-infected macaques. Franchini outlined some of the methodologies employed: key among them is the tetramer assay, which can identify virus-specific CTL based on the exact epitope (basically a tiny slice of a viral protein) that they recognize. Mutations in the virus can alter the epitopes that CTL are targeting, leading to a loss of recognition. Since virus-infected cells are marked for elimination based on the expression of viral epitopes to CTL, such immune escape mutations may be critical in allowing persistent viral replication in the face of an ongoing immune response.

Franchini focused on two well characterized epitopes from SIVmac251: one is a slice of the *tat* protein known as *tat* SL8, the second is from the *gag* protein and called *gag* CM9 (the numbers refer to the number of amino acids that make up the epitope, the letters refer to the amino acids at either end e.g. *tat* SL8 is made up of eight amino acids, with a serine on one end and a leucine at the other). Two cohorts of macaques – acutely and chronically infected – were analyzed for evidence of mutations in these epitopes. The animals were in trials of short course antiretroviral therapy combined with the NYVAC vaccine (with or without additional low-dose IL-2) that Franchini had described in her

earlier presentation. In the acutely infected cohort, one macaque showed evidence of an escape mutation in gag CM9 that severely reduces the ability of CTL to bind to the epitope. In chronic infection, evidence emerged for progressive selection of mutations that initially reduced the ability of CTL to bind the epitope and then, after further amino acid changes, abrogated binding entirely. Franchini felt that given the amount of viral diversification that occurs over the course of infection, such escape was not unexpected.

CTL Escape
Philip Goulder, MGH

Philip Goulder from MGH started his talk on immune escape with a critical observation: that in order to fully understand CTL responses researchers need to sequence the virus from the people who's CTL are being analyzed (autologous virus). Currently, analyses are typically based on consensus viral sequences derived from relatively old HIV isolates, which may be very different from the viruses individuals are infected with today. Goulder also cited the difficulty of tracing the source of a transmitted virus, which the MGH team has addressed by studying mother-infant pairs (Goulder 2001).

Goulder reviewed several observations regarding the role of escape from CTL responses, stressing that all CTL may not be created equal – targeting of certain epitopes may be critical, and mutations in those epitopes may be enough to cause loss of control of viral replication and disease progression. Conversely, escape mutations in less important epitopes may be inconsequential. In support of the idea that the ability to respond to particular epitopes is important, Goulder cited data showing that certain class I HLA genes are associated with protection from infection and disease progression (e.g. B58, B27, B57, B51 and A11) while others are associated with susceptibility and rapid disease progression (A68, B35, B45 and B53). Class I HLA genes produce the specialized molecules (present on all human cells except red blood cells) which capture and present epitopes to CTL, and the structure of these molecules (and thus their ability to present epitopes) is dependent on the HLA genes a person inherits from their parents.

Goulder used the example of an epitope from HIV's *gag* protein (known as KK10) to show how escape from CTL can evolve over time. KK10 is presented by the HLA B27 molecule and is commonly targeted in people who have this HLA type. The epitope is believed to be a functionally important part of the *gag* protein and thus cannot easily mutate without compromising HIV's ability to replicate. Goulder showed that three mutations are required in order for this epitope to escape from CTL (for many epitopes, one mutation is sufficient), and suggested that the additional mutations are "compensatory" – i.e. they help preserve the function of *gag* in the presence of the escape mutation. Similar compensatory mutations are sometimes seen in the setting of antiretroviral drug resistance.

Goulder also outlined the problem of "original antigenic sin" in HIV infection. First described for CTL by Nobel Prize winner Rolf Zinkernagel, original antigenic sin refers to the ability of some mutated epitopes to stimulate the activation and division of CTL, despite the fact that these same CTL can no longer kill infected cells expressing the

mutant epitope. As a possible example of original antigenic sin in HIV infection, Goulder cited the case of a child whose virus developed a CTL escape mutation that was associated with an increase in viral load to over 500,000 copies. Rather than disappear, the CTL targeting the original, unmutated epitope also expanded in parallel with the viral load increase, strongly suggesting that the mutant epitope could trigger expansion of the CTL even though its killing ability had been negated by the escape mutation. Goulder noted that it will likely be very hard, if not impossible, to trigger new CTL responses to a mutated epitope associated with original antigenic sin.

Goulder also believes that this phenomenon may be responsible for the conflicting results obtained by investigators attempting to correlate CTL responses with viral load in untreated HIV infection – a negative correlation may be seen if escape has not occurred, but in the case of original antigenic sin-type escape, the correlation is likely to be positive. Goulder's research team is continuing to study the consequences of CTL escape, particularly the transmission of escape mutations from mother to child. His group is also conducting an STI study in children born with HIV infection in Durban, South Africa.

Immune Control in SIV and HIV Infection
Franco Lori, Research Institute for Genetic and Human Therapy (RIGHT)

Franco Lori was the lead author on the seminal *New England Journal of Medicine* paper describing the "Berlin Patient," an individual who was treated for eight months during acute HIV infection and maintained control of viral load after interrupting therapy. Lori reviewed his randomized trial of STIs in acute SIV infection (Lori 2000), which demonstrated that repeated STIs (three weeks on therapy/three weeks off) led to control of viremia after treatment withdrawal. In contrast, a control group of macaques that were continuously treated did not control viral load when their antiretroviral treatment was withdrawn. Echoing results seen in humans in the MGH cohort, restriction of viremia was associated with potent virus-specific CD4 and CD8 T cell responses (as measured by RIGHT's intracellular cytokine staining assay). Lori attempted a similar study in macaques chronically infected with the pathogenic isolate SIVmac251, but there was no evidence of control of viral load rebound in this setting. This finding led Lori and the co-director of RIGHT, Julianna Lisziewicz, to explore the potential of therapeutic immunization in the chronically infected macaques. RIGHT have developed a DNA vaccine (christened Dermavir) containing an almost complete viral genome, conjugated to polyethylene glycol, which targets the construct to dendritic cells (Lisziewicz 2001). The vaccine is applied directly to an exfoliated patch of skin. Administration of Dermavir to three macaques (during an extension of the chronic infection STI study) led to reductions in viral load rebound during the three week "off" periods. The RIGHT team is now developing Dermavir for a phase I study in HIV-infected individuals.

Open Group Discussion

The group discussion provided an opportunity for researchers to address additional topics, and featured the first discussion of the potential role of antibodies in improving immune control of HIV.

John Moore, Cornell University

The redoubtable antibody expert John Moore posed the question: "Do Antibodies Really Suck?" Moore reminded workshop participants that monoclonal antibodies exist (currently b12, 2G12, 2F5 and 4E10) which can neutralize a broad range of HIV isolates. The reason that these antibodies are rare relates to the poor immunogenicity of HIV's sugar-coated envelope. Moore cited data showing that passive transfer of the monoclonal antibody b12 can protect macaques from SIV infection, and suggested that infusions of antibodies could be used as an adjunctive therapy during STIs. In support of his proposal, Moore noted that the monoclonal antibodies are relatively inexpensive and have a long half-life of around 14 days. Annette Oxenius from the UK reported that this type of study is being considered by the researcher Hermann Katinger, and Kendall Smith proposed that a trial could be conducted at Cornell University.

David Montefiori, Duke University

While most analyses of immune responses in STI studies have focused on CD4 and CD8 T cells, David Montefiori has taken a different tack and investigated virus-specific antibody responses. Montefiori was interested in ascertaining whether STIs increased the magnitude of the HIV-specific antibody response and/or enhanced the ability of antibodies to neutralize the virus. He noted that use of HAART in chronic infection typically leads to a waning of the antibody response, while treatment of acute infection is associated with a lack of maturation of the antibody response (maturation refers to a shift from IgM- to IgG-type antibodies and the affinity maturation of the latter). Montefiori reported data derived from two cohorts: the MGH acute infection group (described earlier by Marcus Altfeld) and a group with chronic infection participating in an STI study at Duke. One individual in the MGH study developed a strong neutralizing antibody (NAb) response against his autologous virus, and this was associated with control of viral load off HAART. Virus-specific CD8 T cell responses were very weak at this time, suggesting a major functional role for the neutralizing antibodies. The neutralizing activity was highly strain-specific; only five of 50 additional primary HIV isolates tested could be neutralized by these antibodies. Montefiori identified two other individuals in this study whose antibodies displayed some degree of neutralizing activity against their own virus, while six individuals who are controlling viral load off therapy show no evidence of NAbs.

In the Duke study, Montefiori was surprised to find that after three STIs, 7/8 participants developed antibodies that could neutralize autologous viral isolates (sampled at the same time as the antibodies) in standard neutralization assays. Such activity is rarely seen in chronic infection, where antibodies typically only neutralize viral isolates sampled several months earlier – in other words, neutralizing activity lags several months behind, as if the immune response is failing to keep pace with the virus. But, despite the appearance of NAbs, none of the Duke study participants were able to control their viral load when HAART was temporarily suspended. In an attempt to explain this finding, Montefiori employed a TCID50 reduction assay that quantifies the amount of virus that

the antibodies are capable of neutralizing. In the MGH study participant whose NAbs were associated with control of viral load, a significant reduction in the amount of antibody required to block the same amount of virus had occurred over time. In contrast, this effect was not seen in chronically infected individuals. Montefiori hypothesized that these differences may relate to the more complex viral quasispecies that evolves in chronic infection, along with defects in affinity maturation (a process by which B cells usually generate more effective antibodies over time). He also noted that the TCID50 reduction assay appears more predictive of beneficial antibody responses than standard neutralization tests.

Danny Douek, Vaccine Research Center, NIH

HIV's tropism for CD4 cells is unusual among viruses, and raises the issue of whether the HIV-specific CD4 cells may be preferential targets for infection. Danny Douek presented a first look at this question, isolating HIV-specific and CMV-specific CD4 cells based on interferon-gamma production and then assessing the level of infection by PCR (which can find a single copy of HIV's *gag* DNA in a cell). Douek reported that in all 12 individuals he studied, HIV-specific CD4 cells were more than two to five times more likely to be infected than CD4 cells specific for CMV or any other antigen. Assuming one copy of HIV *gag* DNA per cell, the actual percentage of infected cells was low, representing 0.1 – 1% of all CD4 cells (Douek noted that this percentage will be even lower if there is more than one copy of HIV DNA per cell). In an attempt to ascertain whether HIV-specific memory CD4 cells could become infected throughout the course of infection – as opposed to infection occurring primarily in activated naïve CD4 cells that then remain infected after maturing into memory cells – Douek studied four individuals undergoing an STI. He found that from 1.5% to 56.8% of infected cells were HIV-specific at the peak of viral load rebound. This observation led Douek to caution that STI may in some circumstances add "fuel to the fire" by activating HIV specific CD4 cells that then become targets for infection (Douek 2002).

Luis Montaner, Wistar Institute

Luis Montaner outlined the case for continued investigation of the potential for STIs to auto-immunize individuals with chronic HIV infection. The disappointing results of the Swiss-Spanish Intermittent Treatment Trial (SSITT) have led some researchers to conclude that auto-immunization is impossible in chronic infection, but Montaner made a number of points regarding SSITT:

- It used a fixed schedule of three two-week interruptions followed by eight weeks on therapy, and people who did not re-suppress to <50 copies of viral load within eight weeks were removed from the study
- The criteria for success after a final 12 week treatment interruption was control of viral load to <5,000 copies, which may be overly strict given the recent changes to treatment guidelines

Montaner suggested that a more appropriate endpoint might be a decline in viral load setpoint compared to the pre-HAART baseline. He also highlighted the considerable heterogeneity that exists in terms of when and how viral load rebounds during STI, noting that 15/23 of the SSITT participants that controlled to <5,000 copies did not rebound at all during the two-week interruptions. In terms of defining safe CD4 cell levels off HAART, Montaner has observed that CD8 cells often expand during STIs and cause a decline in CD4 percentage even though the absolute CD4 count remains stable, raising the question of which marker should be utilized when designing studies.

Montaner concluded by describing the design of a randomized study of STIs in chronic infection, currently ongoing at the Wistar Institute. The STI periods are extended over time, with the first lasting two weeks, the second four weeks and the third six weeks. The timing of the STIs is individualized, occurring only when viral load has been successfully re-suppressed to <50 copies. The study concludes with an extended interruption designed to assess the viral load set point. The control arm will receive continuous treatment prior to the extended interruption. As anecdotal examples of the type of trend he is seeing, Montaner presented data from two participants, one from each arm. The first individual in the STI arm has experienced a downward trend in viral load rebound over time, peaking at around 8,000 copies during the final extended interruption. Montaner reported that this individual remained off therapy at the time of the workshop. In comparison, an example from the control arm manifested a peak viral load of over 100,000 copies during the extended interruption period and resumed HAART as a result. The study has enrolled a total of 42 participants and should be completed later in 2002.

Rodney Phillips, Peter Medawar Building for Pathogen Research, University of Oxford

The immunology from the SSITT study was conducted by the UK's Rodney Phillips who reviewed the results and, while dismissing any auto-immunization effect, presented a number of potentially important findings. Phillips is the first researcher to report an inverse correlation between the pre-HAART viral load and the frequency of HIV-specific CD8 T cells detectable (using an ELISpot assay for interferon-gamma production) after HAART initiation. The same held true for HIV-specific CD4 T cells measured by the same assay. Phillips suggested that HIV-specific T cells are typically a mixed population, comprising both effective cells with good antiretroviral activity and ineffective cells. (The interferon-gamma based ELISpot may detect both populations in the setting of untreated infection, but the HIV-specific T cells persisting after HAART initiation could potentially reflect the more effective subset.)

Overall, Phillips felt that the STI approach used in SSITT did not meaningfully boost the effective T cell response. He was able to show some broadening of the CD8 and the CD4 T cell response in terms of the number of epitopes targeted, and the frequency of HIV-specific CD8 T cells as assessed by ELISpot increased from 300 Spot-forming cells (SFC) at baseline to 2000 SFC at the end of the study (week 52). However, viral load setpoints after the extended treatment interruption at the end of SSITT protocol were only an average of 0.4 – 0.5 logs below the pre-HAART baseline. One workshop participant

who dissented from this negative view of the SSITT results was Steve Deeks from San Francisco General, who noted that from a clinician's perspective, this degree of viral load decline is typically considered significant and is associated with clinical benefit. Deeks asked Phillips why SSITT's principal investigator (Bernard Hirschel) felt that the study had buried the auto-immunization hypothesis. Phillips said that in chronic HIV infection the boost seen did not produce lasting control; indeed in follow-up many patients viral control deteriorated. There is no evidence that the boost on cellular responses was sustained. The full immunology evaluation is now in press at PNAS.

Jeff Harris, Gladstone Institute of Virology & Immunology

Jeff Harris provided the final look at STIs in chronically infected individuals with suppressed viral loads. Harris and colleagues at the Gladstone Institute in San Francisco are conducting a study employing repeated cycles of an eight-week STI followed by re-treatment with HAART for six months (or until viral load remains <50 copies for three months). A broad range of parameters are being investigated, including:

- Thymic function as assessed by both computerized tomography of thymic tissue and T cell receptor rearrangement excision circles (TRECs). (The Gladstone group is interested in whether thymic function correlates with the ability to respond to STIs)
- Naïve and memory T cell phenotyping
- T cell repertoire
- HIV-specific T cell responses as measured by intracellular cytokine flow cytometry (CFC)
- Quality of life
- HIV drug resistant genotype
- Size of the viral reservoir

Thirteen individuals are currently enrolled and have undergone one to three STI cycles. In most cases, there is no evidence of control of viral load. Two participants have shown evidence of approximately a log diminution of viral load over consecutive cycles, but no clear correlate of response has yet emerged. HIV-specific CD4 and CD8 T cell activity measured by CFC appears equivalent in both responders and non-responders, and Harris suggested that additional markers are needed that better reflect function (or as he pithily put it: "we should quit measuring just interferon-gamma responses and move on"). Harris also echoed Luis Montaner's observation that, as a consequence of STIs, CD4 percentages can trend down while the absolute counts rise. Two cases of drug resistance have developed in this study, which Harris discussed in more detail later in the workshop. Concluding with the reminder that this study is a work-in-progress using one approach, Harris wondered aloud about the many potential on/off schedules that could be employed in STI studies and how they might influence outcome.

Resistance in Salvage Patients

Overview
Veronica Miller, Forum for Collaborative HIV Research

Veronica Miller introduced the topic of STI in "salvage" therapy. The goals of STI in this setting are distinct from those previously discussed. The approach seeks to ascertain whether drug toxicity can be reduced, quality of life improved and response rates to salvage regimens raised, without the risk of CD4 decline and clinical progression outweighing the benefit. Virologically, the hope is to reduce the population of drug-resistant virus, but as Miller pointed out this result has not consistently been achieved and the risk/benefit of shifting to a "wild-type" viral population has not been established. The field was in fact launched by a pilot study Miller conducted several years ago while at the Klinikum der Johann Wolfgang Goethe-Universitat in Frankfurt, Germany (Miller 2000).

Randomized STI in Patients with MDR HIV: Role of Resistance and Changes in Resistance in Response to Re-initiation of Treatment
Stephanie Dominguez, Pitié-salpetrière Hospital, Paris

One of the more promising studies of STI in the salvage setting was presented last year at the 8th European Conference on Clinical Aspects and Treatment of HIV Infection in Greece. Conducted under the aegis of the ANRS in France, the GigHAART study investigated the use of an 8-week STI in individuals with MDR virus and very low CD4 counts (an average of 27). Participants in the study initiated a regimen of 6-9 antiretrovirals, either immediately (34 individuals) or after the STI (34 individuals). Stephanie Dominguez reported the results as available at week 12 on treatment of the study, which show an average 1.9 log drop in viral load in the STI group compared to 0.4 logs in the immediate arm.

Dominguez presented an analysis of these data based on both drug levels and whether the viral population shifted from drug-resistant to wild-type during the treatment interruption. Individuals with low drug concentrations experienced an average decline in viral load of 0.4 log, whether they switched drugs immediately or after an STI. Participants in the STI arm with normal drug concentrations showed a viral load reduction of 2.6 log if their virus shifted to wild-type, compared to a 2.0 log drop if resistance mutations were persistently detectable. In terms of safety, there was one fatal case of lymphoma, one diagnosis of PCP and one dual diagnosis of cryptosporidiosis and microsporidiosis in the immediate GigHAART group, compared to one case of TB and one of recurrent herpes zoster in the STI group.

Dominguez was questioned about the differences in outcome seen in this trial compared to that of Ruiz and colleagues. The potential confounding factors include the widely divergent treatment regimens and the baseline CD4 counts, which averaged around 350 cells in the Spanish study. The duration of the STI was also longer, at 16 weeks versus 8 weeks used in the GigHAART trial. It was also pointed out that follow-up on both studies

is of relatively short duration, so longer term results will need to be analyzed before any conclusions can be drawn.

Open Group Discussion

The discussion period was limited, but Francois Clavel briefly described the design of a salvage study that does not use STIs. The intent of Clavel's trial is to employ an antiretroviral regimen that is relatively easy tolerate, but that selects for resistance mutations that decrease the fitness of the virus. Seminal work from Steve Deeks and Mike McCune has shown that less fit virus appears less likely to cause a decline in CD4 counts, and Clavel hopes to duplicate this effect using a regimen of 200mg indinavir, 100mg of ritonavir and 150mg of 3TC, all taken twice daily. Some workshop participants questioned Clavel as to whether this really would qualify as a well-tolerated regimen, but supported the overall rationale behind the approach.

Resistance in Virologically Suppressed Patients

Overview
Veronica Miller

The second day of the STI Workshop began with a review of a critical safety issue: the development of drug resistance as a result of treatment interruptions in people who were previously virologically suppressed on HAART. Veronica Miller introduced the topic, noting that this has long been a concern regarding STIs. In fact, many researchers have expressed surprise that drug resistance has so far been a relatively uncommon occurrence in STI trials. Miller cited the concern that cycling on and off HAART will lead to periods of suboptimal levels of drugs in the body, potentially selecting for drug-resistant virus. She also noted that, due to differences in half-life, some drugs may present more of a risk than others. Echoing Mark Dybul, Miller cited the potential for tension between individual and public health benefits, since STIs could theoretically increase transmission of drug resistant HIV.

Risk of Resistance as a Result of Long- or Short-Cycle STI in Chronically Infected Patients
Mark Dybul, NIH

Mark Dybul presented data on the emergence of resistance as a result of STIs in people with chronic HIV infection. He began by summarizing current knowledge regarding the pharmacokinetics of antiretroviral drugs. NRTIs and PIs are typically cleared from the system within a day, although both 3TC and ddI can persist inside cells (intracellularly) for around three days. The NNRTI drugs nevirapine and efavirenz take much longer to be eliminated: around five and six to seven days, respectively.

Dybul went on to discuss cases of drug resistance that have developed during the NIH's "long-cycle" STI study. This protocol employed fixed periods of two-months on HAART followed by a month-long interruption. Four of eight individuals in this study developed

resistance to efavirenz within four to six cycles. In two of these cases, genotypic resistance to 3TC also emerged. Out of 18 participants in the same long-cycle STI trial that were using PI-based combinations, only two developed signs of resistance, also to 3TC. The clearest risk factor that emerged was stopping drugs when the viral load was still detectable, which represented 5/6 of the total cases (for some individuals, the two month on-therapy period was not sufficient to re-suppress viral load to <50 copies). Dybul also reported that early emergence of drug resistance can be associated with "archived" mutations that were already present at levels too low to be detected, which turned out to be the situation for two the three individuals whose virus developed 3TC resistance.

Dybul derived a number of take-home messages from these data:

- In this setting, treatment should not be interrupted until viral load is re-suppressed to <50 copies
- NNRTIs and 3TC should be used with caution
- Archived drug resistance can exist in individuals despite a viral load of <50 copies at study entry, and these mutations may re-emerge during STIs

Resistance as a Result of STI in Chronically Infected Patients
Jeff Harris, Gladstone Institute of Virology & Immunology

Jeff Harris proffered details regarding the development of drug resistance in the Gladstone Institute STI trial, as discussed in the immune control session. At least two individuals have developed resistance to 3TC over the course of the study to date. The first had a history of antiretroviral therapy dating back to May 1996 when AZT/3TC was initiated as dual therapy. AZT was replaced by d4T two months later, and the protease inhibitor indinavir added in August 1996. The 184V mutation emerged in this individual at the end of the second STI, and Harris was uncertain whether it represented newly developed resistance or the reappearance of an archived virus Upon restarting treatment, HIV replication was re-suppressed to undetectable levels by week six, followed by an uptick to around 1,000 copies. Surprisingly, the 184V mutation could no longer be detected. Nevertheless, abacavir was substituted for 3TC and viral load again declined to <50 copies. . In the second case, the 184V mutation was detectable at the time of the first STI, but as viral load rebounded the proportion of mutant virus declined compared to wild-type.

Harris raised a number of questions regarding the assessment of resistance in the setting of STI:

- When is the best time to test for resistance? (e.g. early, after stopping therapy vs. later, immediately after restarting)
- Does the relative fitness of the resistant virus matter?
- Does the intra-individual variability of drug pharmacokinetics affect the risk of developing resistance?

- Could staggering the withdrawal of individual drugs based on their pharmacokinetic profile help prevent resistance?
- What is the role of the immune response in controlling resistant virus?

Evolution of M184V in STI Cycles: Characterization of Emerging Viral Populations During STI
Javier Martinez-Picado, irsiCaixa Foundation

Javier Martinez-Picado described the emergence of 3TC resistance in a Spanish STI trial. The study enrolled 12 people with chronic HIV infection who had maintained viral load at <50 copies for over two years and a CD4/CD8 ratio of >1 for at least 6 months. Treatment was interrupted for 30 days or until viral load increased to over 3,000 copies on two separate measurements. HAART was then resumed for at least 90 days, with viral load required to reach <50 copies. This protocol was then repeated for a total of four STI cycles. Martinez-Picado reported that two participants developed the 184V mutation that can confer 3TC resistance. In one case, an individual receiving d4T, 3TC and indinavir developed the mutation during the second and third STIs. However, viral load was re-suppressed to <50 copies without a change in regimen. The second case involved an individual on the same regimen who developed 184V during the third STI. Martinez-Picado's conclusion was that drug-resistant viruses can be selected during STIs, especially when only one mutation results in high levels of phenotypic resistance (as seen with the M184V mutation and 3TC). With each STI there was a stepwise increase in the number of viral clones containing the M184V mutation that paralleled a decrease in drug susceptibility. Martinez-Picado believes that repeated STIs led to an increase in the size of the resistant virus population over time, despite the fact that the emergence of the 184V mutation is associated with a decrease in the replication capacity of these specific virus isolates. Martinez-Picado's group has recently published a mathematical model that supports this hypothesis (Martinez-Picado 2002).

Virus Evolution and Development of Resistance During STI in Patients with Acute Infection
Cecile Tremblay, MGH

Cecile Tremblay is a collaborator on the MGH acute infection study described earlier in the workshop by Marcus Altfeld. Tremblay has studied the cohort for evidence of viral evolution (including the evolution of drug resistance) and changes in the size of the HIV reservoir over the course of repeated STIs. One individual displayed the 184V mutation at the first STI. This person had initiated treatment with AZT, 3TC and nelfinavir, but required a switch to d4T, 3TC and indinavir due to AZT side effects (anemia and fatigue). After the first STI, the regimen was changed again, to efavirenz, abacavir and 3TC and viral load was successfully suppressed to <50 copies. Tremblay hypothesized that resistance had developed during the first few days after stopping treatment, perhaps due to the long intracellular half-life of 3TC. In terms of the size of the HIV reservoir, Tremblay could not detect an increase over the course of the study.

Open Group Discussion

Jose Gatell, University of Barcelona

Gatell gave a brief round-up of resistance data from four STI studies that included a total of 71 treatment-naïve participants. Treatment regimens included two NRTIs and a single PI, with or without adjunctive therapies (hydroxyurea, IL-2 or mycophenolate mofetil). Out of 224 samples, no cases of genotypic resistance to antiretrovirals have been detected. There have also been no cases of virologic failure reported subsequent to treatment reinitiation. Gatell noted, however, that NNRTIs were not used in any of these trials, and that STIs were never conducted before viral loads reached <50 copies.

Catherine Fagard, University Hospital, Geneva

Resistance data from the SSITT study was reported by Catherine Fagard. Ten participants showed evidence of resistance mutations in reverse transcriptase, and in nine of these cases it was either 184I or 184V. One study participant had a mutation associated with resistance to the protease inhibitor nelfinavir. This was the only case that required a change in regimen in order to re-suppress viral load to undetectable levels.

Charlie Gilks, Liverpool School of Tropical Medicine

Session moderator Veronica Miller asked Charlie Gilks for his thoughts on the resistance data, and what it might mean for the use of STIs in resource-poor setting. Gilks responded that it was difficult to reach conclusions, since the presentations represented detailed analysis of individual cases as opposed to studies of the impact of STIs on drug resistance at the population level. He noted that it was as yet unknown whether STIs increase the risk of developing drug resistance compared to the continuous use of antiretrovirals, which is also associated with the development of drug resistance in a significant number of individuals. He stressed the importance of assessing the behavioral implications of STIs, particularly in terms of whether they might be associated with increased adherence during periods of drug therapy. Gilks also felt it would be important to understand the impact on transmission (including transmission of drug resistant viruses) and overall survival. He pointed out that STIs are very attractive both at the policy and individual level, for reasons of cost and convenience. Gilks is currently involved in the DART study, which plans to investigate the use of STIs in resource poor settings. The design of this trial was discussed in the closing session of the workshop.

Drug-Sparing Strategies/Toxicity Reduction

Overview
Mark Dybul, NIH

Mark Dybul introduced the final topic of the workshop with the observation that it represented the least controversial aspect of the use STIs: to simply minimize the time on antiretroviral therapy and thus reduce the burden of drug-associated toxicities. While

many researchers - Dybul included - remain highly skeptical about the potential for STIs to produce an auto-immunization effect in chronic infection, the notion that interruption of antiretroviral therapy can be employed as part of a clinical management strategy is gaining wider acceptance.

Assays to Evaluate HAART-Related Toxicities
Kathy Mulligan, UCSF-San Francisco General Hospital

Kathy Mulligan, a researcher in endocrinology and metabolism, shifted the discussion from the theoretical to the practical with an overview of assays for measuring drug toxicity. Mulligan listed a number of important parameters to measure in people on HAART, including:

- Glucose metabolism
- Lipids
- Fat distribution
- Bone mass
- Lactate

Mulligan emphasized the importance of distinguishing between acute and chronic toxicities and assessing the sum of risk factors for any given complication. Some assessments may be easier than others, for example cardiovascular disease risk factors are well established and can be assessed using lipid measurements (including fasting triglycerides, HDL, LDL and total cholesterol) and other biochemical markers (e.g. Lp[a], hsCRP, homocysteine, fibrinogen, PAI-1 and IPA antigen). Additional non-invasive techniques can also be employed to look for evidence of arteriosclerosis (artery wall thickening), such as carotid intima media thickness and brachial artery reactivity. Mulligan reminded workshop participants that coronary heart disease risk is affected by many factors beyond just lipids, and that many of these factors are potentially modifiable.

Problems with glucose metabolism can be identified with a variety of techniques. Basic measures include fasting glucose and insulin levels, which can be supplemented with the oral glucose tolerance test, c-peptide levels and pro-insulin levels. The “gold standard” tests are the intravenous (IV) glucose tolerance test and the IV insulin resistance test. Mulligan pointed out that both of these latter techniques are highly intensive (they require inpatient administration in the presence of a doctor) and are thus inappropriate for use in large-scale clinical trials. Looking at the available data, Mulligan reported that impaired glucose tolerance is relatively common in HIV, but appears to be exacerbated in the setting of lipodystrophy (Hadigan 2001). A recently published study found that administration of the protease inhibitor indinavir to HIV-negative individuals is associated with an acute impairment of glucose metabolism: insulin-mediated glucose uptake decreased 34% after a single dose of the drug (Noor 2001). Mulligan called this an emerging story, and it remains unclear whether this effect is common to all protease inhibitors. Other non-drug factors that may be associated with metabolic disturbances include generalized obesity, excess visceral fat, lipoatrophy, liver disease, and family history.

Moving on to techniques for assessing changes in fat distribution, Mulligan described several common approaches. Dual energy X-ray absorptiometry (DEXA) scanning is gaining in popularity due to its relatively low cost, wide availability and ease of use. Originally designed solely for measuring bone mineral density, DEXA can also be used to look at regional fat. Limitations are that it cannot distinguish between visceral and subcutaneous fat in the abdominal region and has difficulty characterizing dorsal fat pads or "buffalo humps." More detailed analyses of fat distribution can be conducted with the more expensive and intensive techniques of magnetic resonance imaging (MRI) and computerized tomography (CT). The least technical methodology is what Mulligan called "good ol' anthropometry," which employs measurements of body dimensions and parameters such as skinfolds. While Mulligan feels anthropometry can be useful if performed carefully and repeated longitudinally, she noted that DEXA can often be easier and less time consuming (although its cost, while cheap compared to MRI and CT, is still considerably greater than anthropometry).

Mulligan outlined several continuing challenges for researchers, including measuring changes in facial fat, breast size and buffalo humps in a straightforward and reproducible manner. The use of DEXA for measuring bone mineral density in people with HIV is also in its infancy. Lactate measurements are under investigation for their potential to provide an early warning of lactic acidosis, but their utility is still uncertain. Mulligan cited new data indicating that frozen lactate samples are stable for at least a year if collected and processed properly, which suggests that this technique can be employed in trials.

In closing, Mulligan suggested that a number of assays provide useful information on HAART-related metabolic toxicities and could be gainfully employed in STI studies. She also offered a reminder of the hard clinical outcomes that should be evaluated when looking at toxicity reduction strategies, including coronary artery disease, bone fractures, avascular necrosis and lactic acidosis.

NIH Studies
Mark Dybul, NIH

Mark Dybul reviewed toxicity data from STI studies conducted at the NIH, starting with the seven day on/seven day off "short-cycle" STI study (Dybul 2001). Eight individuals have been followed for 68-100 weeks and triglycerides have fallen from an average of 270 to 160 by week 80. Dybul reported that triglyceride levels tended to decline over the first 24 weeks and subsequently reached a stable plateau for the duration of follow-up. Cholesterol levels have shown the same pattern, although a few individuals had levels edge back up after an initial decline. In the long-cycle study (two months on/one month off), triglycerides declined less dramatically, from around 180 at baseline to 140 at week 48. Participants in this study who were randomized to receive continuous HAART showed an ongoing increase in triglyceride levels. Dybul concluded with the observation that, depending on the approach, STIs may be able to slow or prevent toxicities but not necessarily reverse them.

Immunosuppressants as HAART-Sparing Strategies
Jose Gatell, University of Barcelona

Switching to drug-sparing strategies, Jose Gatell presented data on the use of immunosuppressant drugs in the context of STIs. The rationale is, that drugs that inhibit lymphocyte proliferation, such as hydroxyurea and mycophenolate mofetil (MPA), could theoretically limit the number of activated CD4 cells available to support HIV replication. Gatell's first study randomized participants to receive HAART or HAART with hydroxyurea. The design employed several cycles of month-long treatment interruptions followed by re-treatment until viral load was controlled to <50 copies. The last interruption was open ended in order to assess the viral load set point in the absence of HAART. Hydroxyurea treatment was interrupted during the first three STIs, but was subsequently used continuously, including during the open ended HAART interruption at the end of the study. Gatell reported that 8/9 of the people in the hydroxyurea arm maintained a viral load of <5,000 copies after twelve weeks off HAART, compared to only 4/10 in the control group, a statistically significant difference. HIV-specific CD4 T cells directed against p24 were assessed by lymphoproliferative assay, and while these responses were detectable in participants from both groups, there was no clear correlation with control of viral load. CD4 cell counts remained above baseline in all participants, with a slight decline compared to the peak value on HAART.

Gatell next described a similarly designed study that administered MPA (250mg twice-daily) in place of hydroxyurea (although in this case, MPA was given continuously during all HAART interruptions). So far, only one STI has been completed by participants in this trial. Thirty days after stopping HAART, 3/9 individuals receiving MPA showed a rebound in viral load levels, compared to 6/6 in the HAART alone group. Additional STI cycles are now underway. Cal Cohen questioned whether prolonged therapy with drugs like hydroxyurea and MPA would ultimately be less toxic than HAART, and this remains an open question. Linda Grinberg reported that FAIR is funding studies of this type of strategy that will hopefully help provide answers.

STACCATO
Catherine Fagard, University Hospital, Geneva

Catherine Fagard described a recently commenced international trial (involving Australia, Canada, Switzerland and Thailand) of drug-sparing treatment strategies. Participants will be randomized to one of three arms: continuous HAART, seven days on/seven days off, or CD4-driven HAART (wherein treatment is only given when the CD4 count drops below 350, and interrupted if there is a sustained rise above that threshold). The criteria for treatment failure include a viral load >500 copies at any time, in the first two arms, or after 12 weeks of HAART in the CD4-driven arm. The study endpoints -- amount of drugs used, CD4 count, viral load, incidence of hyperlactemia and lipodystrophy -- will be evaluated at weeks 96 and 108. Fagard also outlined several substudies that the research team is conducting. One will look at effects of intermittent HAART on transmission in serodiscordant Thai couples. Another substudy will investigate the utility

of a cheap p24 antigen assay as a substitute for viral load monitoring. Recruitment for STACCATO began in January 2001 and is scheduled to be completed by 2005.

Italian PART Study
Lucia Palmisano, Istituto Superiore di Sanita

In Italy, there is an ongoing study of pulsed antiretroviral therapy (PART). Lucia Palmisano presented details on the design and current status of PART, which has so far enrolled 200 of 600 planned participants. Criteria for entry include a viral load <400 copies for at least six months and a CD4 counts >350 (and no history of a CD4 count <100). The trial compares continuous HAART to cycles of three months of treatment followed by lengthening interruptions (the first two STIs last a month, the second two last two months, and the final interruption is for three months). Monitoring of viral load and CD4 counts occurs every three months in the continuous arm and every month in the STI arm. Resistance testing was originally slated to be performed 15 days into each STI, but Palmisano has found that a significant proportion of participants do not show evidence of viral load rebound in the peripheral blood at this timepoint. In these subjects, genotyping is performed at day 30 of the STI. In addition, genotyping is now performed if viral load has declined by less than a log after two months of re-treatment. In recognition of concerns regarding the long half-lives of NNRTIs (discussed earlier in the workshop), the PART design stipulates that nevirapine be stopped three days ahead of other drugs in a combination; efavirenz is stopped six days in advance. In accordance with the manufacturer's instructions, the 14-day "lead in" dose for nevirapine is used every time the drug is restarted. Although data is preliminary, so far all the individuals in the STI arm have re-suppressed viral load to <400 copies after re-initiation of HAART.

DART
Charlie Gilks, Liverpool School of Tropical Medicine

Charlie Gilks gave an overview of the "Developing Antiretroviral Therapy" (DART) trial, which has been in the planning stages for five years and is now slated to start October 1, 2002. The design involves 3,000 participants, evenly divided between two sites in Uganda and one in Zimbabwe. The study employs two randomizations: the first assigning the type of monitoring a participant will receive (either CD4 count and viral load or clinical monitoring only), the second assigning continuous or intermittent therapy. All participants begin with 6 – 12 months of triple drug therapy (two nucleoside analogs with either nevirapine, abacavir or tenofovir), followed by three months on/three months off cycles for those randomized to the intermittent therapy arm. Full enrollment of the intermittent arm will not occur until independent trial steering committee has conducted a detailed open-label evaluation of 3 months off therapy followed by three months on therapy in 100 participants. A 600-person substudy of abacavir hypersensitivity will also be conducted. The primary endpoint for DART is the development of stage clinical disease or death, secondary endpoints include incidence of opportunistic infections, adherence, quality of life and drug resistance. While the hope is that intermittent therapy will provide a substantial clinical benefit in this setting, Gilks emphasized the unknowns associated with the protocol, including the ideal length of STI durations, the potential for

rapid development of resistance to nevirapine, and the dangers of monitoring antiretroviral therapy based on clinical markers alone.

When to Stop?
Joel Gallant, John Hopkins University

Joel Gallant reviewed data on the effects of simply discontinuing HAART, derived from the observational database at Johns Hopkins University. Ninety-one cases of clinician-sanctioned treatment interruption were identified from the database for Gallant's analysis. Out of these individuals, the major reasons for interrupting HAART were starting too early based on new treatment guidelines (40%), non-adherence (16%) and toxicity (17%). HAART was resumed by 26/91 after an average break of 20 weeks; people that chose not to reinitiate have been off HAART for an average of 47 weeks. Gallant outlined several differences between these two groups, which he christened "resumers" and "non-resumers."

	Resumers	Non-Resumers
CD4 count	283	507
Viral load	129,723	22,831
CD4 count nadir	293	431
Peak CD4 count on HAART	680	956
CD4 count change during interruption (cells/yr)	-314	-91

The most common reasons for HAART resumption were a rising viral load (33%), declining CD4 counts (17%) and nervousness about being off therapy (13%).

Based on these data, Gallant and colleagues used a statistical regression technique to calculate the average length of time it would take for the CD4 count to drop into the danger zone of less than 200.

	Resumers	Non-Resumers
Median	0.7 years	3.8 years
Mean	3.4 years	6.7 years

Gallant believes that this may in fact be an underestimate, since the initial CD4 count decline during treatment interruptions is typically steep, but followed by a plateau, and the regression line may be skewed as a result. While Gallant acknowledged the potential shortcomings of the observational data, he suggested that individuals with low baseline viral loads and a good CD4 count increase on HAART are likely to be the best candidates for extended treatment interruptions. However, the increasing trend for deferring HAART is likely to reduce the number of individuals in this category.

Open Group Discussion

Jim Braun, Liberty Medical

Jim Braun briefly discussed results from another observational database, the national GlaxoSmithKline-sponsored CHORUS Project. The analysis was distinct from Gallant's in that inclusion criteria involved an interruption of more than 14 days *and* re-initiation of therapy, so individuals remaining off HAART were excluded. The data spanned a long time period, from August 28, 1996 to July 5, 2001, and included 488 individuals that met study criteria and for whom appropriate laboratory values were available. The main outcome measures involved response to treatment after interruption, with a successful immunologic outcome defined as regaining 90% of the pre-interruption CD4 count and virologic success defined as achieving a viral load of <500 copies. Braun reported that three-quarters of the individuals analyzed experienced a successful immunological outcome, around two-thirds controlled viral load to <500 copies, but only half met both the immunological and virological measures of success. When this analysis was split based on whether viral load was >500 copies at the time of interruption, a divergent pattern emerged: only 50% of those with >500 copies successfully suppressed their viral load after interrupting, compared to 90% of those that were <500 copies to begin with. However, the ability to regain CD4 cells was roughly equivalent, with about three quarters of the individuals in each group reaching 90% of their baseline count.

Closing out the discussion, two additional ongoing STI studies involving IL-2 were mentioned. One is ICARUS, a substudy of a large clinical endpoint IL-2 trial known as ESPRIT. The second study is called TILT. Both intend to investigate whether IL-2 improves the preservation of CD4 counts during treatment interruptions, potentially allowing for longer and safer breaks from HAART.

Wrap Up and Next Steps
Veronica Miller
Alan Landay, Rush Presbyterian Medical Center

Wrapping up the workshop, Veronica Miller and Alan Landay provided a summary of the data presentations and offered suggestions for next steps for STI research. They recapitulated Mark Dybul's introductory separation of STI research into several fields, adding a sub-category for the use of STIs as an "analytical tool" in therapeutic vaccine research. For each field, Landay sketched a vertiginous staircase of next steps:

Field	Next Steps
Therapeutic Immunization & Immune Modulation	• New therapeutic vaccine candidates • Standardization of read-out systems • Immune correlates of success • Researching role of CTL escape • Cytokines and novel adjuvants to help induce new T cell responses • Researching role of neutralizing antibodies, antibody escape • Assays to measure immune responses to autologous virus • Definition of optimal endpoints • Randomized controlled clinical trials
Resistance & Salvage Setting	• Defining role of baseline resistance vs. resistance after STI • Understanding the role of the potential shift to wild-type • Defining best assays to use • Investigate potential for optimizing salvage options
Drug Sparing/Toxicity	• Assessing impact and incidence of resistance development in virologically suppressed individuals • Understanding timing and source of resistant virus • Defining risks and benefits for both public health and individual health • Coordinate storage of samples for future studies • Long term studies to assess impact on metabolic toxicities • Define role of immune suppressants • Evaluation of IL-2 • Further utilization of observational databases

Landay also mentioned a number of areas that the workshop has yet to address, including the use of STI in pediatric HIV infection, the effects of STI in individuals with hepatitis B and C coinfections and the role of emerging fields of genetic research such as proteomics, genomics and pharmacogenomics.

Concluding with some thoughts on where the meeting might be going, Landay suggested a variety of roles that the STI workshop could play including: fostering research collaborations, sharing data, making recommendations for future studies, building consensus and providing guidance on policy issues. The diversity of experience and expertise among workshop participants was cited as rich source for input into all these areas. Thus the workshop as a whole and the energy and excitement it generated stand as a testament to the dedication and commitment of Linda Grinberg, who played the leading role in creating and coordinating the International Workshops on STIs.

References

Altfeld M, Rosenberg ES, Shankarappa R, *et al.* Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. J Exp Med 193(2):169-80, 2001

Altfeld M, van Lunzen J, Frahm N, *et al.* Expansion of pre-existing, lymph node-localized CD8+ T cells during supervised treatment interruptions in chronic HIV-1 infection. J Clin Invest 109(6):837-43, 2002

Appay V, Nixon DF, Donahoe SM, *et al.* HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. J Exp Med 192(1):63-75, 2000

Betts MR, Ambrozak DR, Douek DC, *et al.* Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. J Virol 75(24):11983-91, 2001

Champagne P, Ogg GS, King AS, *et al.* Skewed maturation of memory HIV-specific CD8 T lymphocytes. Nature 410(6824):106-11, 2001

Douek DC, Brenchley JM, Betts MR, *et al.* HIV preferentially infects HIV-specific CD4+ T cells. Nature 417(6884):95-8, 2002

Dybul M, Chun TW, Yoder C, *et al.* Short-cycle structured intermittent treatment of chronic HIV infection with highly active antiretroviral therapy: effects on virologic, immunologic, and toxicity parameters. Proc Natl Acad Sci U S A. 98(26):15161-6, 2001

Goulder PJ, Brander C, Tang Y, *et al.* Evolution and transmission of stable CTL escape mutations in HIV infection. Nature 412(6844):334-8, 2001

Gupta K, Hudgens M, Corey L, *et al.* Safety and immunogenicity of a high-titered canarypox vaccine in combination with rgp120 in a diverse population of HIV-1-uninfected adults: AIDS Vaccine Evaluation Group Protocol 022A. J Acquir Immune Defic Syndr 29(3):254-61, 2002

Hadigan C, Meigs JB, Corcoran C, *et al.* Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy. Clin Infect Dis 32(1):130-9, 2001

Jin X, Ramanathan M Jr, Barsoum S, *et al.* Safety and immunogenicity of ALVAC vCP1452 and recombinant gp160 in newly human immunodeficiency virus type 1-infected patients treated with prolonged highly active antiretroviral therapy. J Virol 76(5):2206-16, 2002

Koup RA, Safrit JT, Cao Y, *et al.* Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 68(7):4650-5, 1994

Koup RA. Virus escape from CTL recognition. J Exp Med 180(3):779-82, 1994a

Lifson JD, Rossio JL, Piatak M Jr, *et al.* Role of CD8(+) lymphocytes in control of simian immunodeficiency virus infection and resistance to rechallenge after transient early antiretroviral treatment. J Virol 75(21):10187-99, 2001

Lisziewicz J, Gabrilovich DI, Varga G, *et al.* Induction of potent human immunodeficiency virus type 1-specific T-cell-restricted immunity by genetically modified dendritic cells. J Virol 75(16):7621-8, 2001

Lori F, Lewis MG, Xu J, *et al.* Control of SIV rebound through structured treatment interruptions during early infection. Science 290(5496):1591-3, 2000

Martinez-Picado J, Morales-Lopetegi K, Wrin T, *et al.* Selection of drug-resistant HIV-1 mutants in response to repeated structured treatment interruptions. AIDS 16(6):895-9, 2002

Miller V, Sabin C, Hertogs K, *et al.* Virological and immunological effects of treatment interruptions in HIV-1 infected patients with treatment failure. AIDS 14(18):2857-67, 2000

Noor MA, Lo JC, Mulligan K, *et al.* Metabolic effects of indinavir in healthy HIV-seronegative men. AIDS 15(7):F11-8, 2001

Ogg GS, Jin X, Bonhoeffer S, *et al*. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science 279(5359):2103-6, 1998

Ostrowski MA, Justement SJ, Ehler L, *et al.* The role of CD4+ T cell help and CD40 ligand in the in vitro expansion of HIV-1-specific memory cytotoxic CD8+ T cell responses. J Immunol 165(11):6133-41, 2000

Quinn TC, Wawer MJ, Sewankambo N, *et al.* Viral Load and Heterosexual Transmission of Human Immunodeficiency Virus Type 1. N Engl J Med 342:921-929, 2000

Rosenberg ES, Altfeld M, Poon SH, *et al.* Immune control of HIV-1 after early treatment of acute infection. Nature 407(6803):523-6, 2000

Schmitz JE, Kuroda MJ, Santra S, *et al.* Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 283(5403):857-60, 1999

Smith KA, Jacobson EL, Sohn T, *et al.* In vivo assessment of antiviral reactivity in chronic HIV infection. HIV Clin Trials 1(3):16-22, 2000

Turner SJ, Cross R, Xie W, *et al.* Concurrent Naive and Memory CD8+ T Cell Responses to an Influenza A Virus. J Immunol 167: 2753-2758, 2001

Steering Committee

Ben Cheng
Steven Deeks
Martin Delaney
Mark Dybul (co-chair)
Jose Gatell
Gregg Gonsalves
Linda Grinberg
Roy (“Trip”) Gulick
Mark Harrington
Mike McCune,
Veronica Miller (co-chair)
Doug Nixon