STi 2000

Structured Treatment Interruptions Workshop Summary

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STi2000

STRUCTURED TREATMENT INTERRUPTIONS WORKSHOP

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STRUCTURED TREATMENT INTERRUPTIONS WORKSHOP

Workshop Summary

The investigation of Structured Treatment Interruption (STI) has fluid borders. Interruption is considered a treatment strategy *per se*, an adjunct to treatment, as well as the removal of treatment. Though first proposed as an immune-based treatment strategy, STI is now being explored as a research tool for basic science and clinical investigation, an option for clinical management, a tactic for toxicity relief, a way of reducing the cost of treatment, an aid to improving patient quality of life and as a method for guiding viral evolution. Recently, research on STI has begun as a resource conservation practice for large health networks or low-resource settings such as in developing countries. In addition, many clinicians and people on therapy have simply perceived a need to study the safety of what is already an established phenomenon — the "drug holiday." Although fears about the danger of stopping treatment have been allayed and the popularity of treatment interruption has soared, there is still no firm consensus about the safety of STI.

> ### STI as Treatment Research
>
> - *to stimulate response to autologous virus*
> - *to boost after acute infection*
> - *to boost after exogenous vaccine*
>
> - *to relieve drug toxicity*
> - *to limit drug exposure*
> - *for improved quality of life*
>
> - *until virus reverts to WT*
> - *to suppress virus with fewer resources*
> - *to reduce the cost of treatment*
>
> ### STI as Pathogenesis Research
>
> - *to elucidate science of immune response*
> - *to elucidate viral and host immune dynamics*
> - *to allow readout of clinical benefits from immune-based therapies*

The second STI Workshop assembled a diverse group of clinicians, researchers and community advocates from multiple disciplines. Investigator interest in structured treatment interruption is varied. Workshop attendees are leaders in

exploring STI as a way to potentially enhance HIV-1-specific immune responses, limit drug exposure, prevent and recover from drug-related toxicity, allow a shift to a more drug-susceptible "wild type virus"(WT) in the absence of drug pressure, and improve quality of life for people with HIV. For two and a half days, participants candidly shared observations, data and speculation about the state and future directions of STI research.

The Workshop agenda was structured by presentations that addressed issues specific to these patient population categories: 1) primary infection, 2) chronic suppressed infection, and 3) chronic, unsuppressed drug resistant infection. A suite of *Interlude Talks* allowed participants to appreciate some interesting perspectives on new assays, mathematical modeling, insights from animal models and bench work. This was followed by a comprehensive overview of the immunologic, virologic and clinical hypotheses behind STI and the state of the evidence supporting them. Looking ahead, participants presented a range of new protocol designs. Finally, the meeting was summarized and task lists were drawn up.

Two years after news of the "Berlin Patient" launched the field, the promise of STI remains greater than its proven benefits. But this assessment varies by the population treated and by the benefit sought. Despite initial excitement about possible disease remission by STI-mediated immune control, it's been the critical need for better quality-of-life while on long-term therapy that has driven the popularity of STI among patients.

Progress in STI Research –
Outstanding Clinical Issues

Clinical Risks

- The risks of clinical events during treatment interruption (TI) are small if CD4+ counts remain above 200 cells/mm^3. For those with lower CD4+ counts, good medical practice is crucial to prevent breakthrough infections.

- The risk of developing resistance during interruption is very low but cases have been observed.

- The risk of viral transmission during interruption is of concern but not quantified.

- The risk of affecting the pharmacokinetics of other drugs by inducing or inhibiting liver enzymes after starting or stopping certain antiretrovirals could be significant.

- Recrudescence of an acute retroviral syndrome (ARS) during viral rebound may require clinical attention.

- Discontinuous treatment schedules may undermine adherence habits.

- Pulsed therapy may have risks of toxicity different from those seen with chronic dosing.

- Some patients may experience rashes or other drug sensitivity reactions when restarting therapy.

Relief of Toxicity and other Benefits

- Preventing metabolic toxicity: It may be possible to delay but not prevent toxicity. (It's still not clear if the drugs are responsible for all of the toxicity – some of it may be due to HIV)

- Reducing or reversing lipodystrophy: Hatano, et al. (and Lori, et al. in SIV monkeys) observed improvement in blood lipid levels but not in insulin resistance profiles or body shape measurements. Muscle cell abnormalities appear early with drug exposure.

- Improving quality of life (QOL): QOL is difficult to assess, available instruments are not used in a standard way, and cultural variables are uncertain. QOL measures are not routinely performed and there is little accepted data on which QOL deficits are a problem and when they resolve. For example, many patients note the reduction of fatigue following TI although some complain of headaches and increased fatigue for a few weeks after interruption, possibly due to a recurrence of acute retroviral syndrome (ARS).

Progress in STI Research
The Population View

Primary Infection

The most encouraging development in STI research is the possibility of durably modulating the intensity of an HIV infection by lowering the viral load setpoint for patients able to be treated at or near the time of seroconversion. Because this approach will be available only to the relatively few individuals diagnosed during primary HIV infection (PHI), the direct public health benefit of this research may not be large. Nevertheless, this work has other important implications. A beneficial intervention during primary infection will affect the design of preventive vaccine trials and may ethically restrict the observation of secondary endpoints after breakthrough infections. Potential clinical benefits aside, STI studies have opened a window onto the HIV disease process by manipulating the dynamics of immune responses that take place soon after infection. Despite this promise, much difficult work remains before the correlates of immune function and the meaning of HIV-1-specific immune activation assays are understood.

Chronic Infection

For individuals with an established, chronic infection, the goal of bolstering immune response by vaccination with autologous virus after repeated cycles of STI remains elusive. There may be an emerging sense that, since durable HIV-1-specific immune stimulation has been difficult to replicate in chronically infected individuals, autovaccination may be a flawed approach to stimulating immune control. It could be that the pathogenic influence of HIV-1 on antigen presentation or cytokine production necessarily undermines the immune response to HIV in the presence of HIV. If this contradiction is not resolvable, then, as has been suggested, vaccination with exogenous antigens while remaining suppressed on antiretroviral therapy may give better results. In this context, vaccination may be used as a "prime" and STI as a "boost" approach.

A better-established clinical benefit of TI is for individuals who need a break from antiretroviral drugs due to toxicity, fatigue or an inability to adhere. A body of anecdotal experience, several prospective cohort studies, and a few small, randomized trials support the clinical safety of STI. Very few cases of resistance or breakthrough clinical events have been observed. Other risks need study before TI can be prescribed as easily as medications are. These include the risk of drug reactions on re-challenge and secondary acute retroviral syndrome (ARS). The current advice that STI should be undertaken in the context of clinical research remains prudent.

The development of metabolic abnormalities, fat redistribution, liver toxicities, and nerve damage has pressed the need for reducing or temporarily stopping drug exposure. Although some laboratory markers of toxicity seem to resolve after STI, it appears that symptoms of fat redistribution are refractory.

Intermittent Therapy

Clinical trials of intermittent treatment during chronic infection with the primary goal of reducing drug exposure and conserving resources are underway. Early reports suggest that good viral control can be maintained on several schedules of periodic dosing.

The effects of pulsed dosing on toxicity and drug clearance rates, the practicability of adherence to discontinuous schedules, and the risks of transmission while unsuppressed require careful study. Questions have also been raised about the cost savings of intermittent therapy if additional monitoring is required.

Chronic Unsuppressed

For a minority of individuals with multi-drug resistant (MDR) virus, a shift to the drug-susceptible wild type (WT) virus after TI is occasionally sustainable. However, most who restart treatment after shifting to WT soon experience treatment failure unless they have switched to previously untried drugs to which their virus is susceptible. Proposed studies of this intervention typically take the form of an immediate versus deferred treatment trial, with one arm waiting for a specified period before starting a new regimen and the other arm switching right away.

In some cases, however, the MDR virus may be less fit or less pathogenic than the WT and it may be as or more effective to remain on the failing regimen as it is to switch or take a break. Of course, for individuals who need to stop therapy due to toxicity, there are no clear solutions. Often, CD4+ cell loss speeds up dramatically after the viral population shifts to WT – well before the effects of toxicity have abated.

Data Presentations

Primary Infection

Discontinuing Prolonged HAART

Martin Markowitz, MD

Aaron Diamond AIDS Research Center, New York, NY

A prospective study observed 8 patients who started treatment when newly infected (within 54 days of symptoms) and had been on HAART with suppressed VL (no more than one blip per year) for about 3 years when treatment was interrupted. Four patients had received ALVAC vaccination.

The pre-treatment median HIV RNA was 5.0 log copies/mL and CD4+ cell count was 498/mm^3. At interruption, all patients had HIV RNA <50 copies/mL and the mean CD4+ count was 823 cell/mm^3.

After interruption, all experienced viral rebound, one patient with a peak VL of 4.3 log copies/mL.

In five of eight patients, genetic characteristics of the rebound virus were identical to virus present during the individual's primary infection, and to virus isolated from latent reservoirs during treatment. Genetic characteristics of rebounding virus from three other individuals diverged from virus isolated during primary infection, and from latent reservoirs, but corresponded with minor viral variants detected in lymphoid tissue while on treatment.

Even after prolonged periods of near or complete suppression, rebound virus can arise from either archives of the founder strain in latent reservoirs or from inadequately suppressed strains that evolved in protected compartments in lymphoid tissue.

Augmentation of HIV-1-specific Immune Responses after STI

Marcus Altfeld, MD

Partners AIDS Research Center, Massachusetts General Hospital, Charlestown, Massachusetts

HIV-1 specific cytotoxic T lymphocytes (CTL) respond weakly to a narrow range of epitopes during primary infection. After treatment with HAART, the frequencies of CTL increase but the strength of response remains weak. The breadth of epitope recognition remained narrow in individuals treated before and after seroconversion, although a broader response was observed in individuals treated at the time chronic infection was established. Individuals treated during primary infection preserved HIV-1-specific CD4+ T cell responses as well as a more homogenous viral population compared to those who were treated later.

Treatment interruptions were performed to determine if HIV-1 specific immune responses could be enhanced to levels sufficient to control viremia. Five individuals treated during primary infection who had suppressed viral load <50 copies/mL for over eight months with no evidence of drug resistance were offered interruption. Treatment was restarted if viral load exceeded 5000 copies/mL on three weekly determinations or reached 50,000 copies/mL on one determination.

All five patients experienced rebound and restarted treatment during the first interruption of treatment. After a second interruption, all patients spontaneously controlled viremia for as long as four months. HIV-1-specific CTL responses were stronger, with new epitopes recognized during the interruptions. These responses were preserved after restarting treatment. All viral load setpoints were well below the median observed from the MACS cohort.

STI resulted in viral rebound and augmentation of HIV-1 specific immune responses that appeared to improve control of viral load during subsequent interruptions.

Cf. recent papers in *Nature* and *J. Exp. Med.*

Chronic Infection: Suppressed Viremia

SSITT (Spanish/Swiss Intermittent Therapy Trial)

Bernard Hirschel, MD

University Hospital, Geneva, Switzerland

A large prospective observational study of STI enrolled 128 patients receiving HAART who had viral load <50 copies/mL for more than six months. Four two-week interruptions were scheduled at weeks 0, 10, 20 and 30. At week 40, treatment is discontinued for all patients; the primary endpoint is viral load at 52 weeks. Eighty patients have completed four interruptions; 73 have reached 52 weeks.

Median pre-therapy baseline CD4+ count was 388 cells/mm³; HIV RNA was 4.5 log copies/mL. Four patients had a VL < 5000 pre-HAART. The median duration of therapy at entry was 25.5 months with no changes due to virologic failure. Median CD4+ count at week 0 was 727 cells/mm³; viral load <50 copies/mL.

Patients were excluded if viral load did not promptly fall to below 50 after re-treatment during weeks 0-40.

	Week 2	Week 12	Week 22	Week 32
Number	128	116	104	80
Viral load (median Logs)	2.7	2.9	3.0	2.9
No rebound	24%	18%	20%	21%
High rebound (>5.0 log)	12.5%	5%	5%	2.5%
Excluded	*9 (7%)*	*8 (7%)*	*4 (4%)*	*3 (4%)*

Patients are scheduled to stop treatment at week 40 and are observed at week 52

·For 73 patients enrolled more than 52 weeks:

Failed before week 52 --------------------------------21 (30%)

Restarted before week 52 -------------------------- 10 (14%)

VL > 5000 at week 52 ------------------------------ 28 (39%)

VL < 5000 at week 52 ---------------------------- 15 (21%)

95 percent confidence interval 9-30 %)

Those who had VL <5000 at week 52 tended to have started HAART relatively early during their infection (7% during primary HIV infection, 38% during chronic HIV infection but within 24 months after infection); had little or no rebound on study up to week 40; had low levels of proviral DNA at the start of the trial; and had lower VL before HAART.

STOP EARTH

Jose Gatell, MD, PhD

University of Barcelona, Barcelona, Spain

STOP EARTH was a 52-week pilot study of STI for chronic infection in 10 patients and 20 matched controls. Treated patients were rolled-over from earlier randomized regimen comparison trials.

Baseline HIV RNA levels were above 5,000 or 10,000 copies/mL; CD4 counts were >500 cells/mm^3.

Controls received no therapy and were monitored for 52 weeks. Treated patients received HAART for 52 weeks; if suppressed >20 copies/mL, patients underwent three cycles of 4 week interruptions. After the 3rd cycle, STI patients were continued off treatment and observed. Nine patients completed the study.

> VL above baseline --- 1
> VL same as bsaeline --- 1
> \> 0.5 log drop from baseline; > 5000 copies ------------------ 4
> \> 0.5 log drop from baseline; < 5000 copies ------------------ 3

Patients who had spontaneous VL drops at stops 2 or 3 also had improved recovery of cytotoxic T-lymphocytes and increased CD4+ lymphoproliferative responses. With >12 months off therapy, all patients had CD4+ counts higher than pre-HAART levels.

STOP EARTH 4

Felipe Garcia, MD, PhD

University of Barcelona, Barcelona, Spain

Twenty patients received HAART with or without hydroxyurea (HU) Two-week treatment interruptions were performed at weeks 10, 20 and 30. All patients stopped HAART at week 40 until restart was triggered by viral load. Patients continued hydroxyurea during the STI at week 40.

	Stop 1		Stop 5	
	ART	HU	ART	HU
Peak VL >5000	*	*	6/8	1/5
VL decrease from baseline	*	*	-0.85	-1.1
VL < baseline	*	*	6/8	4/5
T helper response	0/8	1/5	5/8	5/5
CTL – no response	3/8	2/5	0/8	0/5
CTL – strong response	1/8	1/5	8/8	5/5

Hydroxyurea continued during STI may limit viral rebound peaks without impairing CTL and CD4 responses. STI may induce CTL and CD4 responses capable of controlling virus.

Is Treatment Interruption Safe? Can Interruption Augment Antiviral Immunity in Chronic Infection?

Gabriel Ortiz

Aaron Diamond AIDS Research Center, New York, NY

This is a randomized trial in 12 patients with stable HIV RNA <400 copies/mL and CD4+ counts >400 for more than six months.

Four control patients received continuous HAART; eight patients received HAART for

4 weeks then interrupted for 4 weeks; repeated the cycle; then continued off treatment from week 16 through 28. The schedule is based on a prime/boost model.

Treatment is restarted if CD4+ counts fall to more than 50% of baseline or <200 cells/mm^3; or if viral load is >5.0 log copies/mL on two consecutive visits after week 16.

STI patients	
HIV RNA -0.5 log below baseline at last TI	1 of 8
CD4+ count declines >5% two times	7 of 8
Removed due to CD4+ <50% of baseline	2 of 8
Increased CTL but not CD4	8 of 8
Increased neut. Ab. titer to autologous virus	2 of 8

Control patients maintained HIV RNA <400 copies/mL and had stable CD4+ counts. No CTL enhancement was observed.

- Interruption may enhance CTL and neutralizing antibody titers to autologous virus strains, yet not provide viral suppression for most patients.

- CTL responses are durable up to 22 weeks after restarting suppressive therapy.

- STI may result in a significant (>50%) CD4 decline from "On-HAART" baseline.

Restoration of HIV-1-specific CTL and Investigation of HIV Dynamics after STI in Chronic Infection

Bonaventura Clotet, MD, PhD

Hospital Universitari "Germans Trias i Pujol" Badalona, Spain

- HIV-infected patients with at least 2 years of viral suppression during antiretroviral therapy and a CD4/CD8 ratio>1 were randomized to interrupt HAART three times (n=12) or to continue with HAART (n=14). Each treatment interruption was for a maximum period of 30 days. HAART was resumed after each TI for 90 days until the next STI cycle.

- Plasma virus doubling time (t_d) was shorter during the first STI than t_d in the second and third STIs.

- The area under the curve (AUC), an indirect measure of total viral replication over the interruption was significantly lower in the third STI than in the second for all patients, indicating a progressive although moderate enhancement of viral control.

- The viral reservoir increased after the 2nd and 3rd interruptions

- The average frequency of HIV-1-specific CD8+ T-cells in the TI patients was significantly higher at the end of the third TI cycle than at baseline and at the end of the first interruption.

- A substantial increase in HIV-1-specific CD8+ T-cell frequencies was found in 5 interrupter patients while there were no changes in all 14 non-interrupter individuals.

The Philadelphia Cohort

Luis J. Montaner, DVM, MSc, Dphil

The Wistar Institute, Philadelphia, Pennsylvania

An observational study compared five untreated chronically infected patients with five chronically infected patients who interrupted treatment. Real time HIV RNA and CD4+ counts were available to their provider every two weeks to guide treatment. Restart timing was structured by scheduled visits.

During rebounds after 30-60 day interruption:

- CD4 lymphoproliferative responses (LPR) to p24 were increased

- CD4 LPR to gp160 was not enhanced

- CD8 CTL ELISPOT and tetramer staining was correlated with increased viral replication

- No adverse effects or changes in CD4 count were observed

Intermittent Therapy

Mark Dybul, MD

NIAID, NIH, Bethesda, Maryland

Can viral suppression be maintained with less cumulative drug exposure though a permanent on/off schedule of treatment? If intermittent therapy is effective for suppressing HIV and maintaining CD4+ cell counts, it could possibly reduce toxicity and cost and improve adherence.

A planned 22-month trial of continuous versus intermittent therapy has randomized 40 patients (70 planned) to receive either continuous HAART or to follow a cyclic regimen of HAART for one month followed by one month without treatment. All patients must have a current CD4+ T cell count > 300 cells/mm^3 and a plasma HIV RNA < 50 copies/ml. The mean pre-therapy viral load for 13 patients reported through 12 weeks was 31,600 copies/mL. The mean CD4+ T cell count at enrollment was 725 cells/mm^3.

Number of patients with viral load <50 copies/mL

	Week 0	Week 12	Week 24
Continuous	4/13	2/13	2/8
Intermittent	5/13	1/13	2/8

Of 13 patients on study for 2-4 cycles, all patients had detectable plasma viremia during each of the off-HAART periods. Overall there was no difference in mean plasma viremia between the 1st and 3rd cycles off-HAART. There was a decrease in the mean CD4+ T cell count of 17, 3, and 9% with the 1st, 2nd and 3rd cycles, respectively. However, the mean CD4+ T cell count returned to baseline after 4 weeks of restarting HAART with each of the first 3 cycles.

Because all 13 patients on this schedule of intermittent therapy had detectable viral rebound while off drug, concerns were raised about possible transmissibility. A short-cycle schedule was designed to maintain rebound viral load <1500 copies during rebound. A seven-day cycle was chosen to minimize the chance of resistance and transmission due to significant replication while off therapy.

Ten patients were assigned to a six-month trial of a cyclic regimen of 7 days on HAART followed by 7 days with no treatment. Failure is considered viral load >500 copies/mL at the end of the off-drug period. To date, three patients have reached 6 months with viral load <50 copies/mL. CD4+, CD8+ and CD38+ counts were stable.

Although intermittent schedules may not reverse treatment-associated toxicity, they may lower its incidence. In this highly selected and compliant population, intermittent schedules have been well accepted by patients.

Chronic Infection: Unsuppressed Viremia

The Immunologic and Virologic Consequences of Treatment Interruption in Clinical Practice

Ray Chen, MD

University of Alabama at Birmingham, Birmingham, Alabama

 A clinic cohort of 75 patients on HAART who had interrupted treatment for more than 30 days was retrospectively analyzed. Patients had experience with a median of three prior regimens. The maximum pre-interruption viral load peak was a median 145,000 copies/mL; minimum pre-TI CD4+ count was a median 85 cells/mm^3.

Patients had a median 573 days on HAART. This was followed by a median 67 days of interruption then by a median 171 days on HAART.

	At TI	After TI
HIV RNA	11,456	404
CD4+ count	230	231

The post-TI median CD4+ count returned to baseline in 92 days; 59% recovered 90% of their pre-TI CD4+ count and 77% had viral load within 0.3 log of baseline.

STI in a Multi-drug Resistant, Unsuppressed Population

Veronica Miller, PhD

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Response to re-initiation of therapy after treatment interruption was examined in the original Frankfurt STI cohort and in an expanded set of patients. In the original cohort, 33/48 patients responded with viral load < 500 copies. Twenty-four of thirty-three patients experienced rebound by a median 78 days. Those who responded with a shift to wild type virus had a more durable response.

In an expanded set of 163 patients, 104/163 (63.8%) had a virologic response. Response was associated with the change in viral load during interruption. Eighty-six percent of these had rebound during interruption. Rebound was associated with baseline CD4+ cell count, CD4 nadir, the number of drugs and the number of drug classes exposed to.

Outcome of 40 patients from the original cohort who are currently on treatment:

Viral load	<500	500-5000	>5000	
Patients on tx	15 of 40	11 of 40	12 of 40	

CD4+ Cell Count	<50	50-200	200-500	>500
Patients on tx	5 of 40	11 of 40	17 of 40	5 of 40

Twenty-six of the forty patients had four or more treatment changes after ending the TI period. Thus, studies of response to re-initiation of treatment are complicated by the frequent treatment changes due to tolerability problems in this patient population.

Safety, Resistance and Efficacy of TI in Patients with Multiple Failures of Antiretroviral Therapy Regimens

Christine Katlama, MD

Hopital Pitie-Salpetriere, Paris, France

Twenty patients with a median HIV RNA of 160,000 copies/mL and CD4+ count of 77 cells/mm^3 were enrolled in a pilot study to observe genotypic viral shifts during treatment interruption. The endpoint of genotypic shift was defined as the disappearance of all major resistance mutations to one drug class. Viral genotype was evaluated every 4 weeks.

At baseline, there were a median of 5 mutations conferring resistance to NRTI, 2 to NNRTI and 4 to the PI class. Sixteen patients had more than one major mutation in each drug class.

After a median interruption of eight weeks, genotypic shift was observed in 11/20 patients (to one class in 5 patients; two classes in 2 patients; three classes in 4 patients).

Shifting to WT was associated with the duration of TI with shifts occurring between 8 to 10 weeks. (cf. Deeks cohort, 10-12 weeks)

Both those who experienced a shift to WT and those who did not experienced small viral load increases during TI and reductions of -2.5 log copies/mL two months after restarting treatment. By six months, the reduction was -2.0 log copies/mL. During the interruption, median CD4+ counts dropped by about 15 cells/mm^3 for each group.

Clinical events (Candida esophagitis, CMV retinitis, progression of KS) occurred during interruption in patients with CD4+ counts <30 cells/mm^3 at baseline.

Within three months after restarting therapy, baseline resistance mutation patterns had reemerged in 8/11 patients that shifted to WT.

Preserving CD4+ T Cells during Prolonged Virologic Failure: The Effect of Treatment Interruption

Steven Deeks, MD

San Francisco General Hospital, San Francisco, California

A cross-sectional study of all clinic visitors during one week with CD4+ counts <350 cells/mm^3 evaluated CD4 activation, Ki67 cell cycle (a proliferation assay), and cell turnover rate (k).

	Untreated	Treated, unsuppressed (VL >2500)	Treated, suppressed (VL <50)
Activated CD4	Increased (highest)	Decreased (low)	Decreased (low)
Cell cycle (Ki67)	Increased	Decreased (lower)	Decreased (lower)
Turnover (k)	Increased	Decreased (lower)	Decreased (lower)

A subsequent study evaluated phenotypic drug susceptibility on a weekly basis in 18 heavily pretreated and drug-resistant patients who underwent treatment interruption.

A rapid return to drug susceptibility occurred in the majority of patients soon after their treatment interruption. Although the shift to wild type susceptibility was observed at different times for different patients, in each the shift was rapid when it occurred. The time of the shift was set as Day 0.

HIV RNA levels increased slowly until Day 0, then increased sharply.

CD4+ counts decreased slowly until Day 0, then decreased sharply.

Activation markers increased after interruption and this was associated with a drop in total, memory and naïve CD4 cells. CD8+ counts were stable. CD4 cell turnover increased in some patients with a decrease in the relative half-life after interruption.

Among patients with preserved CD4 cells despite virologic failure, the dynamics of T-cell production, destruction and activation are similar to those observed in treated, suppressed patients. No increase in CD4 cell production was observed after interruption.

This suggests that the wild type virus has a greater inherent fitness or replicative capacity than virus with drug resistant mutations. An increase in replicative capacity prior to week 12 was associated with increased viral load.

Patients who shift rapidly after interruption may have a less fit virus – and therefore have the most to lose from interruption. Patients with low CD4+ counts on therapy with unsuppressed virus are at the greatest risk from disease progression during treatment interruption.

Observational Databases

Short and Longer-Term Safety Experience from Observational Databases

Caroline Sabin, PhD

Royal Free and University College Medical School, London, UK

Veronica Miller, PhD

Klinikum der J.W. Goethe Universitat, Frankfurt, Germany

What happens during an interruption and what happens when treatment is re-started? What factors are predictive of poor outcomes?

	At TI	After TI	Change
CD4+ (cells/mm^3)	207	93	-180
CD8+ (cell/mm^3)	914	690	-156
HIV RNA (log copies/mL)	4.84	5.53	+0.45

A retrospective review was performed of 252 cases of treatment interruption lasting two months or longer that were initiated due to failing regimens. Data was collected from participants in the Frankfort HIV Cohort, the Royal Free Hospital, the San Francisco Cohort, ICONA and the Southern Alberta cohort.

Patients with the highest CD4+ counts and lowest pre-treatment CD4+ nadirs experienced the greatest loss of CD4+ cells during the interruption. CD4+ cell loss was correlated with HIV RNA increase during the interruption.

Of 182 patients who re-challenged and were followed-up, 98/182 (53.8%) achieved HIV RNA <500 copies/mL. Success of viral control after re-challenge was correlated with baseline CD4+ cell count and with the magnitude of CD4+ cell loss while on interruption.

The San Francisco Cohort: Who is Interrupting Treatment?

Jody Lawrence, MD

University of California, San Francisco, San Francisco, California

In a preliminary analysis of a cohort of 1000 patients, 56 have interrupted treatment for longer than one month. Eighty percent interrupted due to treatment failure or toxicity. Twenty-five percent had CD4+ counts <50 cells/mm^3.

Individuals with higher baseline viral load (>100,000) or lower CD4+ counts experienced the smallest changes during interruption. Those with higher CD4+ counts had the greatest changes during TI

Genoveffa Franchini, MD

National Cancer Institute, NIH, Bethesda, Maryland

Twenty-four macaques were infected with SIV_{251} then divided into 3 groups of 8 animals each. Groups A and B received HAART; Group C received no antiretroviral therapy. Groups B and C received vaccinations with NYVAC–SIV-gpe; Group A received a mock vaccine.

A vaccinated macaque infected with SIV_{251} can continuously suppress viral replication. However, even slow progressors will still develop disease.

- Can HAART reconstitute an immune response to SIV?
- Can vaccination enhance the immune response?
- Can vaccination contribute to viral containment after HAART?

If treated during primary infection:

HAART alone (Group A) ------------------------ *Poor suppression off drug*

HAART plus vaccination (Group B) ---------- *Better suppression off drug*

Vaccination alone (Group C) -------------------- *OK suppression off drug [better than HAART alone, worse than HAART+vaccine]*

By a tetramer staining assay, 5.7% of CD8+ T-cells responded to gag epitopes. Vaccination expanded gag-specific responses even when virus was suppressed. The results were inversely correlated with viremia: Better vaccine response was associated with better viral suppression. After interruption, better control of viremia was associated with better suppression while on HAART.

If treated during chronic infection:

- Only animals with >1000 CD4 cell counts responded, suggesting that IL-2 may be needed to enhance immunogenicity.
- IL-2 increased the number of CD8+ effector cells producing IFN-gamma when stimulated by vaccination (NYVAC).
- IL-2 increased the number of functional virus-specific CD8+ cells. Low dose IL-2 contributed to suppression of viremia.
- Vaccines may be more effective than endogenous vaccination if replication in the gut is enhancing the response.

Franco Lori, MD

RIGHT Institute, Washington, D.C.

A comparison of three treatment strategies for SIV infection in macaques makes clear the advantages of animal models for demonstrating concepts.

In primary SIV infection but not chronic infection, STI resulted in durable viral control after treatment discontinuation.

Within six weeks of infection, animals were treated with either continuous HAART, HAART on a 3 weeks on/3 weeks off schedule, or left untreated.

Continuous HAART-treated and intermittent HAART-treated monkeys had equivalent viral load and CD4+ percentages after several cycles of STI.

During the first interruption, all of the HAART/STI monkeys had viral rebound; by the fourth interruption, no monkeys rebounded. After permanent discontinuation of HAART, continuously treated animals had immediate rebound while HAART/STI animals remained suppressed at 41 days. Continuous HAART animals experienced insulin resistance and pancreatitis; HAART/STI animals had no serious toxicity.

A similar study in chronically infected macaques was less encouraging. Continuously treated animals remained suppressed while on HAART but animals treated with a HAART/STI schedule experienced rebound after each interruption.

Deborah Persaud, MD

Johns Hopkins University, Baltimore, Maryland

Reservoirs of HIV-1 exist despite effective suppression on HAART. Resting CD4+ cells hold an archive of viral mutations. Those on sub-optimal therapy probably have some resistant virus archived. Resting CD4+ cells can divide and release archived virus to the plasma even without antigenic stimulation. This explains the persistence of founder viral strains and also explains observations that each viral bloom can be genomically different.

One clinical implication of the persistence of viral mutants is that recycling drugs to treat a rebounding wild type virus after interruption will probably fail in most cases.

Progress in STI Research -
The Outlook for Immune Response

Hypotheses for STI

Primary Infection:

Hypothesis	Evidence	Next Step
Treat briefly to lower the viral setpoint and allow people to live longer with less drug exposure.	We have anecdotal evidence that this is possible.	Determine long-term benefit and safety.

Chronic Infection, suppressed virus

Hypothesis	Evidence	Next Step
Attain remission through HIV-1-specific immune control.	There is evidence that this occurs in some patients.	Identify responders and increase the proportion that responds.
Get acceptable clinical results with less drug exposure and prevent death and disease.	There is evidence that abnormal lab values can recover during interruption; there is little evidence that drug-related symptoms recover, at least during the short interruption periods studied so far.	Identify patients for whom this is safe.

Chronic Infection, unsuppressed virus

Hypothesis	Evidence	Next Step
Reduce toxicity by periodically stopping drug treatments.	There is evidence that abnormal lab values can recover during interruption; there is evidence that fat redistribution does not improve.	Practice careful medical management to minimize the risk of clinical events in patients with low and declining CD4+ cell counts.
Re-sensitize the virus to antiretroviral drugs so patients can avoid death and disease by returning to therapy and suppressing virus. The goal should be to maintain CD4+ cell counts and preserve immune repertoire.	There is evidence that shift to WT virus can occur— but drug susceptibility is eventually lost when therapy is reintroduced. Time required for shift to WT usually results in significant CD4+ cell count declines. These declines are greatest in those with higher CD4+ counts.	Identify patients for whom this is safe. Identify patients for whom this can be predicted.

Outstanding Immunology Issues

There is a crucial need to **standardize**:

- Immune-related HIV assays [HIV strains, viral peptides, VIR]
- Cell collection, storage and shipping protocols.

CORRELATE new assays with standard assays

- Correlate new immune markers (other than CD4+) with clinical endpoints and with virology endpoints.
- Fund immunology substudies of large clinical trials to standardize assays
- Correlate assay results with clinical events
- Correlate assay results in different populations
- Take advantage of the DAIDS repository and immunology QA funding.
- Use chip technology for multiple assay analysis.
- Collect samples and save cells.
- Obtain informed consent to save cells for future testing, including genetic analysis.

EXPAND the use of animal models

- To investigate the ideal duration of STI in primary infection.
- To correlate memory and naive cell numbers/ratios with disease progression or remission.
- Are enough animals available and accessible?

INVESTIGATE

- Host genomics
- Humoral immunity
- Cell collection, storage and shipping protocols.
- Investigate how memory/effector cell ratio influences the outcome of STI.
- Continue to look for ways to stimulate immune control during chronic infection.
- Continue to investigate cytokines and therapeutic vaccines.

Outstanding Virology Issues

What are the virologic determinants for HIV-1-specific immune stimulation?

- Amount (dose) of viral antigen

- Duration of exposure to antigen

- Viral genotypic and phenotypic variation during the interruption. What is the impact of viral evolution during STI?

- What are the best doses and durations for ´prime-boost' approaches? While there may be a lower and higher level of antigen necessary for these responses, they remain undefined. 50,000 should be enough in PHI (Altfeld), there is less data for CHI.

- Should durations be set by patient-oriented schedules, by fixed schedules or by fixed thresholds? Treat as viremia rises, allow VL to peak then go down, or treat when the CD4+ threshold is reached?

- Ongoing trials and animal models may help pinpoint these numbers.

- Could you predict the rebound magnitude based on pre-STI replication rates?

- Do immune-suppressive viral proteins that accompany the viral rebound affect immune response functions and complicate existing models?

Virus rebound – Immunogenic stimulus

- What degree of viral control should be observed to accept the hypothesis that HIV-1-specific immunity can be stimulated? <50, <500, <5000 copies/ mL? A relative drop? -1.0 log, -2.0 log? A lower setpoint than the pre-therapy setpoint? (Pre-therapy setpoints are difficult to determine.)

- In PHI there is no pretreatment setpoint to compare with.

- The risk thresholds for CD4+ or VL vary by opinions on "when to start" in CHI. How long does one wait to see VL rise then spontaneously fall? (Some suggest waiting 12 weeks off drug). Is there a viral threshold considered too risky to continue beyond? (Peak viremia is usually reached by week 7.)

- Why would one use an arbitrary viral load copy number as a trigger to restart treatment rather than CD4+ cell counts? It may depend on the governing hypothesis – or on what one's IRB will allow.

Virus rebound – Antigenic stimulus

- What is the rebounding virus population?

- Does the cellular source of the rebound virus matter in terms of "immunogenicity" and/or "antigenicity"? There is usually more heterogeneity in the viral populations during CHI than in PHI. Reservoirs are critical for reseeding the rebound. (ADARC and NIAID are looking at this.)

- Is viral control on HAART associated with an SI-NSI switch? This could be measured.

- Do chemokine receptor expression, activation markers, cytokines, or antibodies affect or reflect response?

- Can there be an expansion of pre-existing drug-resistant minorities?

Drug exposed virus – Drug pressure

- Viral evolution continues if drug pressure is maintained

- One benefit of STI is to stop the further accumulation of resistance mutations.

- It is possible to allow the outgrowth of WT virus. But who shifts from drug resistant to WT virus and what are the predictors of this switch?

- What is the impact on virus fitness, virulence, or pathogenicity? Are *in vitro* fitness or competitive outgrowth assays relevant?

- Do so-called compensatory mutations actually compensate?

- MDR HIV is highly impaired in replicative capacity but the virology is unclear. Should treatment be terminated? In which patients, for how long?

- Yet MDR HIV can be transmitted and replicate to a high level.

- What is the immunologic and clinical impact of a shift to WT as the dominant population?

- What are the costs of this switch in terms of CD4+ cell loss and clinical endpoints?

Interlude Talks – II

Gabriel Ortiz

Aaron Diamond AIDS Research Center, New York, NY

Question:

How much antigenic stimulation is required to get augmentation of HIV-1-specific CD8+ response during primary and chronic infection?

Method:

Compare the results of a study of 50 patients (38 on HAART) treated soon after infection with results from a study of 22 patients (all on HAART) with chronic infection.

Results:

Individuals with more than two residual viral replication episodes (blips) had an associated increase in HIV-1-specific CD8+ T-cell response. Patients treated in primary infection experienced HIV-1-specific CD8+ response at a lower threshold of viral load.

Antigen requirements for CD8 stimulation

HIV negative ---------------------Sensitive to antigen stimulation

Primary infection --------------Needs more antigen

Chronic infection --------------Needs much more antigen

Conclusion:

Relatively few viremic episodes (blips between 50 and 500 copies/ mL) could be sufficient to augment immune responses during PHI but not during CHI. STI trials for PHI don't need to let RNA rise higher than 500 [or 5,000 – Altfeld]. This is safer and should be effective (although longer interruptions seem to improve CD8+ stimulation and may be needed to affect the viral setpoint).

Rafi Ahmed, PhD

Professor Ahmed, an investigator of the dynamics of the murine antiviral immune response who works outside of the HIV field, gave some much needed guidance for dealing with the myriad combinations of TI schedules, durations and restart triggers.

"What you're trying to do here," he said, "is reset the clock." The goal is to reset the balance between the number of HIV-1-specific T and B cells a patient has when starting HAART at peak viral load, and the number of HIV-1-specific cells they have when they interrupt treatment after a period of suppression. Ideally, there will be a greater number of HIV-1-specific T and B cells at interruption that control the viral rebound "You don't want to recapitulate the primary infection or you're back at square one."

Ahmed outlined some rules for performing effective prime/boost vaccinations.

Weak	+	Strong	=	Still works (in some cases)
Strong	+	Weak	=	Boost does little
Weak	+	Weak	=	Why bother?

A strong booster is essential, and the key to a strong boost is the amount of antigen. "The greater the antigen, the greater the boost." But, Ahmed warned, "The duration of the boost must be limited. For T-cells, the ideal is unlimited antigen for a limited period of time. If you continue the antigen too long you're going to destroy what you activated."

The amount of antigen present during the boost determines the extent of memory and naive T-cell recruitment. Studies that measure the number of memory cells recruited to become effector cells show that complete recruitment only occurs at the highest antigen doses.

The duration of antigen stimulation is governed by proliferation. "When you recruit a T-cell to divide – be it naive or memory – it goes through the entire cell cycle in about six hours. Very fast." At these rates of division, proliferation can't continue indefinitely or you would explode! After 10 to 20 divisions, the cells are driven to apoptosis or some other form of non-functionality. For mice, the limit is 6 to 8 days.

The ideal is to stimulate cells capable of massive expansion and rapid proliferation. You don't want to stimulate activated effector cells. If you are trying to auto-vaccinate with antigen during a treatment interruption, you need to interrupt when you have memory cells, not activated effector cells. Memory CD8 T-cells are not found during acute infection but appear only after a period of rest, during chronic infection. Effector CD8 T-cells have already gone though many

cycles of replication and will not continue to proliferate very much. Memory CD8 T-cells, however, will proliferate for 4 to 6 cycles, slightly better than naive cells. The transition from activated effector to memory cell happens best about 15 days after antigenic stimulation subsides.

"So clearly a period of rest is necessary for cells to re-acquire the ability to proliferate in response to antigen." Continued cellular differentiation depends on the upregulation of new and additional genes.

Naïve Cells proliferate/differentiate → + *antigen*	Effector Cells further diff. → + *rest (no antigen)*	Memory Cells

How would this understanding influence the design of STI schedules? If HAART is initiated during primary infection, the system is not pushed too far. HAART removes the antigenic stimulation. This allows the cells rest and lets some of them to turn into memory cells. The next time therapy is stopped; the memory cell population is boosted, which helps to expand it. Some of the most successful results with STI appear to have followed this scenario.

In chronic infection, the situation is more complex. Effector cells may be retained in the presence of antigen but, if they are functionally exhausted, they may not go on to become memory cells, even if HAART subsequently reduces antigen. For a person with chronic infection on a first interruption, the initial response may be a naive cell response rather than a boosted memory response. "If a first STI produces a naive response, then you are not starting off that much better than when the person was first infected. If the response improves after the second or third TI, then you may be generating some memory cells. Since you don't want to push the system too far in chronic infection, it may be best to keep the durations of TI shorter. If the TI is too long you risk over-stimulating the effector cells to the point they are not able to become memory cells. "A prolonged, chronic stimulus is the worst thing you can do if you want to generate a good memory response."

Discussion - Ahmed

The meeting became activated as participants peppered Dr. Ahmed with questions about the implications of his data on the design and feasibility of STI protocols.

Q: *In the mouse you find the peak of viral replication at 5-7 days and the T-cell response a bit later. A mouse lives about two years if it's lucky. How does that timing relate to humans? Would two days look like four weeks?*

RA: Look at the case of EBV in infectious mononucleosis. A tremendous CD8+ response is generated, which thereafter controls the infection for life.

Q: *So one suggestion for planning STIs might be a short first interruption with longer 2nd and 3rd interruptions?*

RA: Yes. For the first interruption, restart treatment after maybe 2 or 3 weeks, even if the RNA hasn't gone up that much. You may see the effect on the 2nd or 3rd STI.

Q: *Is there any hope for those with chronic infection?*

RA: This would be more difficult because the pool of memory cells is going to be lower. There could also be destruction of the dendritic cells, a lack of costimulation. Have you considered pulsing them with DCs during the STI?

Q: *Would it be wise to select CHI patients who have regenerated naive cells during HAART in the hope that you could stimulate new memory cells during an STI?*

RA: Well, is the repertoire the same as it was before infection or have you been left with holes in the repertoire? In animals we've found that the most effective effectors are deleted during chronic stimulation.

Q *It seems that those with a broader immune reconstitution would have an improved ability to restimulate. But, people have been on effective suppression for varying periods of time. Your work has shown that LCMV memory cells can persist for a year (which is half the life of a mouse), but is the memory component stable?*

RA: If you look at antigen-specific cells in a vaccine setting they remain stable.

Q *So would the duration of effective HAART prior to entry into the protocol be relevant? Is a shorter duration of HAART better?*

RA: Yes.

Q *The rest period is important for expanding the memory subset. But coming into a study with the effector cells engaged is also important. Would the best strategy be to do small interruptions at the start followed by longer ones?*

RA: If the effector cells are too activated you won't be able to expand them.

Q: *Could you use cytokines to stimulate memory cell production?*

RA: If you have the same frequency for a given epitope within naive and memory cells, you'll have more memory cells after cytokine stimulation because memory cells out-compete naive cells, which take longer to get started. We don't have a memory cell-specific cytokine at this time.

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Progress in STI Research – New Methods and Assays

Interlude Talks - III

Rodney Phillips, MDDS, FRCP, MD, MA

John Radcliffe University, Oxford, UK

Immunology from SSITT (Swiss/Spanish Study Intermittent Therapy Trial): "STI: Impact on HIV-1-specific cellular immunity: A preview of plans for immunology analysis of data from 120 patients in the SSIT study."

Antigen-specific T cells can be visually identified and counted with tetramers but this doesn't tell you what the T cells can *do*. In our analysis, we describe optimal CTL epitope peptides with known HLA restrictions, and then screen 10 to 30 peptides at each time point of analysis.

We've observed enhancements in some patients. One patient started with very little reactivity, but with viral recrudescence, there was some stimulation. When virus was re-suppressed, CD8+ cell counts went down. Other responses were not boosted. Using tetramers, we observed the expression of a specific CTL clone increase from 0.2% to 7% – "nearly the highest I've ever seen in my work at Oxford. Whether it's doing any good is another question."

Another patient started with a very limited response to a number of peptides. Each STI resulted in boosted responses; by the end of the fourth interruption we saw many more responses with a very respectable ELISPOT result. We looked at tetramers to three epitopes in the same patient and observed respectable levels, indicating that the SSITT protocol is boosting the frequencies of CTLs that give only a modest ELISPOT functional response. We're seeing a discrepancy between boosted numbers versus boosted function.

In a third patient with 400 CD4+ cells and low viral load we saw no viral recrudescence and no CD8+ boosting. "Some times you see no benefit at all."

Certainly the results to date are far from definitive.

Julianna Lisziewicz, PhD

RIGHT Institute, Washington, D.C.

An experimental assay to quantify HIV-1 Virus-specific Immune Response (VIR) mimics viral rebound *in vitro* then measures IFN-gamma expression of CD4+ and CD8+ cells. VIR correlates well with treated, untreated and intermittently treated patients in cases when viral load and CD4+ count do not. In SIV_{251} infected macaques, VIR is absent when viremia is controlled by HAART and VIR is present when STI is performed.

The "Washington Patient" was treated then had five interruptions with an increasing duration off drug. VIR increased with each successive interruption from 2% to 5.7%. CD4+ and C8+ cell counts did not correlate with the interruptions. VIR detects functional (perforin producing) CD8+ cells and correlates with the duration of immune control.

- VIR determines the absolute number and percentage of functional HIV-1 specific T-cells.
- The quantity of VIR correlates with the duration of immune control of HIV-1
- VIR detects functional HIV-1-specific CTL
- VIR analyzes IFN-gamma production by T-cell subsets (CD4/CD8)
- VIR does not depend on HLA or peptides (unlike tetramers)
- Small samples are sufficient (10mL EDTA)

Sebastian Bonhoeffer, PhD

Friedrich Miescher Institut, Basel, Switzerland

A population dynamics model of HIV replication kinetics suggests that STI can produce immune control without inducing drug resistance under certain conditions.

Prior to treatment with HAART, there is equilibrium between infected and susceptible CD4+ cell population counts. The ratio of CTL to infected CD4+ cells is also steady.

During treatment, this balance is shifted by an increase in virus-susceptible uninfected CD4+ cells and a dramatic decrease in the number of infected CD4+ cells. CTLs also decrease slightly and the ratio of CTL to infected CD4+ cells is shifted in favor of CTLs.

If, after interruption of treatment, HIV-1-specific effector cells outnumber infected cells, the model suggests that immune control of viral growth can be achieved. A negative viral growth rate results when the infected cells are removed by cell death or immune-mediated killing at a faster rate than they are produced.

Growth rate = infection – death – immune killing

For this to occur, **the number of effectors must increase during treatment** to a stable level higher than baseline at the start of treatment. At the time of TI the number of effector cells should more than outnumber new CD4+ target cells.

The model also addresses concerns about developing resistance during STI and concerns that viral reservoirs will be repopulated during viral rebound. A greater risk of resistance arises from drug-resistant mutants present in the viral population at the start of therapy than from newly generated mutants after rebound, as long as the viral load remains below baseline during STI. Similarly, refilling of latent reservoirs is unlikely if the rebound viral load during STI remains below the baseline pre-treatment level.

To evaluate clinical data from STI trials, it will be crucial to have accurate measurements of effector cell populations before, during and after therapy.

Progress in STI Research – Next Steps

Summary of Full Group Discussion

The fundamental goals of STI research are varied, studied for immune augmentation, safety, and drug avoidance.

Patients who have received less cumulative drug can have successful outcomes with less resistance, less toxicity, better QOL, and - possibly - immune benefit. These are reasons to rapidly move ahead with larger, randomized trials. The outstanding question should be: "Is TI better, the same, or worse than continuous treatment?"

Other participants immediately pointed out the difficulties in designing the next wave of trials. "As far as immune response goes, I don't see a clear group of patients who benefit." The heterogeneity of results from the many small studies may call for stratifying the planned trials by age, nadir CD4+ count, pre-treatment viral load or any number of other factors. Such diverse populations will require multicenter cooperation to enroll.

Phase III trials of immune benefit for chronic infection can't be considered until some key parameters of STI are better defined: Which populations to treat; how to schedule interruptions; and which endpoints to use.

One researcher suggested that study endpoints should perhaps be a composite of immune, viral and QOL measures. Still, we lack objective data about QOL and newer immunological markers have not standardized.

"What do we do with the 70% who don't benefit from the STI? People with chronic infection don't generate consistent responses." "I'm stuck between the poor predictive power of the immune responses and the variable data on those parameters. Some patients have had striking improvements – but we don't know why."

One researcher participating in a large trial believes only clinical outcomes will be meaningful as primary endpoints for phase III study. But this should not preclude nesting basic virology and immunology research within clinical endpoint studies, although new alliances need to be formed: "… we need to (collect samples and) get the large trials linked to scientists." It also should not rule out doing lab-based surrogate marker phase I-II studies, depending on hypothesis, to set the stage for phase III studies.

Another participant brought the discussion full circle by stressing the need for better treatment strategies and the lure of STI: "If you can get the same viral load or CD4 result with less drug exposure, that would be enough for any disease – except for HIV. The safety of STI has been demonstrated, more or less, let's roll with randomized controlled trials."

Putting immune benefit aside, the period of interruption to be studied can vary depending on the goal. Toxicity reduction may call for a two to three month interruption if it is possible at all. Maintaining viral load and CD4 levels stable with less drug exposure may require more frequent on/off cycles. [Or this may vary by person.] The only large trial of STI to date (SSITT) found QOL gains reported only during the first interruption.

The economic benefits of interruption and intermittent therapy also need to be critically examined. "If people can't go to work because they're feeling lousy or going to the doctor all the time, these are costs." There may be hidden costs to TI, such as an increased number of clinical events and hospitalizations, or more frequent monitoring while off therapy. "So let's think about all these kinds of costs and assess them in our effectiveness studies."

Use of the term "interruption" supposes that continuous treatment is the natural order. Perhaps we now know enough to overturn this model and begin talking about periods of treatment administration that interrupt and modulate the natural history of this disease.

Brainstorming Session – What needs to be done?

Toxicity and Quality of Life

- Study metabolic abnormalities
- Create an adverse events web page for surveillance and education
- Establish a working group to study reversal of toxicities
- Standardize QOL questionnaires

Immunology

- Press NIH to ask immunologic questions using the STI model
- Investigate immunologic correlates
- Standardize immune function assays
- Move quickly from animal models to the clinic

Clinical Observation

- Study sex-linked differences
- Extend observational databases to additional populations
- Find funding for storage of plasma and cells
- Collect tissues – lymphoid tissue, CSF, seminal, vaginal fluids
- Collaborate to perform meta-analysis
- Collect data in clinics and private practices

Clinical Research

- Develop protocol concepts
- Coordinate development of STI duration and rechallenge triggers
- Establish a working group to define treatment failure
- Interest companies and foundations in therapeutic vaccination
- Establish an acute infection network to help recruit seroconverters
- Foster collaboration between networks (acute infection network, ACTG, CPCRA, international)

Long-term Management

- Study long-term approaches to therapy that incorporate STI
- Study when & how to start treatment and when to stop
- Compare costs of different strategies
- Develop strategies for sparing treatment; collaborate with developing nations

Designing Trials to Demonstrate Immune Benefit from STI

The aggregate of experience from many small studies, anecdotal reports and observational databases provides what we know so far about the safety of STI. Despite this experience, reproducible HIV-1-specific immune control has not yet been demonstrated.

The number of variables that must be considered when designing trials to evaluate potential immune benefits are daunting: The duration of a scheduled interruption may be determined by:

- HIV RNA copy number trigger
- CD4 cell count trigger
- A hybrid of RNA and CD4 count
- By regularly scheduled clinic visits

(CD4+ or RNA triggers can be defined as absolute numbers or as relative changes from a setpoint.)

The choice of number and duration of subsequent interruptions multiplies the variables:

- Fixed periods of equal duration
- Fixed periods of progressive duration
- Individualized periods - CD4+ decline
- Individualized periods - VL increase
- Individualized periods - VL plateau

The duration of treatment after interruption is another consideration:

- Treat until suppressed (<400; <200; <50, or <20 copies/mL)
- Treat until CD4+ counts have recovered
- Treat for a fixed period

Hypotheses exist to support each of these approaches, and evidence from animal models can be used to argue for one or another. Short of hitting on a lucky combination in another round of small trials, much basic science and animal research may be needed before interruption schedules for large Phase III trials can be confidently selected. Appendix 2

Protocol Designs for Clinical Research

Table 1 **Primary Infection**

Study Title/Sponsor	ANRS 1000 "PRIMSTOP"
Population	Primary Infection (within 4 weeks of symptoms)
Study Type	Pilot - no controls.
Regimen	HAART + HU
Size	24 patients
Comparison	None
Plan	3 STI of increasing length (2, 4 and 8 weeks) at 34, 48 and 72 weeks for patients with suppressed VL (<20 copies).
Duration	82 weeks
Follow-up	Until 108 weeks
Primary Endpoint	HIV RNA < 20 copies for at least 6 months from last TI.
Secondary Endpoints	1. Resistance 2. Proviral DNA 3. Cellular RNA 4. HIV-1-specific T-lymphocyte response.
Enrollment started:	5/2000

Table 2: **Chronic Suppressed**

Study Title/Sponsor	TIBET	AUTOVAC II
Population	Chronic Suppressed <50 for 1 year; CD4 >500 for 6 months	Chronic suppressed <50 for 2 years
Study Type	Pilot, no controls	Randomized
Regimen	HAART	HAART w/without IL-2
Size	10 patients	50 patients
Comparison	None	Continuous versus STI w/without IL-2
Plan of Interruptions	Stop treatment until CD4+ count drops <350. Restart and continue until resuppressed.	Cycle between 2 weeks off and 4 weeks on treatment w/without IL-2. After 6 cycles, stop treatment in all arms and observe until CD4 drops <350.
Duration	-	9 months
Follow-up	-	-
Primary Endpoint	Safety	Time off treatment.
Secondary Endpoints	Time off treatment, cost, QOL, immune monitoring.	Immune monitoring.
Enrollment started:	-	-

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Table 3: **Chronic Suppressed**

Study Title/Sponsor	Philadelphia Wistar Institute	Gladstone Institute	Garcia/Gatell
Population	Chronic Suppressed Prior VL > 10,000; current VL < 50. Nadir CD4 count > 100 cells; current CD4 count > 400.	Chronic Suppressed	Chronic Suppressed
Study Type	Individualized Protocol - Randomized.	Pilot - no controls.	Randomized
Regimen	HAART	HAART	Multiple strategies
Size	52 patients	20 patients	175 patients
Comparison	IT by group under continued ART versus group with previous periodic TI.	None	Multiple strategies: Continuing treatment vs. STI w/without HU; w/without Immune mod. w/without immunogen.
Plan of Interruptions	Continuous ART for 40 weeks - (non-adherent patients will be excluded). Stop (comparison TI). Versus 1) Stop for 2 weeks (priming TI). 2) Restart and treat with ART until RNA <50 for 4 weeks. 3) Following suppression for 4 weeks, stop for 4 weeks (boost TI). 4) Restart and treat until RNA < 50 for four weeks. 5) Following suppression for 4 weeks, stop for 6 weeks (CD8 boost TI). 6) Restart and treat until RNA <50 for four weeks. 7) Following suppression for 4 weeks, open-ended TI (comparison TI).	After suppressed on HAART for 3 months: 1. Stop treatment for 2 months. 2. Restart and treat for 6 months. Repeat cycle 3 times.	1) Continue HAART for 1 year, then stop. 2) HAART/STI 3) HAART/STI + HU 4) HAART/STO + general immune modulator 5) HAART/STI + immunogen.
Duration	12 - 18 months	~ 3 years	18 months
Follow-up	~48 months	~ 3 years	-
Primary Endpoint	Time to RNA > 5000 during comparison TI. Demonstrated adherence by electronic monitors required.	Viral load levels	Proportion with VL set point < 10,000 after 6 months off treatment.
Secondary Endpoints	1. CD4+ proliferation. 2. CD8+ response. 3. Amplitude of successive VL rebounds. 4. T-cell phenotype; resistance; thymic function. 5. Quality of life	Intensive immuno-logic monitoring. Quality of life.	HIV-1 specific CTL response. HIV-1 specific T-help response.
Enrollment started:	Underway	Underway	Proposal

Table 4: **Chronic, Previously Untreated**

Study Title/Sponsor	ACTG A5102	SITACI
Population	Patients on first ART regimen; HIV RNA < 200 copies; CD4+ > 500 cells.	Chronic, previously untreated.
Study Type	Randomized.	Randomized
Regimen	HAART with or without IL-2	HAART
Size	80 patients	10 patients
Comparison	IL-2 versus no IL-2	Continuous versus STI
Plan of Interruptions	1) Treat w/without IL-2 for 18 weeks. 2) If CD4 > 500 then stop until CD4 drops < 350 (2x). 3) Restart and treat with ART for 6 months 4) If RNA < 200 continue w/without IL-2 for 18 weeks. 5) If CD4 > 500, stop until CD4 drops < 350 (2x). 6) Restart and treat with ART for 6 months 7) If RNA < 200, continue w/without IL-2 for 18 weeks.	Cycle between 3 months on treatment and 1 month off. At 16 months, stop treatment in both arms and observe until CD4 drops <350.
Duration	~ 18 months to primary endpoint (end of step 2)	16 months +
Follow-up	~ 5 years	-
Primary Endpoint	CD4+ cell count at end of first 18 weeks w/without IL-2; rate of CD4 decline during TI.	Safety
Secondary Endpoints	1. Duration of 1st & 2nd TI. 2. Rate of resuppression. 3. Replicative capacity, fitness, resistance 4. Others	Resistance, time off treatment, immune monitoring
Enrollment started:	12/2000	Proposed

Table 5 **Chronic, Unsuppressed or MDR**

Study Title/Sponsor	ANRS 097 "GigaHAART"	OPTIMA Tri-national study	ACTG A5086	CPCRA 064
Population	Chronic unsuppressed. VL > 75,000; CD4+ < 200.	Chronic unsuppressed. MDR, have failed 2 regimes including 3 classes.	Chronic unsuppressed. VL > 10,000; CD4 > 150. At least one prior virologic failure; heavily pretreated.	Chronic unsuppressed. MDR virus. VL > 10,000.
Study Type	Randomized	Randomized	Randomized	Randomized
Regimen	3-4 NRTI, an NNRTI, HU, RTV+APV or IDV, SQV, NFV	MegaHAART is > 5 drugs. Retreatment based on baseline genotype.	Best available regimen based on baseline viral genotype, phenotype and treatment history.	Regimen selected based on genotyping/ phenotyping at baseline.
Size	90 patients	1300 patients	220 patients	480 patients
Comparison	Begin "Giga-HAART" immediately or wait 8 weeks to begin.	Continue, stop or switch to Mega-HAART	Begin new regimen immediately or wait 16 weeks to begin.	Begin new regimen immediately or wait 16 weeks to begin.
Plan of Interruptions	Deferred group has an 8-week washout before starting.	3 to 6 months drug-free period for interruption arm.	Deferred arm waits 16 weeks to begin new regimen.	Deferred arm waits 16 weeks to begin new regimen.
Duration	-	2 years	64 weeks	24 months
Follow-up	-	3 years	-	-
Primary Endpoint	Virological response = > 1.0 log reduction in VL at weeks 12 and 24.	Time to AIDS or death.	Proportion with VL < 400 at 48 weeks.	Progression to AIDS or death.
Secondary endpoints and monitoring.	Toxicity, genotype, PI plasma concentrations.	Toxicity, illness, QOL, standard markers, economics.	VL < 50 at 24, 48, 64 weeks. Adherence; Stratify above and below VL= 100,000; CD4+ above and below 200. Resistance and Immunology substudies proposed.	Genotype, VL, CD4+, drug levels, fitness assays. QOL adherence. Stored plasma and cells.
Enrollment started:	Underway.	Proposed.	Proposed.	Underway.

Appendix 1

Assays used to detect HIV-1-specific CTL

Kaul and Rowland-Jones

	CTL Culture	Elispot	Tetramer Staining
What it does	Bulk PBMC are co-cultured with HIV-1 antigens, which clonally expands the antigen-specific CTL in the sample.	Anti-cytokine antibodies on a plate reveal individual cytokine-producing cells as single spots that are counted by eye or by computer.	HLA/peptide complexes are organized into a stable tetrameric structure that specifically binds to T-cells expressing the appropriate T-cell receptor for the HLA/antigen complex.
What it measures	When the expanded CTL lyse Cr51 pulsed target cells, radiation is released indicating the presence of functional CTL.	INF-gamma release by CTL in response to antigen correlates well with CTL frequencies measured with lysis assays.	Tetramer staining directly marks antigen-specific CD8 T-cells that can then be counted by flow cytometry.
Advantages	Directly indicates lysis by CTL.	Allows screening for response to multiple epitopes in a single assay.	Allows virus-specific analysis. Allows sorting of antigen-specific cells for subsequent cloning. Allows analysis of co-expressed surface antigens.
Disadvantages	Only detects CTL able to expand in vitro; undercounts exhausted, resting and terminally differentiated CTL.	Not a direct measure of CTL function; uses IFN-gamma release as a marker for lytic capacity.	Not an assay of CTL function. Requires precise knowledge of both epitope and MHC type; not suitable for screening.
Relation to environment in vivo	Extended artificial stimulation in vitro means the frequency and phenotype of CTL are removed from conditions under which lysis occurs in vivo.	Detects antigen-specific cells without in vitro expansion.	Cells are stained directly ex vivo.
Quantitative?	Quantitation is possible with limiting dilution analysis (LDA). Undercounts compared to other techniques.	Indirect counting. Undercounts compared to tetramer staining.	Direct counting of CTL
Sensitivity	LDA can be a highly sensitive technique for detecting low frequency CTL.	More sensitive than tetramer staining.	Least sensitive technique for detecting low frequency CTL.
Time required	Several days	Overnight	Hours

Appendix 2

STI Workshop II Working Groups

* denotes STI Steering Committee Member

Protocol Working Group: Definitions, Endpoints, Outcomes

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