



HIV VACCINE  
TRIALS NETWORK

## HVTN Scientific Agenda

**DRAFT** / May 16, 2008

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## 1. Introduction

### 1.1. Mission of the HVTN

The mission of the HIV Vaccine Trials Network (HVTN) is to enhance the discovery, and drive the development, of a safe and globally effective vaccine for the prevention of HIV through well-designed clinical research trials which objectively and ethically address the critical questions of the field. This is done using an objective clinical trial platform to evaluate safety, immunogenicity and efficacy of candidate vaccines, as well as designing clinical trials that will provide insight on ways to enhance the effectiveness of subsequent vaccines. Candidates include T-cell-mediated vaccines, vaccines that elicit neutralizing

antibodies (nAb) as well as those that elicit mucosal immune responses, and novel adjuvants to improve the breadth, quality, and magnitude of the immune response to HIV-1 immunogens.

## 1.2. Reassessment of top priorities

Between 2005 and 2007, the HVTN initiated 2 test-of-concept trials (Phase 2B) for T-cell-based vaccines: HVTN 502 (STEP) and HVTN 503 (Phambili). Both trials tested the Merck Ad5 gag/pol/nef vaccine. A third test-of-concept trial, PAVE 101, testing a VRC multiclade DNA Ad5 vaccine regimen, was expected to open in September 2007. But in September 2007, at the planned interim analyses of the STEP trial, the Data and Safety Monitoring Board notified the sponsoring partners — Merck, HVTN and the NIH Division of AIDS (DAIDS) — that the Merck Ad5 gag/pol/nef vaccine had no effect on HIV acquisition or set-point viral load. Further analyses indicated a potential increased risk in HIV-1 acquisition in vaccinees, especially in men who had pre-existing neutralizing antibodies against Ad5 and in men who were not circumcised. A series of detailed analyses involving the epidemiological and laboratory data, probing this lack of effectiveness, was subsequently undertaken, in order to maximize the information the field can achieve from the STEP and Phambili trials as a means for designing and developing immunogens with improved efficacy.

As a result, the scientific agenda of the HVTN has in 2008 been significantly revised. Top priorities are:

- To better understand the outcome data from STEP and Phambili, and to use these data to help define and evaluate conceptual improvements in T-cell-based vaccines.
- To foster an iterative process between human and nonhuman primate studies that should allow the field to evaluate such conceptual improvements.

Specific priorities include:

- To fully analyze STEP and Phambili samples to define potential association between post-vaccination and post-infection immune responses and viral load set point and disease progression.
- To use genomic and proteomic technologies to more fully explore the “leads” suggested by correlation of ELISpot responses with viral load post acquisition.
- To evaluate if the potential increased HIV acquisition in STEP and Phambili is associated with Ad5 serology and/or circumcision status.
- To continue follow-up of the STEP and Phambili cohorts to determine if increased risk of HIV acquisition varies over time.
- To follow up subjects who acquired HIV on trial to determine if vaccination altered course of infection.

### 1.3. Major components of scientific agenda

The scientific agenda sets the Network's course in 3 major areas:

- **Role of clinical trials in vaccine discovery and related HIV research fields.** The HVTN standard clinical trials platform enables greater coordination of progress in the discovery and development of candidate HIV vaccines, not only through efficient trial design and implementation, but also through interchange with basic and preclinical research. (Section 2)
- **Clinical trials platform.** As critical questions in HIV vaccine research are modified in the light of new data from human testing, the importance of a standard infrastructure for such testing has continued to be evident. The HVTN was created to provide such a standardized platform for the prompt systematic evaluation of candidate HIV-1 immunogens. It has both shared its clinical trials operations procedures and initiated collaborations with other organizations involved in HIV vaccine and HIV prevention research. The HVTN's infrastructure and collaborations provide a platform from which to drive innovations needed to improve vaccine development. (Section 3)
- **Efficacy trials.** Clinical trial designs and resources need to be directed at defining potential correlates of immune protection, control of viral replication post acquisition, and risk. In addition, cofactors that affect efficacy should be better understood and taken into consideration in efficacy trial design. Ultimately, an efficacious vaccine would need to be evaluated in a variety of populations. (Section 4)

## 2. Role of clinical trials in vaccine discovery and related HIV research fields

Clinical trials are an essential component in addressing the critical questions facing the HIV vaccine field; much of the work of the HVTN has been an integral part of the discovery process of HIV vaccine research. To date all candidate vaccines that have gone forward into advanced clinical trials have been redesigned as a result of data obtained in human clinical trials. Although nonhuman preclinical data are among the criteria used for a vaccine candidate's entry into the HVTN clinical trials program, the ability of the preclinical data to predict the breadth, magnitude, and in vivo functional activity of human immune responses has been limited. Understanding the biological mechanisms behind these limitations requires more study of the linkages between nonhuman primate and human immune responses to candidate immunogens.

The significant contributions made to the field of HIV vaccine research through the standardized clinical trials development platform (Section 3) will contribute to answering critical questions in the field of HIV vaccine research, including:

- Understanding the results of the STEP study; in particular, understanding why the vaccine had no effect on reducing viral load set point in HIV-1-

infected cases, and why there is an apparent increased risk for HIV-1 acquisition in vaccine recipients vs. placebo recipients.

- Defining approaches to increase the breadth (epitope diversity), magnitude, and potential efficacy of T cell immune responses after vaccination. Approaches may include use of HIV gene inserts or proteins that will enhance antigen processing, reduce immunodominance and/or target specific cell types or receptors that influence subsequent memory or T-cell effector functions after vaccination.
- Refining the evaluation of immunological endpoints to incorporate breadth, magnitude and polyfunctionality of the immune response.
- Exploring the mucosal immune response and how that can be improved through vaccine design and/or delivery.
- How adjuvants can be incorporated into vaccine constructs to improve the immune response.
- The role of vector immunity.
- The role of the innate immune system.
- How to expand the effectiveness of the humoral immune response in vaccine design.

The role of HVTN research in addressing each of these questions is described in more detail in the following sections.

## **2.1. Post-STEP analyses**

Recent findings from the STEP trial indicate that the MRK Ad5 HIV trivalent vaccine lacks efficacy in reducing HIV-1 acquisition or post-infection viral load. Additional subgroup analyses reveal a trend of increased risk of HIV-1 acquisition in vaccinees with baseline Ad5 neutralizing antibody titer >200. The partners of the STEP trial — HVTN, Merck, and the NIH Division of AIDS (DAIDS) — in concert with the outside scientific community, will investigate the critical issues that may explain the findings of the STEP trial. These investigations will address 2 scientific questions:

- Why did the vaccine have no effect on reducing viral load set point in HIV-1-infected cases?
- Why is there an apparent increased risk for HIV-1 acquisition in vaccine recipients vs placebo recipients?

The overall research plan will be formulated through the guidance of the external Scientific Review Committee and executed through the components of the HVTN:

- The HVTN Laboratory Program, in collaboration with the Merck Research Laboratory, will facilitate the experimental studies, either performing investigations within its core laboratory or coordinating investigations among expert laboratories in the field.

- The HVTN Core will undertake new studies or amend current protocols to increase understanding of epidemiologic covariates that have significantly affected the STEP findings.
- The Statistical Center for HIV/AIDS Research and Prevention (SCHARP) — the HVTN's Statistical and Data Management Center — will provide study design statistical considerations, conduct study analyses, and coordinate findings for rapid dissemination to guide future vaccine development.

We believe that many of the scientific questions can be addressed, at least in part, by laboratory studies utilizing stored participant specimens and reagents from the STEP trial, as well as other HVTN trials (Phambili, 050, 071) that evaluated the Merck Ad5 HIV vaccines. For this reason, the HVTN Laboratory will establish a new repository for specimen tracking and distribution to laboratories proposing research studies with these specimens. In addition, samples from the STEP study have been prioritized in order to maximize the information that can be obtained from these limited samples. The most important sample set is the case-control cohort containing samples from all study participants who became infected during the trial (cases) and several participants who did not become infected (controls) per case. In addition, a subset of samples have been used to establish concordance between immunogenicity assays used at Merck and the HVTN.

#### 2.1.1. Viral genetics

A primary objective of the overall scientific agenda for the STEP follow-up laboratory studies is to elucidate why the vaccine was not effective at inhibiting acquisition of infection or reducing viral load set point. In that regard, it is imperative that we determine whether the vaccine induces immune responses that exert selection pressure on the infecting virus strain with respect to acquisition of infection and immune escape. Therefore, studies have been designed to compare viral sequences obtained from vaccine and placebo recipients at early and late timepoints following infection.

The laboratories of Dr. Jim Mullins at the University of Washington and Dr. Francine McCutchan at the Henry M. Jackson Foundation will determine viral genome sequences of plasma HIV-1 from the cases identified in the STEP trial. The 2 laboratories will sequence 5 whole genomes at the earliest isolate on the 82 cases, and 10 whole genomes from an additional isolate after approximately 6 months of infection in those who presented with acute infection (n=41).

From the viral sequence data obtained, the genotypic/phenotypic differences between the infecting HIV-1 strains and the HIV-1 strains represented in the vaccine construct will be evaluated. The sequence distance will be defined for each breakthrough infection relative to each HIV-1 sequence represented in the vaccine for *gag*, *nef*, and *pol*. The viral sequence data will also be compared with the HIV-epitope-specific cytotoxic T cell responses detected at 4 weeks after the last vaccination (week 30), the primary immunogenicity time point.

At study inception, a cross-laboratory standardization exercise will be performed, using duplicate aliquots of 3 different HIV-1 positive plasma samples. Using a shared protocol, the 2 laboratories will each derive 5 sequences from each sample. The sequences and the separate and combined phylogenetic trees will be analyzed at SCHARP for consistency.

The primary goals of these studies are to identify the initial replicating strain and to identify patterns of viral evolution, in particular:

- Determine if the infecting HIV strain had gag, nef and pol sequences that were represented in the vaccine.
- Evaluate the early viral evolution and CTL reversion/escape in vaccine and placebo recipients.

Additional goals include:

- Measure viral diversity over time and define any potential differences in vaccine vs placebo and in Ad5 neutralizing antibody titer (Ad 5<18 vs Ad5>18).
- Determine if immune selection is greater or lesser in vaccinated persons.
- Determine which empirically determined vaccine-induced epitopes coincide with viral isolates defined in silico and experimentally (cloned viruses or biological clones).
- Evaluate the hypothesis that, if susceptibility to acquisition increased by vaccination, dual infections would be seen more commonly in the vaccinated group than in the placebo group, or even in those who received 2 doses of vaccine versus 3 (time/exposure adjustment for this latter idea is difficult).
- Determine if viruses cluster by site and acquisition. Are the infecting strains in local areas similar enough to say there are common source partners, or to define if there are strains to which inserts should be directed? Are there strains that are not on our potential T cell epitope (PTE) screening peptides such that they may have unique virulence from an acquisition point of view?
- Determine if HIV epitopes recognized from vaccination were present in the infecting strains. How well did the PTE peptides predict what the infecting strains were?

### 2.1.2. Host genetics

As part of the research to explain the STEP outcome, we will need to elucidate the relationship between host genetics and immunologic outcomes seen in the trial. The data are expected to be exploratory and hypothesis-generating; the research may reveal new information that can enable improved design for future vaccines.

MHC class I and II molecules present peptides to T cells. Therefore, the genetic variability in the HLA genotype is fundamental to the host T cell response induced by HIV infection and HIV vaccines. KIR genes encode for a family of

receptors that recognize MHC class I. The combinational diversity in the KIR and HLA systems lead to functional diversity within the NK and T cell populations. In addition, many other genes, such as CCR5, APOBEC, MIP-1alpha have diversity within the gene or copy number for which investigators have demonstrated a role in host response to HIV.

MHC class I sequencing has been completed for 75 cases. Also, about 300 participants for whom we have immunogenicity data (from Merck/HVTN concordance study) have been HLA genotyped to date. As vials are thawed for cellular studies in the immunogenicity and case control cohorts, the unused remaining cells are saved for DNA isolation for HLA analysis.

Investigators will examine the role of HLA, KIR and other genes in the immune responses induced by the vaccine in the STEP study. Planned research includes:

- Analysis of HLA type and association with risk of HIV infection: Comparisons of the HLA types restricting immune responses in cases and non-cases that received vaccine will be made to determine if HLA type may have contributed to acquisition.
- Analysis of HLA type and set-point viral load: Using the HLA data obtained for all cases within the vaccinee group, a comparison will be made to determine if there is a relationship between HLA type and viral load set point. This analysis may be performed as a comparison with published studies (comparing HLA and viral load).
- Analysis of HLA type and cellular immunogenicity studies: Immunogenicity assessments (ELISpot and/or flow cytometry) will be conducted on the same set of vaccine recipient cases and non-cases described above. This will allow an overview analysis of the relationship between HLA type and the magnitude of immune responses.
- The potential relationships between KIR and HLA class I, HIV viral load, Ad5 nAb serostatus and vaccine-specific cellular immune responses will be examined.
- The relationship between other targeted genes (e.g., CCR5, APOBEC, MIP-1alpha) and HIV viral load, Ad5 nAb serostatus and vaccine-specific cellular immune responses may be examined as well.

### **PLANNED HVTN RESEARCH**

**Title:** *A phase I clinical trial to examine the role of host genetics in determining the cellular immune response to HIV vaccination in humans*

**Hypothesis:** *Host genetic background determines CTL targeting specificities and response magnitudes, the polyfunctionality spectrum of insert-specific T-cells, the pattern and magnitude of systemic cytokine expression associated with innate immunity, and the magnitude of the HIV-specific neutralizing antibody response.*

**Design:** *25 pairs of monozygotic twins and 25 pairs of dizygotic twins (N=100) will be divided into 4 groups. In Groups 1 (mz) and 2 (dz), 20 pairs will receive a DNA prime expressing clade B Gag-Pol-Nef and clade A/B/C Env at 0, 1, and 3 months and a multiclade rAd5 boost expressing clade B Gag-Pol and clade A/B/C Env at 6 months;*

*in Groups 3 (mz) and 4 (dz), 5 pairs will receive the rAd5 vaccine at 0, 1, 3, and 6 months.*

### 2.1.3. Acquisition among Ad5 nAb seropositive persons

The HVTN has initiated several protocols and protocol modifications to evaluate the mechanisms of possible increased acquisition among Ad5 nAb seropositive persons. The protocol modifications include:

- Amendments to HVTN 502 (STEP): Extended follow-up (to the end of 2009) to allow more precise estimation of the risk associated with vaccination; additional PBMC collections have been proposed at weeks 130, 156, 208 for all vaccinees.
- Amendments to HVTN 503 (Phambili): Extended follow-up (3.5 years from time of enrollment for participants who remain HIV uninfected); more frequent follow-up and increased blood collection.
- Amendment to HVTN 071: Extend leukapheresis to all sites and collect PBMCs as close to a peak time point after vaccination and a durability time point. Leukapheresis provides sufficient number of PBMCs to be able to perform an array of assays aimed at helping elucidate factors involved that lead to the observed results in the STEP Study.

### **PLANNED HVTN RESEARCH**

**Title:** *Collection of peripheral blood specimens from participants in the STEP Study and related trials of the Merck trivalent Ad5 vaccine*

**Hypothesis:** *Many hypotheses have also been proposed to explain the ineffectiveness of the STEP study vaccine in providing protection. A specimen repository will be created and specimens allocated for the conduct of approved research proposals.*

**Design:** *Peripheral blood mononuclear cells collected by leukapheresis and phlebotomy from 36 participants.*

**Title:** *Evaluation of cellular immune responses at mucosal sites following immunization with HIV antigens delivered using an adenovirus serotype 5 vector*

*See Section 2.3 for details.*

## **2.2. Breadth of T cell response**

The extensive sequence variation exhibited by HIV is arguably the most difficult hurdle to overcome in developing an effective AIDS vaccine. Such variability is one of the major obstacles to the induction of both cellular and humoral responses. T cell responses seem more likely to be cross-clade but even these responses are limited in this capacity. Several approaches have been proposed to try to induce more broadly reactive immune responses.

### 2.2.1. Heterologous inserts

The key role that HIV-1 specific CD8 T cells have during acute HIV infection has been highlighted by the temporal association of the first emergence of these cells

to the decline of HIV viremia from high levels to the viral set point, and the resolution of clinical symptoms of the acute retroviral syndrome. In most cases these CD8+ T cells are narrowly directed against a small number of immunodominant epitopes.

Despite the vast diversity of the global HIV gene pool and the capacity of HIV genes to evolve under selective pressure, HIV gene loci encoding 9-mer peptide T cell epitopes nevertheless exist on a spectrum ranging from relatively conserved to highly variable. Conserved and variable epitopes reflect sites that are relatively intolerant or tolerant of change, and therefore relatively less or more capable of mutational escape from host immune responses. Thus an immune response directed predominantly against conserved T cell epitopes would be a highly desirable outcome after vaccination, since such a response would be expected to provide optimal protection from a diverse viral challenge in uninfected individuals, and offer the most robust antiviral effect in individuals with established infection. In agreement with these considerations, CTL responses among individuals with HLA B57 and B27 (haplotypes associated with delayed disease progression) have been shown to target conserved epitopes.

These observations suggest that the optimal CTL-based vaccine should contain mostly conserved epitopes and few variable epitopes. One approach would be to include only conserved epitopes within an entirely engineered immunogenic insert. An alternative approach would be to capitalize on the adaptive properties of the CTL response through the use of a heterologous-insert prime-boost regimen. Following initial antigenic exposure, anamnestic T-cell responses persist at low levels. After any additional crossreactive antigenic exposure, memory responses preferentially re-expand and suppress the formation of new CTL responses. Therefore, when using overlapping non-identical immunogenic inserts (for example, gene inserts from different strains) are used in a prime-boost regimen, epitopes common to both priming and boosting inserts may be preferentially expanded. Without further stimulation at the boosting stage, priming insert-specific responses will eventually be lost, while responses to epitopes present only in the boost insert are actively suppressed. In this manner only the shared epitope responses are selectively expanded as they are stimulated at both the priming and boosting stages.

Because epitopes shared between two divergent strains are generally conserved epitopes, heterologous insert prime-boost vaccination regimens should enhance the epitope “coverage” over traditional homologous insert prime-boost vaccination strategies, by eliciting responses directed at epitopes that are highly prevalent within the epidemic.

#### **PLANNED HVTN RESEARCH**

**Title:** *A Phase Ib clinical trial to evaluate the safety and immunogenicity of recombinant adenoviral serotype 35 (rAd35) that encodes for the HIV-1 Clade A Env followed by rAd5 that encodes for the HIV-1 Clade A or B Env glycoprotein in healthy, HIV-1 uninfected adult participants*

**Hypothesis:** *A heterologous-insert prime-boost vaccine regimen can preferentially elicit CTL responses against conserved HIV epitopes.*

**Design:** 72 participants will be divided equally into 2 groups. Both groups will be given an Ad35 vector with clade A envelope insert, followed at 6 months by an Ad5 vector with either clade A (Group 1) or clade B (Group 2) envelope insert.

### 2.2.2. Heterologous vectors

Data from studies in nonhuman primates as well as clinical trials in humans indicate that vaccine regimens that employ the same vector twice (as a prime-boost regimen or as a repeated boost) may not be optimal for eliciting strong responses to insert sequences. This likely occurs because strong immune responses are induced to vector epitopes by the initial exposure and these responses limit vector replication\expression after subsequent exposures. An example of the superior responses seen with heterologous prime-boosting was seen in HVTN 055, which assessed the immunogenicity of 3 regimens:

- 5 doses of fowlpox (rFPV#1 – gag/pol and rFPV#2 –tat/nef/rev-RT),
- 5 doses of rMVA (rMVA#1 – gag/pol and rMVA#2 –tat/nef/rev-RT),
- 2 doses of rMVA followed by 3 doses of fowlpox.

The IFN- $\gamma$  ELISpot responses clearly demonstrate the superiority of the heterologous prime-boost regimen over the 2 purely homologous regimens.

The magnitude of all responses, even those elicited by the heterologous regimen, was relatively low. It is possible that cross-reactivity between the 2 vectors exists. Antivector responses elicited by the poxvirus prime may limit responses to the heterologous poxvirus boost, albeit to a lesser extent than for the homologous prime-boost regimen.

Another potential approach, which could further reduce the possibility of attenuation of booster immunization responses, would be to boost with vaccine vectors from another class. This approach has been explored in a nonhuman primate model using pox and adenovirus vectors with *gag* inserts by Casimiro et al. In these experiments, Ad5 vaccination at 0, 1, and 6 months elicited relatively high level responses (129-449 SFC/  $10^6$  PBMC), but boosting with Ad5 did not increase responses over respective peak post-prime responses. Homologous prime-boosting with MVA at 0, 1, and 6 months gave responses close to background. However, heterologous boosting with MVA at 6 months after Ad5 prime (at 0, 1 month) resulted in responses that were roughly 10-fold higher (901-1,562 SFC/  $10^6$  PBMC) than the homologous Ad5 regimen.

In addition to increasing the frequency and magnitude of responses, using different vectors may also affect the phenotypic and functional characteristics of the immune response.

## **PLANNED HVTN RESEARCH**

**Title:** A Phase Ib clinical trial to evaluate the safety and immunogenicity of vaccine regimens with DNA prime followed by MVA- and Ad5-vectored boost used singly or sequentially (as heterologous boosts) in healthy, HIV-1 uninfected adult participants

**Hypothesis:** Boosting with a vector of a different class will improve the immunogenicity (as defined by response rate, functionality, magnitude, and breadth of response) over that using the same vector class, and that the order of the vectors will affect immunogenicity.

**Design:** 180 participants will be divided equally into 6 groups. 4 groups will receive a 2-dose DNA prime (with gag insert) followed at 6 months by either Ad5 (with gag-pol insert) – Group 1; MVA (gag-pol-env) – Group 2; MVA at 3 months followed by Ad5 at 6 months – Group 3; or Ad5 at 3 months followed by MVA at 6 months – Group 4. The remaining two groups will receive either MVA or Ad5 without DNA, followed by the alternate vector boost at 6 months – Groups 5 and 6, respectively.

**Title:** A phase 1b clinical trial to evaluate the safety and immunogenicity of recombinant adenoviral subtype 35 (rAd35) and subtype 5 (rAd5) HIV-1 vaccines when given as a heterologous prime-boost regimen or as boosts to a recombinant DNA vaccine in healthy, Ad5-naïve and Ad5-exposed, low risk, HIV-1 uninfected adult participants (HVTN 077)

**Hypothesis:** An adenovirus subtype 35 vector, when used in combination with DNA or Ad5-based vaccines, will demonstrate substantial immunogenicity (magnitude and frequency of response), while potentially circumventing the issues raised by Ad5 pre-existing immunity; moreover, pre-existing immunity to Ad5 will affect immunogenicity.

**Design:** 192 participants will be divided into 4 groups. Group 1 (34 + 6 placebo) will receive an Ad 35 vaccine encoding HIV-1 clade A Env glycoprotein followed at 6 months by an Ad 5 vaccine encoding HIV-1 clade A Env glycoprotein. The remaining groups will receive a recombinant DNA plasmid encoding HIV-1 clade A Env glycoprotein, repeated at 1, 2, and 3 months; Group 2 (48 + 8 placebo) will receive the Ad 5 vaccine at 6 months; Group 3 (48 + 8 placebo) and Group 4 (34 + 6 placebo) will receive the Ad35 vaccine at 6 months, but Group 4 will have pre-existing Ad5 nAb.

**Title:** A Phase I clinical trial to evaluate the safety and immunogenicity of a prime-boost HIV vaccine using two recombinant adeno-associated virus serotypes in healthy, HIV-1 uninfected adult participants

**Hypothesis:** A regimen of 2 adeno-associated virus vaccines containing clade B gag, protease and reverse transcriptase proteins and clade A envelope, given in different prime/boost combinations, is safe and tolerable in HIV-uninfected healthy adults.

**Design:** 108 participants (90 vaccine, 18 placebo) will be divided into 5 groups. Groups 1-3 (10 + 2 placebo) will receive increasing doses of an adeno-associated virus vaccine containing clade B gag, protease and reverse transcriptase proteins and clade A envelope (AAV1). If it is safe to proceed, Group 4 (30 + 6 placebo) will be given the maximally tolerated dose of AAV1, boosted at 4 months by a similar adeno-associated virus vaccine (AAV2); Group 5 (30 + 6 placebo) will be given an AAV2 prime and AAV1 boost at the same time points.

### 2.2.3. Immunodominance and administration of vaccine at heterologous sites

The extensive sequence variation exhibited by HIV is arguably the most difficult hurdle to overcome in developing an effective vaccine. Such variability is one of the major obstacles to the induction of both cellular and antibody responses. T

cells seem to be more able to generate cross-clade responses but even these are limited in this capacity. Several approaches have been proposed to try to induce more broadly reactive cellular and humoral immune responses. Mosaic vaccines are artificial sequences that are computationally designed to maximally cover potential circulating epitopes, resulting in a vaccine cocktail containing multiple mosaic sequences. It has been theoretically demonstrated that a mosaic vaccine approach can be used as a universal vaccine; however, this assumes that a vaccinated individual will respond to all components of the vaccine. A complementary approach is the use of a vaccine that contains separate inserts corresponding to natural HIV strains representative of different clades. The success of either of these approaches to induce more broadly reactive immune responses will ultimately be affected by immunodominance: will the addition of epitopes to a vaccine result in the induction of new immune responses to divergent epitopes or merely a shift in the specific epitopes recognized without a true broadening of the response? The answer to this question is critically important to understand whether a mosaic or multiclade vaccine strategy will be able to induce cross-reactive T cell response more effectively than a single component vaccine.

Studies in nonhuman primates have demonstrated that monkeys receiving a multiclade Env immunization developed robust immune responses to all vaccine antigens and a greater breadth of Env recognition than monkeys immunized with vaccines including a single Env immunogen. These data suggest that a multicomponent vaccine encoding Env protein from multiple clades of HIV-1 can generate broad Env-specific T lymphocyte and antibody responses without antigenic interference.

One of the significant limitations in attempting to address issues related to immunodominance in human clinical trials has been the relative lack of appropriate vaccines that express either single or multiple matched inserts and are known to be immunogenic. The NIH Vaccine Research Center (VRC) multicomponent DNA/rAd5 prime/boost regimen, which includes envelope inserts from HIV-1 subtypes A, B, and C, provides such a platform. Comparison of a vaccine regimen consisting of either the VRC DNA plasmids encoding the Env ABC proteins, followed by boosting with the corresponding rAd5 vectors, with a prime-boost regimen with Env A alone will permit analysis of whether a multiclade vaccination is able to increase the breadth of vaccine-induced T cell responses. This increased breadth of T cell responses might be reflected in either a larger number of epitopes being recognized, an increased breadth of recognition of variant sequences by T cells specific for a given epitope, or a combination of both mechanisms. The distribution of shared epitopes within the VRC Env inserts indicates minimal levels of PTE overlap, suggesting that each Env immunogen may have unique epitope determinants that are not shared allowing for an increased breadth of T cell responses. While it is likely that responses induced by Env A alone will be somewhat cross-reactive, we hypothesize that the inclusion of multiple Env immunogens will result in a significant increase in the ability of the vaccine-elicited immune responses to recognize a broader range of HIV variants.

We also hypothesize that vaccination with a multiclade Env DNA prime/Ad5 boost at separate anatomical sites will result in increased magnitude or breadth

of T cell responses compared with those induced by vaccination with a multiclade vaccine administered at a single site. Studies in mice have shown that anatomic separation strategies utilizing epitope-modified DNA vaccines can be utilized to expand the breadth of vaccine-elicited cellular immune responses.

#### **PLANNED HVTN RESEARCH**

**Title:** *A Phase I clinical trial to compare the safety and immunogenicity of a recombinant adenoviral serotype 5 (rAd5) that encodes for the HIV-1 Clade A, B, and C Env glycoprotein with a rAd5 that encodes for the HIV-1 Clade A glycoprotein alone in healthy, HIV-1 uninfected adult participants*

**Hypothesis:** *A multiclade vaccine strategy will be able to induce cross-reactive T-cell response more effectively than a single-component vaccine; the inclusion of multiple Env immunogens will result in a significant increase in the ability of the vaccine-elicited immune responses to recognize a broader range of HIV variants.*

**Design:** *90 participants will be divided equally into 3 groups. Each group will be given a DNA vaccine encoding Env protein at 0, 1, 2 months, followed by an Ad5 vaccine encoding Env protein at 6 months. Vaccines given to Group 1 encode clade A Env protein; those given to the other two groups encode Env proteins from clade A, B, and C. Injections of the 3 Env vectors in group 3 will be separated by site of administration: 1 injection in the right deltoid, 1 in the left deltoid, and 1 in thigh muscle.*

**Title:** *A Phase I clinical trial to assess the impact of timing and site of vaccination on immunogenicity in healthy, HIV-1 uninfected adult participants*

**Hypothesis:** *Vaccination with a multiclade Env vaccine with each clade antigen administered at a separate anatomic sites can increase either the magnitude or breadth of T cell responses over vaccination with a multiclade Env vaccine administered at a single site multiple times.*

**Design:** *Participants will be divided into 2 groups. Group 1 will be given a DNA vaccine encoding Env protein at 0 months (in the right arm on day 0 and in the left leg on day 2) and at 1 month (in the right arm on day 30 and in the left leg on day 32), followed by an Ad5 boost at 4 months. Group 2 will receive the same vaccine regimen as Group 1, but all injections will be given in the right arm.*

#### 2.2.4. Intracellular processing and T cell response

Two related trials will test related DNA constructs to determine whether intracellular processing can be manipulated in order to shift the balance of the T cell response. These experiments will test 2 DNA constructs expressing HIV-1 proteins Gag, PR, RT, Env, Tat, Rev, and Vpu. JS7 largely expresses virus-like particles (VLPs) whereas JS2 expresses Gag in intracellular aggregates. JS7 differs from JS2 by an inactivating point mutation in protease that results in VLP formation and prevents the premature cleavage of over-expressed Gag leading to aggregate formation. Initial studies in humans of JS7 DNA boosted by MVA found lower CD8+ responses than had been seen in preclinical testing in nonhuman primates.

The hypothesis under investigation is whether the JS2 aggregated Gag (a misfolded protein) enters proteasome pathways that lead to class I presentation more efficiently than Gag in VLP, and whether this will lead to enhanced frequency and breadth of CD8+ responses in humans.

## **PLANNED HVTN RESEARCH**

**Title:** A phase 2a clinical trial to evaluate the safety and immunogenicity of a prime-boost vaccine regimen of pGA2/JS7 DNA and MVA/HIV62, in healthy, HIV uninfected vaccinia-naïve adult participants (HVTN 205)

**Hypothesis:** The heterologous DNA/rMVA prime-boost combination will elicit high titer T cell and antibody (Ab) responses. The DNA prime, expressing only HIV proteins, serves to prime a HIV-focused immune response; the rMVA boost, which expresses both HIV and MVA proteins, preferentially boosts the focused response.

**Design:** 225 participants (150 vaccine, 75 placebo) will be given pGA2/JS7 DNA vaccine at 0 and 2 months, boosted by MVA/HIV62 at 4 and 6 months.

**Title:** A Phase I clinical trial to evaluate the safety and immunogenicity of a prime-boost vaccine regimen of pGA2/JS2 DNA and MVA/HIV62, in healthy, vaccinia-naïve, HIV-1 uninfected adult participants

**Hypothesis:** JS2 DNA, which expresses aggregates of Gag, followed by a MVA62 boost can elicit a more balanced CD4+ and CD8+ T cell response to Gag than priming with the closely related JS7 DNA, which expresses virus-like particles.

**Design:** 36 participants (30 vaccine, 6 placebo) will be given pGA2/JS2 DNA vaccine at 0 and 2 months, boosted by MVA/HIV62 at 4 and 6 months.

### **2.3. Mucosal immune response**

One of the goals of the HVTN is to develop and evaluate vaccine approaches designed to optimize mucosal immune responses. This may include the definition and quantitation of T cells that home to mucosal surfaces, as well as cellular, antibody and innate responses that may inhibit the spread of HIV across genital mucosal surfaces or contain HIV replication within gut lymphoid tissues. HVTN studies which address this issue have become especially important in the aftermath of the STEP trial.

Hypotheses advanced to explain the ineffectiveness of the Merck Ad5 vaccine in providing protection generally posit that cytotoxic responses are of insufficient magnitude or fail to target appropriate epitopes necessary to effectively suppress early viral replication in mucosal tissues. To explain higher rates of infection among Ad5 nAb seropositive vaccinees, it has been hypothesized that recruitment of activated target cells to mucosal sites in men with prior natural adenoviral infection results in increased susceptibility to infection after exposure.

The hypothesis is consistent with ongoing studies that indicate adenoviruses are commonly shed in the stool of healthy higher-order primates and humans. In addition, adenovirus-specific T cell responses are seen in colorectal lamina propria and intraepithelial lymphocytes in surgical specimens from patients without evidence of active adenovirus infection (Jim Wilson, unpublished observations). These observations suggest that prior natural infection may result in persistent expression of adenovirus antigens in the gut and homing of adenovirus-specific cells to the gut. (It is not known whether natural infection results in homing of adenovirus-specific cells to genital tract mucosa.) Vaccination with Ad vectors subsequently may result in increases in activated adenovirus-specific cells which traffic to gut mucosa. This phenomenon likely did not occur in Ad-naïve participants who are boosted with Ad vectors after

receiving Ad-vectored vaccines intramuscularly because the vectors differ from natural infection with respect to imprinting and mucosal homing.

The focus of the investigations proposed in an ancillary study is to elucidate vaccine-induced immune processes at mucosal surfaces that may have rendered vaccinees more susceptible to HIV acquisition. Experiments exploring this question would delineate lymphocyte populations at mucosal sites (lymphocyte types and numbers, T cell subsets, activation states), and antigen specificities of mucosal effector cells, in vaccinees and placebo recipients. The primary focus of these studies will be on rectal mucosal immunity; a limited number of parallel studies will be conducted in genital mucosal samples to evaluate whether homing to these tissues occurs as well. These responses will be compared to those in peripheral blood.

Laboratory assays to carry out these studies are limited at present by the number of viable cells that can be obtained by biopsy. For example, mucosal tissues obtained by anoscopy and biopsy of rectal mucosa typically yield between  $10^4$  and  $10^5$  viable CD3+ cells, which is not consistently sufficient for more than a single ELISpot assay. In contrast, flexible sigmoidoscopy offers the possibility of safely obtaining a larger number of tissue biopsies from sites within the sigmoid colon and the rectum. Pooling of samples from multiple biopsies may yield upwards of  $\sim 10^6$  CD3+ cells, allowing for comprehensive qualitative and functional assessment of tissue-specific lymphocytes. Because flexible sigmoidoscopy is routinely used in studies of this kind, has a well-established safety history, and does not require patient sedation, we propose to carry out our studies (particularly functional studies measuring anti-vector responses) in the gastrointestinal tract using this technique. Specimens obtained by anoscopy can be fixed or frozen for assessments of lymphocyte populations (by immunohistochemistry) or used to generate single cell preparations for assessment of bulk activation by flow cytometry. Similarly, single cell preparations from cytobrush specimens or semen will be used to assess target cell populations and activation in the genital tract. Peripheral blood will be collected at the same visit for comparison of responses in the periphery and at mucosal sites. An amendment to the STEP study is underway to obtain tissue (flexible sigmoidoscopy) on 20 persons/group Ad5 nAb seropositive (vaccine + placebo).

#### **PLANNED HVTN RESEARCH**

**Title:** *A phase 1b clinical trial to evaluate mucosal immune responses to a DNA plasmid vaccine prime followed by an HIV-1 adenoviral vector boost in healthy adenovirus type 5 seronegative HIV-1-uninfected adults (HVTN 076)*

**Hypothesis:** *DNA priming and boosting with an adenovirus vector will improve cellular immune response at mucosal surfaces and in the periphery targeted to mucosal surfaces.*

**Design:** *45 participants will receive a DNA multiclade 6-plasmid vaccine (clade B gag-pol-nef, clade A/B/C env), followed by the same vaccine at 1 and 2 months and a multiclade Ad 5 vaccine (clade B gag-pol fusion, clade A/B/C env) at 6 months. Responses will be measured in peripheral blood, in semen, and in cervical and rectal mucosa.*

**Title:** *Evaluation of cellular immune responses at mucosal sites following immunization with HIV antigens delivered using an adenovirus serotype 5 vector*

**Hypothesis:** *Adenovirus-specific T-cell responses in the mucosa are greater in individuals with preexisting immunity to Ad5 than in those without, and greater in vaccine recipients than in placebo recipients.*

**Design:** *Exploratory cross-sectional analysis of participants who received the MRK trivalent Ad5 HIV vaccine or placebo in other trials. Mucosal, semen, foreskin, and blood specimens will be collected.*

**Title:** *Evaluation of Cellular Immune Responses and Activation at Peripheral, Mucosal and Genital Tract Sites Following Immunization with an Adenovirus Serotype 5 Vected Vaccine*

**Hypothesis:** *Vaccination with an Ad 5-vectored vaccine will show improved immunogenicity (frequency and activation) in individuals without prior natural Ad5 immunity over individuals with prior immunity.*

**Design:** *40 participants will be divided into 2 equal groups: 1 with immunity to Ad5 (titer > 18) and 1 without (Ad 5 titer ≤ 18); all participants will be given an Ad 5-vectored vaccine at a single timepoint. Responses (activation and adaptive immune responses to vector and insert) will be measured in PBMC and in cervical and rectal mucosa.*

## 2.4. Adjuvants

To date, only a handful of adjuvants (alum, MF-59, and most recently MTP) have been licensed for use in humans. Recent studies of both malaria sporozoite and HSV antigens indicate that improvements in adjuvant design can make critical differences in improving potential efficacy of recombinant proteins.

A goal of the HVTN is to perform clinical trials of novel adjuvants, assess the unique innate or adaptive immune response elicited by such adjuvants and utilize these adjuvants with HIV-1 immunogens to improve the breadth, quality, and magnitude of the immunological responses to HIV-1 vaccines and to assess if such responses improve vaccine efficacy, acceptability and cost.

HVTN has conducted 7 studies of adjuvants in the last 5 years:

- DNA + IL-2 (HVTN 044)
- PLG + MF59 (HVTN 049)
- DNA + IL-12 (HVTN 060)
- DNA, DNA + IL-15, DNA + IL-12 + IL 15 (HVTN 063)
- DNA + IL-12, DNA + IL-15 (HVTN 070)
- Peptides + RC529 SE + GCMSF (HVTN 056/061)

All of these studies showed ineffectiveness (near inertness) of cytokine adjuvants.

Recent advances in understanding innate immunity have started to elucidate the potential mechanisms by which adjuvants may enhance vaccine effectiveness. These studies suggest that the innate immune system, particularly dendritic cells

(DCs) and pathogen recognition receptors (PRRs), such as toll-like receptors (TLRs), play critical roles in initiating adaptive immune responses, and in controlling the strength and quality of the adaptive immune response. Work in the last decade has elucidated the mechanisms by which the immune system can recognize microbes or vaccines. Many of these processes are modulated through a series PRRs expressed in and on DCs. An important family of PRRs are the TLRs. Mammalian TLRs, of which 13 have been described so far, are widely expressed on a variety of innate immune cells, including DCs.

#### 2.4.1. Resiquimod

Recent studies indicate that conjugation of a recombinant protein with an optimal TLR agonist may markedly enhance subsequent adaptive T cell responses.

#### **PLANNED HVTN RESEARCH**

**Title:** *A phase I clinical trial to compare the safety, tolerability, and immunogenicity of an HIV-1 DNA plasmid vaccine administered intradermally with a topical resiquimod gel.*

**Hypothesis:** *A topically applied TLR 7/8 agonist (Resiquimod) will augment the adaptive immune response to a DNA vaccine; the route of vaccine administration (intradermal or intramuscular) and the timing of application will affect the performance of the adjuvant.*

**Design:** *96 participants (78 + 18 placebo) will be divided equally into 3 groups. Each group will receive DNA vaccine at Months 0 and 1; Group 1 will receive topical resiquimod immediately after vaccination; Group 2 will receive topical resiquimod 24 hours after vaccination; Group 3 will receive the vaccination only.*

#### 2.4.2. Electroporation

One of the recent innovations in vaccine design, especially with DNA vaccines has been the use of electroporation or chemical methods to increase the immunogenicity of plasmid DNA vaccines. Much of the reduced immunogenicity of DNA vaccines appears to be in the lack of uptake in antigen presenting cells. Preclinical studies have used electroporation as an adjuvant to DNA.

Electroporation creates a significant increase in the electrical conductivity and permeability of the cell plasma membrane by externally applying an electrical field. For vaccine research, electroporation would involve delivering brief electrical pulses to the muscle after injection of DNA vaccines. These pulses are believed to induce transient pore formation in cell membranes, facilitating entry of the DNA and the production of vaccine-encoded antigens. Electroporation is also believed to attract inflammatory cells, including antigen-presenting cells, to the site of immunization.

The electroporation technology has been shown to substantially increase delivery of DNA to cells, resulting in increased expression and elevated immune responses in nonhuman primates.

Among the variety of delivery methods of DNA vaccines explored to date, electroporation appears to be one of the most efficient. Numerous animal studies

have outlined the effectiveness of electroporation for enhancing the uptake, and consequently, the expression of plasmids encoding antigens, as well as inducing more potent cell mediated immune responses. Confirmation of the effectiveness of electroporation awaits results from the first ongoing Phase I clinical trials.

#### **PLANNED HVTN RESEARCH**

**Title:** *A Phase I clinical trial to evaluate the safety and immunogenicity of PENNVAX-B (gag, pol, env) with IL-12 DNA plasmid and electroporation, in healthy, HIV-1 uninfected adult participants*

**Hypothesis:** *Electroporation, when used as adjuvant to a DNA vaccine plus IL-12 DNA, is safe and tolerable in HIV-uninfected healthy adults. Electroporation will increase the magnitude of cellular immune responses to the vaccine.*

**Design:** *36 participants will be divided into 2 groups. In Group 1, 12 participants (10 + 2 placebo) will be given a multiantigen HIV DNA vaccine (gag, pol, env) with IL-12 DNA and electroporation at 0, 1, and 3 months. If it is safe to proceed with Group 2, 24 participants (20 + 4 placebo) will be given the same regimen.*

### **2.5. Innate immunity**

Many viruses and bacteria encode proteins that inhibit intracellular signaling by the TLR pathway. Vaccinia viruses encode A4GR, A52R, and NIL proteins which act as inhibitors of TLR signaling; A46R inhibits IL1 signaling and A52R inhibits both IL1 and IL-12 pathway and NIL inhibits signaling to NF $\kappa$ B through TLR-2, TLR-3 and TLR-4. As such, understanding the effects of viral vectors on the innate immune system and better understanding of how such effects influence subsequent adaptive immune responses are areas of increasing importance to the HIV vaccine field. For example, while it is recognized that human adenovirus type 5 influences the maturity of immune DCs and triggers the rapid release of IL-6, IL-12 and TNF- $\alpha$  in nonhuman primates, little information is available on the effects in human cells, and whether alterations in the vector backbone can help improve immunogenicity through specific adaptations in the innate immune response. While most studies on DCs have focused on their ability to trigger T cell responses, there is also evidence to suggest that DCs may influence other immune effectors including B cells. Indeed, myeloid DCs have been shown to trigger B cell growth and differentiation through the release of soluble factors such as IL-12 and IL-6, and/or membrane molecules such as BAFF/APRIL. A recent study suggests that virus-triggered plasma DCs induce, through IFN- $\alpha$  and IL-6 release, the generation of plasma cells secreting virus-specific antibodies.

The HVTN has established collaborations to unravel the precise molecular pathways of innate immunity that adjuvants and vectors stimulate. In so doing, we intend to provide in vivo data that will be used to improve the design of vaccine candidates. Specifically, we will help standardize and implement an in vitro platform using human PBMC to screen and fully evaluate the innate immune responses induced by vectors and adjuvants alone and in combination; then assess the impact of these innate responses on the phenotype, function and magnitude of HIV-specific T cell responses induced. We will also help elucidate how adjuvants and the innate responses shape the quality and persistence of

memory T cells in vivo. And for select adjuvants or combinations of adjuvants, we will perform clinical phase I trials administering adjuvants/immunogen combinations to evaluate the predictive value of in vitro findings to identify innate markers that correlate with reactogenicity and enhanced HIV specific immunity, including innate responses that may inhibit the spread of HIV. Lastly, we have collaborations with a wide range of investigators involving studies to evaluate transcriptional patterns of innate immunity after vaccination, which will be integral to the approaches mentioned above.

## 2.6. Antibodies

The ideal preventive HIV vaccine will elicit both T-cell-mediated immune responses and broadly nAb responses against HIV. Initially, nAbs were thought to be sufficient for protection against HIV, although data from clinical trials showed that nAbs from immunization with monomeric HIV gp120 were inadequate. The subsequent focus on the elicitation of CTL-based strategies has also been questioned, and thus eliciting a combination of humoral and cellular immune responses against HIV is likely to be the most effective strategy to prevent and/or control HIV infection.

Better understanding of Env structure and function, and of the structure of the core region of gp120, has led to an understanding of the mechanisms of neutralization and the target on the virus, presumably the Env trimer. Genetic information based on sequence diversity provides insight into regions that are dispensable for Env function and which may also serve to mask recognition of HIV Env in vivo. Still, approaches for eliciting antibodies that neutralize diverse HIV-1 isolates remain elusive.

One possible approach is a multiclade candidate vaccine. A multiclade immune response should help to reduce the likelihood of viral escape, both from CTL and antibodies. It has been suggested that diverse isolates show similar sensitivity to neutralizing antibodies, and that many HIV-1 strains share common antigenic determinants in their envelope glycoproteins. But recent primary isolates from Africa (clade C and clade A) appear to be more resistant to neutralization. The precise differences between disparate clades are unknown. Also unknown is the relationship of clade diversity to immunity.

Another possible approach is passive immunization with polyclonal and monoclonal antibodies (mAbs) — routinely used in cancer therapy and diagnostics, in autoimmune disorders, as antitoxins, and in the treatment or prevention of viral, bacterial, or parasitic infections. Recent studies suggest that targeting conserved epitopes may be protective, and that it could be possible to design an immunogen capable of inducing broadly reactive neutralizing mAbs by active vaccination. However, these immunogens are not currently available. Therefore, prevention of HIV by passive immunization should be thoroughly explored in the clinic to test whether these antibodies will actually protect against HIV infection. The HVTN proposes to evaluate passive immunization approaches in HIV-uninfected persons with pre-IND discussions with the FDA, Phase I clinical trials, and, if warranted, test-of-concept efficacy studies.

The initial Phase I studies will focus on safety and pharmacokinetics. Safety requirements are high for antibody therapeutics, but these hurdles have been overcome as over 20 mAbs and immunoglobulin Fc fusion proteins have received FDA approval. Safety requirements for prophylactic evaluation will be particularly rigorous, but extensive pre-IND discussions with the FDA and a thorough safety assessment plan will address the strategy for comprehensive safety evaluation of passive immunization by the HVTN. In formal pharmacokinetics studies, these antibodies showed distribution and elimination kinetics similar to those seen for other human-like mAbs, although now only two studies with HIV-infected patients, and a few studies in other disease settings, have established the pharmacokinetics of antibodies after long-term multiple-dose administration in humans. The HVTN will evaluate mAb by dose escalation, multiple infusion intervals, and variation in the duration of follow-up to establish the predictive efficacy of candidate HIV neutralizing mAbs for the prevention of HIV infection in adults. Ultimately, a test-of-concept study of the best candidate neutralizing mAb in high-risk populations for prevention of HIV infection will be designed, developed, and implemented by the HVTN.

## **2.7. Interchange between human and animal model research**

Various nonhuman primate models have been used to explore protection conferred to vaccinated animals against challenge, with inconsistent results. Coordination of macaque and human studies has been variable, especially with respect to doses, schedules, and routes of challenge. Moreover, identical vectors and inserts have rarely been tested in macaques and humans. At present, the results of vaccine studies in nonhuman primates cannot be extrapolated to predict vaccine immunogenicity or efficacy in humans.

Such results have been indeed been poor predictors of vaccine candidate immunogenicity in humans; over 70% of vaccines tested by the HVTN, though highly immunogenic in nonhuman primates, exhibited poor immunity in humans. After the results of the STEP study (which induced protection in preclinical testing using a SHIV 89.6 challenge, often in animals with a greater predisposition to an immunodominant response (MAMU-A1)), it is clear that different nonhuman primate challenge models are needed. This iteration between clinical efficacy trials and nonhuman primate studies is an integral part of improving the predictive efficacy of preclinical evaluation of HIV vaccines.

In 2006, the HVTN and DAIDS convened a workshop to determine if a more standardized approach of preclinical evaluation, especially in the nonhuman primate model, could improve preclinical design and develop better correlates between NHP and human clinical studies. The discussion at this workshop is described in a manuscript in press in PLoS Medicine.

Nonhuman primate models have allowed critical insights in HIV immunobiology. However, the variety of SIV models, HIV and SIV's varied pathogenesis, and the varied endpoints used in NHP models have not led to a validated model of protection of infection or disease progression. The need for interchange between clinical and animal model research has never been greater. The HVTN will continue to reach out to the animal model research community to establish

linkages that will not only enrich our understanding of SIV and HIV, but also provide the incentives that will attract young investigators to the field.

### 3. Clinical trials platform

As critical questions in HIV vaccine research are modified in the light of new data from human testing, the importance of a standard infrastructure for such testing is evident. This infrastructure is provided by the HVTN, which was created to provide a standardized platform for the prompt systematic evaluation of candidate HIV-1 immunogens.

Over the past 8 years, the HVTN has conducted 7 phase 1a and 13 phase 1a/1b trials (including 17 trials in which product or regimen was first-in-humans); 7 phase 1b trials (defining dose and schedule), 3 phase 2a trials, and 2 phase 2b test-of-concept trials. Vaccine constructs from academia/government (10), biotechnology (7) and pharmaceutical companies (10) have been evaluated.

Over half of the clinical trials conducted by the HVTN since its inception have been the initial evaluation of vaccine candidates for their immunogenicity and safety in humans. These studies have (unfortunately) shown that small animal models (mice/rabbits) and even NHP studies have been poor predictors of vaccine candidate immunogenicity in humans (Section 2.7).

In contrast, all candidate vectors that have gone forward into advanced clinical trials to date have been redesigned in some fashion after their initial Phase I trial. This has been the case for VaxGen gp120 (addition of GNE8/A244), ALVAC vectors (vCP205 to vCP1452), the VRC DNA (4 vs 6 plasmid DNA), GeoVax DNA (codon optimized and promoters altered) and MRK Ad5 gag to MRK Ad5 trivalent constructs (redesigned vector to increase stability of the transgene). Hence, much of the work of the HVTN has been an integral part of the discovery process of HIV vaccines.

The HVTN has built a standardized clinical trials platform that is flexible enough to drive the innovations needed to improve vaccine development and withstand the rigors and scrutiny that accompany all clinical trials. The HVTN has evaluated, and will continue to evaluate, vaccine candidates at all phases of development including first-in-human trials establishing initial safety and immunogenicity, and regimen optimization, through efficacy trials for worldwide registration of the first preventive HIV vaccine.

#### 3.1. Standard assays

The HVTN Laboratory Program has established a series of validated immunological assays for evaluation of the immunogenicity of candidate vaccines in humans, including serologic assays to define antibody responses to vaccination and a unique series of validated assays to measure T cell immunity to HIV genes. Assays to define vector-induced immunity and a variety of assays to define and confirm HIV infections after vaccination are also part of the laboratory's repertoire. Collaborations with a wide range of investigators involving

studies to evaluate genetic aspects of vaccine induced responses and protection from infection as well as transcriptional patterns of innate and adaptive immunity after vaccination are also part of the HVTN program.

The HVTN laboratories have developed a wide range of standardized and validated assays to measure antibody and T cell responses. The use of standardized validated assays is critical; however, we must learn from the data from each clinical trial and use these results to drive scientific innovation. In this section we will describe the standardized assays and how recent data have fostered further innovation as the field evolves. This is an important part of the flexibility of the HVTN clinical trials platform.

At present, 2 specific immunologic assays with the greatest utility in evaluating T cell responses elicited by HIV vaccine candidates are the IFN- $\gamma$  ELISpot and the flow cytometry assays. Together, these 2 assays are capable of reproducibly measuring both quantitative and qualitative parameters of antigen-specific T cell responses. Additionally, these assays can be performed with cryopreserved PBMC samples, thus permitting batch testing as well as retrospective immunogenicity studies. The HVTN has conducted extensive studies to optimize most of the steps that are common to the 2 assays, such as PBMC collection, processing, and cell counting of the samples. Extensive validation studies have been conducted for both assays.

The HVTN program has formally validated the IFN- $\gamma$  ELISpot assay and successfully implemented it in determining the immunologic endpoints in more than a dozen vaccine trials. In comparison with other assays frequently used to detect CD8+ T cell responses, the IFN- $\gamma$  ELISpot assay has proven to perform equally well to the ICS assay, and may be more sensitive than ICS for detection of low-frequency responses. IFN- $\gamma$  producing CD8+ T cells are a primary endpoint for many HVTN studies.

### 3.1.1. Role of current assays for future development

The recent data from the STEP trial indicate that IFN- $\gamma$  ELISpots are unlikely to predict protection against infection or even post-acquisition viremia among candidate HIV vaccines. Hence, as of this writing no immunologic assay predictive of clinical efficacy exists. However, the flow cytometry and IFN- $\gamma$  ELISpot platform have defined incremental improvements between vaccine candidates in immunogenicity and protection against experimental challenge in NHP and can be used in concert with other data to define the immunological characteristics of candidate vaccines.

### 3.1.2. New assays needed

The HVTN Laboratory Program has an extensive program focused on the development of new assays and validation of immune monitoring assays. Optimization of flow cytometry conditions requires at a minimum development of a variety of panels, including: IFN- $\gamma$ , IL-2, TNF- $\alpha$ , perforin, granzyme B and CD57; a panel with the same cytokines as above plus additional memory markers (e.g. CD45RA, CD27, CCR7); a larger bulk memory panel with more

markers; and bulk and Ag-specific panels that include CCR5 and other activation markers (e.g. KI67, Bcl2). Additional work is also needed to determine how to best measure vector specific immunity, including acquiring and validating peptides corresponding to Ad5 protein sequences for use in cellular assays, using whole Ad5 as stimulation for cellular assays, and developing stimulation methods for pox vector cellular assays. We are also focused on the development of a T cell neutralization assay to evaluate in vitro killing of laboratory and autologous isolates induced by vaccination, as well as post-acquisition responses.

The need to identify and quantify epitopes, and ensure that they are mapped accurately, has never been greater. Characterizing the epitopes to be conserved or variable, and measuring the overall breadth of coverage of circulating isolates by vaccine induced epitopes, will assist in vaccine insert design strategies.

As mentioned in Section 2.2.4, laboratory assays to carry out mucosal immunogenicity studies are limited at present by the number of viable cells that can be obtained by biopsy. How we characterize mucosal immunity will require developing procedures for collection and preservation of mucosal specimens for immunohistochemistry, as well as tested methods to use immunohistochemistry to quantify activated T cells, macrophages and dendritic cells in pre- and post-vaccination specimens. To meet these requirements the HVTN is working with multiple collaborators to establish an immunohistochemistry lab. We are also developing procedures for collection and preservation of mucosal specimens for cellular immunogenicity and activation assays to look for the presence of insert-specific, vector-specific and bulk responses.

### 3.1.3. Feeding information from assays back to the discovery process

Immunogenicity assays will likely provide the link between HIV pathogenesis and vaccine efficacy when we are able to identify immune responses that correlate with and/or directly protect against HIV infection. The ICS and  $\gamma$ IFN ELISpot assays, as well as the assays currently under development, will be used to inform the vaccine discovery process. Additionally, identifying genomic and/or proteomic patterns that innately alter susceptibility to SIV and/or HIV infection, whose encoded function can be manipulated for potential anti-viral and immunological therapeutics, will be applied to future studies.

The HVTN is working closely with the Pacific Northwest Correlates Consortium (McElrath, PI). This consortium thinks that at present, the best strategies to elucidate the correlates of immunity are to identify (1) how vaccines mediate protection against SIV infection in the nonhuman primate model, and (2) how persons exposed to HIV-1 exhibit resistance or unusual control of infection. This focus on discovering the immune correlates of SIV protection in the NHP and, by extension, providing the framework to confirm these findings in humans exposed to HIV-1 by sexual contact, will also guide us in the use of these assays in the clinical research and discovery process.

#### 3.1.4. Potential T cell epitopes

One challenge in the development of a standardized platform for assessing T cell response to vaccines is to be able to assess multiple vaccine candidates with differing inserts, and to measure immune responses to HIV-1 targets that reflect responses to real-world HIV-1 isolates. The HVTN has developed and standardized a panel of peptides with amino acid sequences that, to date, define the broadest range of immune response to circulating and infecting viruses. Dr. Fusheng Li, a faculty statistician with SCHARP, working with Drs. McElrath and Self, utilized the Los Alamos database to construct amino acid sequence information on all HIV isolates in the database. They then identified all PTEs in the database — that is, all possible unique amino acid sequences (9-mers) contained in the virus sequences. As the typical minimum epitope length is 9 amino acids, this has greater relevance than the percent difference in amino acids between proteins or immunogens. For a given virus isolate and a candidate antigen, the PTE distance is the proportion of PTEs in the isolate not covered by the candidate antigen, and PTE coverage is  $1 - \text{PTE distance}$ . Thus, for 2 antigens with no common potential epitopes the PTE distance is 1 and the PTE coverage is 0, likewise for 2 antigens with identical sequences the PTE distance is 0 and PTE coverage is 1.

### 3.2. Use of clinical trial platform to advance candidate development

At the core of the HVTN clinical trials platform is a coordinated development and implementation process. This process includes consistent protocol design, clearly defined endpoints for both safety and immunogenicity, rigorous safety monitoring, and the use of standardized assays. The outcome is data that are clear, interpretable and contribute to the field of HIV vaccine research in terms of the specific outcomes of the trials, as well as assessments of comparative immunogenicity and setting priorities for future research.

HVTN phase 1 protocols are usually designed as a single protocol with 2 separate parts; Part A which consists of an initial safety evaluation, followed by a selection (Part B or Phase IB) design. With all current assays (whether ELISpot, flow cytometry, or neutralization antibodies) the confidence limits among any immunological measurements from the Part A trial design are too wide to be useful to define if a vaccine is immunogenic or not. Thus, the HVTN has developed a “selection design” approach to evaluate a set of regimens constructed by varying vaccine formulation, schedule and route of administration. The goals of this design are to obtain a reasonably accurate assessment of immunogenicity for all of the regimens identified for initial evaluation and to select the most immunogenic regimen for further evaluation. The selection design used in the HVTN typically consists of 3-5 groups with approximately 30 participants in each vaccine arm and total placebo control group of approximately 20% of the total vaccine arms (eg, 30 vaccinees and 6 placebo/group). Vaccines with ample prior safety data are tested in Phase 1b trials without Part A.

The independent platform that the HVTN has developed, including the application of specific criteria for the advancement of HIV vaccine candidates, is one way of ensuring that the candidates with the best opportunity for success are efficiently developed. These criteria however must evolve and be consistent with

the current state of the field. The criteria guiding the decision to take a product into a phase 1 clinical trial have been:

- preclinical safety data that meet safety requirements deemed likely to achieve relevant regulatory agency approval,
- Candidate must show CTL and T helper activity and or neutralizing antibody activity against HIV in one or more relevant animal models, and
- If efficacy in non human primates is achieved in the absence of the above described T or B cell responses a product may also be considered if a scientific rationale for the efficacy is presented.

These criteria should and will be reevaluated following the results of the Step trial. Certainly the use of SHIV 86.9P alone is no longer considered a stiff challenge for a vaccine candidate.

What are the criteria we will use to advance T cell candidates to Phase 2B? Should we rely solely on protection in NHP studies using a mac 239 or other types of challenges?

The HVTN clinical trial platform can also be used to conduct head-to-head comparisons of candidate vaccines to determine whether there are competitive advantages in safety and/or immunogenicity between the candidates, especially products that fall into a class of agents such as poxvirus vectors, replication-defective adenovirus vectors, or DNA plasmids.

A critical role for the HVTN in maximizing the benefit that can be gained from a given portfolio of vaccine candidates is the network's role of fostering collaborations to ensure the best candidate vaccines are appropriately developed. The HVTN is uniquely positioned to identify vaccine candidates or parts of vaccine regimens, and to provide a collaboration framework for organizations with potential candidates that require clinical evaluation, to feed back into their discovery process. Only through this iterative process are we likely to identify the critical components of an effective HIV vaccine.

### **3.3. Portfolio of current trials**

- HVTN 060: A Phase I clinical trial to evaluate the safety and immunogenicity of an HIV-1 *gag* DNA vaccine with or without *IL-12* DNA adjuvant, boosted with homologous plasmids or with HIV CTL multiepitope peptide vaccine / RC529-SE plus GM-CSF, in healthy, HIV-1 uninfected adult participants
- HVTN 063: A Phase I clinical trial to evaluate the safety and immunogenicity of HIV-1 *gag* DNA vaccine alone or with *IL-15* DNA, boosted with HIV-1 *gag* DNA + *IL-15* DNA, or HIV-1 *gag* DNA + *IL-12* DNA, in healthy, HIV-1 uninfected adult participants
- HVTN 065: A Phase I clinical trial to evaluate the safety and immunogenicity of pGA2/JS7 DNA vaccine and recombinant modified vaccinia Ankara/HIV62 vaccine in healthy, HIV-1-uninfected adult participants

- HVTN 067: A Phase I clinical trial to evaluate the safety and immunogenicity of DNA vaccine EP-1233 and recombinant MVA-HIV polytope vaccine MVA-mBN32, separately and in a combined prime-boost regimen, when given to healthy, vaccinia-naive, HIV-1-uninfected adults
- HVTN 068: A phase Ib clinical trial to evaluate immune response kinetics and safety of two different primes, adenoviral vector vaccine (VRC-HIVADV014-00-VP) and DNA vaccine (VRC-HIVDNA009-00-VP), each followed by adenoviral vector boost in healthy, HIV-1 uninfected adults
- HVTN 069: A phase Ib clinical trial to compare the safety, tolerability, and immunogenicity of an HIV-1 adenoviral vector boost administered intramuscularly, intradermally, or subcutaneously after an HIV-1 DNA plasmid vaccine prime administered intramuscularly to healthy adenovirus type 5 seropositive HIV-1-uninfected adults
- HVTN 070: A Phase I clinical trial to evaluate the safety and immunogenicity of PENNVAX-B (*gag, pol, env*) given alone, with *IL-12* DNA, or with a dose escalation of *IL-15* DNA, in healthy, HIV-1–uninfected adult participants
- HVTN 071: A phase 1B open-label clinical trial to expand the characterization of the immune responses to the Merck Adenovirus serotype 5 HIV-1 *gag/pol/nef* vaccine in healthy, HIV-1–uninfected adult participants
- HVTN 072: A Phase 1B clinical trial to evaluate the safety and immunogenicity of recombinant adenoviral serotype 35 (rAd35) and serotype 5 (rAd5) HIV-1 vaccines when given in heterologous prime-boost regimens or as a boost to a recombinant DNA vaccine in healthy, HIV-1–uninfected adult participants with pre-existing immunity to adenovirus serotype 5 infection
- HVTN 204: A Phase II clinical trial to evaluate the safety and immunogenicity of a multiclade HIV-1 DNA plasmid vaccine, VRC-HIVDNA016-00-VP, followed by a multiclade recombinant adenoviral vector HIV-1 vaccine boost, VRC-HIVADV014-00-VP, in HIV-1 uninfected adult participants
- HVTN 502: A Multicenter, Double-Blind, Randomized, Placebo-Controlled Phase II Proof-of-Concept Study to Evaluate the Safety and Efficacy of a 3-Dose Regimen of the Merck Adenovirus Serotype 5 HIV-1 *gag/pol/nef* Vaccine (MRKAd5 HIV-1 *gag/pol/nef*) in Adults at High Risk of HIV-1 Infection
- HVTN 503: A multicenter double-blind randomized placebo-controlled Phase IIB test-of-concept study to evaluate the safety and efficacy of a 3-dose regimen of the Clade B-based Merck Adenovirus serotype 5 HIV-1 *gag/pol/nef* vaccine in HIV-1-uninfected adults in South Africa

## 4. Efficacy trials

Human efficacy trials, especially for T-cell-mediated vaccines, are a central part of the vaccine discovery process. The design of HIV efficacy trials involves several challenges. First, trials must assess multiple types of vaccine efficacy in terms of the ability to block infection and/or to delay disease progression and/or to reduce infectiousness. Second, since immunologic and virologic correlates of protection are unknown, efficacy trials will need to incorporate secondary objectives aimed at identifying such correlates. Third, the global nature of the epidemic and the genetic diversity of HIV mean that trials must accommodate heterogeneity in study populations and in infecting virus strains, both of which may affect efficacy. The HVTN is well poised to address these challenges.

The HVTN efficacy trials program currently employs the Phase IIB or test-of-concept trial design. The STEP and Phambili trials were designed as test-of-concept trials. The overarching design goals were to minimize the number of participants and time needed to advance an efficacious cell-mediated-immunity-based vaccine to phase 3 testing, based on the following characteristics: (1) restricting the study population to those with greatest chance of demonstrating efficacy; (2) assessing HIV infection and set-point viral load as co-primary endpoints; (3) combining separate statistical tests of the 2 endpoints, as opposed to a single test based on an endpoint that assigns zero viral load (or best rank) to uninfected subjects; and (4) using an interim analysis of efficacy after 30 infections and a final analysis after 50 infections. The HVTN Biostatistical Program, in collaboration with statisticians in industry and at the NIH, have been instrumental in designing these trials to make most efficient use of resources while rapidly advancing the science.

Besides providing the expertise in vaccine trial design, one of the major programs in the HVTN is to develop methods to analyze novel immunological assays as well as to develop standardized reagents to evaluate a diverse array of HIV vaccine platforms and their genetic inserts. The HVTN Biostatistical/Data Management Program, led by Dr. Steve Self, has written extensively on the methodologies to define ELISpot and flow cytometry responses after vaccination as well as the design of a unique set overlapping peptides that are representative of circulating strains of HIV regionally and globally.

It is critically important to use information gained from human efficacy testing to refine and redefine preclinical testing. While the NHP model has made critical insights in HIV immunobiology, the variety of SIV models, the varied pathogenesis between HIV and SIV's, and the varied endpoints used in NHP models have not led to a validated model of protection of infection or disease progression for HIV. Prior to 2006, the most common evaluation of HIV-1 immunogens was using a SHIV 89.6 challenge, often in animals with a greater predisposition to an immunodominant response (MAMU-A01). The vaccine construct used in STEP induced protection in this model. Despite the disappointing results, STEP also made some unique scientific contributions including demonstration that different nonhuman primate challenge models need to be developed for preclinical studies of vaccine efficacy. This iteration between clinical efficacy trials and nonhuman primate trials is an integral part of improving

the predictive efficacy of preclinical evaluation of HIV vaccines. Another important contribution of the Step trial was to demonstrate that a test-of-concept trial of an HIV vaccine candidate can provide clear, interpretable data in a relatively short period of time. One critical factor is having a network that is prepared to implement a global efficacy trial as soon as the data warrant.

#### **4.1. Trials to address correlates: immunity, protection, risk**

Identification of a vaccine-induced immune response (or responses) that correlates with protection from infection or disease is a central goal of HIV vaccine research. Correlates of protective immunity are useful for guiding vaccine development, improving immunogens iteratively between basic and clinical research, providing a basis for regulatory decisions, and guiding vaccine policy. A correlate of protective immunity can be used to build a bridge to predict vaccine effects in a new setting based on vaccine effects observed in a different setting (ie, in an efficacy trial). Different immunological measurements may build different kinds of bridges, having different prediction targets, for example across vaccine lots, human populations, or viral populations.

The term “correlate of protective immunity” means different things to different people, and therefore for clarity it is necessary to define some terminology. Often what is meant by a correlate of protection is an immunologic measurement that correlates with the rate of infection or disease in a population immunized with a vaccine that has some efficacy. The word “protection” in this definition may be a misnomer, because the immunologic measurement may only correlate with risk but have no causal effect on protection. Accordingly, an immunologic measurement that is associated with risk among vaccinees is referred to as a correlate of risk.

There are many potential immunologic and virologic factors that could influence the effectiveness of an HIV vaccine. These include prior immunity to the vaccine vector, HLA class I and II alleles, homozygosity or heterozygosity to specific alleles, and host genetic factors that influence a wide variety of HIV functions. All of these functions are present on enrollment and while likely in a randomized trial to be evenly distributed between vaccine and placebo recipients, may differ markedly in those who have high vs low set point levels of viremia.

Immunological correlates of protection are generally evaluated at a specified timepoint, eg, the peak immunogenicity timepoint post vaccination, or in the sample taken immediately prior to acquisition. Several conceptual types of assay and approaches can be undertaken. These include validated assays such as number of IFN- $\gamma$  producing CD8+ T cells or breadth of the T cell response as defined by the number of proteins, epitopes, or peptide pools to which positive responses are seen.

Studies of such correlates of risk are discussed in Section 2.1.

#### **4.2. Population preparation**

One critical component on the path to an approved HIV vaccine is testing in a variety of target populations. In addition to current and planned trials involving

men who have sex with men (MSM), the HVTN is actively working to design research targeting other populations affected by the epidemic and for whom critical questions have not yet been answered. Research development for such populations includes:

- Two planned observational studies (one US and one international trial) involving women considered at high risk for HIV acquisition
- Working group designing a research path for studies in injection drug users (IDU)
- Working group designing a research path for studies in adolescents

It will be important to determine the safety and immunogenicity profiles of candidate HIV vaccines in each of these populations.

#### 4.2.1. Women considered at high risk for HIV acquisition

Women at heterosexual risk in the Americas remain an important population for participation in HIV vaccine trials. From a public health perspective, women in the US account for more than one quarter of all new HIV/AIDS diagnoses. The Caribbean is the second-most affected region in the world after Africa, where the predominant route of HIV transmission is heterosexual contact. From a biological perspective, gender may influence the vaccine efficacy and interpretation of endpoints.

The primary objectives of HVTN research for this population are to define novel recruitment strategies that would enroll and retain women with an HIV acquisition risk that is compatible with conducting a subsequent test-of-concept efficacy trial (e.g., 1.5% annual incidence rate). Study designs which are locale specific and which utilize known epidemiologic characteristics of high risk women are being drafted for implementation in 2008.

#### **PLANNED HVTN RESEARCH**

**Title:** Longitudinal study of women at high risk for HIV-1 infection to inform HIV vaccine trial participation (HVTN 906)

**Hypothesis:** Recruiting and retaining women at high risk of HIV infection, with a focus on women who (1) reside or engage in risk behavior in local risk pockets and/or (2) are partners of men who are from subgroups with a high prevalence of HIV, is feasible.

**Design:** Multicenter observational study

**Title:** Longitudinal study among female sex workers at high risk for HIV-1 infection to inform HIV vaccine trial participation (HVTN 907)

**Hypothesis:** Recruiting and retaining women at high risk of HIV infection, with a focus on female sex workers with demographic, behavioral, or other social factors associated with high prevalence of HIV is feasible.

**Design:** Multicenter observational study

#### 4.2.2. Injection drug users

Our proposed clinical research for the IDU population is focused on addressing several central questions that are of general importance to HIV vaccine development, including:

- Will CTL mediated vaccines be less effective in reducing acquisition in persons who acquire HIV through routes other than sexual transmission (ie, through intravenous drug use)?
- How effective will CTL-mediated vaccines be in controlling virus and stabilizing CD4+ T cell counts in persons who acquire HIV through intravenous drug use?
- Which will provide greater efficacy: vaccines which seek to concentrate the immune response using inserts closely matched to circulating strains, or vaccines which target responses to more divergent strains of HIV?
- Will antivector immunity influence the effectiveness of the vaccine candidate?

The IDU working group is evaluating the possibility of testing vaccines among IDU and has several members who have experience working with IDU populations in the US and internationally.

#### 4.2.3. Adolescents

An HVTN/ATN (Adolescent Trials Network) collaborative effort is now in place to systematically evaluate and enhance currently available risk reduction tools for their efficacy among unique adolescent risk and to develop modules that can be used to effectively counsel adolescents against disinhibition in the context of a clinical trial.

### 4.3. **Cofactors that affect efficacy**

Three key biological cofactors have been identified to help explain the extent of the HIV epidemic and the differential spread of HIV across different regions in sub-Saharan Africa: lack of male circumcision, genital herpes, and HIV coinfections some of which induce higher HIV virus concentration in the blood such as malaria. Several randomized trials have been designed to examine the efficacy of targeting these biological cofactors on HIV acquisition and transmission. The results of these studies, as well as those proposed by the HVTN, will provide further guidance for future HIV vaccine clinical trials where these biological cofactors impact primary infection endpoints and/or vaccine efficacy.

Three trials examined the efficacy of male circumcision on HIV acquisition and reported a large protective effect of about 60%. Several ongoing trials are examining the efficacy of treating genital herpes on HIV acquisition, transmission, virus concentration levels, and disease progression. The role of herpes treatment in reducing HIV transmission to susceptible partners is currently being investigated by a multi-center trial funded by the Bill and Melinda Gates Foundation (BMGF) which is expected to report its results by 2009. Meanwhile,

one of the trials examining the role of herpes treatment on reducing HIV acquisition reported negative results at the CROI 2008 meeting. Positive outcomes in the other trial would establish the utility of herpes control by acyclovir, or herpes vaccination, as a potent tool in reducing HIV acquisition and transmission and/or delaying the onset of AIDS.

Growing evidence has also been affirming the role of coinfections, such as malaria, herpes, tuberculosis, Helminth infections, leishmaniasis and bacterial pneumonia in causing bouts of elevated HIV virus concentration in the blood. This evidence indicates the need to evaluate HIV coinfections in the context of HIV vaccine clinical trials when HIV virus concentrations are endpoints of these studies.

In addition to the role of coinfections in inducing higher HIV viral load, multiple studies of HIV and parasitic infection interactions such as schistosomiasis, lymphatic filariasis, intestinal helminths, and leishmaniasis, suggest the biological possibility of a role for parasitic infections in HIV epidemiology. It is becoming increasingly plausible that parasitic infections can enhance susceptibility to HIV infection, increase HIV transmissibility, and alter HIV disease progression. Conversely, HIV infection appears to affect the natural history, impedes the diagnosis, and reduces the efficacy of treatment of parasitic infections. These interactions must be taken into account when HIV vaccine trials are conducted in regions where these coinfections occur.

There is also evidence that cellular and humoral immune responses elicited by vaccines may be suppressed in malaria-endemic areas, as well as in areas with other coinfections. A number of factors could be responsible for this immunosuppression and the subsequent potential impact on vaccine induced immune responses and vaccine efficacy. The HVTN will assess the impact of malaria-induced immunosuppression on vaccine immune responses in the populations at risk.

Other factors may also suppress vaccine induced immune responses — eg, malnourishment or concurrent helminth infections. The impact of these factors on immune responses elicited by HIV vaccines warrants further investigation.

In summary, these and other factors may affect HIV acquisition and/or viral load, and some affect response to vaccines, including presumably HIV vaccine candidates. Factors likely to affect immune responses should be considered during phase 1-2 testing and could lead to regional differences in response to vaccine. During efficacy testing, one anticipates that these factors will be distributed equally among vaccine and placebo recipients. Reduced immune responses among vaccinees could lead to reductions in estimated vaccine efficacy compared to populations without these factors. The influence of factors affecting HIV acquisition and viral load will vary depending on their distribution and how the effect of each factor is influenced by the vaccine. They should be considered, nonetheless, as potential confounders or effect-modifiers of vaccine efficacy.