HIV VACCINE
TRIALS NETWORK

Protocol

HVTN 505

Phase 2b, randomized, placebo-controlled test-of-concept trial to evaluate the safety and efficacy of a multiclade HIV-1 DNA plasmid vaccine followed by a multiclade HIV-1 recombinant adenoviral vector vaccine in HIV-uninfected, adenovirus type 5 neutralizing antibody negative, circumcised men and male-to-female (MTF) transgender persons, who have sex with men

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At the first planned interim analysis for efficacy futility, conducted under Version 4 of the protocol on April 22, 2013, the DSMB recommended that the trial be stopped for efficacy futility. A total of 71 HIV infections had been diagnosed in the MITT cohort (41 among vaccine recipients, 30 among placebo recipients). Of these, 48 constituted primary endpoints (Week 28+ HIV infections diagnosed on or after Day 196 post-enrollment); 27 occurred among vaccine recipients and 21 among placebo recipients. There was no statistically significant difference in the rate of HIV infection between treatment arms, either in the Week 28+ cohort (estimated hazard ratio = 1.25; 95% CI: 0.71 to 2.20; p = 0.446) or in the MITT cohort (hazard ratio = 1.33; 95% CI: 0.83 to 2.13; p = 0.230). However, given that the number of HIV infections was larger in the vaccine arm, and given that there was a trend toward the hazard ratio increasing over time since enrollment (p = 0.09), the DSMB recommended, and the study team agreed, to continue to follow all participants beyond the Month 24 visit (the terminal visit in Version 4 of the protocol). Under Version 5 of the protocol, participants were followed post-unblinding to 48 months post-enrollment. Additional interim analyses were conducted at six-month intervals to evaluate the HIV-1 acquisition rate in the two treatment arms, and to evaluate conditional power to detect an increased rate of acquisition in the vaccine arm as compared to the placebo arm. At the second interim analysis, conducted on March 24, 2014 under Version 5 of the protocol, the study oversight group recommended that the protocol be revised to reduce the frequency of post-unblinding follow-up visits. A total of 109 HIV infections had been diagnosed in the MITT cohort to 48 months post-enrollment (53 among vaccine recipients and 56 among placebo recipients). There was no statistically significant difference in the rate of HIV-1 infection between treatment arms, either including all follow-up to 48 months post-enrollment (estimated hazard ratio = 0.92; 95% CI: 0.63 to 1.34; p = 0.65), or restricting follow-up to 24 months post-enrollment (estimated hazard ratio = 1.09; 95% CI: 0.72 to 1.66; p = 0.68). Given these data, there was very low power (< 1%) to detect an increased rate of HIV-1 infection in the vaccine vs. placebo arm, if the study were to continue following participants as specified under Version 5 of the protocol. As described in Sections 8.2 and 8.2.1, under Version 6 of the protocol participants will continue to be followed to 48 months post-enrollment, with an additional health contact at 60 months, but with study visits only annually following Month 24. The goal of this extended follow-up is to continue to monitor the rate of HIV-1 acquisition in the two treatment arms. To this end, the rate of study dropout will also continue to be evaluated in each treatment arm. The objectives of the study have been modified accordingly. Assessing the rates of study dropout and of HIV infection in the vaccine vs. placebo arms are now the primary objectives (see Section 5.2). Assessing the impact of vaccination on post-infection endpoints and on immunogenicity; assessing modification of vaccine effects by host immune genetic and other factors; and assessing immune correlates of risk are now exploratory objectives (see Section 5.4).

Contents

1 Ethical considerations

Multiple candidate HIV vaccines will need to be studied simultaneously in different populations around the world before a successful HIV preventive vaccine is found. It is critical that universally accepted ethical guidelines are followed at all sites involved in the conduct of these clinical trials. The HIV Vaccine Trials Network (HVTN) has addressed ethical concerns in the following ways:

HVTN trials are designed and conducted to enhance the knowledge base necessary to find a preventive vaccine, using methods that are scientifically rigorous and valid, and in accordance with Good Clinical Practice (GCP) guidelines.

HVTN scientists and operational staff incorporate the philosophies underlying major codes [1-3], declarations, and other guidance documents relevant to human subjects research into the design and conduct of HIV vaccine clinical trials.

HVTN scientists and operational staff are committed to substantive community input—into the planning, conduct, and follow-up of its research—to help ensure that locally appropriate cultural and linguistic needs of study populations are met.

HVTN clinical trial staff members counsel each participant at each study visit on how to reduce HIV risk. Participants who become HIV-infected during the trial are provided counseling on notifying their partners and about HIV infection according to local guidelines. HVTN clinical trial staff members will also counsel them about reducing their risk of transmitting HIV to others.

Participants who become HIV-infected during the trial are referred to medical practitioners to manage their HIV infection and to identify potential clinical trials they may want to join.

The HVTN provides training so that all participating sites similarly ensure fair participant selection, protect the privacy of research participants, and obtain meaningful informed consent. Participants should have their privacy protected, the opportunity to withdraw, and their well-being monitored.

Prior to implementation, HVTN trials are rigorously reviewed by scientists who are not involved in the conduct of the trials under consideration.

HVTN trials are reviewed by local and national regulatory bodies and are conducted in compliance with all applicable national and local regulations.

The HVTN designs its research to minimize risk and maximize benefit to both study participants and their local communities. For example, HVTN protocols provide enhancement of participants' knowledge of HIV and HIV prevention, as well as counseling, guidance, and assistance with any social impacts that may result from research participation. HVTN protocols also include careful medical review of each research participant's health conditions and reactions to study products while in the study.

HVTN research aims to benefit local communities by directly addressing the health HIV prevention needs of those communities and by strengthening the capacity of the communities through training, support, shared knowledge, and equipment. Researchers involved in HVTN trials are able to conduct other critical research in their local research settings.

The HVTN recognizes the importance of institutional review and values the role of in-country Institutional Review Boards (IRBs) and Independent Ethics Committees (IECs) as custodians responsible for ensuring the ethical conduct of research in each local setting.

The HVTN recognizes the importance of diversity in all research. The requirement for participants in this study to be circumcised may reduce participation from some ethnic or racial groups. While unfortunate, this is necessary in order to address the safety issues that were discovered from the results of the Step Study.

As new HIV prevention strategies become available and are scientifically validated, the HVTN will inform participants.

2 IRB/IEC review considerations

The US Food and Drug Administration (FDA) and other US federal regulations require IRBs or IECs to ensure that certain requirements are satisfied on initial and continuing review of research (Title 45, Code of Federal Regulations (CFR), Part 46.111(a) 1-7; 21 CFR 56.111(a) 1-7). The following section highlights how procedures in this protocol address each of these research requirements. Each HVTN Investigator welcomes IRB/IEC questions or concerns regarding these items.

2.1 Risks to participants

45 CFR 46.111 (a) 1 and 21 CFR 56.111 (a) 1: Risks to subjects are minimized.

This protocol minimizes risks to participants by (a) correctly and promptly informing participants about risks so that they can join in partnership with the researcher in recognizing and reporting harms; (b) respecting local/national blood draw limits; (c) having staff properly trained in administering study procedures that may cause physical harm or psychological distress, such as blood draws, HIV testing and counseling, and HIV risk reduction counseling; (e) providing HIV risk reduction counseling; and (f) providing safety monitoring.

2.2 Risk/benefit balance

45 CFR 46.111 (a) 2 and 21 CFR 56.111 (a) 2: Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result.

In public health research, the risk-benefit ratio may be difficult to assess because the benefits to a healthy participant are not as apparent as they would be in treatment protocols, where a study participant may be ill and may have exhausted all conventional treatment options. However, this protocol is designed to minimize the risks to participants while maximizing the potential value of the knowledge it is designed to generate.

Researchers have seen different patterns of HIV risk associated with some experimental HIV vaccines. Researchers need more information to make sense of these patterns.

In one study in Thailand (RV144), the vaccine lowered the risk of getting HIV by about 31%. The study was in people at lower risk of getting HIV.

Post hoc analyses in a previous trial (Merck V520 Protocol 023 / HVTN 502, also called the Step Study) of another adenovirus serotype 5 (Ad5) vector vaccine showed a potential increased risk of HIV infection in uncircumcised men who have sex with men (MSM) who had existing neutralizing antibodies (nAb) against naturally occurring Ad5 prior to receiving the study vaccine (see Section 4.1.3). The VRC rAd5 vaccine differs from the Merck-rAd5 vaccine that was used in the Step Study in the antigen content, vector platforms, immunization schedule, rAd5 vector construction and manufacturing substrate, and pattern of immune responses induced (see Section 4.3.2 for complete information). Also, the VRC rAd5 vaccine in this study has been administered at a lower dose than the

Ad5 vaccine evaluated in the Step Study. In addition, the HVTN 505 study limits enrollment to participants who share characteristics (circumcision and no detectable nAb to Ad5) with the subset of the vaccinated population in the Step Study that showed no increase in the rate of HIV infection compared to placebo (see Section 4.1.3 and Table 4-2). In this way, the HVTN has minimized the risk to HVTN 505 study participants while continuing to develop information important to the development of T-cell based HIV vaccines.

Results are now available for a second study using the same vaccine as the Step Study (see Section 4.1.3.6). That study, called Phambili, was conducted in South Africa and enrolled men and women at high risk for HIV. The Phambili study stopped further injections as soon as the Step study injections were stopped. The study results are showing more HIV infections in those who received the study vaccine. The increase in risk became significant about 2 ½ years after the first vaccination. In that study, men who were circumcised and who had never been infected with adenovirus type 5 were at increased risk of HIV infection like everyone else. What these results mean is not yet clear. Researchers are asking HVTN 503 participants to return to the clinic for more testing.

The HVTN 505 study stopped study injections on April 23, 2013 due to efficacy futility. Data presented to the DSMB showed that, through March 22, 2013, there were 27 Week 28+ endpoint infections in the vaccine arm and 21 in the placebo arm (HR 1.25 [95% CI 0.71, 2.20]). In the MITT population (defined as all participants who were enrolled and HIV-negative at baseline), there were 41 HIV infections in the vaccine arm and 30 in the placebo arm. While these differences are not statistically significant (P = 0.446 for Week 28+ infections, the primary endpoint cohort), numerically the observed infection rate is higher in participants who received the study vaccine.

Since efficacy futility criteria have been met, HVTN 505 has transitioned from a proof-of-concept efficacy trial to an observational study of the effect of the VRC DNA/rAd5 vaccine regimen on the rate of HIV-1 acquisition compared to placebo.

The HVTN will now monitor the participants to ascertain whether vaccine effects on the rate of HIV infection can be detected during an extended period of follow-up. We will continue with state-of-the art, individually tailored risk reduction counseling to ensure the safety of each study participant.

2.3 Subject selection

45 CFR 46.111 (a) 3 and 21 CFR 56.111 (a) 3; Subject selection is equitable

This protocol has specific inclusion and exclusion criteria for investigators to follow in admitting participants into the protocol. Participants are selected because of these criteria and not because of positions of vulnerability or privilege. Investigators are required to maintain screening and enrollment logs to document volunteers who screened into and out of the protocol and for what reasons.

2.4 Informed consent

45 CFR 46.111 (a) 4 & 5 and 21 CFR 56.111 (a) 4 & 5: Informed consent is sought from each prospective subject or the subject's legally authorized representative as required by 45 CFR 46.416; informed consent is appropriately documented as required by 45 CFR 46.417

The protocol specifies that informed consent must be obtained before any study procedures are initiated and assessed throughout the trial (see Section 9.1). Each clinical trial site in HVTN 505 uses an Assessment of Understanding to test each participant's understanding before enrollment into the study. The Assessment of Understanding (AOU) is a series of statements that a participant is asked to judge as "true" or "false." The content of the AOU relates to some of the basic elements of informed consent, such as the participant's ability to withdraw at any time without penalty. It also asks about the purpose of the study, whether the study vaccines can give someone HIV, the potential for testing vaccine-induced antibody (Ab) positive after receiving the study vaccine(s), and the need for a study participant to continue to reduce his or her risk of HIV while in the study. The HVTN 505 AOU also tests a participant's awareness of the Step Study results. Study staff review the answers with the participant and participants must verbalize the correct response to the answers that they got wrong in order to join the trial. Although there is no formal schedule for assessing continued understanding during the trial, the protocol makes it clear that "key study concepts should be reviewed periodically with the participant and the review should be documented" (see Section 8.1).

Each site is provided training in informed consent by the HVTN as part of its entering the HVTN. The HVTN requires a signed consent document for documentation, in addition to chart notes or a consent checklist.

2.5 Safety monitoring

45 CFR 46.111 (a) 6 and 21 CFR 56.111 (a) 6: There is adequate provision for monitoring the data collected to ensure the safety of subjects.

This protocol has safety monitoring in place (see Section 12). Safety is monitored regularly by clinical safety staff and routinely by the Protocol Safety Review Team (PSRT) (see Section 11.3).

As part of the protocol conduct, each participant will receive HIV risk reduction counseling at each study visit (see Section 8.4). HIV risk reduction counseling training has been provided to key study site staff members. These trained staff members then train other counselors at their sites.

2.6 Privacy/confidentiality

45 CFR 46.111 (a) 7 and 21 CFR 56.111 (a) 7: There are adequate provisions to protect the privacy of subjects and maintain the confidentiality of data.

Privacy refers to an individual's right to be free from unauthorized or unreasonable intrusion into his/her private life and the right to control access to individually identifiable information about him/her. The term "privacy" concerns research participants

or potential research participants as individuals whereas the term “confidentiality” is used to refer to the treatment of information about those individuals. This protocol respects the privacy of participants by informing them about who will have access to their personal information and study data (see Appendix A, Sections 8 and 18). The privacy of participants is protected by assigning unique identifiers in place of the participant’s name on study data and specimens. In the United States, research participants in HVTN protocols are protected by a Certificate of Confidentiality from the US National Institutes of Health (NIH), which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form. In addition, each staff member at each study site in this protocol signs a Confidentiality Agreement with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

Phase 2b, randomized, placebo-controlled test-of-concept trial to evaluate the safety and efficacy of a multiclade HIV-1 DNA plasmid vaccine followed by a multiclade HIV-1 recombinant adenoviral vector vaccine in HIV-uninfected, adenovirus type 5 neutralizing antibody negative, circumcised men and male-to-female (MTF) transgender persons, who have sex with men

Primary objective

- To evaluate the rate of study dropout in vaccine and placebo recipients
- To evaluate the effect of the VRC DNA/rAd5 vaccine regimen on the rate of HIV-1 acquisition compared to placebo

Study products and routes of administration

Note

All study participants have received at least some of the following products.

- **DNA vaccine**: Recombinant DNA plasmid (VRC-HIVDNA016-00-VP) composed of 6 closed, circular DNA plasmids that encode for HIV-1 Gag, Pol and Nef proteins (from clade B) and Env glycoproteins from clade A, clade B, and clade C. The dose is 4 mg, delivered intramuscularly (IM) via Biojector®.
- **rAd5 vaccine**: Recombinant adenoviral serotype 5 (rAd5) vector vaccine (VRC-HIVADV014-00-VP) composed of 4 recombinant non-replicating adenoviral vectors that encode for HIV-1 Gag-Pol polyproteins (from clade B) and Env glycoproteins from clade A, clade B, and clade C. The dose is 1×10^{10} particle units (PU), delivered IM via needle and syringe.
- **PBS**: The placebo for the DNA vaccine is sterile phosphate-buffered saline (PBS), VRC-PBSPLA043-00-VP delivered IM via Biojector®.
- **FFB**: The placebo for the adenoviral vector vaccine is the adenoviral final formulation buffer (FFB), VRC-DILUENT013-DIL-VP, delivered IM via needle and syringe.

Note

Study injections were discontinued as of April 23, 2013 after site notification of the April 22, 2013 DSMB recommendations. Subsequently, study participants have been unblinded to their treatment assignments (vaccine or placebo).

Table 3-1 Schema

		Injection schedule in months (days)			
		Prime			Boost
Group	N*	0 (0)	1 (28)	2 (56)	6 (168)
1	1250	4 mg DNA	4 mg DNA	4 mg DNA	10^{10} PU rAd5
2	1250	PBS	PBS	PBS	FFB

*Due to the randomization scheme, the numbers of vaccine and placebo recipients may differ slightly.

Participants

2504 healthy, HIV-uninfected, Ad5 nAb negative, circumcised US MSM and male-to-female [MTF] transgender persons, ages 18-50 years, at risk for HIV-1 infection through sexual exposure. The study was fully enrolled as of March 27, 2013. 1253 participants were enrolled into the active vaccine arm and 1251 participants were enrolled into the placebo arm.

Design

Extended unblinded follow-up of participants enrolled in the multicenter, randomized, placebo-controlled, (originally) double-blinded trial

Duration per participant

60 months for HIV-1–uninfected participants (48 months of clinic visits plus a participant health contact at Month 60). 6 months for participants diagnosed with HIV infection.

Estimated total study duration

Enrollment spanned June 2009 to March 2013. Given 60 months of scheduled follow-up for the final enrolled participant and potentially 6 months of additional visits should that participant be diagnosed with HIV infection at Month 60, the total duration from first enrollment to last follow-up visit is estimated at 9.25 years.

Investigational New Drug (IND) sponsor

DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)

Vaccine provider

- All products provided by the VRC (Bethesda, Maryland, USA)

Core operations

HVTN Vaccine Leadership Group/Core Operations Center, Fred Hutchinson Cancer Research Center (FHCRC) (Seattle, Washington, USA)

Statistical and data management center (SDMC)

Statistical Center for HIV/AIDS Research and Prevention (SCHARP), FHCRC

HIV diagnostic laboratory

University of Washington Virology Specialty Laboratory (UW/VSL) (Seattle, Washington, USA)

Endpoint assay laboratories

- UW/VSL (Seattle, Washington, USA)
- Duke University Medical Center (Durham, North Carolina, USA)
- FHCRC/University of Washington (Seattle, Washington, USA)
- NIAID Vaccine Immune T-Cell and Antibody Laboratory (NVITAL) (Gaithersburg, Maryland, USA)
- University of Washington (Seattle, Washington, USA)
- Henry Jackson Foundation (Rockville, Maryland, USA)
- UW-MRL = University of Washington Molecular Retrovirology Laboratory (Seattle, Washington, USA)

Study sites

US HVTN clinical research sites (CRSs) specified in the Protocol Opening Notice and subsequent Site Announcement Memos.

Safety monitoring

HVTN 505 Protocol PSRT; Protocol Team

3.1 Protocol Team

Protocol leadership

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4 Background and rationale

4.1 HIV epidemic and epidemiology

In 2007 the Joint United Nations Programme on HIV/AIDS (UNAIDS) reported a lower estimated number of persons living with HIV/AIDS than in previous years. However, the difference in the estimates result from changes in methods used in formulating estimates, not trends in the epidemic itself. In December 2007, UNAIDS estimated that 33.2 million (30.6 to 36.1) million people were living with HIV/AIDS globally. The 2007 global incidence was estimated to be 2.5 million (1.8 to 4.1 million) new cases per year. The estimated number of deaths due to AIDS in 2007 was 2.1 million (1.9 to 2.4 million) worldwide [4]. While the estimated number of deaths due to AIDS continues to decline, the number of people living with HIV continues to grow due to population growth and longer life expectancy from improved access to antiretroviral (ARV) medications [5].

On the basis of results from the Step Study, first reported in 2007 (see Section 4.1.3), HVTN 505 will enroll a study population comprising US MSM[1]. The current status of the HIV epidemic among MSM in the US, therefore, is directly relevant to HVTN 505. The US HIV prevalence data reported in October 2008 by the Centers for Disease Control and Prevention (CDC) are based on data gathered in 2006 [6]. This report estimates that 1.1 million adults and adolescents (prevalence rate: 447.8 per 100,000 population) were living with diagnosed or undiagnosed HIV infection in the United States at the end of 2006. Nearly half of all US HIV infections (48.1%) were in MSM. These estimates are based on 40 states that submitted data on both HIV and AIDS diagnoses, but of note several high-morbidity areas, including California, Illinois, Maryland, and the District of Columbia, did not contribute data about new HIV diagnoses. The June 2007 CDC fact sheet on HIV/AIDS among MSM reports that MSM accounted for 71% of new HIV infections among male adults and adolescents in 2005 even though only an estimated 5-7% of males identify themselves as MSM. The number of new HIV diagnoses decreased during the 1990s but more recent surveillance reports indicate an increase in HIV diagnoses among MSM. Diagnoses of HIV/AIDS among MSM increased 11% from 2001 through 2005, although it is not certain if this increase is due to more testing or to actual increased incidence [7]. A study of young MSM indicated that 77% who tested positive for HIV did not know they were infected [8]. The CDC has recommended greater emphasis on voluntary testing and counseling with the expectation that knowledge of HIV serostatus has the potential to reduce risk behaviors and the transmission rate of HIV infection [9]. A study conducted in June 2004-April 2005 in 5 large US cities found HIV prevalence among black MSM was 46% and among white MSM was 21% [10].

Given the difficulty of maintaining behaviors that prevent HIV transmission over a lifetime and the occurrence of non-consensual sex, the need for a safe and effective vaccine is clear. In 2009, results from RV 144, a large phase 3 clinical trial in Thailand, provided encouraging evidence that a preventive vaccine might reduce HIV acquisition [11] (see Section 4.1.2). In addition, models predict that even a vaccine that did not prevent infection but reduced VL and, therefore, reduced disease progression and transmission rates, would also have an impact on the HIV epidemic (see Section 4.5).

[1] In descriptions of the HVTN 505 study population, "MSM" is taken to include MTF transgender persons who otherwise meet the eligibility criteria (see sections 7.1 and 7.2). This definition of "MSM" does not necessarily apply in other contexts (eg, descriptions of previous research).

Implementation of a disease-modifying vaccination strategy would present unique challenges and would need to be part of a comprehensive multi-modality HIV-prevention program [12]. Multi-modality prevention is an important public health goal and current investigational approaches include improved strategies for testing and risk reduction counseling, better access to ARV treatment, circumcision, pre- and post-exposure prophylaxis (PrEP and PEP), development of topical microbicides that would inhibit transmission across mucosal surfaces, and reduction in outbreaks of concomitant sexually transmitted diseases that increase the risk of HIV transmission [13-15].

The VRC, DAIDS (NIAID, NIH), and the NIAID-funded HVTN are committed to the development of safe, effective vaccines to prevent HIV infection.

4.1.1 HIV vaccine development

In more than 2 decades of work on development of preventive HIV vaccine, a variety of strategies have been tested. Early candidate vaccines were protein immunogens that induced antibodies (Ab), but not broadly neutralizing antibodies (nAb). With a better understanding of the structural and immunological factors that make induction of nAb to HIV-1 envelope so difficult, more sophisticated approaches are now being developed. However, no candidate HIV-1 vaccines with known ability to induce broadly nAb are in clinical trials at this time. T-cell responses are believed to be critical in controlling HIV-1 replication and for the last several years, the development of candidate vaccines has emphasized T-cell immunity as well [16].

In the absence of natural immunity to infection with HIV-1, the design of a vaccination strategy has been based on knowledge of viral pathogenesis, including the study of infected individuals who are long-term nonprogressors (LTNP) [16] and the overall immune response to HIV infection in relation to infection parameters [17]. Cytotoxic T lymphocyte (CTL) responses specific to HIV contribute to reduction in VL during acute infection [18,19] and may be involved in protection against HIV disease progression. High-frequency CTL responses to HIV-1 are correlated with low VL and slow disease progression in chronically infected individuals [20].

Based on the premise that LTNPs have an immune response that is superior to those who have progressive disease and that this may serve as a model for desirable immune responses in vaccine candidates, a study was undertaken to characterize the T-cell functional profiles of 79 HIV-infected individuals and compare these profiles to those of 9 LTNPs. Flow cytometry was used to simultaneously evaluate 5 CD8+ T-cell functional markers: degranulation (CD107a), cytokine production (interferon gamma [IFN-γ]), tumor necrosis factor alpha (TNF-α), interleukin-2 (IL-2), and chemokine production (MIP-1β). LTNPs had a fundamentally different profile with enhanced CD8+ T-cell functionality. There was a dramatic loss of 5-function CD8+ T-cells in the progressors and both the frequency and proportion of 5-function CD8+ T-cells correlated inversely with VL in progressors. Diminished T-cell function in progressors was not a result of overall immunosuppression as polyfunctional responses to other viral infections (eg, Epstein-Barr virus, cytomegalovirus, and influenza) may be retained even in the presence of high HIV VL. In addition, the study data suggested that the functional quality rather than the quantity of HIV-specific CD8+ T cells is a correlate of protection from disease progression [21]. The VRC candidate vaccines being evaluated in HVTN 505 were developed to induce a polyfunctional T-cell immune response as well as Ab to the HIV-1 envelope protein.

To date, only 3 candidate vaccines have advanced into efficacy studies. VaxGen (San Francisco, CA) completed phase 3 efficacy studies of a gp120 protein immunogen that was known to induce binding, but not broadly nAb; no protective efficacy was observed [22,23]. A large phase 3 trial in Thailand of a recombinant canary pox vector prime with a gp120 protein boost was completed and the primary results, indicating a modest protective effect, have been published [11]. The third candidate, developed by Merck & Co., Inc. (Whitehouse Station, NJ) was a rAd5 vector encoding for the HIV proteins Gag, Pol and Nef. This vaccine was designed to elicit a T-cell response and was evaluated in phase 2b efficacy studies, known as the Step and Phambili Studies. It was found to not prevent HIV-1 acquisition and to not reduce the VL after HIV-1 infection [24]. An unexpected outcome of the Step trial was the observation of more HIV infections in the vaccine group than in the placebo group overall (see Section 4.1.3).

The outcome of the Step Study is sobering and has been considered carefully in the design of HVTN 505. The VRC HIV vaccine regimen comprising multiple DNA primes followed by a single boost with recombinant E1-, E3- (partial), and E4-deleted rAd5 encoding for the HIV proteins EnvA, EnvB, EnvC, Gag, Pol, and Nef is also designed to induce T-cell mediated protection. The differences in platforms, antigenic content, preclinical data, and immunological characteristics in phase 1 and 2 clinical trials have been carefully reviewed and judged to merit additional clinical evaluation of the VRC vaccine regimen.

4.1.2 RV 144 and rationale for sample size increase in HVTN 505

The efficacy of the prime-boost regimen of ALVAC-HIV (vCP1521) and AIDSVAX B/E for preventing HIV infection or modulating HIV viral load post-infection was examined in the RV 144 trial [11]. This study was conducted in two provinces in Thailand as a collaboration between the Thai Ministry of Public Health and the US Military HIV Research Program. It was designed as a multicentered, randomized, double-blind, placebo-controlled, community-based trial and enrolled 18 to 30 year old men and women without regard to individual risk for HIV infection. A total of 26,676 volunteers were screened and 16,402 were randomized. The vaccine regimen was safe and well-tolerated. After 42 months and 52,985 person-years of follow-up, 132 individuals acquired HIV-1 infection (56 in the vaccine group and 76 in the placebo group). Three analyses were conducted: intention-to-treat (ITT), modified intention-to-treat (MITT) and per protocol. The MITT analysis is thought by most observers to be the most appropriate because it excludes persons who were infected at study entry prior to receiving the first dose of vaccine ($n = 7$ in RV 144). Vaccine efficacies in the 3 analyses were 26.4% ($P=0.08$), 31.2% ($p = 0.04$) and 26.2% ($p = 0.16$), respectively, with the differences evident early post-vaccination in the Kaplan-Meier plots. Much debate concerning the analytic approaches and the interpretation of the p values took place in the scientific community but the trends were concordant across the analyses and consistent with a modest degree of protective efficacy [25]. Neither viral load nor CD4+ T-cell counts post-acquisition was different between the vaccine and placebo groups.

RV 144 remains the only vaccine study to demonstrate any protective efficacy for HIV-1 acquisition and it reignited the field. No immunologic correlate of protection was demonstrated on initial testing and questions concerning the mechanism of protection remain. AIDSVAX B/E had failed to protect when tested alone in a phase 3 trial [26] but as a boost to the ALVAC-HIV prime may have induced a protective effect through the production of binding antibodies to gp120 in this context. That the protective effect was mediated by antibody dependent cellular cytotoxicity (ADCC) remains one hypothesis. The

investigators and their collaborators are working with existing specimens and planning new studies to explain and replicate this result in higher risk populations.

The results of RV 144 are relevant to HVTN 505 from a number of perspectives and support elevating prevention of HIV-1 acquisition to a co-primary endpoint. RV 144 once again demonstrates the value of later phase testing of products in humans as part of the discovery process. The VRC regimen being tested in HVTN 505 elicits strong binding antibodies to Env, comparable at least to the levels induced by the RV 144 regimen [27]. Although the frequency of vaccine-elicited CD4+ T-cell responses were similar between the 2 vaccine regimens, the VRC regimen induced a higher frequency of HIV-specific CD8+ T-cell responses [28]. A greater number of samples are being stored prospectively in HVTN 505 on an individual participant level so the Team will be in a good position to determine the mechanism of protection, and possibly identify a correlate, should a positive result be seen. Taken together with the non-human primate data detailed in Section 4.4.1, these results support increasing the sample size to provide the power to examine HIV-1 acquisition in the 7-24 month post vaccination timeframe. [Note: On July 31, 2012, the HVTN 505 Oversight Group recommended that the study overenroll by 14% (300 participants) to bring the total N to 2500. This action was taken to insure that the study maintains the power for its key objectives.]

4.1.3 Results of the Step Study and rationale for the HVTN 505 study population

The Step Study (also known as Merck V520 Protocol 023 or HVTN 502) was developed as an efficacy trial in 3000 participants. It tested a rAd5-vector-based HIV vaccine encoding Gag, Pol, and Nef antigens developed by Merck & Co., Inc. (Whitehouse Station, NJ). While there are substantial differences between the Merck and VRC vaccine regimens (see Section 4.3.2), the Step Study is, to date, the largest clinical trial of an HIV-1 vaccine incorporating a recombinant adenoviral vector. As such, safety results from that trial have been considered in the development of HVTN 505. In particular, the Step Study results have been examined with respect to adverse reactions to vaccine, increase in risk of HIV infection, and potential exacerbation of HIV disease in vaccine recipients who acquire HIV infection [29,30].

In the Step Study, side effects of the vaccine were mild and similar to those reported in previous smaller studies of the Merck rAd5 vaccine [31]. There were no clinically significant differences in safety laboratory results between vaccine and placebo recipients. Of 40 serious adverse events (SAEs) reported by blinded study investigators, only 2 (fever, rigors) that were reported in the vaccine group were deemed related to study vaccine [29]. The comparable general safety data, showing no SAEs attributable to the VRC HIV vaccines and that they are generally well tolerated, are summarized in Section 4.6.1.

For the second and third potential safety issues, the HVTN 505 protocol team has carefully considered the results of the Step trial. We note that there are no human data that provide a statistically powered comparison of the HIV acquisition or HIV disease progression endpoints for recipients of the VRC HIV vaccine product and an appropriate control group. Therefore, to inform safety issues in HVTN 505 development the relevant, publicly reported Step Study results based upon the HIV infections diagnosed through October 17, 2007 [29] were considered and evaluated as supporting a conclusion that in circumcised, Ad5 nAb negative men, the Merck rAd5 vaccine did not elevate the rate of HIV infection. We note that less rAd5 vector will be administered in HVTN 505 than was the case in the Step Study (respectively, 1 dose of VRC rAd5 at 1×10^{10} PU determined by spectrophotometry compared to 3 doses of Merck-rAd5 at 3×10^{10} viral particles

determined by polymerase chain reaction [PCR]). An inference of vaccine safety in the Step Study sub-population supports a premise of vaccine safety in the HVTN 505 population, because HVTN 505 restricts enrollment to the sub-population in the Step Study for whom there was no evidence of increased HIV infection risk from exposure to the vaccine as assessed through the statistical analysis that follows.

At the first interim analysis in September 2007, the Step Study was found to have met the pre-specified futility boundaries. The study was fully enrolled and the majority of study vaccinations had been administered, but additional injections were stopped at that time. Additional exploratory analyses of the vaccine effect on HIV infection rate and on postinfection endpoints were conducted using all available data through October 17, 2007. Participants were blinded to vaccination assignment up to this date, implying that the amount of HIV exposure is expected to be the same in the vaccine and placebo groups. Because only 1 HIV infection occurred in female participants, the analyses were restricted to male participants. Credibility of the results is supported by the high rate of protocol adherence, with 94% of the vaccine and placebo groups receiving all 3 study injections, and by the high rate of retention, with 93.5% of vaccine recipients and 94.2% of placebo recipients staying in the study (participants had approximately 1 year of follow-up on average).

Univariate Cox proportional hazards models were used to quantify vaccine effects for various Step Study participant subgroups defined by demographic and/or baseline behavioral risk factors. The demographic factors included baseline Ad5 nAb titer (> 18, ≤ 18), self-reported circumcision status (circumcised, uncircumcised), age (≤ 30, > 30 years), race (White, Other), and region (North America, Other), while the behavioral risk factors included self-reports of the practice of unprotected receptive anal sex (URAS: yes, no), unprotected insertive anal sex (UIAS: yes, no), number of male sex partners (> 4, ≤ 4), and history of any drug use (yes, no), all in the 6 months prior to study entry. The time-to-event variable for the Cox model analyses was defined as the time from initial vaccination to the midpoint between the date of the last HIV seronegative visit and the date of the first evidence of HIV infection, as determined by the blinded Endpoint Adjudication Committee. Participants who never showed any evidence of HIV infection were right-censored on the date of their last study visit prior to October 17, 2007.

Multivariate Cox models were used to estimate the treatment effect after adjusting for potential confounding variables. Candidate confounders were pre-selected on the basis of their plausibility to impact HIV infection risk, and included the aforementioned demographic and baseline behavioral risk factors, with additional specificity for some variables. For example, the URAS and UIAS variables were each expanded to 3 variables defined by the corresponding risk behavior with an HIV seropositive (HIV+), HIV Ab negative (HIV-), or HIV status unknown partner; likewise, the history of any drug use was expanded to 2 variables, use of amyl nitrates (poppers) or use of amphetamines. The candidate confounders were all dichotomous for simplicity, stabilizing the model fitting, and reducing the modeling assumptions.

The Cox multivariate regression modeling was structured hierarchically into 4 sequential steps. First, separate interaction tests for each of 9 baseline variables with treatment were conducted. Based on the outcome of the univariate interaction tests, the multivariate base model 1 was defined as the model with independent variables treatment, Ad5 (> 18, ≤ 18), circumcision, and the interactions of treatment with Ad5 and treatment with circumcision. Model 2 included model 1 variables plus the candidate confounders that stayed in a backwards elimination model selection procedure that successively removed the variable

with the greatest p-value. Model 3 included model 2 variables plus any interactions of treatment with candidate confounders that stayed in the model after backwards elimination. Model 4 included model 3 variables plus the 2 interactions of circumcision with UIAS with an HIV+ partner and with UIAS with an HIV status unknown partner, if they stayed in the model after backwards elimination. The backwards elimination procedure used a Wald p-value threshold of 0.15 for removing variables; similar results were observed using a threshold of 0.10. To assess consistency of results, treatment effects for relevant subgroups were estimated using each of the 4 models.

Possible imbalance in HIV exposure between the vaccine and placebo groups was assessed. For each risk behavior variable, a generalized linear model for longitudinal binary data with logit link [32] was used to evaluate if and how treatment impacted the frequency of the reported behavior during 6-18 months after randomization. The baseline behavior status, age, and race were included as covariates to enhance the precision of the analysis and to increase compliance with standard modeling assumptions.

Analyses were conducted on the MITT population, which consisted of all randomized participants who received at least 1 dose of vaccine or placebo, except those who had a positive HIV screening test prior to randomization. Similar results were obtained for the per-protocol population (all randomized participants who received the first 2 doses of either vaccine or placebo, except those who were either diagnosed with HIV-1 infection before or at week 12 (ie, 8 weeks post dose 2) and/or were identified as protocol violators based on predefined criteria).

4.1.3.1 Univariate analyses of the vaccine effect on HIV infection

Forty-nine of the 914 male vaccine recipients became HIV infected (annual HIV incidence 4.6%, 95% confidence interval (CI) 3.4 to 6.1) and 33 of the 922 male placebo recipients became HIV infected (annual incidence 3.1%, 95% CI 2.1 to 4.3). The overall treatment effect HR (vaccine/placebo) from the univariate Cox model was 1.5 (95% CI 0.97 to 2.3, p = 0.07). Because the randomization was stratified within each of 4 pre-specified baseline Ad5 neutralization titer strata using the Merck Laboratories Ad5 nAb assay (see Section 4.2) (≤ 18, 19 - 200, 201 - 1000, > 1000), the vaccine effect within the Ad5 nAb negative stratum (Ad5 ≤ 18) and within the Ad5 seropositive stratum (Ad5 > 18) can be validly estimated, with the full protection of randomization from potential confounding bias. The vaccine effect can also be validly estimated within subgroups defined by self-reported circumcision status and within subgroups defined by Ad5 nAb status cross-classified with circumcision status, because circumcision status was measured prior to randomization and was independent of treatment assignment.

For subgroups defined by either Ad5 nAb status or circumcision status, HIV incidence estimates during 3 semi-annual periods from the time of enrollment, separately for the vaccine and placebo groups, were calculated as nonparametric estimates of discrete hazard functions. For Ad5 nAb negative men, the estimated HIV incidence was slightly lower in the vaccine group than the placebo group in all 3 time intervals; the result is the same for circumcised men. Using all of the available follow-up information, of the 776 Ad5 nAb negative men, the annual HIV incidence estimates were nearly identical in the vaccine group (20 infections, estimated annual incidence 4.1%) and the placebo group (20 infections, estimated annual incidence 4.0%) (Table 4-1). Similarly, of the 999 circumcised men, the annual HIV incidence estimates were nearly identical in the vaccine group (26 infections, estimated annual incidence 4.1%) and the placebo group (26 infections, estimated annual incidence 4.2%) (Table 4-1). In the 578 men who were both Ad5 nAb

negative and circumcised, there were 12 infections in the vaccine group (estimated annual incidence 3.2%) and 18 infections in the placebo group (estimated annual incidence 4.6%) (Table 4-1). In this subgroup the estimated HR (vaccine/placebo) was 0.7 (95% CI 0.3 to 1.4) (Table 4-2), indicating that within this subgroup it is unlikely the risk of infection was greater in the vaccine group.

Table 4-1 HRs of HIV infection for male subgroups defined by demographic and baseline behavioral risk factors (univariate Cox model analyses)

MITT Population	N	Number of HIV infections		HIV infection rate (% per year)		HR (Vaccine/Placebo) (95% CI)		Interaction p-value[1]
		Vaccine	Placebo	Vaccine	Placebo			
Demographic factors								
Ad5– (titer ≤ 18)	**776**	**20**	**20**	**4.1**	**4.0**	**1.0**	**(0.5 to 1.9)**	**0.08**
Ad5+ (titer > 18)	**1060**	**29**	**13**	**5.1**	**2.2**	**2.3**	**(1.2 to 4.3)**	
Circumcised	**999[2]**	**26**	**26**	**4.1**	**4.2**	**1.0**	**(0.6 to 1.7)**	**0.01**
Uncircumcised	**788**	**22**	**6**	**5.2**	**1.4**	**3.8**	**(1.5 to 9.3)**	
Whites	907	24	18	4.4	3.2	1.4	(0.8 to 2.6)	0.71
Non-Whites	929	25	15	4.8	2.9	1.6	(0.9 to 3.1)	
Age ≤ 30 yrs	970	28	19	5.0	3.5	1.4	(0.8 to 2.6)	0.81
Age > 30 yrs	866	21	14	4.1	2.6	1.6	(0.8 to 3.1)	
North America	1171	37	29	5.2	4.0	1.3	(0.8 to 2.1)	0.18
Others	665	12	4	3.4	1.1	3.0	(1.0 to 9.4)	
Behavioral risk factors[3]								
UIAS[4]: yes	1097	36	25	5.6	3.9	1.4	(0.9 to 2.4)	0.75
UIAS: no	739	13	8	3.1	1.8	1.7	(0.7 to 4.1)	
URAS[5]: yes	916	37	25	7.2	4.7	1.5	(0.9 to 2.5)	0.99
URAS: no	920	12	8	2.2	1.5	1.5	(0.6 to 3.7)	
Any drug use: yes	792	29	19	6.2	4.3	1.5	(0.8 to 2.6)	0.96
Any drug use: no	1044	20	14	3.3	2.2	1.5	(0.8 to 3.0)	
> 4 male sex partners	1101	32	23	5.1	3.5	1.5	(0.9 to 2.5)	0.88
≤ 4 male sex partners	735	17	10	3.9	2.4	1.6	(0.7 to 3.5)	

[1] 2-tailed p-value for a test of difference between the HRs for the 2 subgroups, not corrected for multiplicity

[2] circumcision data unknown for 49/1836 males, including 1 infected male from each of the vaccine and placebo groups

[3] behavioral risk data are based on self-reported behavior within 6 months prior to randomization

[4] UIAS = unprotected insertive anal sex

[5] URAS = unprotected receptive anal sex

Table 4-2 HRs (vaccine/placebo) (95% CIs) of HIV infection using univariate and multivariate Cox models for subgroups defined by baseline Ad5 nAb status and/or circumcision status

	Baseline Ad5 nAb titer regardless of circumcision status		Circumcision status regardless of baseline Ad5 nAb titer	
Analysis	**Ad5–**	**Ad5+**	**Circumcised**	**Uncircumcised**
Univariate	1.0 (0.5 to 1.9)	2.3 (1.2 to4.3)	1.0 (0.6 to 1.7)	3.8 (1.5 to 9.3)
Multivariate				
Model 1	1.0 (0.5 to 1.8)	2.4 (1.2 to4.7)	1.0 (0.6 to 1.7)	3.8 (1.5 to 9.3)
Model 2	1.1 (0.6 to 2.0)	2.7 (1.3 to 5.5)	1.0 (0.6 to 1.8)	3.8 (1.6 to 9.5)
Model 3	1.1 (0.6 to 2.0)	3.1 (1.5 to 6.5)	1.1 (0.6 to 2.0)	4.1 (1.6 to 10.4)
Model 4	0.8 (0.4 to 1.6)	2.6 (1.3 to 5.4)	0.9 (0.5 to 1.6)	3.4 (1.4 to 8.4)

Analysis	**Circumcised and Ad5–**	**Circumcised and Ad5+**	**Uncircumcised and Ad5–**	**Uncircumcised and Ad5+**
Univariate	0.7 (0.3 to 1.4)	1.6 (0.7 to 3.8)	3.3 (0.7 to 15.8)	3.9 (1.3 to 11.9)
Multivariate				
Model 1	0.8 (0.4 to 1.6)	1.4 (0.6 to 3.2)	2.5 (0.8 to 8.0)	4.3 (1.7 to 11.0)
Model 2	0.8 (0.4 to 1.7)	1.7 (0.7 to 3.8)	2.4 (0.8 to 7.3)	4.8 (1.8 to 12.6)
Model 3	0.6 (0.3 to 1.2)	1.3 (0.6 to 3.0)	2.0 (0.6 to 6.3)	4.6 (1.8 to 12)
Model 4	0.6 (0.3 to 1.2)	1.4 (0.6 to 3.1)	2.1 (0.7 to 6.6)	4.2 (1.6 to 11.1)

Ad5– and Ad5+ are baseline Ad5 nAb negative (≤ 18) and seropositive (> 18), respectively; 18 is the lower quantification limit of the Merck Laboratories Ad5 nAb titer assay; models 1-4 are of increasing complexity and differ in the number and type of baseline factors adjusted for in the Cox regression analysis (see Section 4.1.3).

4.1.3.2 Evaluation of effect modification by Ad5 nAb status and circumcision status

Given that the overall HR was 1.5 (95% CI 0.97 to 2.3), which trended toward statistical significance (p = 0.07), it is possible that the vaccine elevated the risk of infection uniformly, and the apparent safety in the Ad5 nAb negative circumcised subgroup could be due to statistical variations of the HR estimates across subgroups. However, this possibility is made less likely by the following additional results. First, based on interaction tests from a Cox model, there was statistical evidence that the HR was higher in Ad5 seropositive men than Ad5 nAb negative men (HR 2.3 versus 1.0 respectively, interaction test p = 0.08), and was higher in uncircumcised men than circumcised men (HR = 3.8 vs. 1.0, interaction test p = 0.01) (Table 4-1). In addition, there was evidence that the HR increased with $\log_{10}$ (Ad5) neutralization titer (univariate Cox model trend test p-value = 0.06).

Second, the univariate results did not materially change after adjusting for other baseline and demographic covariates in multivariate models. This result was obtained by estimating HRs adjusting for the significant independent predictors of HIV infection in multivariable models 2, 3, and/or 4. The variables age ≤ 30, non-White race, North American, URAS with an HIV+ partner, and URAS with an HIV status unknown partner were significant in all three models 2 through 4, whereas other risk behavior variables were independent risk

factors in only one of the models 2 through 4. Interactions of treatment with popper use and with UIAS anal sex with an HIV+ partner were evident in both models 3 and 4.

The multivariate analyses showed a consistent pattern of treatment effects by Ad5 nAb status and circumcision status across the 4 models, which were also similar to the univariate treatment effects. Furthermore, there was evidence that the HR increased with $\log_{10}$ (Ad5) neutralization titer even after adjusting for potential confounding variables (multivariate Cox model trend test p-value = 0.03). The covariate-adjusted treatment effect HRs for Ad5 nAb negative men and for circumcised men ranged from 0.8 to 1.1 (see Table 4-1). The similarity of the univariate and multivariate results support that the randomization and blinding were effective in eliminating confounding from the measured baseline variables, and that any increased risk of infection by vaccine was restricted to Ad5 nAb positive and/or uncircumcised men.

4.1.3.3 Comparison of risk behavior data between the vaccine and placebo groups

It is possible that, due to chance, exposure to HIV was greater in the placebo group for Ad5 nAb negative men and/or for circumcised men. This could render the estimated HR an underestimate of the true HR, in which case there would be less evidence for vaccine safety in Ad5 nAb negative circumcised men. To address whether this chance-imbalance may have occurred, the risk behavioral data were compared between vaccine and placebo recipients through 18 months of follow-up. For Ad5 nAb negative men rates of UIAS, URAS with an HIV+ or HIV status unknown partner, and any drug use trended statistically higher in the vaccine group, when averaged over the 6-18 month time period, and other risk behaviors had similar frequencies in the 2 treatment groups. For circumcised men the frequencies of the risk behaviors were generally similar in the 2 treatment groups. These results support that a chance-imbalance of greater HIV exposure in the placebo group for Ad5 nAb negative men and/or circumcised men did not occur. In addition, the trend of higher risk behavior in the vaccine group for circumcised men further support an inference of no elevation of HIV infection risk due to vaccination in Ad5 nAb negative circumcised men.

In sum, all of the Step Study analyses are consistent with a premise that the Step Study vaccine did not elevate the risk of HIV infection in Ad5 nAb negative circumcised men.

4.1.3.4 Vaccine effect on postinfection endpoints in Step Study male HIV-infected volunteers

Postinfection follow-up of HIV infected participants ranged from 0 to 1115 days (mean = 359 days). VL setpoints (using the pre-specified definition of the primary endpoint) were not materially different between vaccine and placebo recipients in the overall group of infected participants or within any subgroup defined by Ad5 nAb status or by circumcision status. Twenty-two participants initiated antiretroviral therapy (ART) (14 vaccine recipients and 8 placebos), all but 1 from North America. The estimated probability of initiating anti-retroviral therapy (ART) within 1 year of diagnosis was 0.23 for infected placebo recipients and 0.38 for infected vaccine recipients; time to ART initiation did not differ between treatment groups (log-rank p = 0.38). Longitudinal pre-ART VL and CD4+ T cell trajectories were also similar between treatment groups (p = 0.38 and 0.43, respectively). In sum, the vaccine did not have a measurable impact on postinfection outcomes, supporting that it did not exacerbate the course of HIV-1 disease progression. These data support a premise that the rAd5 component of the VRC product is expected to not exacerbate HIV-1 disease.

4.1.3.5 Step Study data through December 31, 2009

Vaccinations were stopped in the Step Study on Sept 19, 2007, after the DSMB's first interim efficacy analysis. Study participants were unblinded as to treatment assignment beginning in November 2007. Results from these early analyses have been published and included data through October 17, 2007 [29]. Follow-up of study participants was continued in the Step Study and (subsequently) HVTN 504 until 4 years from first study injection or until December 31, 2009. Because few HIV infections were detected among women over the entire follow-up period (2 prior to unblinding and 13 post-unblinding), analysis has been limited to men. There were 172 infections among 1836 men during the entire follow-up period; 88 before 18 months and 84 afterwards. For the entire follow-up period, there was a higher risk of HIV infection among the male MITT (modified intent to treat) vaccine recipients versus placebo recipients; the covariate-adjusted HR was 1.44 (95% CI: [1.05, 1.97], p = 0.03). Analyses of vaccine effects on HIV infection over time suggest that, in those subgroups that showed elevated risk associated with vaccine receipt (ie, Ad5 seropositive and/or uncircumcised men), the vaccine-associated risk appeared to be highest shortly after vaccination and to decrease after 18 months, possibly due to a waning vaccine effect [33,34]}. Note that circumcised men who were Ad5 seronegative at baseline showed no evidence of elevated infection risk at any time.

Analysis of follow-up data through September 22, 2009 on male Step Study participants who became HIV-infected prior to unblinding found no differences in CD4+ T cell count, plasma viral load, time to initiation of ART, or time to an AIDS-defining disease between vaccine and placebo recipients [35]. These results are consistent with the primary analysis of the Step Study, conducted at the time of unblinding [29].

4.1.3.6 HVTN 503 (Phambili) results

At the time vaccinations were stopped in the Step Study, enrollment was underway in HVTN 503 (Phambili), a phase 2b efficacy study of the same vaccine regimen in South Africa. 801 of a planned 3000 participants had been enrolled at that point. Vaccinations in HVTN 503 were stopped immediately and participants were unblinded to their treatment assignments. Among those assigned to the active treatment arm, 112 participants had received 1 vaccination, 259 had received 2 vaccinations, and only 29 had received all three planned vaccinations. Visit schedules were revised to call for clinic visits every 3 months for 42 months (3.5 years). At the time HVTN 503 was unblinded, the few endpoint HIV infections that had accrued were relatively evenly divided between the vaccine and placebo arms. A subsequent analysis when Phambili participants had been followed for an average of ~18 months found no significant difference in infections between vaccine and placebo arms. However, the final analysis following 42 months of follow-up for all participants found that, of 100 study participants who became HIV-infected, 63 had received the study vaccine and 37 had received placebo injections [36]. The increased number of infections among vaccinees was greatest among men and more pronounced toward the end of follow-up, that is, roughly 30 months or more following initial vaccination. In this study, vaccinees were more likely than placebo recipients to become HIV-1–infected irrespective of their circumcision status or whether they were Ad5 seropositive at baseline. While the difference in HIV infections between vaccinees and placebo recipients is statistically significant (estimated HR 1.70, 95% CI 1.13 – 2.55, p = 0.01), there were differences in retention between the two study arms and more than 85% of follow-up was post-unblinding, both of which potentially introduce bias and make interpretation of the observed results problematic [36]. Based on these concerns, investigators recalled former study participants to the clinical sites for further testing

[37]. Of 695 participants who were HIV-1–uninfected at their final Phambili study visit, 464 received follow-up HIV testing, including 69 of the 189 participants who had terminated from Phambili early. In total, 36 additional HIV infections were detected. Including the additional follow-up time, the adjusted HR was 1.53 (95% CI: 1.08-2.16) vs 1.70 (95% CI 1.13 – 2.55) seen at 3.5 years of follow-up.

4.1.4 iPrEx study results

The primary analysis of iPrEx study data was reported in the 4th quarter of 2010. This study represents the first efficacy evaluation of systemic pre-exposure prophylaxis (PrEP) with once-daily oral emtricitabine/tenofovir (FTC/TDF) to prevent HIV acquisition [38]. The study was conducted in South America, the US, Thailand, and South Africa among 2,499 HIV-negative male and male-to-female transgender participants who reported sex with a man. As part of the study, participants received HIV testing, risk reduction counseling, and PrEP adherence counseling every 4 weeks, and STI screening and treatment every 24 weeks. The median length of follow-up was 1.2 years with a maximum of 2.8 years. It should be noted that only 10% of the study participants were enrolled in US clinical sites (Boston and San Francisco). For this reason, the study was not powered to detect differences based on region or to determine the effect of this intervention on HIV-1 incidence among US MSM.

In the analysis based on data from visits through May 1, 2010 (excluding 10 infections diagnosed at enrollment), 36 incident infections were identified in the FTC/TDF group and 64 in the placebo group, for a relative risk reduction in HIV acquisition of 44% (95% CI of 15% to 63%). Prespecified subgroup analyses did not reveal any significant differences in efficacy based on region, race/ethnicity, male circumcision status, alcohol use, or age. Risk behavior was assessed every 12 weeks; there were no overall differences in reported risk behavior between the two groups and there was no evidence of risk compensation during follow up. Importantly, there were no significant differences in the number of episodes of other sexually transmitted infections (eg, gonorrhea, syphilis) during follow up.

Reduction of HIV-1 acquisition correlated with adherence to the PrEP regimen. For example, a 50% (95% CI, 18%-70%) reduction in HIV-1 incidence was noted at visits where participants had taken FTC/TDF on 50% or more of days since their last visit as measured by self-report and pill count/dispensing. In *post hoc* analyses, pill use on 90% or more of days was associated with an incidence reduction of 73% (95% CI, 41%–88%). Drug level testing [plasma and peripheral-blood mononuclear cells (PBMCs)] was performed for all incident HIV-1 cases and a matched subset of uninfected controls. It was noted that only 8% of HIV-infected and 54% of uninfected controls who were considered on treatment on more than 50% of days had detectable study drug in plasma or PBMC samples. In the FTC/TDF arm, individuals with a detectable drug level had a relative reduction in HIV risk of 92% (95% CI, 40-99%) compared to those with no detectable drug. These results indicate that most of the incident infections within the FTC/TDF group occurred when there was no detectable drug present in plasma or PBMCs. Follow-up data on infections through November 2010 indicate that the daily use of FTC/TDF decreased HIV acquisition by 42%, preventing an expected 35 infections in the FTC/TDF arm. As in the interim analysis, more consistent use of FTC/TDF correlated with protection from HIV acquisition [39,40].

There were no differences in severe or life threatening laboratory events and the drug was generally well tolerated with some exceptions. For example, there was a trend toward

elevations in serum creatinine levels in the FTC/TDF group (defined as creatinine elevation at least 1.1 times the upper limit of normal or > 1.5 times the baseline level). Specifically, there were 26 instances of creatinine elevations in the FTC/TDF group and 15 in the placebo group (p=0.08). Overall, however, this resulted in only a total of 10 discontinuations of the study agents (7 in the active arm and 3 in the placebo group) which were restarted in 9 individuals. Similarly, moderate nausea and unintentional weight loss of 5% were reported more frequently in the FTC/TDF group compared to placebo. The differences were most pronounced in the beginning of the study. For example, nausea was more common during the first 4 weeks after enrollment (9% vs. 5%, $p < 0.001$) and thereafter decreased to comparable levels in both groups. Similarly, differences in weight between the groups equilibrated after week 12.

Among those who seroconverted in the study, there were no differences in viral load setpoint and CD4+ T cell count between participants in the FTC/TDF and placebo group. Drug resistance was evaluated among the 8 individuals randomized to placebo and 2 randomized to FTC/TDF who on subsequent testing were plasma HIV-1 RNA positive at enrollment. There was one case of primary or transmitted resistance detected in the placebo group (multidrug resistance conferred by K103N, M184V, and T215Y mutations). Among 2 individuals assigned to the FTC/TDF group, both had FTC resistance, one was confirmed as acquired during the first 4 weeks of FTC/TDF use; in the second individual the enrollment HIV-1 RNA level was too low to allow detection of drug resistance, thus it was not possible to determine if this was the case of acquired or primary drug resistance. These data highlight the need for a careful evaluation of at-risk individuals for possible acute retroviral syndrome, particularly in presence of signs or symptoms consistent with a viral syndrome. Importantly, there was no FTC or TDF resistance reported among individuals who seroconverted on-study.

4.1.5 Additional efficacy trials of Truvada® as PrEP

In addition to iPrEX summarized above, four trials (Partners PrEP, TDF2, FEM PrEP and VOICE [MTN-003]) have evaluated the safety and efficacy of Truvada® (FTC/TDF) among HIV-uninfected, heterosexually active adults [41-44].

Partners PrEP, demonstrated the efficacy of tenofovir or emtricitabine/tenofovir in serodiscordant heterosexual couples in sub-Saharan Africa. The study demonstrated 67% (95% CI 44–81%, $p < 0.0001$) and 75% (95% CI 55–87%, $p < 0.0001$) reductions in HIV acquisition in the tenofovir and emtricitabine/tenofovir arms, respectively; the Data and Safety Monitoring Board (DSMB) overseeing the trial reviewed the data and stopped the placebo arm early. The TDF2 trial, an extended safety trial that enrolled 1219 HIV-negative sexually active men and women in Botswana, announced similar results. In the TDF2 trial, the arm assigned to daily emtricitabine/tenofovir reported 62.6% fewer HIV-1 infections than the placebo arm (95% CI 21.5–83.4%, $p = 0.0133$). A second analysis that excluded individuals who became infected more than 30 days after the last reported study drug/placebo dose determined that FTC/TDF reduced HIV-1 acquisition risk by 77.9% (95% CI 41.2–93.6%, $p = 0.0053$).

The FEM-PrEP trial and the oral TDF portion of the VOICE trial conducted in high risk women were stopped early by their data safety monitoring boards when they concluded that no evidence of efficacy would be found. Although self-reported adherence to the medication was high in FEM-PrEP, measurements of drug levels in plasma revealed that adherence was <50%.

Primary results for the VOICE trial were announced in March 2013 [45]. The study found no evidence that the oral Truvada® regimen was efficacious in preventing HIV infection. Notably, while self-reports and pill counts pointed to high levels of adherence to the study drug regimen, laboratory assays found low systemic levels of study drugs, suggesting that adherence was, in fact, very low, especially among young women who were at highest risk of HIV infection.

No serious toxicities were identified in any of the trials evaluating daily oral FTC/TDF; a notable exception was nausea and vomiting which occurred more commonly in those receiving FTC/TDF than in those receiving placebo in the first 1–2 months on medication.

Based on the strength of the evidence from iPrEx and Partners PrEP, on July 16, 2012, the U.S. Food and Drug Administration approved Truvada (emtricitabine/tenofovir disoproxil fumarate) for PrEP in combination with safer sex practices to reduce the risk of sexually acquired HIV-infection in adults at high risk [46,47]. Specifically, once daily Truvada is indicated in combination with a comprehensive HIV prevention strategy (including condoms, HIV testing, and risk reduction counseling) among men and transgender women who have sex with men and women in serodiscordant relationships.

4.1.5.1 Monitoring for ARV use for HIV-1 prophylaxis

The results of the iPrEx study demonstrated that combination emtricitabine and tenofovir taken orally every day by men and male-to-female transgender persons who have sex with men can provide moderate protection from HIV acquisition. This intervention was tested in combination with proven risk-reduction strategies such as frequent HIV testing, STI screening and treatment, condom use and risk reduction counseling. Many questions still remain about the applicability of these results to diverse communities of MSM, transwomen, and other groups at risk for HIV acquisition, about long-term safety of this regimen, and about development of resistance to FTC/TDF, which is an important component of the preferred initial antiretroviral regimen in the US [48].

At present, it remains to be seen what the uptake of PrEP will be in the communities of men who have sex with men and transgender individuals in the US but it will likely depend on factors such as perception of risk, barriers to adherence, access to drug, cost, and perceived side effect profile [49,50]. Prior to release of iPrEx results, surveys among at-risk MSM from San Francisco and Boston demonstrated that knowledge about and use of PrEP are rare at present but that there is interest in considering its use [51,52]. A web-based survey conducted among HVTN 505 participants (n = 376) indicated that many considered iPrEx results very important either to them personally or to their community (35% and 66%, respectively); 31% of responders would consider taking PrEP in the next year and only a minority indicated that taking PrEP would affect their willingness to stay in the study (9%), or willingness of others to enroll in HVTN 505 (16%) [53]. Knowledge and attitudes towards use of ARVs as prophylaxis will likely evolve as more data come from long-term follow up of iPrEx participants and as normative guidelines are issued. Likewise, as more clinical data emerge about the optimal timing and frequency of dosing of ART around high-risk exposure, the distinction between PrEP and PEP may become less clear. In this context, monitoring for use of ARVs as prophylaxis against HIV acquisition among 505 participants becomes crucially important.

The availability of partially effective oral PrEP raises a number of important questions that are important to consider in the context of HVTN 505. For example, since HVTN

505 evaluates the effect of the vaccine regimen on setpoint viral load and HIV acquisition, ARVs used as pre or post-exposure prophylaxis may influence parameters such as HIV incidence. Use of PEP or PrEP may also influence post-infection disease course such as early virologic and immunologic marker trajectories and development of drug resistance. Furthermore, evaluating patterns and predictors of such ARV use may yield important information about their effects on study participation, risk behavior over time, and perception of risk, all of which are key issues for the vaccine and prevention fields in general.

To evaluate these questions, prophylactic use of ARVs in HVTN 505 will be assessed through both FTC/TDF plasma drug level testing and self-report.

4.2 Ad5 nAb assays

Merck Laboratories developed and validated an Ad5 nAb assay (hereafter termed the "Merck assay") that has been used to assess Ad5 nAb titers in Merck-sponsored HIV vaccine trials, including the Step Study. NVITAL, in conjunction with Crucell Co., has also produced an assay to measure Ad5 nAb. The NVITAL assay has been used to monitor Ad5 nAb for clinical trials using the VRC products. The dilution range of the Merck assay is 1:18 to 1:4608 while the dilution range of the NVITAL assay is 1:12 to 1:8748. Hence, a sample that tests < 18 in the Merck assay is evaluated as Ad5 nAb negative, whereas a value of < 12 marks a sample as Ad5 nAb negative in the NVITAL assay. Samples testing ≥18 (Merck) and ≥ 12 (NVITAL) are judged Ad5 nAb positive in the 2 assays. Because the selection of the study population for HVTN 505 has been guided by the results of the Step Study (see Section 4.1.3) and we want to interpret the results of HVTN 505 in relation to the Step Study Ad5 nAb screening cutoff, we will utilize the Merck assay for HVTN 505.

4.3 Study vaccines

In 2001, the World Health Organization UNAIDS HIV Vaccine Advisory Committee recommended that candidate HIV vaccines design should be based upon the strains prevalent in the country in which trials are to be conducted [54]. The VRC and the World Health Organization-UNAIDS organized a meeting focused on the genetic diversity of HIV and strategies to develop vaccine candidates. A consensus was reached that generation of multiclade candidate vaccines was an international scientific priority [55]. This approach was the foundation for the design of the VRC vaccine candidates. The ensuing work from 2002 through 2007 was directed toward the goal of first evaluating safety and immunogenicity in a diverse international population and, if the vaccine candidates were found to be safe and immunogenic, to proceed to a global efficacy study in both men and women. Much work towards this goal was accomplished and a global study was ready to open in late September 2007. Following public release of the Step Study results on September 21, 2007, the plans were revised. The HVTN 505 study will not include women and is limited to the subset of the Step Study vaccinated population that did not have increased rate of HIV infection compared to placebo. There is not an identified cohort of high-risk women in a population with a relatively low prevalence of Ad5 nAb seropositivity. Although the study population for HVTN 505 is in the clade B region only, the multiclade vaccine is still considered to be relevant as it includes encoded sequences for clade B Env, Gag, Pol and Nef.

The regimen is comprised of 3 injections of a 6-plasmid multiclade HIV-1 deoxyribonucleic acid (DNA) vaccine, VRC-HIVDNA016-00-VP, followed by a single booster injection of a multiclade HIV-1 rAd5 vector vaccine, VRC-HIVADV014-00-VP, and the study population includes HIV-uninfected MSM participants who are at risk for HIV-1 infection primarily through sexual exposure. The participants will be randomized in equal numbers to the vaccination regimen or to placebo injections.

4.3.1 DNA vaccine

The investigational DNA vaccine, VRC-HIVDNA016-00-VP, is composed of 6 closed circular plasmid DNA macromolecules in a 1:1:1:1:1:1 ratio. Plasmids VRC-4401, VRC-4409, and VRC-4404 are designed to express clade B HIV-1 Gag, Pol, or Nef, respectively. VRC-5736, VRC-5737, and VRC-5738 are designed to express HIV-1 Env glycoprotein from clade A, clade B, and clade C, respectively. The construction of these plasmids has been published [56] and is described in the Investigators' Brochure. The VRC, NIAID, Vaccine Pilot Plant (VRC/NIAID/VPP) manufactured the vaccine DNA plasmids and performed the final formulation, fill and packaging.

The dosage to be administered is based on experience with several VRC DNA vaccines, including specifically a dose escalation evaluation with a similar HIV DNA vaccine. A phase 1 study (VRC 004) of a similar 4-plasmid DNA vaccine (VRC-HIVDNA009-00-VP) was initiated in 2002 and was conducted as a single site study at the NIH Clinical Center (Bethesda, MD). Dosages up to 8 mg of DNA were well-tolerated and assessed as safe for further evaluation [57]. The 4 mg dosage was chosen for further evaluation of the 4-plasmid DNA vaccine alone in a multicenter US study, HVTN 052 (n = 180). With the goal of improving the immunogenicity of the DNA plasmid constructs, the DNA vaccine was redesigned using a different promoter and 3 separate plasmids for the Gag, Pol and Nef encoded sequences; also an additional 68 amino acids were encoded in the *gag* plasmid (reconstituting the p6 region at the carboxy terminus). This approach to improving immunogenicity was supported by a small bridging study, VRC 007 [58] and the 6-plasmid DNA was assessed as the leading candidate DNA vaccine in prime-boost HIV vaccine studies conducted from 2005-2007.

4.3.2 rAd5 vaccine

The investigational rAd5 vaccine, VRC-HIVADV014-00-VP, is a 3:1:1:1 ratio of the adenoviral vectors that encode for HIV-1 Gag/Pol polyprotein from clade B and HIV-1 Env glycoproteins from clades A, B, and C, respectively. DNA plasmids developed by the VRC/NIAID/NIH (Bethesda, MD) were used to construct the adenoviral vector clinical seed stocks used to produce the vaccine. The construction of the adenoviral vectors has been published [59,60] and is described in the Investigators' Brochure. The dosage is specified in PU. PU are the number of viral particles, active or not, found in the product as determined by spectrophotometry. The adenoviral vectors were manufactured for the VRC by GenVec, Inc. (Gaithersburg, MD) at a contract manufacturer, Molecular Medicine (San Diego, CA). The FFB was custom manufactured by Cambrex (Walkerville, MD). Final formulation, fill, and packaging were performed by the VRC/NIAID/VPP, operated by SAIC-Frederick, MD.

The 10^{10} PU dosage of the VRC rAd5 vaccine was selected initially based on two phase 1 dose escalation studies. The VRC 006 study (n=36) was initiated in July 2004 at the NIH Clinical Center, Bethesda, MD as the first phase 1 randomized, placebo-controlled, dose-escalation study of the rAd5 vaccine. Dosages evaluated included 10^9 PU (n=10), 10^{10} PU

(n=10) and 10^{11} PU (n=10), with the 10^{10} PU dosage evaluated as offering good immunogenicity with less reactogenicity [61].

The HVTN 054 study (n=48; 40 vaccinees and 8 placebo recipients) was initiated in April 2005 in multiple US sites as the second phase 1 study of the rAd5 vaccine in uninfected, Ad5 nAb negative adult study participants. The HVTN 054 safety and immunogenicity data also supported the selection of 10^{10} PU for the VRC rAd5 vaccine [62].

Given the concern about all Ad5 vaccines that was raised by the observation of more HIV infections in the vaccine group in the Step Study, it is important to note that the VRC rAd5 vaccine differs from the Merck-rAd5 vaccine that was used in the Step Study in the antigen content, vector platforms, immunization schedule, rAd5 vector construction, and manufacturing substrate [59,60,63], and pattern of immune responses induced [31,61]. The character of immunity generated by DNA prime/rAd5 boost differs from consecutive injections of homologous rAd5 vaccine [64,65]. Emerging data from studies in mice, nonhuman primates (NHP), and humans suggest that high doses of rAd5 vaccine vectors generate a population of effector T-cells which are predominantly differentiated with low proliferative capacity. In contrast, lower rAd5 doses, or rAd5 given after a primary immunization with a heterologous vaccine vector, generates CD4+ T cell help and diversification of the CD4+ T-cell responses, which results in more balanced CD4+ and potent CD8+ T cell effector responses [66-69]. Studies of the VRC DNA and rAd5 vectors in preclinical and phase 1 and 2 studies are consistent with these findings. Compared to rAd5 alone, DNA/rAd5 elicits a higher magnitude of CD4+ and CD8+ T-cell responses (see Section 4.6.2.2) and a higher proportion of multi-functional cells comprising the total cytokine response [70]. While the biological significance of these differences in humans is unknown, *in vivo* animal model systems show that such qualitative immunological differences can affect vaccine-elicited protection [71,72].

The VRC rAd5 and Merck-rAd5 vaccines express different antigens. The VRC DNA and rAd5 vectors express clades A, B, and C Env antigens that elicit both T and B cell responses. The Merck-rAd5 vaccine expressed Gag, Pol and Nef, but not Env. The VRC vaccine also includes Gag and Pol; T-cell responses to Gag have a slightly lower magnitude than those to Env while Pol-specific responses are much lower. Nef is included in the DNA, but not in the rAd5. Thus, the predominant immune responses induced by the Merck vaccine are to Gag and Pol, while the VRC vaccine elicits predominant responses to Gag and Env. Since circulating HIV-1 strains in North America are largely clade B, it is reasonable to expect that the Gag and Env responses will recognize some epitopes in many or most circulating strains. While the vaccine-induced immune responses have not been evaluated side-by-side, the total magnitude of the IFN-γ enzyme-linked immunospot (ELISpot) responses to the 2 vaccines is similar, but the specificity of the responses is different [62].

Vector construction of the Merck and VRC rAd5 vaccines is different. The VRC rAd5 vector includes deletions of the Ad5 E1 and E4 regions with partial deletion of E3 region, while the Merck rAd5 vector has an E1 deletion, but intact E3 and E4 regions [59,60,63]. Studies of the VRC rAd5 vector show that the E4 gene deletion results in markedly reduced expression of Ad5 structural and non-structural proteins, while expression of the HIV-1 gene inserts is maintained. Compared to the Merck rAd5 3 injection regimen, the VRC DNA prime- rAd5 boost vaccine regimen should generate a more favorable ratio of immunity to the encoded HIV antigen versus immunity to the Ad5 vector, and the

heterologous prime-boost approach compared to homologous boosting has theoretical advantages for T-cell function, based on nonclinical studies [65,67].

4.3.3 Placebos

In this study the placebo for the DNA vaccine is designated VRC-PBSPLA043-00-VP and composed of PBS and the placebo for rAd5 vaccine is designated VRC-DILUENT013-DIL-VP and composed of the FFB. Both types of placebo were filled and packaged by the VRC/NIAID/VPP, operated by SAIC-Frederick, MD.

4.4 Non-clinical and natural history data that provide evidence of potential vaccine activity

The following section provides a summary of findings from non-clinical studies that are pertinent to study vaccine activity endpoints. "Vaccine activity" is defined in the context of this section as the ability to modulate HIV-1 acquisition and VL post-acquisition (see primary objectives 1 and 2, Section 5.1). Refer to Investigators' Brochure (IB) for a more extensive summary of non-clinical studies that provide more information about the safety and immunogenicity of the vaccination regimen. Section 4.5 provides a summary of human natural history data that suggest desirable immunological characteristics of a preventive HIV-1 vaccine regimen.

4.4.1 Non-clinical data

Over 2 decades of work on HIV vaccine concepts has led to the prevailing belief that control of the AIDS epidemic through a vaccination strategy would require the development of a vaccination regimen against HIV-1 that elicits potent cellular and humoral immune responses which recognize divergent viral strains. The vaccine regimen in this protocol is a multiclade DNA vaccine prime followed by a multiclade adenoviral vector vaccine boost. Earlier prime-boost vaccination regimens developed by others showed promise in NHP models of HIV infection with the potential for raising high levels of immune responses [73-75]. In these studies, attenuation of a pathogenic simian human immunodeficiency virus (SHIV) infection in rhesus macaques was attributed to the generation of a CD8+ CTL response. Compelling evidence of an antiviral effect of CD8+ T cells was demonstrated in controlled studies in macaques, in which CD8+ cells were depleted *in vivo* using a monoclonal Ab. The VLs in these animals increased or decreased as the CD8+ T cells were depleted or reappeared, respectively [76,77]. Therefore, induction of a CTL response specific to the viral proteins may represent a desirable response in an HIV-1 vaccine.

More recently, studies have been conducted in NHP using vaccines modeled on the VRC vaccine candidates, which encode a simian immunodeficiency virus (SIV) Gag-Pol-Nef or SIV Gag-Pol polyprotein in combination with either a single HIV-1 Env or a mixture of clade A, clade B and clade C HIV-1 Env. Monkeys receiving multiclade Env immunization developed robust cellular and humoral immune responses to all vaccine antigens and a greater breadth of Env recognition than monkeys immunized with a single Env immunogen. After challenge with a pathogenic SHIV strain (SHIV-89.6P), all groups of vaccinated monkeys demonstrated a lower setpoint VL than control monkeys. The monkey data indicate that a multiclade vaccination regimen can generate broad Env-specific T-lymphocyte and Ab responses without antigenic interference from the presence of multiple immunogens [78].

The NHP studies have also attempted to evaluate whether a DNA prime-rAd5 boost vaccination strategy against HIV-1 offers a survival advantage over placebo in a SIV challenge model. The data suggest that even if the HIV vaccination regimen offers transient reduction in VL, there could be a more prolonged survival advantage in subjects infected with HIV despite vaccination [73,74]. In the monkey model, the vaccine regimen induced broad CD4+ and CD8+ T-cell responses and antibodies that neutralized the primary isolate of SIV used in the challenge model [74]. Although the regimen did not prevent the SIV infection, vaccinated animals had prolonged survival compared to control animals [68]. The NHP studies demonstrated that the best predictor of survival of vaccinated animals after exposure to SIV was preservation of the central memory CD4+ T lymphocytes [68,69]. In theses studies the VRC DNA/rAd5 vaccine platform demonstrated a beneficial virologic and clinical effect in the SIV_{mac251} challenge model using Mamu A*01 negative animals. Compared to controls, vaccinated animals had a 30-fold decrease in peak VL and a statistically significant increase in AIDS-free survival. The Merck rAd5 SIV Gag vaccine did not demonstrate these effects in Mamu A*01 negative animals. Improved AIDS-free survival is a key goal of vaccination. We do not know how these SIV data will translate to heterologous virus protection or to protection against HIV-1 in humans. Nor do we know if high-dose intravenous SIV challenge resulting in AIDS in a median of 1 year is a predictive model for the low-dose mucosal exposure more common in humans.

In follow-up to the above noted studies, an NHP study in a larger number of animals was initiated to expand upon and further characterize these observations. Mamu-A*01 animals were excluded. Four groups of 13 monkeys each were included with the following types of DNA prime-rAd5 boost vaccine constructs administered: (1) *gag/pol* SIV_{mac239}; (2) *env* SIV_{mac239}; (3) *gag/pol* + *env* SIV_{mac239}; and, (4) sham DNA with empty vector Ad5. Preliminary data (also excluding the few Mamu-B*17 and B*08 animals in the study) on the peak (14 days post challenge with SIV_{mac251} IV) VLs by group are available (Figure 4-1), along with VL data in the semen in the groups receiving the *gag/pol* immunogens (not shown). The following conclusions have been drawn from these preliminary data: while the groups receiving either Env alone or Gag/Pol alone have some effect on peak VL, the group receiving all the immunogens had the maximal effect on peak plasma VL (see Figure 4-1). This effect was not mediated by nAb, as no neutralizing activity was detected by week 20 post challenge in a pseudo-virion neutralization assay. There was significant decrease in the VL with statistical significance in the first 80 days post challenge. Since much transmission of HIV is believed to occur during the high viremia of acute infection, reduction in peak VL in the plasma and in the semen during this acute period may result in decreased transmission, providing an indirect public health benefit from vaccination. The study continues and effects on VL at setpoint will be evaluated, as well as T-cell responses. In addition, plans to conduct a study to evaluate the potential of the prime-boost regimen to impact acquisition of SIV infection in a repeated low-dose mucosal challenge model are planned.

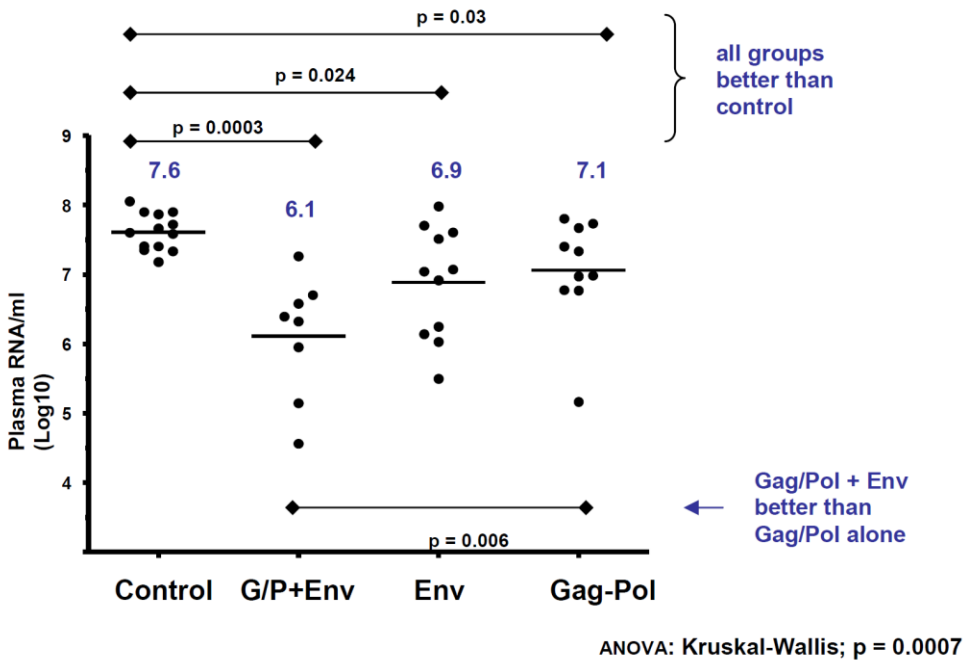


Figure 4-1 DNA prime/rAd5 boost effect on VL after SIV challenge

The NHP data support the hypothesis that the VRC prime-boost vaccination regimen may be able to induce an immune response that is associated with an improved outcome if HIV-1 infection should occur compared to what would be expected in the absence of vaccine-induced immunity.

To address the question of whether the DNA/rAd5 regimen expressing SIVmac239 *env* and *gag*-pol genes could prevent acquisition when macaques were subjected to repeated rectal challenge, a model system was developed using either SIVmac251 or heterologous SIVsmE660 stocks titrated to infect about 50% of animals following each exposure. Rhesus macaques challenged weekly for 12 weeks intra-rectally are uniformly infected. Studies were performed in 129 animals stratified by MHC alleles. 20 vaccinees and 20 sham vaccinated Mamu A*01- animals were challenged intrarectally with SIVmac251 and 25 vaccinees and 25 sham vaccinated Mamu A*01- animals were challenged intrarectally with SIVsmE660. An additional 20 vaccinees and 19 placebo recipients that were Mamu A*01+ were also challenged intra-rectally with SIVsmE660. In the SIVmac251 challenged animals there was no protection from acquisition, but as had been noted in prior IV challenge experiments, there was a significantly lower peak VL in vaccinees post-challenge ($p = 0.001$, Wilcoxon rank-sum test). In Mamu A*01- animals challenged with SIVsmE660 there was a 50% reduction in acquisition ($p=0.001$, Logrank test) but no effect on peak VL. However, in Mamu A*01+ animals both a 50% reduction in acquisition ($p = 0.009$) and a reduced peak VL ($p = 0.07$) were seen in vaccinees. When the 45 vaccinees and 44 placebo recipients with all MHC phenotypes that were challenged with SIVsmE660 were combined, the vaccine conferred a statistically significant effect on both acquisition and VL (Figure 4-2)

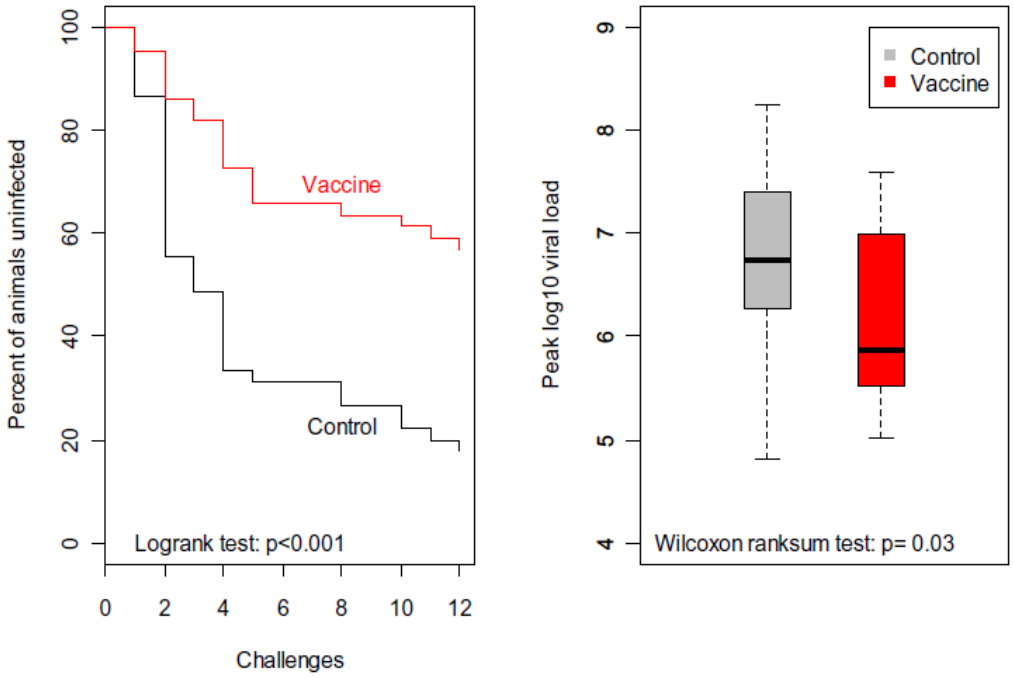


Figure 4-2 DNA prime/rAd5 boost effect on acquisition and VL following heterologous SIV challenge

These data indicate that the VRC DNA/rAd5 regimen has the capacity to prevent acquisition from a mucosal heterologous lentivirus challenge, and can induce CD8+ T cells (particularly in subjects with certain MHC 1 alleles) that can reduce VL in lentivirus-infected vaccinees. Although protection was not achieved against SIVmac251, 50% of vaccinated monkeys were protected from infection with SIVsmE660 [79].

4.5 Potential benefit of VL reduction as a vaccination outcome

There are substantial data to suggest that, even in the absence of sterilizing immunity, a vaccine capable of reducing VL in vaccinated participants would provide clinical benefit to the individual (ie, delay the time to the onset of AIDS or initiation of antiretroviral therapy) and be a valuable public health intervention (ie, decrease the risk of transmission). However, it is unclear at this time whether the predictive value of a vaccine-induced reduction in VL is comparable to that seen in natural infection.

Data generated from large natural history cohort studies suggest that plasma VL is strongly predictive of the risk of HIV disease progression. For example, in the Multicenter AIDS Cohort Study (MACS), levels of plasma VL, even those measured early after infection, discriminated risk at all levels of CD4+ T lymphocyte counts and predicted their subsequent rate of decline, risk of progression to AIDS (Figure 4-3), and death from AIDS [80,81]. Other cohort studies have demonstrated a similar association [82].

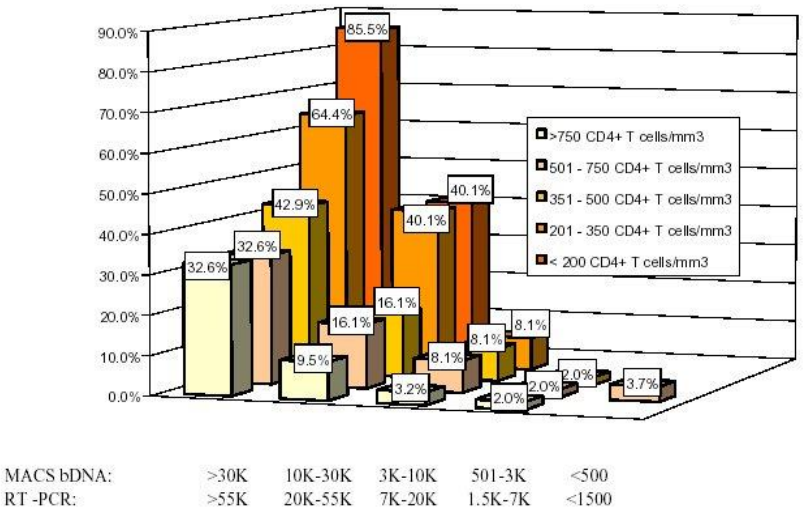


Figure 4-3 Likelihood of developing AIDS within 3 years in the MACS cohort [80]

Similarly, multiple analyses have shown a statistically significant dose-response type association between decreases in plasma viremia in a number of antiretroviral treatment trials and improved clinical outcome [83].

Based on the data described above, a vaccine effect resulting in a sustained reduction in VL could be predicted to translate into a clinically significant delay in the time to progress to AIDS or initiation of antiretroviral therapy.

A vaccine capable of reducing VL in vaccinated recipients would be expected to decrease the risk of secondary transmission. Support for this comes from a community-based study in a rural district of Uganda where each $\log_{10}$ increment in the VL of an infected individual was associated with a 2.45-fold increase in the risk of secondary transmission (Figure 4-4) [81].

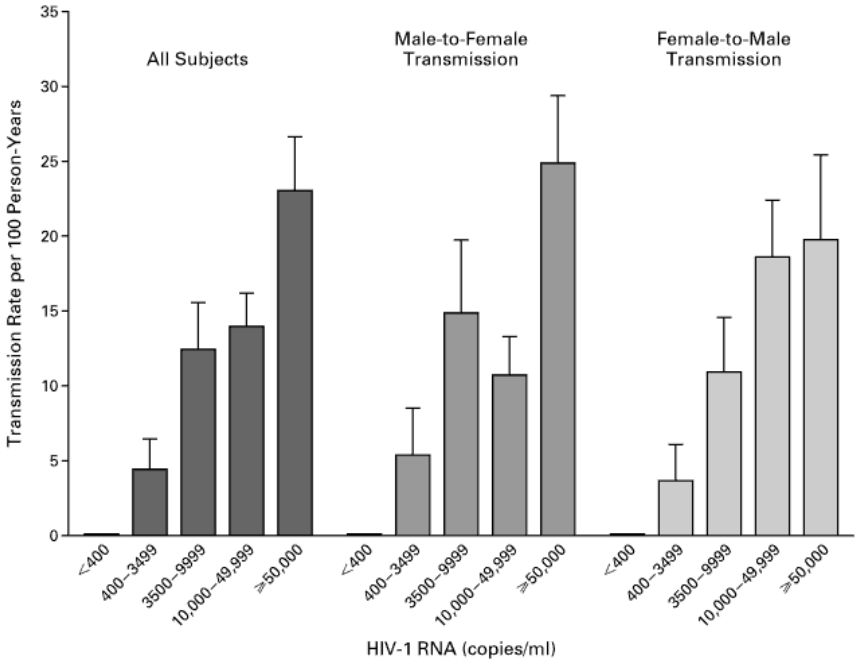


Figure 4-4 Mean (+SE) rate of heterosexual transmission of HIV-1 among 415 discordant couples, according to the sex and the serum HIV-1 RNA level of the HIV-1–positive partner [84] [Copyright ©2000 *Massachusetts Medical Society. All rights reserved.]*

Formal demonstration of an effect on secondary transmission will require large studies of long duration. However, mathematical models and natural history data suggest that observing a difference in VL could have substantial public health benefit.

Modjarrad, *et al*. [85] recently completed a systematic review of studies that evaluated associations between small differences in VL changes and rates of heterosexual HIV transmission, and also between VL and progression to AIDS or death. Based on these studies, the authors estimated the relative risks (RR) of HIV transmission and disease progression associated with 0.3, 0.5, and 1.0 $\log_{10}$ differences in VL. The calculations indicate that every 0.3 $\log_{10}$ increase in VL increases the likelihood of transmitting HIV-1 (heterosexually) by 20% and increases the risk of progression to AIDS or death by 25%; For every 1.0 $\log_{10}$ increase in VL, the relative risk of HIV heterosexual transmission was 2.0 and progression to an AIDS-defining event was 2.13 (see Table 4-3, adapted from Modjarrad, *et al.* [85]). Comparable published data for MSM transmission of HIV-1 are not available.

Table 4-3 RR of HIV transmission and disease progression per 1.0 log_{10} increase in plasma HIV RNA

RR of heterosexual transmission of HIV between serodiscordant couples per 1.0 log_{10} increment of plasma HIV RNA concentration

Reference	Risk ratio per 1.0 log_{10} Δ HIV RNA
Quinn et al., 2000 [84]	2.45 (1.85, 3.26)
Fideli et al., 2001 [86]	
• Female-to-male	2.5 (1.5, 4.0)
• Male-to-female	1.8 (1.2, 2.8)
Hisada et al., 2000 [87]	1.31 (0.94, 1.84)
Tovanabutra et al., 2002 [88]	1.81 (1.33, 2.48)
Weighted mean	2.0 (1.4, 2.8)

RR of progression to an AIDS-defining event or AIDS-related death per 1.0 log_{10} increment of plasma HIV RNA concentration.

Reference	Risk ratio per 1.0 log_{10} Δ HIV RNA
Welles et al., 1996 [68]	2.79 (1.44, 5.38)
Coombs et al., 1996 [89]	2.26 (1.10, 5.13)
Sterling et al., 2001 [90]	1.55 (0.95, 2.52)
Phillips et al., 2004 [91]	2.03 (1.68, 2.46)
Lavreys et al., 2006 [92]	2.28 (1.36, 3.59)
Weighted mean	2.13 (1.33, 3.62)

This review supports the conclusion that even modest reductions in VL contribute to reducing the risk of transmission and disease progression

4.6 Experience with the VRC vaccine regimen

A series of phase 1 and 2 clinical trials sponsored in collaboration with DAIDS, NIAID, NIH have been conducted by several clinical trials research groups, including the VRC, HVTN, International AIDS Vaccine Initiative (IAVI), and US Military HIV Research Program (USMHRP). These clinical trials have included 1 of 2 similar multiclade DNA vaccines and/or the rAd5 vaccine in single agent and combination vaccination regimens. Table 4-4 shows the clinical trials with these vaccines conducted through 5 INDs for which HIV prevention was the intended indication. All studies in this table are closed to accrual and have completed subject clinical follow-up although several studies are proceeding with long-term follow-up contacts.

Table 4-4 Clinical trials with VRC multiclade HIV vaccines for uninfected participants

IND	Product	Protocol Number	Total Accrual
BB10681	VRC-HIVDNA009-00-VP (4 plasmid DNA)	VRC 004	50
		RV 156	31
		HVTN 052	180
BB11661	VRC-HIVADV014-00-VP (Adenoviral vector)	VRC 006	36
		HVTN 054	48
		VRC 015	31
BB11750	VRC-HIVDNA016-00-VP (6 plasmid DNA)	VRC 007	15
BB11894	VRC-HIVDNA009-00-VP VRC-HIVADV014-00-VP	HVTN 057	70
		VRC 009	10
		HVTN 068	66
		HVTN 069	90
		RV156A	18
BB12326	VRC-HIVDNA016-00-VP VRC-HIVADV014-00-VP	VRC 008	40
		VRC 010	4
		VRC 011	60
		IAVI V001	114
		RV 172	326
		HVTN 204	480

The VRC studies were conducted at the National Institutes of Health, Bethesda, MD. HVTN 204 was an international study in the Americas and South Africa; other HVTN studies were multicenter studies with all or the majority of participants at US sites. USMHRP (RV) studies and the IAVI V001 study are in Eastern Africa. The last 3 studies listed (IAVI V001, RV 172, and HVTN 204) are known as the "Triad" studies and were designed to provide an international phase 1 and 2 experience among partner organization with the intention of conducting a large, international phase 2b efficacy study with safety, HIV acquisition, and early VL as endpoints. Following the announcement of Step Study results in 2007, plans for a large international efficacy study were canceled. With consideration given to input from a number of advisory groups, including a public NIAID summit [93], development of HVTN 505 then ensued with a focus on the subject population from the Step Study in whom there is equipoise for evaluating an rAd5 vector-containing vaccine regimen despite the increased risk of HIV acquisition among Step vaccinees as a whole. The eligible study population (circumcised MSM who are Ad5 nAb negative) was not at increased risk of HIV acquisition in the Step Study [29]. As originally designed, vaccine safety and the potential effect of vaccine on the VL setpoint were the primary endpoints in HVTN 505 when it opened to accrual in June 2009. As discussed in Section 4.1.2, subsequent information and events provide support for expansion of the trial to test as a co-primary objective the ability of the vaccine regimen to prevent HIV-1 acquisition.

BB-IND 12326 includes the same regimen as HVTN 505. Prior to the opening of HVTN 505, studies in this IND provided more than 700 person years of safety data on the prime-

boost regimen in the phase 1 and 2 Triad studies with the VRC vaccine. Across the 3 INDs (BB IND 11661, BB IND 11750, and BB IND 12326) in which the 2 specific vaccine products in HVTN 505 have been previously evaluated, cumulatively 1121 participants have participated in phase 1 and phase 2 studies thus far with 699 receiving vaccine and 422 receiving placebo. As of May 2013, there have been 5 HIV-1 infections in vaccinees and 4 in placebo recipients who received the complete vaccine regimen specified for HVTN 505. Given the very small numbers of infections and that these studies were not designed to determine efficacy, conclusions about the vaccines' effect on the risk of acquiring HIV cannot be made from long-term follow-up.

Because of the concerns raised in the Step trial about the possibility that Ad5 immunity may predispose to infection in individuals receiving the Merck vaccine and because this risk was highest in those who were not circumcised, HVTN 505 includes eligibility criteria that restrict enrollment to circumcised men with no nAb to Ad5. This trial design provides equipoise related to the imputed safety concern observed with the Merck vaccine.

4.6.1 Safety of the 6-plasmid DNA and rAd5 vaccines alone and in prime-boost regimens

The vaccination regimen in HVTN 505 will be 3 priming doses of DNA vaccine at 4 mg each with 1 dose of rAd5 vaccine boost at 10^{10} PU. The safety summary emphasizes experience as of October 2008 for this regimen; there have been no other new studies of the regimen completed since that time and no new risks or serious adverse events attributed to vaccine from smaller ongoing studies with the VRC regimen.

The VRC DNA vaccinations will be administered IM by the needle-free injection device, Biojector® 2000 Needle-Free Injection Management System™, manufactured by Bioject (Tualatin, OR). In the VRC 008 study, which compared needle to Biojector® injection of vaccine, the close, prospective scrutiny of the vaccination sites revealed that a small skin lesion, described as a papule, was commonly observed after DNA injection by Biojector® (38/60 [63.3%]), but was not observed after DNA injection by standard needle. These papules or scabs are infrequently recorded on diary cards completed by study participants and resolve without treatment. Although the Biojector® is associated with pain, redness, swelling and/or bruising at the injection site [94], as well as some self-limited, small skin lesions, this method of administration is well tolerated, and offers the advantage of eliminating needle stick accidents in the clinic. This system has FDA clearance for delivering IM injections of vaccine.

The VRC rAd5 vaccine will be administered IM by needle injection. Several studies have safety data for the rAd5 vaccine alone and as a booster vaccine at both 10^{10} and 10^{11} PU. There is greater frequency and severity of symptoms as dosage is increased. Systemic symptoms typically start 12-16 hours after rAd5 vaccination and diminish in severity within hours. When an acute fever occurs in association with rAd5 vaccination it typically resolves within a few hours. Headache and malaise are the most common symptoms and may persist for a few days, but respond quickly to treatment with acetaminophen or nonsteroidal anti-inflammatory medications. Local reactogenicity (pain, redness, swelling) is usually mild and often begins within 24 hours after vaccination, but in some individuals onset of local injection site reactions may be delayed until 3-5 days after vaccination and may reach moderate severity before resolving spontaneously. The VRC 008 study included both 10^{10} (n=19) and 10^{11} PU (n=20) booster vaccinations of the rAd5 vaccine, with an equal number of participants with low and high Ad5 nAb titers at screening randomized to each dosage. Three cases of

moderate (> 9 x 9 cm) erythema and/or induration in the injected arm had onset postvaccination days 3-5, peaked about days 5-6, and resolved at days 7-9. A large erythema reaction was observed in 1 participant in the VRC 009 study and in 1 subject in HVTN 068 with a similar pattern of onset and resolution. The onset is after the period of solicited reactogenicity in the HVTN, IAVI, and USMHRP studies that have used 3 days reactogenicity collection. The subjects observed to have these reactions in the VRC studies with 5 day solicited reactogenicity have not been alarmed by the reaction and may not have reported it if it had not been solicited; therefore, the incidence is not well defined. This is the basis for including a longer period of solicited local reactogenicity data collection following the booster rAd5 injection in the HVTN 505 study.

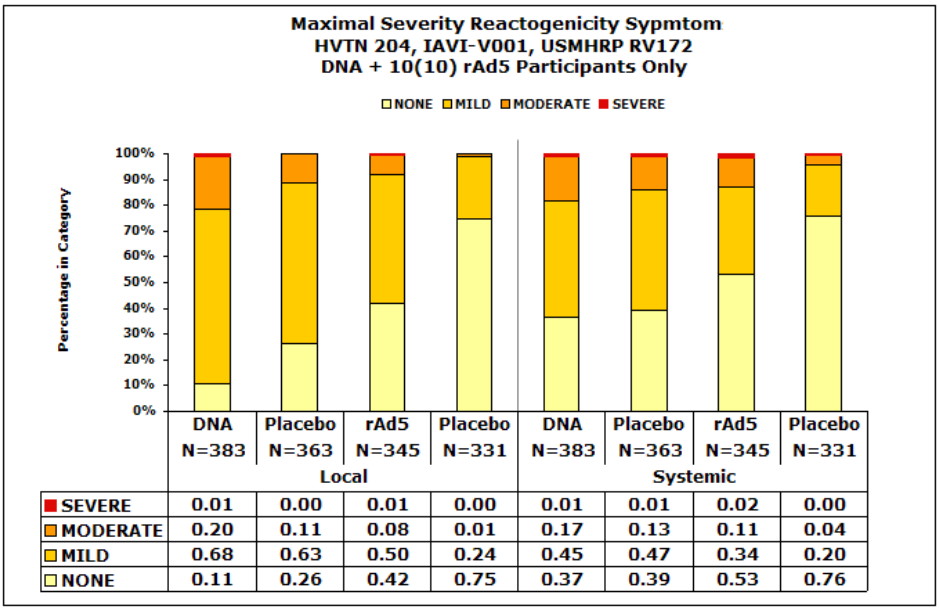
The Triad Studies (HVTN 204 [28], RV 172 [95], and IAVI V001 [96]) were designed to provide an international Phase 1 and 2 safety and immunogenicity evaluation of the VRC regimen towards the original goal of conducting an international efficacy study. The individual results from each of these studies are available [28,92,93]. Prior to initiating the HVTN 505 study, a cross-study summary of the unblinded safety data was completed and is shown in Table 4-5 for the participants randomized to the vaccine and placebo arms for the regimen that will be administered in HVTN 505. This is why the numbers shown add up to N=746 (383 vaccine and 363 placebo recipients) rather than the 918 total for the 3 studies shown in Table 4-4. The summary in Table 4-5 shows incidence for the unsolicited adverse events (AEs) that were reported in >1% of vaccinees for which the incidence was equal to or higher than that in placebo recipients; a statistical analysis has not been applied. There were no SAEs attributable to either the DNA vaccine or the rAd5 vaccine alone or in the combination regimen.

Table 4-5 Triad studies AEs in >1% of vaccine recipients for which there was equal or greater frequency in the vaccine than in the placebo groups, sorted by descending frequency

MedDRA Preferred Term	Vaccine (n=383) *n (%)*	Placebo (N=363) *n (%)*
Number with one or more AE	346 (90.3)	321 (88.4)
Headache	66 (17.2)	53 (14.6)
Neutropenia	48 (12.5)	40 (11)
Malaria	40 (10.4)	27 (7.4)
Nasopharyngitis	40 (10.4)	34 (9.4)
Alanine aminotransferase (ALT) increased	34 (8.9)	30 (8.3)
Neutrophil count decreased	24 (6.3)	18 (5)
Back pain	22 (5.7)	19 (5.2)
Gastroenteritis	20 (5.2)	15 (4.1)
Viral upper respiratory tract infection	19 (5)	16 (4.4)
Arthralgia	16 (4.2)	14 (3.9)
Diarrhea	13 (3.4)	10 (2.8)
Lymphadenopathy	13 (3.4)	11 (3)
Toothache	13 (3.4)	9 (2.5)
Influenza	12 (3.1)	11 (3)
Pharyngitis	12 (3.1)	9 (2.5)
Vomiting	12 (3.1)	6 (1.7)
Weight decreased	12 (3.1)	6 (1.7)
Anemia	11 (2.9)	9 (2.5)
Blood creatinine increased	11 (2.9)	8 (2.2)
Cough	11 (2.9)	4 (1.1)
Dyspepsia	11 (2.9)	8 (2.2)
Influenza like illness	9 (2.3)	7 (1.9)
Dental caries	8 (2.1)	6 (1.7)
Gastritis	8 (2.1)	7 (1.9)
Pharyngolaryngeal pain	8 (2.1)	6 (1.7)
Abdominal discomfort	7 (1.8)	1 (0.3)
Contusion	7 (1.8)	4 (1.1)
Epistaxis	7 (1.8)	4 (1.1)
Furuncle	7 (1.8)	2 (0.6)
Urticaria	7 (1.8)	4 (1.1)
Dermatitis	6 (1.6)	4 (1.1)
Injection site pruritus	6 (1.6)	0 (0)
Leukopenia	6 (1.8)	3 (0.8)
Menorrhagia	6 (1.6)	3 (0.8)
Oral herpes	6 (1.6)	3 (0.8)
Pelvic inflammatory disease	6 (1.6)	4 (1.1)
Soft tissue injury	6 (1.6)	3 (0.8)
Viral infection	6 (1.6)	4 (1.1)
Depression	5 (1.3)	3 (0.8)
Dysmenorrhea	5 (1.3)	2 (0.6)
Excoriation	5 (1.3)	2 (0.6)
Hemorrhoids	5 (1.3)	2 (0.6)
Metrorrhagia	5 (1.3)	2 (0.6)
Otitis media	5 (1.3)	4 (1.1)
Pyrexia	5 (1.3)	2 (0.6)
Sexually transmitted disease	5 (1.3)	0 (0)
Subcutaneous abscess	5 (1.3)	2 (0.6)
Tinea versicolor	5 (1.3)	3 (0.8)

Figure 4-5 shows the worst severity local and systemic reactogenicity across up to 3 DNA primes and following a single 10^{10} PU rAd5 boost for the VRC prime-boost regimen cumulatively across the Triad studies. Among the local reactogenicity parameters, pain and/or tenderness is the most frequently reported symptom for both the

DNA and rAd5 vaccine. The most frequently reported systemic symptoms were headache and malaise/fatigue. Across the diverse population in the Triad studies reactogenicity is reported as none to mild in severity in 70-90% of all vaccine recipients.



	DNA N=383	Placebo N=363	rAd5 N=345	Placebo N=331	DNA N=383	Placebo N=363	rAd5 N=345	Placebo N=331
	Local				Systemic			
SEVERE	0.01	0.00	0.01	0.00	0.01	0.01	0.02	0.00
MODERATE	0.20	0.11	0.08	0.01	0.17	0.13	0.11	0.04
MILD	0.68	0.63	0.50	0.24	0.45	0.47	0.34	0.20
NONE	0.11	0.26	0.42	0.75	0.37	0.39	0.53	0.76

Figure 4-5 Maximal severity reactogenicity symptoms in the Triad studies (HVTN 505 vaccine/control regimen only)

4.6.1.1 Safety of the 6-plasmid DNA prime, rAd5 boost regimen in HVTN 204

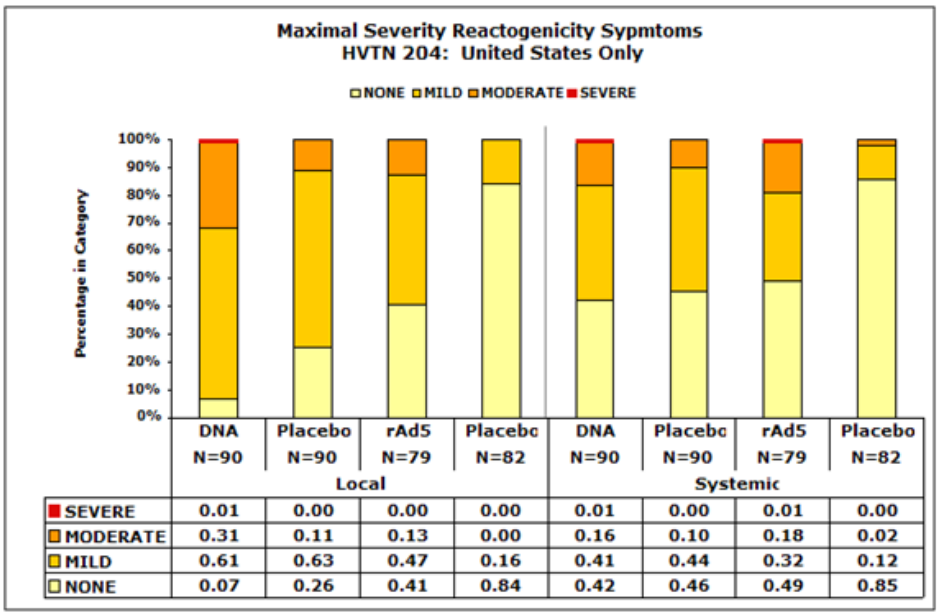
The HVTN's contribution to the Triad studies was HVTN 204, a randomized, placebo-controlled phase 2a study examining the identical vaccine regimen that is being proposed for HVTN 505. The study enrolled 480 participants without regard to their pre-existing Ad5 nAb titer. The study was stratified so that half (N=240) were enrolled at HVTN sites in the Americas (US, Haiti, Jamaica and Brazil) and half (N=240) were enrolled at HVTN sites in South Africa. The stratum enrolled in the Americas was further divided into 180 subjects enrolled at US sites and 60 at non-US sites. In each stratum, an equal number of participants received the candidate vaccines and control preparations. The cohort in the US was enrolled between September 22, 2005 and April 7, 2006, whereas the cohort in South Africa was enrolled between July 17, 2006 and December 18, 2006, and the non-US Americas cohort was enrolled between August 11, 2006 and March 20, 2007. Study participants were followed for local and systemic reactogenicity for 3 days after each vaccination and AEs were recorded for the 12 months duration of the study.

Pre-existing Ad5 nAb (titer > 12 in the NVITAL assay) were found in 118 of 171 (69%) of participants enrolled in the US (9 missing values), 53 of 60 (88%) enrolled at the non-US sites in the Americas and 230 of 238 (97%) enrolled in South Africa (2 missing values). Of the 180 participants enrolled in the US, 27 (15%) were MSM or bisexual, and of these 11 of 25 (44%) were Ad5 nAb negative (2 missing values). Data on circumcision status was not collected in HVTN 204.

A table of AEs constructed similar to Table 4-5 was prepared for US participants only. Although some AEs (eg, malaria) do not appear on a comparable table for US

participants, there were no AE types reported for US participants in HVTN 204 that would suggest any difference in the general safety of the vaccines in this population. The overall reactogenicity profile of participants enrolled in all strata in HVTN 204 was not appreciably different than that seen in the Triad studies as a whole.

The VRC DNA and rAd5 candidate vaccines were also well-tolerated by participants enrolled at US sites, in spite of having lower median Ad5 nAb titers on study entry. Local reactions of mild and moderate intensity were reported by more participants after the DNA candidate vaccines than the Ad5 vaccine (92% vs 60%), while the frequency and intensity of systemic reactions was similar in both groups (57% vs 50%). Only 3 of the 180 study participants in the US reported severe reactions, 1 with severe local pain and tenderness after receiving each DNA vaccine injection, another with severe elevated temperature after the second DNA vaccine injection, and 1 with severe systemic malaise and/or fatigue after receiving the rAd5 boost. In all cases, the reactions diminished within 24 hours and completely resolved by day 5. In addition, AEs reported in US vaccine recipients did not differ significantly from those reported by placebo recipients.



	DNA N=90	Placebo N=90	rAd5 N=79	Placebo N=82	DNA N=90	Placebo N=90	rAd5 N=79	Placebo N=82
	Local				Systemic			
SEVERE	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00
MODERATE	0.31	0.11	0.13	0.00	0.16	0.10	0.18	0.02
MILD	0.61	0.63	0.47	0.16	0.41	0.44	0.32	0.12
NONE	0.07	0.26	0.41	0.84	0.42	0.46	0.49	0.85

Figure 4-6 Maximum severity reactogenicity symptoms in HVTN 204 (US only)

In addition, there were no important differences in AEs reported by US recipients of the DNA+ rAd5 or placebo preparations in HVTN 204.

4.6.1.2 Potential risks of study products and administration

Table 4-6 Summary of potential risks of study products and administration

Known risks associated with the VRC DNA prime-rAd5 boost regimen	
Common	• Mild to moderate injection site pain, tenderness, erythema, or swelling/induration/edema • Malaise/fatigue, myalgia, or headache in the first few days following injection • A vaccine-induced positive HIV Ab test result
Less common or uncommon	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, nausea, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, laceration, or bleeding related to the injection procedure • Papule or scab formation at or near the site of Biojector® injection • Painless induration and erythema inferior to site of the Ad5 vector injection
Potential or theoretical risks of HIV vaccines in general	
General rare risks of vaccination	• Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis
Unknown frequency or theoretical risks	• Muscle damage at the injection site • Autoimmune disease or cancer • Effects on a participant's response to an approved HIV vaccine administered in the future • Effects on susceptibility to HIV, if the participant is exposed to HIV • Effects on the course of HIV infection/disease, if the participant is infected with HIV • Effects on the fetus and on pregnancy

4.6.2 Immunogenicity of DNA vaccine and rAd5 vaccine alone and in combination

The VRC, through evaluations performed by the Immunology Core Laboratory, as well as the NVITAL, HVTN, and IAVI laboratories, has accumulated phase 1 and 2 data indicating that the study vaccines show cellular and humoral immunogenicity comparable to or greater than other investigational HIV vaccines to date [28,62,70,95,96].

4.6.2.1 T-cell immune responses

The VRC plasmid DNA and rAd5 vectors are immunogenic as individual vectors in humans [57,58,61]. DNA delivery by a needle-free Biojector® device results in improved ELISpot and CD8+ T-cell responses compared with when the DNA prime is delivered by needle and syringe [97]. rAd5 boosting after DNA prime also results in a greater proportion of CD4+ and CD8+ T cells secreting multiple cytokines that include IL-2 than after rAd5 alone. Phase 2 evaluation was performed in collaboration with the HVTN,

USMHRP, and IAVI. These studies enrolled a total of 920 participants. T-cell responses were evaluated by VRC and in laboratories from each of the 3 network partners. All laboratories showed T-cell response rates in the range of 70% or higher. Over 50% of participants produced antigen-specific CD8 T-cell responses, and these responses were in volunteers with varied Ad5 nAb titers. The proposed HVTN 505 study would include only Ad5 nAb negative volunteers, a population that would be optimal for induction of immune responses to the vaccine.

4.6.2.2 T-cell immune responses in HVTN 204

The primary HVTN immunogenicity readout in HVTN 204 was IFN-γ ELISpot stimulated by global potential T-cell epitope (PTE-g) peptide pools (ie, pools including all peptides found in 15%+ of sequences in the Los Alamos database [98]) from cryopreserved peripheral blood mononuclear cells (PBMCs) collected 6 weeks after the Ad5-HIV boost. In addition, intracellular cytokine staining (ICS) examining CD4+ vs CD8+ T cell phenotypes and IFN-γ, IL-2, and TNF-α cytokine production in response to global PTE-g was performed using PBMCs collected at that time.

IFN-γ ELISpot responses to any HIV antigen were detected in 55/74 (74.3% [CI 63.3, 82.9]) of vaccine recipients in the US stratum, 15/21 (71.4%; CI 50.0, 86.2]) of vaccine recipients in the non-US Americas stratum and 64/93 (68.8%; CI 58.8, 77.3]) of vaccine recipients in the South African stratum. As shown in Figure 4-7, these responses were primarily directed at Env and Gag peptide pools and positive responders had a mean of 100-250 spot-forming cells (SFC)/10^6 PBMCs for all HIV antigens. In addition, the frequency and intensity of ELISpot responses did not differ significantly between the geographic regions.

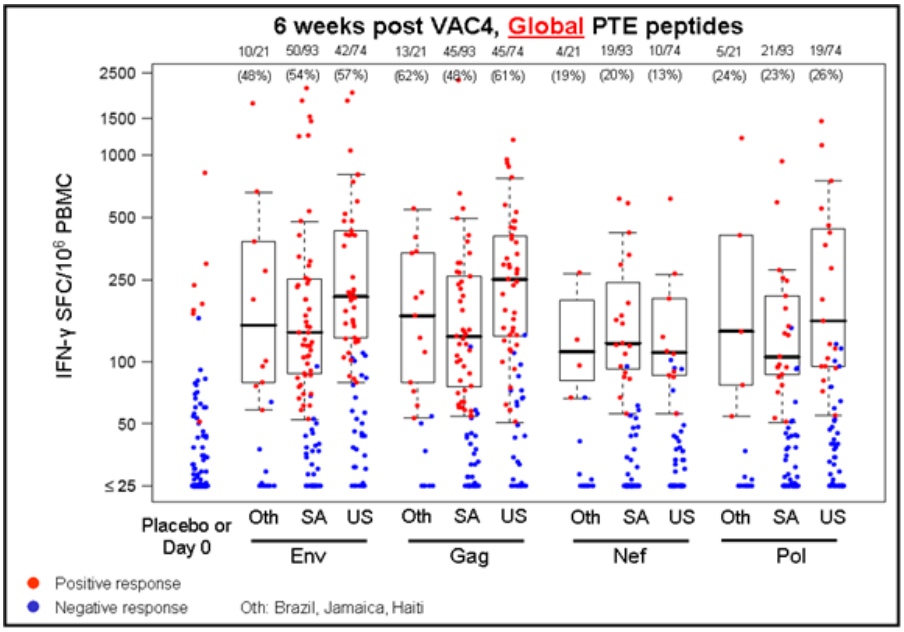


Figure 4-7 IFN-γ ELISpot responses in HVTN 204 by antigen and region

ICS assays were also performed on PBMCs from this timepoint. CD4+ T-cell responses to any HIV antigen were detected in 26/57 (45.6 %; CI 33.4, 58.4) of vaccinees in the US stratum and in 5/24 (20.8%; CI 9.2, 40.5) of vaccines in the South African stratum. CD8+

T-cell responses were seen in 30/56 (53.6%; CI 40.7, 66.0) and 16/38 (42.1%; CI 27.9, 57.8) in the US and South African groups, respectively. CD4+ T-cell responses were most frequently directed at Gag (in 32.4% of vaccine recipients) with a mean of approximately 0.2% cells producing IFN-γ and/or IL-2 (Figure 4-8). CD8+ T-cell responses were most frequently directed at Env (in 31.9% of vaccine recipients) with a mean of approximately 0.2% cells producing IFN-γ and/or IL-2 (Figure 4-9). Of note, CD4+ and CD8+ responses did not differ significantly among vaccine recipients who had pre-existing Ad5 nAb or not.

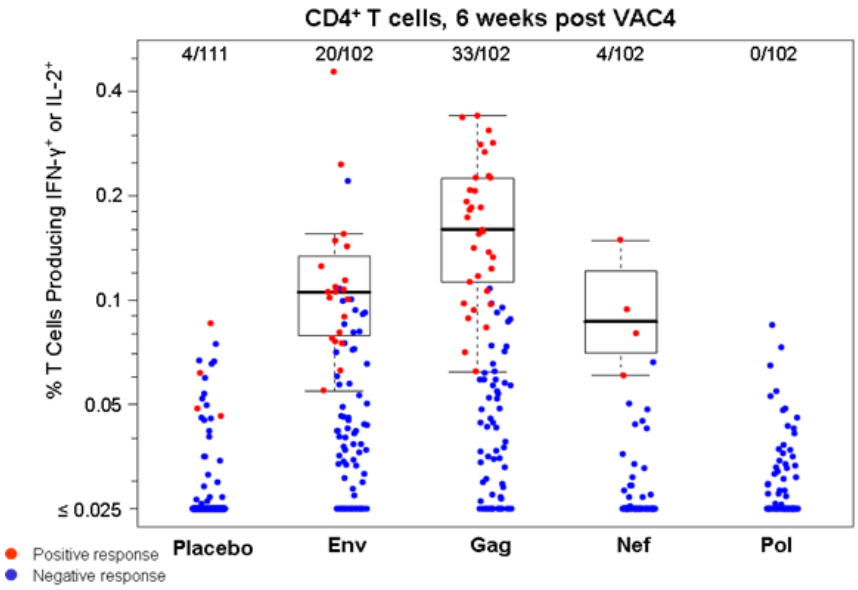


Figure 4-8 CD4+ T-cell cytokine responses by antigen

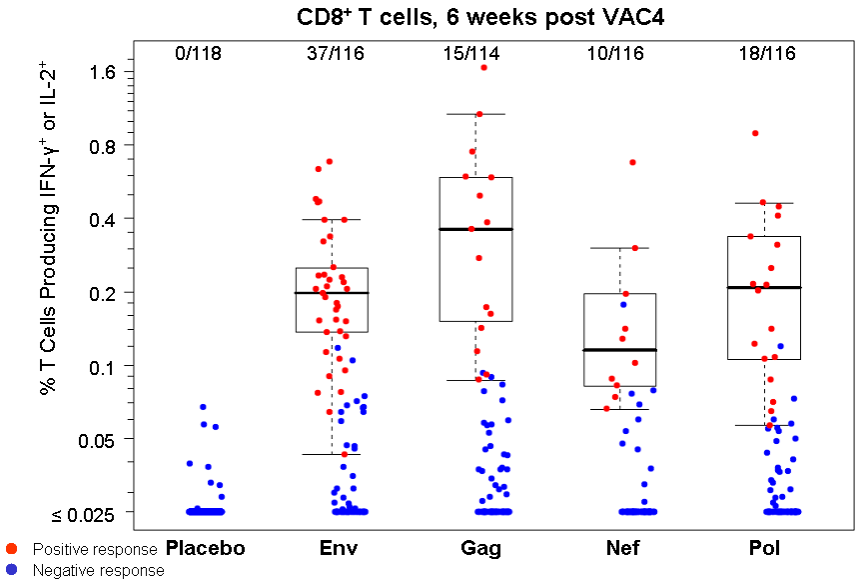


Figure 4-9 CD8+ T-cell cytokine responses by antigen

4.6.2.3 Humoral immunity

Ab responses have been measured by a research enzyme-linked immunosorbent assay (ELISA) that involves use of vaccine-specific purified protein bound to the solid phase. All vaccinees who completed 3 DNA primes and an rAd5 boost evaluated to date from the VRC phase 1 studies, as well as the preliminary Triad sample set, have had vaccine-specific Ab responses. The rAd5 vaccine boosts the Ab response even in the presence of pre-existing Ad5 nAb. The neutralizing activity of the vaccine-induced Ab response is type-specific and limited to laboratory-adapted HIV-1 isolates. An occasional participant sample will neutralize selected primary HIV-1 isolates such as SF162, which are considered relatively neutralization-sensitive, but none of the sera evaluated to date is broadly neutralizing.

4.6.2.4 Immunogenicity evaluations in HVTN 505

Evaluation of HIV-1–specific immunogenicity of the VRC DNA/rAd5 vaccine regimen is currently in process using samples collected pre-unblinding.

5 Objectives and endpoints of the unblinded extended follow-up phase

5.1 Primary objective and endpoints

Primary objective:

To evaluate the rate of study dropout in vaccine and placebo recipients

Primary endpoint:

Study dropout through the Month 48 visit

Primary objective:

To evaluate the effect of the VRC DNA/rAd5 vaccine regimen on the rate of HIV-1 acquisition compared to placebo

Primary endpoints:

HIV-1 infection diagnosed after Day 0 through the Month 24 visit

HIV-1 infection diagnosed after Day 0 including all available follow up through the maximum Month 48 visit

Note

Evaluation of differences in safety parameters between vaccine and placebo recipients, Primary objective 3 in previous versions of HVTN 505, remains as a primary objective for data obtained prior to protocol unblinding. During post-unblinding follow-up, HIV-1–uninfected participants will be monitored for SAEs and safety reports will be generated routinely by the SDMC and will be reviewed by the HVTN 505 PSRT (see Section 11).

5.2 Exploratory objectives

Version 6.0: Scientific priorities and resources will determine which of the following exploratory objectives will be pursued.

Exploratory objectives:

To evaluate the effect of the VRC DNA/rAd5 vaccine regimen on VL at the time of HIV-1 infection diagnosis

To evaluate the effect of the VRC DNA/rAd5 vaccine regimen on CD4+ T cell count and disease progression course

To evaluate HIV-1-specific and Ad5 vector-specific immune responses induced by the vaccine regimen

To evaluate the impact of viral genetic variation, host genetic factors, prophylactic ARV use, and other participant covariates including self-reported risk behavior on vaccine effects on study endpoints

To describe the prevalence of drug (TDF and FTC) resistance mutations after HIV-1 seroconversion among vaccine and placebo recipients reporting prophylactic ARV use

To evaluate immune correlates of risk of HIV-1 infection among vaccine recipients and (possibly) placebo recipients

6 Statistical considerations

6.1 Overview

This study consists of extended unblinded follow-up of participants enrolled in the multicenter, randomized, placebo-controlled, double-blinded trial described under protocol versions 1 through 4. The primary analysis will evaluate and compare the rate of study dropout and HIV-1 acquisition in the vaccine and placebo groups.

6.2 Objectives

At the first planned interim analysis for efficacy futility under Version 4 of the protocol, on April 22, 2013, the DSMB recommended that the trial be stopped for efficacy futility. A total of 71 HIV infections had been diagnosed in the MITT cohort (41 among vaccine recipients, 30 among placebo recipients). Of these, 48 constituted primary endpoints (Week 28+ HIV infections diagnosed on or after Day 196 post-enrollment); 27 occurred among vaccine recipients and 21 among placebo recipients. There was no statistically significant difference in the rate of HIV infection between treatment arms, either in the Week 28+ cohort (estimated hazard ratio = 1.25; 95% CI: 0.71 to 2.20; p = 0.446) or in the MITT cohort (hazard ratio = 1.33; 95% CI: 0.83 to 2.13; p = 0.230). However, given that the number of HIV infections was larger in the vaccine arm, and given that there was a trend toward the hazard ratio increasing over time since enrollment (p = 0.09), the DSMB recommended, and the study team agreed, to continue to follow all participants beyond the Month 24 visit [the terminal visit in Version 4 of the protocol]. Under Version 5 of the protocol, participants were followed post-unblinding to 48 months post-enrollment. Six-monthly interim analyses were conducted to evaluate the HIV-1 acquisition rate in the two treatment arms, and to evaluate conditional power to detect an increased rate of acquisition in the vaccine arm as compared to the placebo arm. At the second interim analysis under Version 5 of the protocol, on March 24, 2014, the study oversight group recommended that the protocol be revised to reduce the frequency of post-unblinding follow-up visits. A total of 109 HIV infections had been diagnosed in the MITT cohort to 48 months post-enrollment (53 among vaccine recipients and 56 among placebo recipients). There was no statistically significant difference in the rate of HIV-1 infection between treatment arms, either including all follow-up to 48 months post-enrollment (estimated hazard ratio = 0.92; 95% CI: 0.63 to 1.34; p = 0.65), or restricting to follow-up to 24 months post-enrollment (estimated hazard ratio = 1.09; 95% CI: 0.72 to 1.66; p = 0.68).

As described in Sections 8.2 and 8.2.1, under Version 6 of the protocol participants will continue to be followed to 48 months post-enrollment, with an additional health contact at 60 months, but with study visits only annually following Month 24. The goal of this extended follow-up is to continue to monitor the rate of HIV-1 acquisition in the two treatment arms. To this end, the rate of study dropout will also continue to be evaluated in each treatment arm. . The objectives of the study have been modified accordingly. Assessing the rates of study dropout and of HIV infection in vaccine vs. placebo arms are now the primary objectives (see Section 5.1). Assessing the impact of vaccination on post-infection endpoints and on immunogenicity; assessing modification of vaccine

effects by host immune genetic and other factors; and assessing immune correlates of risk are now exploratory objectives (see Section 5.2).

6.3 Endpoints

The first primary endpoint is study dropout during the follow-up of the trial. Participants who terminate from the study will be considered to be "dropouts" at the date of their last visit.

The second primary endpoint is diagnosis of HIV-1 infection during the follow-up of the trial. The occurrence of HIV-1 infection will be detected through HIV-1 tests administered at timepoints specified in Appendix C or at interim visits. Participants found to be HIV-1–infected will have additional testing to confirm the diagnosis of HIV-1 infection. The vaccine-induced immune responses may lead to positive HIV-1 tests and difficulty in interpretation. Therefore, the study will continue to use a specialized HIV-1 diagnostic testing algorithm to confirm diagnoses of HIV-1 infection.

Primary analyses of the HIV-1 infection endpoint will include infections diagnosed after enrollment; we refer to these as "MITT infections". The set of all enrolled participants who are HIV-1 negative at Day 0 is referred to as the "MITT population". Versions 1 through 4 of the protocol restrict attention to "Week 28+" infections, defined as those diagnosed on or after Day 196 post-enrollment through the Month 24 visit, for primary analyses. By Day 196, all participants would have been expected to have had their fourth study injection and most would have had time to develop a complete vaccine-induced immune response. This restriction will no longer be applied for primary analyses because of a primary interest in all HIV-1 infections and because a subset of participants had not reached the fourth injection visit when study injections were discontinued on April 23, 2013. In supportive analyses the HIV-1 infection endpoint will be assessed in various subgroups of the MITT population.

We will continue to define the date of diagnosis of HIV-1 infection to be the draw date of the first sample that leads to a positive result by the diagnostic algorithm. The final analysis will take place after the last enrolled participant has reached the end of the Month 48 visit window; we refer to this as the final evaluation time (FET).

6.3.1 Primary endpoints

The primary dropout endpoint is study dropout through the Month 48 visit.

The primary HIV-1 acquisition endpoints are HIV-1 infection diagnosed after Day 0 through the Month 24 visit, and HIV-1 infection diagnosed after Day 0 including all follow-up through the Month 48 visit. Corresponding to these primary endpoints, primary analyses will conduct estimation and inference on the parameter $VE^{MITT}(24)$, the multiplicative reduction in the hazard rate of HIV infection (vaccine versus placebo) by the Month 24 visit, and on the parameter $VE^{MITT}(t)$, the multiplicative reduction by time t post-enrollment for all times through to the Month 48 study visit.

6.3.2 Exploratory endpoints

- VL obtained from samples drawn at the visit at which a study participant is diagnosed with HIV-1 infection

- Post-infection CD4+ T cell count, initiation of ART, clinical events
- Immune responses measured by HIV-1 specific and Ad5-specific multiparameter flow cytometry, epitope mapping, and Ab assays (binding and neutralization)
- Host genetic factors measured in HIV-1 infected participants and a random sample of uninfected participants
- Full-genome HIV-1 sequences measured from each HIV-1 infected subject
- Resistance mutations identified using viral genome sequencing of HIV-1 strains at the first evidence of infection
- Participant risk behavior and prophylactic ARV use as measured by behavioral risk factor and prophylactic ARV use questionnaire as well as plasma ARV drug level testing
- Selected immune response biomarkers measured at baseline and at selected post-baseline visits at which participants test HIV-1 negative
- Social impacts

6.4 Sample size rationale

The size of the study is fixed; all participants enrolled under versions 1 through 4 of the protocol will continue to be followed. In addition, participants brought back into the study under version 5 of the protocol will continue to be followed. This choice ensures that the primary endpoints are estimated with maximal precision.

6.5 Sampling design for assessing prophylactic ARV use

For all participants and at all ARV assessment visits, questionnaires will capture information on prophylactic ARV use. Plasma drug levels may be measured on samples from selected timepoints and subjects, where decisions on which samples to run will be made retrospectively taking into account the questionnaire data and HIV-1 diagnostic data. This sampling strategy was implemented in Version 3.0 of the protocol, as questionnaire data and stored plasma samples were lacking for study participants enrolled previously.

Details of sampling and analysis plans for assessing prophylactic ARV use will be provided in a statistical analysis plan (SAP).

6.6 Statistical analysis

All primary analyses of study dropout will be performed in the MITT population. Analyses will right-censor HIV-1-uninfected participants at the time of their Month 48 visit (or the end of the Month 48 visit window, should that visit be missed).

All primary analyses of HIV-1 acquisition will be performed in the MITT population. Analyses of $VE^{MITT}(24)$ will right-censor HIV-1-uninfected participants at the time of last HIV test result in Months 0-24, Analyses of VE through time t post-entry, VE(t), will right-censor participants at the time of the last HIV test result prior to t. Secondary

analyses of the HIV-1 acquisition endpoint will consider defined subgroups of the MITT population (see Section 6.6.3.3).

6.6.1 Analysis variables

The analysis variables consist of baseline variables, vaccine activity variables, immunogenicity variables, and social impact variables.

6.6.2 Baseline comparability

Treatment groups will be compared on baseline characteristics using descriptive statistics. Variables examined will include potential risk factors such as age, race/ethnicity, and self-reported risk behavior, as well as other demographic characteristics.

6.6.3 Primary analyses

6.6.3.1 Primary analysis: Study dropout

To evaluate the primary endpoint of study dropout, the incidence of study dropout in each treatment arm will be estimated by dividing the number of dropouts by the amount of person-time "at risk", and exact methods will be used to calculate 95% confidence intervals. The dropout incidence will be compared between treatment arms using a two-sided score test assuming a Cox proportional hazards model. Dropout incidence will also be assessed over study time (pooling treatment arms), both pre- and post-study unblinding, using methods described in the SAP. Goodness-of-fit tests will be performed, including the Grambsch and Therneau [99] test based on Schoenfeld residuals, to assess the proportional hazards assumption of the Cox model. Cumulative probabilities of dropout over time will also be estimated in each treatment arm.

6.6.3.2 Primary analysis: HIV-1 acquisition

To evaluate the primary endpoint of HIV-1 acquisition, the incidence of acquired HIV-1 infections ("events") in the vaccine arm will be compared to the incidence in the placebo arm. The vaccine effect will be assessed using a Cox proportional hazards model. Given that vaccine and placebo arms will be compared over a time period when participants are unblinded as to treatment assignment, the potential for confounding will be carefully considered. Specifically, in addition to estimating the marginal HR associated with vaccine assignment, the HR adjusted for potential confounding factors will also be estimated. First, the extent to which baseline participant characteristics and baseline behavioral risk score are predictive of HIV-1 infection risk will be assessed. Variables that, when considered univariately and pooled over treatment arms, predict infection risk with significance $p < 0.10$ based on two-sided score tests in the Cox model will be added to the Cox model relating treatment assignment to HIV-1 infection risk. The baseline variables considered will be age, baseline behavioral risk score, race/ethnicity, HSV-2 serostatus, and BMI. BMI is included in this set because it may predict infection risk among vaccine recipients if it modifies the vaccine effect on HIV-1 acquisition; some biomedical interventions have efficacy that differs by subject BMI [100,101]. Note that HSV-2 serostatus will not be considered if fewer than 95% of participants have measurements; this assay is specified as optional in the protocol. The behavioral risk score has been calculated using baseline data as of April 22, 2013 and is a function of variables measured by the behavioral risk questionnaire, which queries participants about

risk behavior over the last 3 months. The score is a function of an indicator that the number of male sexual partners is greater than three and an indicator of unprotected receptive anal sex. Both unadjusted HRs for vaccination (without adjustment for the baseline covariates) and adjusted HRs (with adjustment for baseline covariates) will be reported.

The method of Lu and Tsiatis [102] will be used for more efficient estimation or standard partial likelihood estimation will be used depending on certain criteria specified in the statistical analysis plan (SAP). Two-sided 0.05-level Wald tests will be used for inference. Goodness-of-fit tests will be performed [including the Grambsch and Therneau [99] test based on Schoenfeld residuals] to assess the proportional hazards assumption of the Cox model.

An additional analysis, especially relevant if the goodness-of-fit diagnostics support violation of the proportional hazards assumption of the Cox model, is estimation of cumulative probabilities of HIV-1 infection over time in each treatment arm, adjusted for potential confounders, and estimation of the additive difference and the ratio of these cumulative probabilities for the vaccine versus placebo group. In addition to the point estimates, 95% confidence intervals about the cumulative probabilities of HIV-1 infection over time for each treatment arm will be computed, as well as 95% confidence intervals about the additive difference and ratios over time. The Cox collaborative targeted maximum likelihood estimation method [103] will be used, which in addition to allowing confounding adjustment can correct for potential bias due to covariate-dependent censoring.

If the goodness-of-fit diagnostics support failure of the proportional hazards assumption, then additional Cox regression modeling analyses will be performed that include time-dependent interactions between the natural logarithm of failure time and treatment arm. In addition, the nonparametric smoothing method of Durham et al. [104] based on Schoenfeld residuals may be used. These analyses will adjust for covariates in the same manner as the Cox model analysis that does not include the time-dependent interactions.

The SAP will specify the details of the methods that will be applied, and will be developed independent of any data collected after April 22, 2013.

6.6.3.3 Secondary analyses of dropout

Secondary analyses of study dropout will restrict attention to follow-up to Month 24 post-enrollment. These analyses will inform the validity of vaccine efficacy analyses restricted to Month 0-24 follow-up.

6.6.3.4 Secondary vaccine efficacy analyses: HIV-1 acquisition

Secondary analyses of vaccine efficacy will consider four subgroups of the MITT population:

(1) The Week 28+ population, those on-study on Day 196 and HIV-negative (ie, not yet diagnosed as infected) prior to that;

(2) The Week 28+ population who received rAd5/FFB;

(3) The Week 28+ population who did not receive rAd5/FFB; and

(4) (Per-protocol) the Week 28+ population who received all four immunizations with correct product administration and within visit windows.

For each subgroup analysis, both VE(24) and VE over all available follow-up time will be evaluated. The subgroups will help to address whether and how vaccine efficacy depends on the rAd5 vaccination.

Additional secondary analyses will assess the possibility of confounding by time-dependent variables as specified in the SAP.

6.7 Monitoring of trial

The study will be monitored, potentially leading to modification or termination of the study. Interim analyses will occur every 6 months, with the first analysis scheduled for approximately September, 2014. The results of the interim analyses will be shared in a report to the Oversight Group (see Section 12.1.3) that will keep the results confidential.

Interim analysis reports will include point estimates, 95% confidence intervals, and 2-sided p-values testing H_0: VE = 0% for $VE^{MITT}(24)$, $VE^{MITT}(t)$ for the latest available time point t, and for these parameters defined for the subgroups defined in Section 6.6.3.3. Point estimates and 95% confidence intervals will also be reported for dropout rates by treatment arm, over study time, and in pre- and post-unblinding follow-up periods.

7 Selection and withdrawal of participants

At the time that the DSMB determined that HVTN 505 had met pre-set criteria for efficacy futility and recommended stopping all further study vaccinations (April 22, 2013), the study was fully enrolled.

At enrollment, participants were healthy, HIV-1–uninfected (Ad5 nAb negative) adults who comprehended the purpose of the study and provided written informed consent. Volunteers were determined to be eligible based on the inclusion and exclusion criteria (see Sections 7.1 and 7.2). Final eligibility determination depended on results of laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions.

In addition, investigators used clinical judgment in considering a volunteer's overall fitness for trial participation.

7.1 Inclusion criteria

1. **Male**[1]**,** age 18 to 50 years, who is fully circumcised (as documented at screening examination) and who, in the 6 months prior to randomization, experienced 1 or both of the following HIV risk criteria:

    - unprotected anal intercourse with 1 or more male or MTF transgender partner(s); or

    - anal intercourse with 2 or more male or MTF transgender partners.

    Note: Volunteers who have been in a monogamous relationship with an HIV-1 seronegative partner for > 1 year are excluded.

2. **Negative HIV-1 and -2 blood test** (FDA-approved enzyme immunoassay [EIA])

3. **Ad5 nAb titer** < 1:18

4. **ALT** ≤ 2.5 upper limit of normal

5. **Within reach of a participating study site** and willing to be followed for the planned duration of the study, including long-term safety surveillance contact for 5 years after enrollment

6. Able and willing to provide **informed consent**

7. **Assessment of understanding**: demonstrates understanding of this study and the Step Study results; completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly

[1] Male-to-female transgender volunteers who have undergone gender reassignment surgery (GRS) are allowed to participate if they provide documentation from a healthcare provider confirming that they were fully circumcised prior to GRS. Male-to-female transgender volunteers who have not undergone GRS are also eligible to participate if they meet all enrollment criteria. Receipt of hormonal therapy does not make a transgender volunteer ineligible.

8. Willing to receive **HIV test results**

9. Willing to discuss **HIV infection risks** and amenable to **risk reduction counseling**

10. **Good general health** as shown by medical history, physical exam, and screening laboratory tests

11. **Agrees not to enroll in another study** of an investigational research agent prior to unblinding of the HVTN 505 study

7.2 Exclusion criteria

1. **Volunteer has received any of the following:**

    - HIV vaccine(s) received in a prior HIV vaccine trial. For potential participants who have received control/placebo in an HIV vaccine trial, the HVTN 505 PSRT will determine eligibility on a case-by-case basis.

    - Immunosuppressive medications received within 168 days before first vaccination. (Not excluded: [1] corticosteroid nasal spray for allergic rhinitis; [2] topical corticosteroids for mild, uncomplicated dermatitis; or [3] oral/parenteral corticosteroids given for non-chronic conditions not expected to recur [length of therapy 10 days or less with completion at least 30 days prior to enrollment].)

    - Blood products within 90 days before first vaccination

    - Immunoglobulin within 90 days before first vaccination

    - Live attenuated vaccines other than influenza vaccine within 30 days before first vaccination or scheduled within 14 days after injection (eg, measles, mumps, and rubella [MMR]; oral polio vaccine [OPV]; varicella; yellow fever)

    - Influenza vaccine or any vaccines that are not live attenuated vaccines within 14 days before first vaccination (eg, tetanus, pneumococcal, Hepatitis A or B)

    - Allergy treatment with antigen injections within 30 days before first vaccination or that are scheduled within 14 days after first vaccination

    - Investigational research agents within 90 days before first vaccination

2. Volunteer has used **antiretroviral drugs (ARVs) for the purpose of HIV-1 prophylaxis** $\geq$ 50% of days during the 3 months prior to first vaccination, or for 30 consecutive days within the 60 days prior to first vaccination

3. Volunteer has been **circumcised within 90 days** prior to first vaccination or displays evidence that surgical site is not fully healed

4. **History of serious adverse reactions to vaccines** including anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain. (Not

excluded: a participant who had a nonanaphylactic adverse reaction to pertussis vaccine as a child.)

5. **Current anti-tuberculosis prophylaxis or therapy**

6. **Clinically significant medical condition,** physical examination findings, clinically significant abnormal laboratory results, or past medical history that, in the judgment of the investigator, has clinically significant implications for current health.

7. **Any medical, psychiatric**, or occupational or other condition that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety or reactogenicity, or a participant's ability to give informed consent

8. **Psychiatric condition** that precludes compliance with the protocol. Specifically excluded are persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years.

9. **Autoimmune disease** (Not excluded: Volunteer with mild, stable and uncomplicated autoimmune disease that does not require immunosuppressive medication and that, in the judgment of the site investigator, is likely not subject to exacerbation and likely not to complicate reactogenicity and AE assessments.)

10. **Immunodeficiency**

11. **Bleeding disorder** diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions). [This exclusion also applies to therapeutic anticoagulation that results in a prolonged prothrombin time/international normalized ratio (PT/INR) or partial thromboplastin time (PTT).]

12. **History of malignancy** (Not excluded: a participant with a surgical excision and subsequent observation period that in the investigator's estimation has a reasonable assurance of sustained cure or is unlikely to recur during the period of the study.)

13. **Seizure disorder** (Not excluded: a participant with a history of seizures who has had no seizures within the past 3 years.)

14. **Asthma other than mild, well-controlled asthma**

15. **Hereditary Angioedema (HAE), Acquired Angioedema (AAE), or idiopathic angioedema**

7.3 Co-enrollment of HVTN 505 participants

Co-enrollment of HVTN 505 participants is allowed. If co-enrollment (including co-enrollment in trials that do not utilize investigational agents) may pose safety risks to the participant (eg, where blood draw limits may be exceeded), the HVTN 505 PSRT should be consulted. Any additional questions regarding co-enrollment should be directed to the HVTN 505 PSRT. Instances of co-enrollment should be recorded and reported to the SDMC on the appropriate case report form (CRF).

7.4 Participant termination from the study

Under certain circumstances, an individual participant may be terminated from participation in this study. Specific events that will result in early termination include:

- Participant refuses further participation,
- Participant relocates and remote follow-up or transfer to another HVTN CRS is not possible,
- HVTN CRS determines that the participant is lost to follow-up, or
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff).

8 Clinical procedures

The schedules of clinic procedures are shown in Appendix E and Appendix F.

8.1 Informed consent

Informed consent is the process of ensuring that participants fully understand what will and may happen to them while participating in a research study. The HVTN informed consent form documents that a participant (1) has been informed about the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in an HVTN study. Informed consent encompasses all written or verbal study information HVTN site staff members provide to the participant, before and during the trial. Study site staff will obtain informed consent of participants according to HVTN policies and procedures.

The informed consent process continues throughout the study. Key study concepts should be reviewed periodically with the participant and the review should be documented. At each study visit, site staff should consider reviewing the procedures and requirements for that visit and for the remaining visits. Additionally, if any new information is learned that might affect the participants' decisions to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign revised informed consent forms.

A study site may employ recruitment efforts prior to the participant consenting. For example, some HVTN CRSs use a telephone script to prescreen people before they come to the clinic for a full screening visit. Participants must sign a screening or protocol-specific consent before any procedures are performed to determine eligibility. Study sites must submit recruitment and prescreening materials to IRBs/IECs for human subjects protection review and approval.

8.1.1 Protocol-specific consent form

The protocol-specific consent form describes the study products to be used and all aspects of protocol participation, including screening and enrollment procedures.

Each study site is responsible for developing a protocol-specific consent form for local use, based on the sample protocol-specific consent form in . The consent form must be developed in accordance with local IRB/IEC requirements and the principles of informed consent as described in Title 45, CFR Part 46 and Title 21 CFR, Part 50, and in International Conference on Harmonisation (ICH) E6 (R1), *Guideline for Good Clinical Practice*: Section 4.8, *Informed consent of trial subjects*. It must be approved by all responsible ethical review bodies before any participants can be deemed to have consented for the study.

Study sites are strongly encouraged to have their local CABs review the sites' protocol-specific consent form. This review should include, but should not be limited to, issues of cultural competence, local language considerations, and the level of understandability.

The sample informed consent form includes interspersed instructions for developing specific content.

An addendum to the sample protocol-specific consent form is located in Appendix A. This form reviews key study information, provides new information, and describes changes to the protocol in Version 6. Each study site is responsible for developing an addendum to the consent form for local use, based on the sample addendum in Appendix A. Informed consent must be obtained prior to performing laboratory and clinic procedures in Version 5.0.

The DAIDS Support Center (RSC) Protocol Registration Office will review all site-specific informed consent forms and approve them for use according to DAIDS policies. The study cannot be initiated at a site until the site is fully registered with the DAIDS RSC Protocol Registration Office and has received written notification of protocol activation from HVTN Regulatory Affairs.

8.2 Follow-up visits for HIV-uninfected participants

The following procedures will be performed at all scheduled clinic visits through Month 48 (see Appendix E):

- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Risk reduction counseling;
- Behavioral risk, prophylactic ARV use, and demographics questionnaire;
- HIV infection assessment, including pre-test information and assessment of signs and symptoms of acute HIV infection. A subsequent follow-up contact is conducted after testing to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriated;
- Assessment of new or unresolved SAEs;
- Social impact assessment;
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, medical insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Assessment of ARV use for purposes of PrEP or PEP [Note: If and only if a participant reports having used ARVs for PrEP or PEP since the last clinic visit, draw blood sample for plasma as indicated in Appendix C];
- Testing for Neisseria gonorrhea (GC), Chlamydia trachomatis (CT), and Syphilis (see Appendix C and Appendix E).
- Specimen collection (see Appendix C).

8.2.1 Month 60 health contact

As indicated in Appendix E, a clinic visit is not required at Month 60. This contact is for purposes of assessing participant vital status and safety surveillance. At this contact, CRS staff will collect the information listed below. Except as noted, clinic visits are not required.

- Confirmation of vital status; if deceased, attempt to learn cause of death
- If participant is alive, record the participant's responses to questions regarding any occurrence of the following events since the last HVTN study contact:
    - Life-threatening adverse experiences;
    - Persistent or significant disability/incapacity;
    - Hospitalizations and reasons;
    - Other important medical events that may jeopardize the participant or may require intervention to prevent 1 of the other outcomes listed above;
    - New chronic conditions requiring medical intervention of more than 30 days;
    - Change in HIV status; and
    - Newly diagnosed or treated STIs.

All such events will be recorded and assessed for relationship to study product except for change in HIV status. HIV infections will be recorded in the database without a relationship assessment. Any participant reporting that they have become HIV-infected will be asked to come to the clinic so that HIV status can be confirmed. If HIV-1 infection is confirmed, the infected participant will be followed under Schedule 7 (see Section 8.3, Appendix D, and Appendix F).

During the window for this contact, an interim clinic visit for purposes of HIV testing may take place at the participant's request.

8.2.2 Unblinding and evaluation of vaccine-induced seroreactivity (EOS) testing

The study has been unblinded and study participants have been informed of their treatment assignments. EOS testing among vaccinees is currently underway (see Section 10.11) and results are being provided to participants as soon as they become available. EOS testing will be repeated on samples obtained from study participants at their Month 48 visits.

8.3 Procedures for HIV-1–infected participants

The following procedures will be performed at all scheduled clinic visits (see Appendix F):

- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- ART assessment;
- Transmission risk reduction counseling;
- Notation of HIV-associated events;
- Social impact assessment; and
- Specimen collection (see Appendix D).

The following procedures will be performed at designated clinic visits (see Appendix F):

- Counseling on HIV testing/diagnosis; and
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, medical insurance, life insurance, educational or employment opportunities, housing, immigration, or travel).

8.4 HIV risk reduction counseling

HIV counseling will be performed in compliance with the CDC's guidelines and other local guidelines for HIV counseling, testing, and referral. Information on PEP and PrEP should be included, including information about HIV testing and risk reduction counseling for individuals taking ARVs for HIV-1 prophylaxis.. Participants will be counseled at all scheduled visits during the trial on the avoidance of HIV infection. Vaccine recipients will be counseled on the potential negative social impacts of testing Ab positive due to the vaccine. They will also be counseled on the risks of HIV Ab testing outside of the study sites during any period of vaccine-induced positive serology.

Study staff will take particular care to inform vaccine recipients of the likelihood of routine HIV testing being offered or performed outside the study site at emergency rooms, clinics, and medical offices. Such testing has become more likely due to the CDC's revised guidelines for HIV counseling and testing, as well as policy changes in many countries to make HIV testing more frequent and routine. Site staff should inform vaccine recipients of their right to opt out of HIV testing outside the study site. Site staff should inform study participants if local and/or state policies and regulations permit medical providers to perform HIV testing without first informing patients. If this is the case, then site staff should advise study vaccine recipients that they may decline testing preemptively. Site staff should provide vaccine recipients with site contact information and should encourage participants to ask medical providers to contact the site. The site can verify that the vaccinee is a participant in an HIV vaccine clinical trial and should only be tested at the study site.

As part of risk reduction counseling, study participants should be informed of and educated about clinical trial results pertaining to HIV-1 prophylaxis (eg, prophylactic ARV use in iPrEx [38]. Participants should be informed of the Food and Drug Administration's approval on July 16, 2012 of the fixed-dose combination of emtricitabine-tenofovir disoproxil fumarate (FTC-TDF; trade name Truvada®) for the prevention of HIV infection in persons at high risk of sexual acquisition of HIV (PrEP), and of the CDC's release, on May 14, 2014, of "*Preexposure Prophylaxis for the Prevention of HIV Infection in the United States -2014: A Clinical Practice Guideline"*. The CDC guidelines provide comprehensive information for the use of daily oral antiretroviral pre-exposure prophylaxis (PrEP) to reduce the risk of acquiring HIV infection in adults. HIV risk reduction counseling by a clinician may help a participant determine what measures are appropriate for that individual's personal circumstances. These may include abstinence, condom use, systemic or topical prophylactic ARV use, behavior modification, etc. As needed or desired by a participant, referrals will be made to appropriate services. A participant interested in PrEP will receive information on where to access this intervention, including, where appropriate, referrals to providers who may prescribe PrEP.

8.5 Visit windows and missed visits

Visit windows are defined in *HVTN* 505 *Study Specific Procedures*. For a visit not performed within the window period, a Missed Visit form is completed. If the missed visit is one that required safety assessments or local safety labs, site staff should attempt to bring the participant in for an interim visit as soon as possible.

Procedures performed at an interim visit are limited to toxicity/safety assessments (including local safety labs) and HIV testing. With the exception of HIV testing, these procedures are performed only if they were required at the missed visit or if clinically indicated. HIV testing may be performed as deemed appropriate by the study staff. Blood samples for immunogenicity assays are not typically collected at interim visits.

8.6 Early termination visit

In the event of early participant termination, site staff should consider if the following safety assessments are appropriate: a final physical examination, social impact assessment, and HIV test.

9 HIV-1 infection assessment and clinical response

9.1 HIV-1 symptom assessment

At each visit and at unscheduled visits due to illness or suspected exposure, if necessary, information will be collected about any signs or symptoms suggestive of acute HIV-1 infection. Participants will be counseled about signs and symptoms of acute HIV infection and at visits following recent high-risk exposure, participants will be queried about any signs/symptoms suggestive of acute HIV-1 infection. Presence of signs/symptoms suggestive of acute HIV-1 infection, an intercurrent illness consistent with acute retroviral syndrome, or history of high-risk exposure would prompt a diagnostic work-up per the protocol-specific algorithm to determine HIV infection.

9.2 HIV-1 testing during post-unblinding follow-up

HIV testing at surveillance visits will be performed using the HVTN HIV testing algorithm (as described in the HVTN Laboratory Manual of Operations), which is able to distinguish vaccine-induced antibody responses from actual HIV infections. Samples to be stored for future immunogenicity or virology studies will also be collected at this time (see Appendix C).

A ‘case’ will be defined as a participant with confirmed detectable HIV-1 nucleic acid PCR on 2 different specimen collection dates. The nucleic acid test will most commonly be the HIV-1 RNA VL PCR test. Confirmation of HIV-1 infection will be determined through use of the protocol-specific HIV testing algorithm (available on the HVTN website). Before issuing an HIV-1 infection report for a participant diagnosed with HIV-1 infection, all testing results will be reviewed by a blinded, independent Endpoint Adjudicator or designee (see Section 9.3).

If a participant is confirmed to have become HIV-1–infected following the initial injection of study product, plasma HIV-1 viral RNA will be measured on archived samples prior to the first positive screening test and at subsequent timepoints indicated in Appendix D.

If a participant had completed scheduled clinic visits and, while in Schedule 3 (*Annual health contacts*), reports having been diagnosed with HIV-1 infection, the participant will be asked to come to the CRS for confirmatory HIV testing.

The HVTN Laboratory Program is responsible for all in-study diagnostic testing.

9.3 Endpoint adjudication

The diagnostic criteria for HIV-1 infection outside the setting of a vaccine trial are well accepted. However, definitive diagnosis of HIV-1 infection in the context of having received a vaccine that is even partially effective may be more difficult. Specifically, if the immune responses elicited by vaccination are capable of completely suppressing viral replication, or if vaccination alters the normal serological response upon exposure to

HIV-1, standard diagnostic tests may be more difficult to assess. For example, CDC guidelines define a positive HIV-1 Western blot as one having reactivity to at least 2 of the following antigens: p24, gp41, gp120/160. However, the HIV vaccines may elicit antibodies to p24 in many participants, so reactivity to this antigen could be due to either vaccination or infection. Therefore, the HVTN will have an endpoint adjudication process to assess all serological and virological testing, in a blinded manner, on each participant in the trial who tests positive per the HVTN 505 HIV-1 diagnostic testing algorithm. The assessment of the Endpoint Adjudicator or designee will be reported to the SDMC and to the HIV diagnostics laboratory.

The Endpoint Adjudicator and/or designee must notify the SDMC within 1 working day of any confirmed HIV-1 infection. The HIV diagnostics lab will inform the clinic of the outcome of the HIV testing algorithm (ie, HIV-infected, HIV-uninfected, or redraw required).

The Endpoint Adjudicator will be an expert in the fields of infectious diseases or laboratory medicine independent of the VRC and clinical investigators participating in this trial. A separate Standard Operating Procedure will govern the activities of the Endpoint Adjudicator.

9.4 HIV-1 infection during the study

Participants who develop HIV-1 infection following the initial injection of study product will be asked to remain in the study for follow-up. Participants who become HIV-1–infected following enrollment will be monitored as indicated in Appendix D and Appendix F. Longer-term follow-up for these participants may be accomplished through enrollment in another protocol. Archived samples from earlier visits may also be tested to determine the earliest date of HIV-1 infection.

9.5 Medical care for participants who become HIV-1–infected

The investigators associated with this trial will refer participants who develop HIV infection while participating in this trial to medical professionals for care.

10 Laboratory

10.1 Study site laboratory procedures

The cross-protocol HVTN *Laboratory Manual of Operations* provides further guidelines for operational issues concerning the clinical laboratories and phlebotomy. The procedures include general specimen collection guidelines, special considerations for blood collection, HIV testing guidelines, guidelines for processing whole blood, and labeling guidelines.

Tube types for blood collection post-unblinding are listed in Appendix C and Appendix D.

In specific situations, the blood collection tubes will be redirected to another laboratory or will require study-specific processing techniques. In these cases, laboratory special instructions will be posted on the protocol-specific section of the HVTN website.

10.2 Total blood volume

Total blood draw volumes per visit for post-unblinding Schedules 6 and 7 are listed in Appendix C and Appendix D. Not shown is any additional blood volume that would be required if HIV testing needs to be repeated. The total blood volume drawn for each participant does not exceed 500 mL in any 56-day (8-week) period.

10.3 VL determination

VL assessments will be conducted in accordance with the HIV diagnostic algorithm implemented for this study. Details can be found in the document entitled *HVTN 505 HIV-1 Diagnostic Testing*.

10.4 Immunogenicity timepoints

The primary immunogenicity timepoint in this study is at visit 7 (Day 196), 4 weeks following the final vaccination. Endpoint assays for humoral and cellular responses will be performed on approximately 8% of participants at baseline (humoral only) and at the primary immunogenicity timepoint. Depending on the number of responders observed, assays for humoral and cellular responses may be performed on additional participants and at other timepoints.

10.5 Immunogenicity assays

10.5.1 Humoral assays

10.5.1.1 Binding antibodies by HIV-1 multiplex Ab assay

HIV-specific binding antibodies against Env may be assessed on plasma/serum samples from study participants taken at the primary immunogenicity timepoint and baseline.

10.5.1.2 HIV-1 nAb assay

HIV-1–specific nAb assays may be performed on serum samples from study participants taken at baseline and at the primary immunogenicity timepoint. The assays will initially test neutralization of HIV-1 MN and SF162.LS strains. Because this is an artificial Envelope immunogen, no corresponding full-length functional gp-160 gene is available for use as a pseudovirus in the assessment of nAb in this clinical trial.

Additionally, the HVTN Laboratory Program may examine nAb responses against a panel of heterologous primary isolates using cross-sectional serum samples. If nAb are detected, a subset of samples with the best neutralization activity will be screened at a single serum dilution for neutralization activity against a panel of heterologous strains.

10.5.1.3 Rectal secretion assays

Frozen rectal secretion samples may be used to identify HIV-specific mucosal antibodies. Env-specific IgG and IgA may be assessed by binding antibody multiplex assay (BAMA). In addition, soluble cytokines and other immunological markers may be assessed by multiplex bead array.

10.5.2 Cellular assays

10.5.2.1 Flow cytometry

Flow cytometry will be used to examine HIV-specific CD4+ and CD8+ T-cell responses using IL-2 and IFN-γ ICS of PBMC following stimulation with vaccine-match peptide sets or PTE-g synthetic HIV peptides sets that span the proteins encoded by the vaccine construct. Data will be reported as percentages of CD4+ or CD8+ T cells recognizing a specific peptide pool. Response rates will be determined using validated positivity criteria for this assay.

As an exploratory assay, other markers included in the flow cytometry assay above may be analyzed (eg, TNF-α, perforin, granzyme B, and CD57). Some of these markers may be validated by the time these assays are performed.

Additionally, the HVTN Laboratory Program may assay PBMCs using a panel of cell surface markers that can distinguish HIV-specific central and effector memory T-cell subsets. These assays will be performed if positive HIV-specific cellular responses are detected at the primary immunogenicity timepoint. Additional cell surface markers or functional markers may also be analyzed.

Multiparameter flow cytometry for Ad5-specific responses will be conducted using the HVTN method, including currently validated markers (IL-2 and IFN-γ). Ad5 empty vectors will be used for antigen stimulation in these assays.

10.5.2.2 T cell viral suppression assay

T cell suppression assays that measure the ability of CTLs to suppress the replication of HIV-1 may be conducted on PBMC specimens at the primary immunogenicity and other timepoints.

10.6 ARV plasma drug level monitoring

Extracellular levels of ARV drugs may be measured from stored plasma samples. The sampling selection will be based on the prophylactic ARV-use questionnaires completed by the participants, the statistical sampling plan, and the HIV-1 diagnostic data.

10.7 Viral sequencing

Viral sequencing may be conducted on the earliest available plasma and semen specimens with positive HIV-1 RNA PCR tests from study participants who are diagnosed with HIV-1 infection.

10.7.1 Testing for HIV-1 drug resistance mutations

Drug resistance genotyping and phenotyping may be conducted on stored plasma and/or semen specimens of select participants with positive HIV-1 RNA PCR tests at select time points.

10.8 Genotyping

Molecular human leukocyte antigen (HLA) typing may be performed on enrolled participants using cryopreserved PBMC collected at baseline or another timepoint. HLA typing may be performed on specimens from participants who demonstrate vaccine-induced T-cell responses at postvaccination timepoints. In addition, HLA typing may be performed on HIV-infected participants regardless of whether they demonstrate vaccine-induced T-cell responses. Other participants (including control recipients) may be HLA-typed to support future studies of immunological interest at the discretion of the protocol chair and the HVTN Laboratory Program. Other markers, such as genes associated with immune responses or HIV-1 may also be tested.

10.9 Testing for STIs

Post-unblinding, testing for Neisseria gonorrhea (GC) and Chlamydia trachomatis (CT) by nucleic acid amplification testing (NAAT) or culture on urine samples and serum testing for syphilis will be conducted on all HIV-uninfected participants (see Appendix C and Appendix E).

Cryopreserved specimens may be used to test for the presence of sexually transmitted infections (eg, herpes simplex virus type 2 [HSV-2]) at baseline and postvaccination timepoints.

10.10 Semen specimens

Semen specimens collected from a subset of HIV-1–uninfected study participants may be used to examine vaccine-induced antibodies and other immunomodulatory factors such as cytokines. Env-specific IgG and IgA may be assessed by binding antibody multiplex assay (BAMA). In addition, soluble cytokines and other immunological markers may be assessed by multiplex bead array.

10.11 EOS testing

Following study unblinding on April 23, 2013, EOS testing was undertaken on samples from HIV-1–uninfected vaccinees for whom this testing had not yet been performed. For HIV-uninfected vaccinees, this testing is repeated at Month 48 (see Section 8.2.2 and Appendix C).

All participants who have positive or indeterminate HIV-1 serology at the last clinic visit (as measured by standard anti-HIV Ab screening tests) may obtain follow-up HIV-1 diagnostic testing periodically and free of charge as medically/socially indicated (approximately every 6 months). This testing will be available until the HIV diagnostic test(s) no longer yields positive or indeterminate results or until HIV infection is confirmed. It may be noted that vaccine induced positive serology may last many years (see the HVTN Laboratory Manual of Operations).

10.12 Ancillary studies

Cryopreserved samples may be used to perform additional assays to further HIV or vaccine research.

10.13 Other use of stored specimens

The HVTN and VRC aim not only to test vaccine candidates but also to continue to explore the correlates of immunity to HIV. In order to do so, specimens from participants will be stored. These samples may be used for other testing and research to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other testing on specimens will only occur, at a minimum, after review and approval by the HVTN and the IRB of the researcher requesting the specimens.

The protocol sample informed consent form is written so that the participant either explicitly allows or does not allow sample storage for other research when signing the form. Participants who initially agree to other use of their samples may rescind their approval once they enter the study; such participants will still remain in this study. If a participant rescinds approval for other use, the study site investigator or designee must notify HVTN Regulatory Affairs in writing. In either case, after study analyses are complete, the HVTN Laboratory Program will request that the repository destroy all specimens with the participant identification numbers of all participants who do not agree to other use of their samples. HVTN Core will report the destruction of relevant specimens to the participants' site PIs.

Study sites must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on the use of stored specimens.

10.14 Biohazard containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the CDC and the NIH or other locally appropriate agencies.

All dangerous goods materials, including Biological Substances, Category A or Category B, must be transported according to instructions detailed in the International Air Transport Association Dangerous Goods Regulations.

11 Safety monitoring and review

11.1 SAE reporting

Through Month 48 during post-unblinding follow-up for HIV-1–uninfected study participants (see Section 8.2 and Appendix E), SAEs regardless of relatedness to study products (per ICH guideline E2A) will be recorded on the appropriate CRF and reported to the SDMC according to procedures listed in the *Safety monitoring* section of the *HVTN 505 Study Specific Procedures*. SAEs that are deemed related are subject to expedited reporting via DAERS (see Section 11.2). Grade 1 through 4 AEs that are not SAEs should not be reported.

All SAEs are graded according to *The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events* (DAIDS AE Grading Table), Version 1.0, December, 2004 (Clarification dated August 2009) (available at http://rsc.tech-res.com/safetyandpharmacovigilance).

11.2 EAE reporting

Requirements, definitions, and methods for expedited reporting of AEs are outlined in Version 2.0 of "The Manual for Expedited Reporting of Adverse Events to DAIDS" (DAIDS EAE Manual), which is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/.

The internet-based DAIDS Adverse Event Reporting System (DAERS) must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AE reports may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact DAIDS-ES at DAIDS-ESSupport@niaid.nih.gov or from within the DAERS application itself.

Sites where DAERS has not been implemented will submit expedited AE reports by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/. For questions about expedited AE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

11.2.1 EAE reporting periods

Through Month 48 in Schedule 6 (see Section 8.2, Appendix C, and Appendix E), report SAEs deemed related to study product.

Following the Month 48 visit in Schedule 6 and for the Month 60 participant health contact (see Section 8.2.1, Appendix C, and Appendix E) report Serious, Unexpected Suspected Adverse Reactions (SUSARs), as defined in Version 2.0 of the DAIDS EAE manual.

11.2.2 Study products for expedited reporting to DAIDS

The study products that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS are recombinant DNA plasmid (VRC-HIVDNA016-00-VP), rAd5 vector vaccine (VRC-HIVADV014-00-VP), placebo for the DNA vaccine (VRC-PBSPLA043-00-VP), and placebo for the rAd5 vector vaccine (VRC-DILUENT013-DIL-VP).

11.3 Review of safety data

Reviews proceed from a standardized set of protocol-specific safety data reports. These reports are produced by the SDMC and include queries to the HVTN CRSs. Events are tracked by internal reports until resolution. Reports will be reviewed periodically by Clinical safety specialist(s) and by the PSRT.

11.3.1 Protocol team review of cumulative safety data

The Protocol Chair or DAIDS Medical Officer may periodically request cumulative summary reports of safety data, which will be made available on a secure website for PSRT review.

12 Protocol conduct

The protocol will be conducted in compliance with the principles of GCP and according to standard DAIDS and HVTN policies and procedures, including procedures for the following:

- Protocol registration, activation, and implementation;
- Informed consent, screening, and enrollment;
- Clinical and safety assessments;
- Safety monitoring and reporting;
- Data collection and documentation;
- Study follow-up and close-out;
- Unblinding of staff and participants;
- Quality control;
- Protocol monitoring and compliance;
- Advocacy and assistance through local and governmental activities to participants regarding social impacts associated with the vaccine trial;
- Risk reduction counseling; and
- Specimen collection, processing, and analysis.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions will be described in the *HVTN 505 Study Specific Procedures* (eg, instructions for randomization specific to this study).

12.1 Protocol governance

12.1.1 Protocol Team

The Protocol Team will be responsible for administrative oversight of the study, provides the overall operational direction for the trial, and is responsible for the conduct of the trial according to the highest scientific and ethical standards, as well as approving revisions and amendments to the protocol.

12.1.2 PSRT

The PSRT will review all safety data during the course of the study.

The HVTN 505 PSRT is composed of the following members:

- Protocol chair and co-chair*
- Core medical monitor*
- Clinical safety specialist
- DAIDS medical officer*

- Vaccine developer representative

* The clinician members of the PSRT, who are responsible for the review of the clinical safety reports, and decisions regarding cancellation of scheduled safety calls.

The protocol team clinic coordinator, project manager, and others may also be included at the request of the HVTN 505 PSRT.

12.1.3 Oversight Group

The Oversight Group is a high-level committee co-chaired by the Director of DAIDS, HVTN PI, and VRC Director along with the Chair and Co-chairs of the Protocol Team. The Oversight Group provides the overall scientific direction for the trial. The Oversight Group must approve all scientific reports concerning the main findings of the trial.

12.2 Overview of data collection methods

Clinical research data will be collected in a secure electronic data management system by the assigned SDMC. Data will be extracted and provided to the protocol statistician for statistical analysis.

12.2.1 Source documents and data entry at sites

Standard GCP will be followed to ensure accurate, reliable, and consistent data collection. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH, GCP, regulatory, network, and institutional requirements for the protection of confidentiality of participants.

HVTN sites will follow the *DAIDS Standard Operating Procedure on Source Documentation*, Version 2 or any later version in managing source documentation for the trial. Source document information may include but is not limited to:

- Signed informed consent documents;
- Dates of visits, including dates of study injections;
- Documentation of the study eligibility evaluation;
- Reported laboratory results;
- SAE evaluations;
- Participant reported concomitant medications; and

CRFs and laboratory reports will be reviewed by the site clinical team responsible for ensuring that they are accurate and complete. Many HVTN CRFs are designed to be used as source documents. HVTN CRSs complete a source documentation table to indicate which CRFs the site will use as source documents for the trial.

12.2.2 Participant confidentiality

Documentation, data, and all other information generated for a participant will be held in strict confidence. No identifying participant information concerning the study or the data

will be released to any unauthorized third party without prior written approval of the participant except as necessary for monitoring by the IRB/IEC, the FDA (if applicable), the study sponsor, the OHRP, and the pharmaceutical supporter(s) (if applicable). Information about a study participant also may be released when required by law. Participants must be made aware in the informed consent document of the occasions when information may be released without their consent. In addition, if information is released, either by accident or deliberately without a participant's consent, the site must attempt to notify the participant of the release, complete a Protocol Event Form (see Section 12.3.2), and notify their IRB/IEC.

US study sites that are at institutions regarded as covered entities under the Health Information Portability and Accountability Act (HIPAA) are expected to take appropriate action to remain in compliance with the legislation.

US sites are covered by an NIH Certificate of Confidentiality (see Section 2.6).

The study database assembled by the SDMC will identify study participants only by a study identification number and will not contain identifying information such as name, address, national identification number (eg, social security number), medical record number, or personal contact information.

12.2.3 Lab data transfer

Data generated at central and regional laboratories will be transferred directly from the laboratory to the SDMC by secure means and with procedures that ensure the integrity of the data.

12.2.4 Storage of source documents and completed CRFs

All study data must be verifiable to the source documentation. A file containing all the source documents will be maintained for each study participant at the study site. Source documentation will be available for review to ensure that the collected data are consistent with the CRFs.

CRFs, source documents, and other supporting documents will be kept in a secure location.

12.3 Study site monitoring

To ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records, site monitors under contract to NIAID may visit participating CRSs to review the individual subject records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, and individuals' hospital charts). The monitors will inspect sites' regulatory files to ensure that regulatory requirements are being followed and may also inspect sites' pharmacies to review product management and storage.

12.3.1 Access to source documents

Because this study is sponsored by NIAID, each site must permit authorized representatives of NIAID and regulatory agencies to examine (and, when required by

applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study's safety and progress.

Additionally, each site must permit representatives of the HVTN, SDMC, and related contractors to examine clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study's safety and progress.

12.3.2 Protocol events

A protocol event is defined as an individual incident or omission in study conduct that results in significant added risk to the participant, or nonadherence to significant protocol requirements, or nonadherence to the *International Conference on Harmonisation E6: Guideline for Good Clinical Practice*.

The nonadherence may be either on the part of a participant, the investigator, or the study site staff.

It is the responsibility of the site to identify and report protocol events according to the guidelines of the IND sponsor and the local IRB/IEC per their guidelines. The site must also report protocol events to the HVTN using the Protocol Event Form. The site PI and study staff are responsible for knowing and adhering to their IRB requirements.

In response to noted protocol events, site personnel are to implement corrective actions promptly.

12.4 Social impacts

Participants in this study risk experiencing discrimination or other personal problems as a result of being in the study or developing a vaccine-induced positive HIV Ab response. The HVTN CRS is obliged to provide advocacy for and assistance to participants regarding negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding how to assist a participant dealing with a social impact, a designated NIAID representative can be contacted.

Social harms are tabulated by the SDMC and subjected to descriptive analysis with a view toward reducing their incidence and enhancing the ability of study staff to mitigate them when possible.

Summary tables of social impact events will be generated weekly and made available for review by the protocol chairs, Protocol Team leader, and the designated NIAID representative.

12.5 Study participant reimbursement

Reimbursement of study participants for attendance at study visits is at the discretion of each study site. Reimbursement should be comparable to the reimbursement offered for similar research in the local community, if possible. The study site is encouraged to confer with its local CAB in deciding appropriate reimbursement.

The study consent submitted to the site IRB/IEC will state the plan for reimbursement (if any). The HVTN relies upon local IRBs/IECs to determine whether the proposed plan for reimbursement meets ethical requirements in the local context. The exact amounts may be modified during the course of the study in consideration of changes in costs such as bus fares, exchange rates, child care, or other factors that affect the ability of a participant to comply with study visit requirements. Reviewing IRBs/IECs must be made aware of the changes in reimbursement before they occur. Study participants will not be charged for study injections, research clinic visits, research-related examinations, or research-related laboratory tests.

The HVTN does not allow reimbursement that induces a study participant to remain in the study against his or her will. A lump sum reimbursement at trial completion or a reimbursement plan that starts with low reimbursement that increases at later study visits solely to encourage retention is unacceptable.

12.6 Compliance with NIH guidelines for research involving products containing recombinant DNA

Because this study is evaluating products containing recombinant DNA, per NIH *Guidelines for Research Involving Recombinant DNA Molecules* the study must be submitted to site Institutional Biosafety Committees (IBC) and must be approved before participants are enrolled at each respective institution. Investigators at each site are responsible for obtaining IBC approval and periodic review of the research per NIH guidelines *Section IV-B07-b-(6)* and *Section IV-B-2-b.* IBC review and approval must be documented by the investigator and submitted as part of initial protocol registration for this trial.

12.7 Emergency communication with study participants

As in all clinical research, this study may generate a need to reach participants quickly to avoid imminent harm or in order to report study findings that may otherwise concern their health or welfare.

In communicating with the trial participants emergently, the clinical trial site will request that its IRB/IEC expedite review of the message. However, if IRB/IEC review cannot be completed in a timeframe consistent with the urgency of communication with study participants, the clinical trial site will contact the participant and then notify the IRB/IEC as soon as possible during normal business hours.

13 Version history

The Protocol Team may modify the original version of the protocol. Modifications are made to HVTN protocols via clarification memos, letters of amendment, or full protocol amendments.

The table below describes the version history of, and modifications to, Protocol HVTN 505.

Protocol history and modifications

Date: July 21, 2014

Protocol version: Version 6.0
Protocol modification: Full Protocol Amendment 5

Item 1	Boxed text added following title page: Summary of extended follow-up results, Version 6 study redesign
Item 2	Revised in Section 5: Study objectives and endpoints
Item 3	Revised in Section 8 and Appendices C, D, E, and F: Visit schedules for HIV-1–uninfected and for HIV-1–infected study participants
Item 4	HVTN 503 (Phambili) results updated in Section 4.1.3.6
Item 5	Revised in Section 6, *Statistical considerations*: Objectives, endpoints, sample size rationale, analysis plans, and study monitoring
Item 6	Deleted: Section 8.1.2, *VISP registry consent form* and former Appendix C: *HVTN VISP registry consent*
Item 7	Clinic procedures revised in Section 8.2, *Follow-up visits for HIV-uninfected participants*
Item 8	Deleted: (former) Section 8.4, Procedures for participants who became HIV-1–infected during annual health contacts
Item 9	Section 8.4, *HIV risk reduction counseling* updated to reference CDC PrEP clinical practice guidelines
Item 10	Description of HVTN HIV testing algorithm updated in Section 9.2, *HIV-1 testing during post-unblinding follow-up* and in Section 10.11, *EOS testing*
Item 11	May 2014 CDC PrEP clinical practice guidelines added to Section 14, *Document references (other than literature citations)*
Item 12	Job titles and institutional affiliations updated in Section 3.1, *Protocol team*, Section 11.3, *Review of safety data*, and Section 12.1.2, *PSRT*
Item 13	Updated in Section 13, *Version history*: Protocol history and modifications
Item 14	Updated: Appendix A, *Addendum to sample informed consent form*
Item 15	Updated in Appendix B, *Tables of procedures (for Sample informed consent form addendum)*

Item 16 Visit schedule revised in Appendix C: *Schedule 6–Post-unblinding laboratory procedures for HIV-uninfected participants* and Appendix E, *Schedule 6—Post-unblinding clinic procedures for HIV-uninfected participants*

Item 17 Visit schedule revised in Appendix D: *Schedule 7–Post-unblinding laboratory procedures for HIV-infected participants* and Appendix F, *Schedule 6—Post-unblinding clinic procedures for HIV-infected participants*

Item 18 Minor errors in grammar, typography, and cross-references have been corrected throughout the protocol document

Date: March 11, 2014

Protocol version: Version 5.0
Protocol modification: Clarification Memo 1

Item 1 Clarified: Safety monitoring functions transition to HVTN Core Clinical Safety Specialists

Item 2 Clarified: Current HVTN laboratory algorithms for HIV diagnostic testing and evaluation of vaccine-induced seroreactivity

Date: July 12, 2013

Protocol version: Version 5.0
Protocol modification: Full Protocol Amendment 4

Item 1 Boxed text added following title page: Vaccinations stopped, treatment assignments unblinded, and study redesigned

Item 2 Revised in Section 5: Study hypothesis, objectives, and endpoints

Item 3 Revised in Section 8 and Appendices D and G: Visit schedule for HIV-1–uninfected participants extended to 48 months of clinic visits with Month 60 participant health contact

Item 4 Revised in Section 8 and Appendices E, F, H, and I: Visit schedules for HIV-1–infected study participants

Item 5 Revised in Section 3, Overview: Primary objective, participants, design, duration per participant, estimated total study duration, safety monitoring

Item 6 Updated in Section 4, Background and rationale: HVTN 503 (Phambili) and VOICE study results, PrEP uptake, and HIV-1 infections in HVTN 204

Item 7 Revised in Section 6, Statistical considerations: Objectives, endpoints, sample size rationale, analysis plans, and study monitoring

Item 8 Updated and modified in Section 7, Selection and withdrawal of participants: Introductory text, co-enrollment, participant departure from vaccination schedule or withdrawal

Item 9 Deleted: (Former) Section 8, Study product preparation and administration

Item 10 Deleted in Section 8, Clinical procedures: Screening consent form and assessment of understanding

Item 11 Deleted in Section 8, Clinical procedures: Subsections concerning pre-enrollment procedures and procedures at enrollment and vaccination visits, procedures for participants discovered to be HIV-infected at enrollment, and reactogenicity assessment

Item 12	Revised in Section 8.2 and Appendices D and G: Clinic and laboratory procedures for HIV-1–uninfected study participants
Item 13	Revised in Sections 8.3 and 8.4 and Appendices E, F, H, and I: Procedures for HIV-1–infected study participants
Item 14	Revised in Section 8.2.2: Unblinding and evaluation of vaccine-induced seroreactivity (EOS) testing
Item 15	Revised in Section 8.5, Risk reduction counseling: Counseling regarding outside testing limited to vaccine recipients
Item 16	Removed in Section 8.7, Visit windows and missed visits: Reference to missed vaccination visits
Item 17	Revised in Section 9: HIV-1 infection and clinical response
Item 18	Revised in Section 10, Laboratory: Specimens, blood volumes, and assays
Item 19	Revised in Section 11, Safety monitoring and review: Safety reporting and safety monitoring
Item 20	Revised in Section 12, Protocol conduct: DSMB oversight and Protocol Team blinding removed, Oversight Committee membership updated,
Item 21	Updated in Section 13, Version history: Protocol history and modifications
Item 22	Added in Section 14, Document references (other than literature citations): ICH E2(A)
Item 23	Literature references updated in Section 16
Item 24	Deleted: (Former) Appendix A, Sample informed consent form
Item 25	Deleted: (Former) Appendix B, Tables of procedures (for sample informed consent form)
Item 26	Added as (new) Appendix A: Addendum to sample informed consent form
Item 27	Added as Appendix B: Tables of procedures (for Sample informed consent form addendum)
Item 28	Renumbered as Appendix C: HVTN VISP registry consent
Item 29	Deleted: (former) Appendix E, Rationale for the primary VL endpoint definition
Item 30	Deleted: (former) Appendix J, Annual health contacts for HIV-uninfected participants
Item 31	Deleted: (former) Appendix M, Sample consent form for rectal fluid and semen collection
Item 32	Updated in Section 3.1: Protocol Team members and contact information
Item 33	Cross-references updated and minor errors corrected

Date: March 5, 2013

Protocol version: Version 4.0
Protocol modification: Clarification Memo 1

Item 1 Clarified in Section 9.4.2, Annual health contacts for HIV-uninfected participants and Section 12.4.4, DSMB review of cumulative safety data: Safety reviews during annual health contact period

Item 2 Formatting error corrected in Section 12.3, EAE reporting

Date: September 12, 2012

Protocol version: Version 4.0
Protocol modification: Full Protocol Amendment 3

Item 1 Sample size increased to 2500

Item 2 Information on use of Truvada® for HIV prevention updated

Item 3 Endpoints and analysis plans corrected in Section 5.3, 6.3.3.4, and 6.6.4.7

Item 4 Extended follow-up for HIV-infected study participants clarified via Letter of Amendment 1 to Version 3.0

Item 5 Optional rectal secretion and semen sampling for HIV-uninfected study participants added via Letter of Amendment 1 to Version 3.0

Item 6 Syphilis testing methods clarified via Clarification Memo 1 to Version 3.0

Date: April 27, 2012

Protocol version: Version 3.0
Protocol modification: Clarification Memo 2

Item 1 Clarified in Sections 9.4.3 and 9.4.4 and in footnotes to Appendices F and H: Mucosal sampling timepoints

Date: April 2, 2012

Protocol version: Version 3.0
Protocol modification: Clarification Memo 1

Item 1 Clarified in Sections 9.4.3, Rectal secretion sampling and 9.4.4, Semen sampling: Sampling timepoints

Item 2 Clarified in Section 11.10, STD testing and footnote to Appendix F, Schedule 1—Laboratory procedures for HIV-uninfected participants: Syphilis testing

Date: January 23, 2012

Protocol version: Version 3.0
Protocol modification: Full Protocol Amendment 2

Item 1 Title revised

Item 2 Enrollment expanded to 2200

Item 3 HIV acquisition elevated to primary endpoint

Item 4 Duration per participant and total study duration revised

Item 5 Eligibility age limit raised from 45 to 50 years

Item 6	Protocol leadership and other contributors to the original protocol revised in Section 3.1, Protocol team
Item 7	HIV epidemiology updated in Section 4.1
Item 8	Description of VRC candidate vaccines revised in Section 4.1.1
Item 9	Information on RV 144 and rationale for sample size increase added as new Section 4.1.2
Item 10	Summary update of Step Study results added as Section 4.1.3.5
Item 11	Added in Sections 4, 5, 6, 7, 9, 11, and Appendices: iPrEx study results and monitoring of ARV use for HIV-1 prophylaxis
Item 12	NHP SIV challenge data added to Section 4.4.1
Item 13	Human experience with VRC vaccine regimen updated in Section 4.6
Item 14	Viral load objectives/endpoints modified in Sections 5.2 and 5.3
Item 15	Exploratory objectives added in Section 5.4
Item 16	Statistical considerations revised in Section 6
Item 17	Exclusion criteria clarified in Section 7.2
Item 18	Co-enrollment of participants in other clinical trials addressed in new Section 7.3
Item 19	Sections 7.4.2, 9.4.2, 12.2.1, 12.3, and Appendix I revised for consistency with version 2.0 of DAIDS EAE manual
Item 20	VISP Registry consent form added in Section 9.1.3 and new Appendix D
Item 21	Unblinding, VISP testing, and provision of VISP test results revised in Sections 9.4.1 and 11.12
Item 22	Annual health contact language updated in Section 9.4.2 and Appendix J
Item 23	STI testing referrals added to risk reduction counseling in Section 9.6
Item 24	References to DAIDS AE grading table updated in Sections 9.7, 12.1, and 15
Item 25	IBC review requirements clarified in Section 13.6
Item 26	Version history table updated in Section 14
Item 27	Reference to DAIDS EAE Manual updated in Section 15
Item 28	Acronyms and abbreviations updated in Section 16
Item 29	Appendix A, Sample informed consent form, revised
Item 30	Visit schedule revised in Appendix B
Item 31	Consent for other uses of study samples revised in Appendix C
Item 32	Laboratory and clinic procedures schedules revised in Appendices F and H
Item 33	Blood samples for viral isolation/sequencing and semen sampling clarified in footnotes to Appendix G
Item 34	Annual health contacts table revised in Appendix J
Item 35	Reference numbers and cross-references to protocol sections and appendices have been updated throughout the document

Item 36 Typographical errors, duplicate references, and deviations from HVTN protocol style conventions have been corrected throughout the document

Date: September 29, 2010

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 4 (new version-specific numbering system)

Item 1 Revised in Sections 3, Overview and 7.1, Inclusion criteria: Upper age limit raised from 45 to 50 years

Date: June 7, 2010

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 3 (new version-specific numbering system)

Item 1 Revised for consistency with version 2.0 of DAIDS EAE manual: Sections 7.3.2, 9.4.2, 12.2.1, 12.3, and Appendix I

Item 2 Updated in Sections 9.7, 12.1, and 15: References to DAIDS AE grading table

Date: March 12, 2010

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 4

Item 1 Replaced in title and text: "Transgender women" by "male-to-female (MTF) transgender persons"

Item 2 Added as new section 4.1.2.5: Summary update of Step Study results

Item 3 Revised and reorganized in Appendix A, Sample informed consent form: Sections 5 – 8, including update to "Step Study" language

Item 4 Revised in Section 7.2, Exclusion criteria: Autoimmune disease exclusion criterion

Date: January 28, 2010

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 3

Item 1 Revised in Appendix A, Sample informed consent form: Section 5 title

Date: December 29, 2009

Protocol version: Version 2.0
Protocol modification: Full Protocol Amendment 1

Item 1 Title, objectives, and participants clarified: MSM and MTF transgender persons included

Item 2 HVTN 505 assigned BB-IND 13971

Item 3 Revised throughout protocol: "Post-week 28 infection" replaces WITT

Item 4 Updated in section 3.1: Protocol Team

Item 5 Updated in section 4.1.1: Reference to the Thai trial

Item 6 Clarified throughout: Study transition points and estimated study duration

Item 7	Clarified in section 6: Primary analysis population and MITT infections, alternative VL setpoint, and trial monitoring
Item 8	Revised in section 7.1: Behavioral risk criteria, circumcision status, and transgender eligibility
Item 9	Revised in section 7, Selection and withdrawal of participants: Influenza vaccine exclusion/delay windows, therapeutic anticoagulation exclusion, and co-enrollment restrictions
Item 10	Deleted in sections 8.3.3, 8.3.4, and 8.4.2: 5 mL syringe option
Item 11	Added to sections 9, 10, Appendix A and Appendix B: Provision for participants discovered to have been HIV-infected at enrollment
Item 12	Clarified in section 9: Clinical procedures timing
Item 13	Clarified in section 11: Samples assayed for secondary and exploratory endpoint data, applicability of VISP testing, and other uses of stored specimens
Item 14	Clarified in section 12: AE/EAE reporting and immediate notification
Item 15	Clarified in section 13: DSMB monitoring for operational futility
Item 16	Corrected in section 15: Document listings
Item 17	Clarified and corrected in Appendix A: Follow-up for HIV-infected participants, participants confirmed to be HIV-infected at enrollment circumcision, semen samples, blood volumes, and minor errors
Item 18	Clarified in Appendix B: Outside testing counseling and follow-up for HIV-infected participants
Item 19	Revised in Appendix C: Genetic testing added and “leftover” deleted
Item 20	Corrected in Appendix E: Sampling volumes, timepoints, lab listings
Item 21	Revised in Appendix F: Ship to and assay location added for semen samples, new lab added, and footnote revised
Item 22	Revised in Appendix G: Final Schedule 1 visit designation, outside testing counseling, and behavior risk assessment timing
Item 23	Revised in Appendix I: Timepoints, contact numbers, and footnote
Item 24	Corrected: Minor errors

Date: October 16, 2009

Protocol version: Version 1.0
Protocol modification: Letter of Amendment 2

Item 1	Added: Provision for participants discovered to have been HIV-infected at enrollment
Item 2	Revised in sections 7.2, Exclusion criteria and 7.3.1, Delaying vaccinations for a participant: Influenza vaccine exclusion/delay windows

Date: April 22, 2009

Protocol version: Version 1.0
Protocol modification: Letter of Amendment 1

Item 1	HVTN 505 assigned BB-IND 13971
Item 2	Clarified in section 7.1, Inclusion criteria and Appendix A, Sample informed consent form: Circumcision status
Item 3	Corrected in section 9, Clinical procedures and Appendix G, Schedule 1—Clinic procedures for HIV-uninfected participants: Timing of initial administration of behavioral risk assessment questionnaire
Item 4	Replaced in sections 9.3.3, 9.4, Appendix B, and Appendix G: Outside testing questionnaire by outside testing counseling
Item 5	Clarified in section 12.2, AE reporting: AE reporting periods for participants who miss the third or fourth vaccination
Item 6	Revised in Appendix E, Schedule 1—Laboratory procedures for HIV-uninfected participants: Blood draws added for viral sequencing and ELISpot/ICS volume increased at visit 5

Date: February 25, 2009

Protocol version: Version 1.0
Protocol modification: Original protocol

14 Document references (other than literature citations)

Other documents referred to in this protocol, and containing information relevant to the conduct of this study, include:

- *Assessment of Understanding*. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines. Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf.
- *Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents*. Available at http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/
- *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events*, Version 1.0, December 2004 (Clarification dated August 2009). Available at http://rsc.tech-res.com/safetyandpharmacovigilance.
- *The Manual for Expedited Reporting of Adverse Events to DAIDS*. Version 2.0, January 2010. Available at http://rsc.tech-res.com/safetyandpharmacovigilance.
- *HVTN 505 HIV-1 Diagnostic Testing*. Available through the HVTN protocol-specific website.
- *HVTN 505 Special Instructions*. Accessible through the HVTN protocol-specific website.
- *HVTN 505 Study Specific Procedures.* Accessible through the HVTN protocol-specific website.
- *HVTN Laboratory Manual of Operations*. Accessible through the HVTN website.
- *HVTN Manual of Operations*. Accessible through the HVTN website.
- International Conference on Harmonisation (ICH) E2 (A), *Clinical Safety Data Management,* section 3B, *Serious Adverse Event or Adverse Drug Reaction*. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E2A/Step4/E2A_Guideline.pdf.
- International Conference on Harmonisation (ICH) E6 (R1), *Guideline for Good Clinical Practice*: section 4.8, *Informed consent of trial subjects*. Available at http://www.emea.europa.eu/pdfs/human/ich/013595en.pdf.
- *NIH Guidelines for Research Involving Recombinant DNA Molecules*. Available at http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html.
- *NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research*. Available at http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html.
- *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*, July 2008.
- *Protocol Registration Policy and Procedure Manual*. Accessible through the HVTN website.

- *Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials.* Available at http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/ClinicalSite.htm
- *Title 21, Code of Federal Regulations, Part 50*. Available at http://www.access.gpo.gov/nara/cfr/waisidx_08/21cfrv1_08.html.
- *Title 45, Code of Federal Regulations, Part 46*. Available at http://www.access.gpo.gov/nara/cfr/waisidx_07/45cfrv1_07.html.
- *Preexposure Prophylaxis for the Prevention of HIV Infection in the United States – 2014 Clinical Practice Guideline*. Available at http://www.cdc.gov/hiv/prevention/research/prep/.

See section 16 for literature cited in the background and statistics sections of this protocol.

15 Acronyms and abbreviations

Ab	antibody
Ad5	adenovirus serotype 5
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANOVA	analysis of variance
AOU	assessment of understanding
ART	(post-HIV diagnosis) antiretroviral therapy
ARV	antiretroviral (drug)
BOI	burden-of-illness
CAB	Community Advisory Board
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	confidence intervals
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
CTL	cytotoxic T lymphocyte
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
DSMB	NIAID Data and Safety Monitoring Board
EAE	expedited adverse event
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
EOS	evaluation of vaccine-induced seroreactivity
FDA	US Food and Drug Administration
FET	final evaluation time
FFB	final formulation buffer
FHCRC	Fred Hutchinson Cancer Research Center
FTC	emtricitabine
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HLA	human leukocyte antigen
HR	hazard ratio
HSV-2	Herpes simplex virus type 2
HVTN	HIV Vaccine Trials Network
IAVI	International AIDS Vaccine Initiative
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee

ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IEC	Independent Ethics Committee
IFN-γ	interferon gamma
IL-2	interleukin 2
IND	Investigational New Drug
IRB	Institutional Review Board
IV	intravenous
LTNP	long-term nonprogressors
MAR	missing at random
MIP1-β	macrophage inflammatory protein 1 beta
MOP	manual of operations
MITT	modified intent-to-treat
MSM	men who have sex with men
MTF	male-to-female
nAb	neutralizing antibody
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NIH	US National Institutes of Health
NVITAL	NIAID Vaccine Immune T-Cell and Antibody Laboratory
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PD	post [HIV-infection] diagnosis
PEP	post-exposure prophylaxis
PET	Primary Evaluation Time
PI	Principal Investigator
PrEP	pre-exposure prophylaxis
PSRT	HVTN Protocol Safety Review Team
PTE-g	global potential T-cell epitope
PT/INR	prothrombin time/international normalized ratio
PTT	partial thromboplastin time
rAd5	recombinant adenovirus serotype 5
RCC	DAIDS Regulatory Compliance Center
RR	relative risk
SAE	serious adverse event
SAP	Statistical Analysis Plan
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
sd	standard deviation
SDMC	statistical and data management center
SFC	spot-forming cell
SIV	simian immunodeficiency virus

SUSAR	sudden unexpected serious adverse reaction
TDF	tenofovir disoproxil fumarate
TNF-α	tumor necrosis factor alpha
UIAS	unprotected insertive anal sex
UNAIDS	Joint United Nations Programme on HIV/AIDS
URAS	unprotected receptive anal sex
USMHRP	US Military HIV Research Program
UW-VSL	University of Washington Virology Specialty Laboratory
VISP	vaccine-induced seropositivity
VL	viral load
VPP	Vaccine Pilot Plant
VRC	Vaccine Research Center (NIAID)

* CRSs were formerly referred to as HIV Vaccine Trial Units (HVTUs). Conversion to use of the term CRS is in process, and some HVTN documents may still refer to HVTUs.

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Appendix A Addendum to sample informed consent form

Protocol HVTN 505, Version 5.01: Phase 2b, randomized, placebo-controlled test-of-concept trial to evaluate the safety and efficacy of a multiclade HIV-1 DNA plasmid vaccine followed by a multiclade HIV-1 recombinant adenoviral vector vaccine in HIV-uninfected, adenovirus type 5 neutralizing antibody negative, circumcised men and male-to-female (MTF) transgender persons, who have sex with men

Short title of study: HVTN 505

Please review this form carefully. The study staff will talk with you about the information in it. You may also ask to review the information in the original study consent form. You are free to ask questions at any time.

To show that you have received and understand this information, we will ask you to sign this form. You will get a copy to keep.

Review of study information

You are a participant in a study called HVTN 505. This is a study of an experimental HIV vaccine regimen that used a DNA vaccine and an adenoviral vector vaccine.

This study was designed to look at two main questions:

- Does this study vaccine regimen prevent HIV infection?
- Does this study vaccine regimen reduce the amount of HIV in a person's body (the HIV viral load) in those who got the vaccine and later developed HIV infection?

The study opened on June 5, 2009. By March 31, 2013 the study was fully enrolled with 2504 participants. 1253 participants received the study vaccines and 1251 received the placebo.

In April 2013, injections in HVTN 505 were stopped after an interim analysis showed that the study vaccines did not prevent HIV infection. This analysis also showed that the study vaccines did not reduce the amount of virus (viral load) in people who later became infected with HIV.

At that time, we asked HVTN 505 participants to stay in the study so we could monitor their health and follow participants who later became infected with HIV. We also wanted to make sure that people who got the study vaccines were not more likely to get infected with HIV.

New information

Since then we have collected more information. This information confirmed the earlier analysis that the study vaccines did not prevent HIV infection or reduce viral load.

This information also showed that people who got the study vaccines did not have more HIV infections than people who got the placebo.

Thanks to our study volunteers, these important questions about the HVTN 505 study vaccines have been answered.

Ongoing laboratory studies of samples collected in HVTN 505 continue to provide information about immune system responses to the vaccines, about factors affecting whether HIV vaccines work or not, and about other factors that make a person more or less likely to get infected with HIV.

Changes to HVTN 505

Even though many questions have been answered, there is still more to learn from HVTN 505. Additional information gathered in this study may help us improve the design of future studies. For this reason, we are asking HVTN 505 study participants to continue in the study.

We want to continue to check on your health and to track any new HIV infections. It is also important to learn whether people will stay enrolled in a study, especially after they have learned whether they received the study vaccines or placebo.

This new information may change how you feel about staying in the study. If you decide to stay in the study, we will ask you to continue visits to the study clinic.

For participants who are HIV-negative, we will ask you to come to the clinic for annual visits. These clinic visits will be scheduled 3 years and 4 years after your first vaccination.

At these annual clinic visits, we will:

- Do regular HIV testing, as well as counseling on your results;
- Perform brief physical exams;
- Collect blood samples;
- Collect urine samples;
- Ask questions about your health;
- Counsel you on avoiding HIV infection;
- Ask about any personal problems or benefits you may have from being in this study;
- Ask questions about any antiretroviral (ARV) drugs you may be taking; and
- Give you a computer questionnaire about ARV drugs, behaviors that could put you at risk for HIV infection, and some aspects of your life situation.

We will use some blood and urine samples to test you for chlamydia, gonorrhea, and syphilis. If you have one of these infections, we will refer you for care and treatment.

After your clinic visits have ended, we will contact you once more to check on your health. This contact will be about 5 years after your first vaccination. This may just be a phone call. If you have had a positive HIV test since your last clinic visit, we will ask you to come to the clinic for testing to confirm your HIV infection.

If you become HIV-infected before completing your scheduled clinic visits, we will ask you to come to the clinic 3 more times over a 6-month period. At these visits, we will:

- Ask questions about your health;
- Perform brief physical exams;
- Ask about any antiretroviral treatments (ART) you may be taking;
- Collect blood samples;
- Counsel you on how to avoid giving HIV to other people; and
- Ask about any personal problems or benefits you may have from being in this study.

We will tell you where to get support, medical care, and HIV treatment if you do not already have these in place.

After your time in this study ends, we may invite you to join another study to follow your health and to see how your body controls your HIV infection. For this separate study there will be a new consent form that we will review with you.

PrEP in HVTN 505

In July 2012, the US FDA approved the use of the antiretroviral medicine Truvada for prevention of HIV infection. This is called pre-exposure prophylaxis (PrEP).

In May 2014, the US Centers for Disease Control and Prevention (CDC) published guidelines for doctors to follow when prescribing PrEP. These guidelines recommend that PrEP be considered as a method of HIV prevention for people who are HIV-negative and at increased risk for HIV infection.

The HVTN, the Division of AIDS, and Gilead Sciences, Inc. (the maker of Truvada) have reached an agreement to provide Truvada as PrEP to study participants in HVTN 505. If you are interested, clinic staff can give you more information about this program and can refer you to providers who can prescribe PrEP.

What if I choose to leave this study?

You can leave this study at any time. If you leave this study, you will not lose any benefits or rights you would normally have.

If you decide to leave this study, please tell the clinic staff. We will ask you to come back to the clinic at least one last time, to check your health and your immune response.

Who should I call if I have questions or problems?

If you have questions about this study, contact
[name and telephone number of the investigator or other study staff].

If you have any symptoms that you think may be related to this study, contact
[name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, contact
[name/title/phone of person on IRB or other appropriate organization].

If you want to leave this study, contact
[name and telephone number of the investigator or other study staff].

Other information

The rest of the information in the consent forms that you signed earlier, about the study purpose, the risks and benefits of participation, your rights and responsibilities, and how your privacy is protected, continues to be important information for you and has not changed. Additional copies of the consent form are available from the clinic staff and we encourage you to read it again.

In addition, other consent forms you have signed previously remain in place. These other forms are for other uses of your study samples and your inclusion in the VISP registry. We will provide copies of these consent forms if you would like them. *[Site: Modify this paragraph as appropriate to reflect local practice and IRB requirements.]*

I agree to continue in this study. Initial_____

I do not want to continue in this study. Initial_____

If you have read this addendum to the consent form (or had it explained to you) and understand it, please sign your name below.

Participant's name (print)	Participant's signature or mark	Date	Time
Study staff conducting consent discussion (print)	Study staff signature	Date	Time

For participants who are unable to read or write, also complete the signature block below:

Witness's name (print)[#]	Witness's signature	Date	Time

[#] Witness is impartial and was present for the consent process.

Appendix B Revised tables of procedures (for Sample informed consent form addendum)

HIV-negative participants

Procedure	Months after first study injection									
	6	9	12	15	18	21	24	36	48	60
Brief physical exam	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	
HIV testing/counseling	√	√	√	√	√	√	√	√	√	
Questions/questionnaire	√	√	√	√	√	√	√	√	√	√*
Risk reduction counseling	√	√	√	√	√	√	√	√	√	
Testing for syphilis, gonorrhea, and chlamydia	√		√		√		√	√	√	

Shaded area indicates visits previously completed by all study participants.
* Clinic visit not required.

Participants who become HIV-infected

Procedure	0*	Weeks after first HVTN test showing HIV infection		
		2	4	24
HIV testing/counseling	√	√	√	
Brief physical (as needed)	√	√	√	√
Risk reduction counseling	√	√	√	√
Questions/questionnaire	√	√	√	√
Blood drawn	√	√	√	√

* If there has been a positive HIV test outside the HVTN, participant comes to site for HIV confirmatory testing.

Appendix C Schedule 6—Post-unblinding laboratory procedures for HIV-uninfected participants

			Visit:	**101**	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**	**110**	**111**	**112**	**113**	**114**	**115**	
			Day:	D168	D273	D364	D455	D546	D637	D728	D819	D909	D1000	D1091	D1182	D1273	D1454	D1818	
			Month	6	9	12	15	18	21	24	27	30	33	36	39	42	48	60	
Procedure	Ship to[1,2]	Assay location[2]	Tube[3]																**Total**
Blood Collection																			
HIV diagnostic test	UW/VSL	UW/VSL	EDTA	10	10	10	10	10	10	10	—	—	—	10	—	—	20	—	**100**
Syphilis	Local Lab	Local lab	SST	—	—	10	—	—	—	10	—	—	—	10	—	—	10	—	**40**
Plasma[4]	CSR	UNC	EDTA	5	5	5	5	5	5	5	—	—	—	5	—	—	5	—	**45**
Specimen storage																			
Serum	CSR		SST	—	—	17	—	—	—	17	—	—	—	—	—	—	—	—	**34**
Visit Total				**15**	**15**	**42**	**15**	**15**	**15**	**42**	**0**	**0**	**0**	**25**	**0**	**0**	**35**	**0**	**219**
56-Day total				**15**	**15**	**42**	**15**	**15**	**15**	**42**	**0**	**0**	**0**	**25**	**0**	**0**	**35**	**0**	
URINE COLLECTION																			
STI Testing																			
GC/Chlamydia by NAAT or culture	Local Lab	Local Lab		X	—	X	—	X	—	X	—	—	—	X	—	—	X		

Shaded visits not required.

[1] CSR = central specimen repository

[2] HVTN Laboratory Program includes endpoint laboratories at UW-VSL. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA). Non-HVTN Laboratories include UNC. UNC = University of North Carolina - Chapel Hill (Chapel Hill, North Carolina, USA).

[3] Local labs may assign appropriate alternative tube types for locally performed tests.

[4] Draw plasma at specified visit only if participant reports having used ARVs for PrEP or PEP since the last clinic visit.

Appendix D Schedule 7—Post-unblinding laboratory procedures for HIV-infected participants

Procedure	Ship to[2,3]	Assay location[3]	Visit / Weeks after Diagnosis / Tube[4]	#.X[1] W2	201 W4	202 W8	203 W12	204 W24	Total
Blood Collection									
Screening or diagnostic assays									
HIV diagnostic EIA/WB/PCR	UW/VSL	UW/VSL	EDTA	15	—	—	—	—	**15**
HIV PCR viral load	UW/VSL	UW/VSL	EDTA	—	—	—	—	—	**0**
CD4+T cell count	Local Lab	Local Lab	EDTA	5	—	—	—	—	**5**
Storage									
Serum	CSR		SST	—	8.5	—	—	8.5	**17**
Total				**20**	**8.5**	**—**	**—**	**8.5**	**37**
56-Day total				**20**	**29**	**—**	**—**	**8.5**	

Shaded visits not required.

[1] Visit #.X = interim visit for the purpose of drawing samples for confirmatory HIV testing

[2] CSR = central specimen repository

[3] HVTN Laboratory Program includes endpoint laboratories at UW-VSL. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA)

[4] Local labs may assign appropriate alternative tube types for locally performed tests.

Appendix E Schedule 6—Post-unblinding clinic procedures for HIV-uninfected participants

Visit:	**101**	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**	**110**	**111**	**112**	**113**	**114**	**115**[a]
Day:	D168	D273	D364	D455	D546	D637	D728	D819	D909	D1000	D1091	D1182	D1273	D1454	D1818
Month:	6	9	12	15	18	21	24	27	30	33	36	39	42	48	60
Procedure															
Study procedures[b]															
Abbreviated physical exam	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
Risk reduction counseling	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
Behavioral risk, prophylactic ARV use, demographics questionnaire	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
Social impact assessment	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
Social impact assessment questionnaire	—	—	X	—	X	—	X	—	—	—	X	—	—	X	—
PEP/PrEP assessment[c]	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
SAE assessment[d]	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
Vital status & safety surveillance	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X[e] [f]
HIV infection assessment[g]	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
Confirm HIV test results provided to participant	X	X	X	X	X	X	X	—	—	—	X	—	—	X	—
Local lab assessment								—	—	—		—	—		—
GC/CT test by NAAT or culture[h]	X	—	X	—	X	—	X	—	—	—	X	—	—	X	—
Syphilis test	—	—	X	—	—	—	X	—	—	—	X	—	—	X	—

Shaded visits not required.

[a] Clinic visit not required (see Section 8.2.1).

[b] For specimen collection requirements, see Appendix C.

[c] If and only if participant reports having used ARVs for PrEP or PEP since the last clinic visit, draw blood sample for plasma as indicated in Appendix C.

[d] Report SAEs only. Do not report Grade 1 through 4 AEs unless they are also SAEs (see Section 11.1.

[e] Any participant reporting that they have become HIV-infected will be asked to come to the clinic so that HIV status can be confirmed.

[f] Report SUSARs only.

[g] Includes pre-test information and assessment of signs and symptoms of acute HIV infection. A subsequent follow-up contact is conducted to provide post-test counseling and to provide test results to the participant.

[h] Urine test (see Appendix C).

Appendix F Schedule 7—Post-unblinding clinic procedures for HIV-infected participants[a]

Visit Number:	#.X[b]	201	202	203	204
Weeks after diagnosis:	2	4	8	12	24
Study procedures[c]					
Counseling on HIV-1 testing/diagnosis	X	X			—
Abbreviated physical exam	X	X			X
ART assessment	X	X			X
HIV-associated events	X	X			X
Transmission risk reduction counseling	X	X			X
Social impact assessment	X	X			X
Social impact assessment questionnaire	—	—			X

Shaded visits not required.

[a] At completion of Schedule 7, HIV-infected participants may be invited to enroll in a separate follow-up protocol.

[b] Visit #.X = interim visit for the purpose of drawing samples for confirmatory HIV testing

[c] For specimen collection requirements, see Appendix D.