HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 096/EV04

A phase 1 double blind placebo-controlled clinical trial to evaluate the safety and to compare the priming ability of NYVAC alone versus NYVAC + AIDSVAX® B/E, and DNA alone versus DNA + AIDSVAX® B/E when followed by NYVAC + AIDSVAX® B/E boosts in healthy, HIV-1-uninfected adult participants

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CLINICAL TRIAL SPONSORED BY

EuroVacc Foundation
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STUDY PRODUCTS PROVIDED BY

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The signatures of Dr. Giuseppe Pantaleo and Dr. Pierre-Alexandre Bart as the Principal Investigators constitute their approval of this protocol and provide assurance that this study will be conducted in the clinical centre according to the stipulations contained within this document, including the statements of confidentiality, provision of indemnity for clinical staff employed by their institutions, and good clinical practice. Mr. Jean-Philippe Rochat's signature on behalf of EuroVacc Foundation provides the assurance of the Sponsors' approval of the protocol including provision of a policy to provide coverage for clinical trial liability other than clinical negligence and negligence during the conduct of the clinical trial.

Signed_____ Date:_____
Dr. Giuseppe Pantaleo

Signed_____ Date:_____
Dr. Pierre-Alexandre Bart

Signed_____ Date:_____
Mr. Jean-Philippe Rochat

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. Community Advisory Boards (CABs) are required by the Division of AIDS (DAIDS) and supported at all HVTN research sites to ensure community input.

- HVTN clinical trial staff counsel study participants routinely 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. Staff members will also counsel them about reducing their risk of transmitting HIV to others.

- The HVTN requires that all international HVTN sites lacking national plans for providing antiretroviral therapy (ART) develop plans for the care and treatment of participants who acquire HIV infection during a trial. Each plan is developed in consultation with representatives of host countries, communities from which potential trial participants will be drawn, sponsors, and the HVTN. Participants will be referred to programs for ART provision when the appropriate criteria for starting ART are met. If a program is not available at a site and ART is needed, a privately established fund will be used to pay for access to treatment to the fullest extent possible.

- 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. During the study, participants will have their wellbeing monitored, and to the fullest extent possible, their privacy protected. Participants may withdraw from the study at any time.

- 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 and 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 Ethics Committees (ECs) as custodians responsible for ensuring the ethical conduct of research in each setting.

2 IRB/EC review considerations

US Food and Drug Administration (FDA) and other US federal regulations require IRBs/ECs 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 this protocol addresses each of these research requirements. Each HVTN Investigator welcomes IRB/EC questions or concerns regarding these research requirements.

2.1 Minimized 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) performing direct observation of participants postvaccination and collecting information regarding side effects for several days postvaccination; (d) having staff properly trained in administering study procedures that may cause physical harm or psychological distress, such as blood draws, vaccinations, HIV testing and counseling and HIV risk reduction counseling; (e) providing HIV risk reduction counseling and checking on contraception use (for women); and (f) providing safety monitoring.

2.2 Reasonable risk/benefit balance

45 CFR 46.111 (a) 2 and 21 CFR 56 (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 all 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.

2.3 Equitable 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 Appropriate informed consent

45 CFR 46.111 (a) 4 and 5 and 21 CFR 56.111 (a) 4 and 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 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 Adequate 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 extensive safety monitoring in place (see Section 11). Safety is monitored daily by clinical affairs staff and routinely by the HVTN 096/EV04 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) or a Data and Safety Monitoring Board (DSMB) periodically reviews study data.

2.6 Protect 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). The privacy of participants is protected by assigning unique identifiers in place of the participant's name on study data and specimens. 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

A phase 1 double blind placebo-controlled clinical trial to evaluate the safety and to compare the priming ability of NYVAC alone versus NYVAC + AIDSVAX® B/E, and DNA alone versus DNA + AIDSVAX® B/E when followed by NYVAC + AIDSVAX® B/E boosts in healthy, HIV-1-uninfected adult participants

Primary objective(s)

- To evaluate the safety and tolerability of i) NYVAC prime plus NYVAC + AIDSVAX® B/E boosts; ii) NYVAC + AIDSVAX® B/E prime plus NYVAC + AIDSVAX® B/E boosts; iii) DNA prime plus NYVAC + AIDSVAX® B/E boosts; iv) DNA + AIDSVAX® B/E prime plus NYVAC + AIDSVAX® B/E boosts in HIV-1-uninfected healthy adults

- To evaluate and compare the immunogenicity of different priming regimens when followed by two doses of NYVAC/AIDSVAX® B/E, comparing between:
  - NYVAC and NYVAC/AIDSVAX® B/E primes (Group 1 versus 2)
  - DNA and DNA/AIDSVAX® B/E primes (Group 3 versus 4)

- To evaluate and compare the durability of the vaccine-induced antibody (Ab) response for different priming regimens when followed by two doses of NYVAC/AIDSVAX® B/E, comparing between:
  - NYVAC and NYVAC/AIDSVAX® B/E primes (Group 1 versus 2)
  - DNA and DNA/AIDSVAX® B/E primes (Group 3 versus 4)

Study products and routes of administration

- **DNA**: DNA-HIV-PT123: 4 mg of DNA encoding clade C ZM96 Gag and gp140, CN54 Pol-Nef, administered intramuscularly (IM)

- **NYVAC:** NYVAC-HIV-PT1 and NYVAC-HIV-PT4: $\geq 5x10^6$ PFU each encoding clade C ZM96 gp140 and ZM96 Gag and CN54 Pol-Nef, administered IM

- **AIDSVAX® B/E**: AIDSVAX® B/E: 300mcg of subtype B (MN) HIV gp120 glycoprotein and 300mcg of subtype E (A244) HIV gp120 glycoprotein absorbed onto 600mcg of aluminum hydroxide gel adjuvant, administered IM.

- **Placebo for DNA/NYVAC**: Sodium Chloride for injection, 0.9% administered IM

- **Placebo for AIDSVAX® B/E:** 600 mcg of aluminum hydroxide adjuvant, administered IM

Table 3-1 Schema

Group	N		Month 0 (Day 0)	Month 1 (Day 28)	Month 3 (Day 84)	Month 6 (Day 168)
1 (T1)	20	Left	NYVAC (2)	NYVAC (2)	NYVAC (2)	NYVAC (2)
		Right	placebo*	placebo*	AIDSVAX® B/E	AIDSVAX® B/E
(C1)	4	Left	placebo (2)	placebo (2)	placebo (2)	placebo (2)
		Right	placebo*	placebo*	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E
2 (T2)	20	Left	NYVAC (2)	NYVAC (2)	NYVAC (2)	NYVAC (2)
		Right	AIDSVAX® B/E	AIDSVAX® B/E	AIDSVAX® B/E	AIDSVAX® B/E
(C2)	4	Left	placebo (2)	placebo (2)	placebo (2)	placebo (2)
		Right	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E
3 (T3)	20	Left	DNA+placebo*	DNA+placebo*	NYVAC (2)	NYVAC (2)
		Right	placebo*	placebo*	AIDSVAX® B/E	AIDSVAX® B/E
(C3)	4	Left	placebo (2)*	placebo (2)*	placebo (2)	placebo (2)
		Right	placebo*	placebo*	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E
4 (T4)	20	Left	DNA+placebo*	DNA+placebo*	NYVAC (2)	NYVAC (2)
		Right	AIDSVAX® B/E	AIDSVAX® B/E	AIDSVAX® B/E	AIDSVAX® B/E
(C4)	4	Left	placebo (2)*	placebo (2)*	placebo (2)	placebo (2)
		Right	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E	placebo for AIDSVAX® B/E
Total	96 (80/16)					

(2) indicates two injections

*Sodium Chloride for injection, 0.9% is being used to equalize the number of injections among groups.

Note: All groups will enroll simultaneously. Enrollment will be restricted to a maximum of 2 participants per day, with no more than (1) participant in group 1 or 2 and (1) participant in group 3 or 4 until 20 participants have been enrolled with 5 in each group (4 vaccine recipients and 1 placebo recipient in each group). The HVTN 096 PSRT will review the safety and reactogenicity data reported for the first 168 hours postvaccination on each of these participants and will determine whether it is safe to proceed with full enrollment.

Participants

96 healthy, HIV-1-uninfected volunteers aged 18 to 50 years; 80 vaccinees, 16 placebo recipients

Design

Single site, randomized, placebo-controlled, double-blind trial

Duration per participant

18 months of scheduled clinic visits (main study) followed by annual participant health contacts to a total of 5 years following initial study injection

Estimated total study duration

66 months (includes enrollment, follow-up, and annual health contacts)

Study sponsor

EuroVacc Foundation (Lausanne, Switzerland)

Study product providers

- DNA: IPPOX Foundation (Lausanne, Switzerland)
- NYVAC: EuroVacc Foundation (Lausanne, Switzerland)
- DNA and NYVAC placebo: Saline solution commercially available to site
- AIDSVAX® B/E: US Military HIV Research Program (USMHRP) (Bethesda, Maryland, USA)
- Placebo for AIDSVAX® B/E: Global Solutions for Infectious Diseases (GSID) (South San Francisco, CA, 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 (Seattle, Washington, USA)

HIV diagnostic laboratory

University of Washington Virology Specialty Laboratory (Seattle, Washington, USA)

Endpoint assay laboratories

- Duke University Medical Center (Durham, North Carolina, USA)
- FHCRC/University of Washington (Seattle, Washington, USA)

Study sites

HVTN Clinical Research Site (HVTN CRS) in Lausanne, Switzerland

Safety monitoring

HVTN 096/EV04 PSRT; HVTN SMB

3.1 Protocol Team

Protocol leadership

Chair	Giuseppe Pantaleo Centre Hospitalier Universitaire Vaudois Hospices (CHUV)	*Statistician*	Holly Janes SCHARP, FHCRC
Cochair	Pierre-Alexandre Bart CHUV	*DAIDS Medical Liaison*	G. Laissa Ouedraogo DAIDS, NIAID
Protocol Team leader	Shelly Karuna HVTN Core, FHCRC	*EuroVacc Medical Officer*	Edna Venneker EuroVacc Foundation

Other contributors to the original protocol

Core medical monitor	Shelly Karuna HVTN Core, FHCRC	*Clinical affairs*	Yevgeny Grigoriev SCHARP, FHCRC
Vaccine developer representative	Song Ding EuroVacc	*Clinical trials manager*	Tamra Madenwald HVTN Core, FHCRC
Vaccine developer representative	Carter Lee GSID	*Project manager*	Lisa Sunner SCHARP, FHCRC
Vaccine developer representative	Charla Andrews USMHRP		
Laboratory Program representative	Eva Chung HVTN Laboratory Program, FHCRC	*Statistical Research Associate*	Maggie Wang SCHARP, FHCRC
Regulatory affairs	Renee Holt HVTN Core, FHCRC	*Protocol development coordinator*	Ramey Fair HVTN Core, FHCRC
Clinic coordinator	Nils Rettby Lausanne, Switzerland CRS	*Community engagement unit representative*	Carrie Schonwald HVTN Core, FHCRC
Community Advisory Board (CAB) members	Diego Lindlau Lausanne, Switzerland CRS	*Community educator/recruiter*	Nils Rettby Lausanne, Switzerland CRS
	Ana Maria Begle Lausanne, Switzerland CAB	*Technical editor*	Adi Ferrara HVTN Core, FHCRC
DAIDS protocol pharmacist	Scharla G. Estep DAIDS, NIAID	*DAIDS PRDB Officer*	Michael Pensiero DAIDS, NIAID

4 Background

4.1 Rationale for trial concept

The RV144 efficacy trial indicated that a recombinant canarypox vector, ALVAC® HIV vaccine vCP1521, expressing Env, Gag, and Pro, boosted with a gp120 protein, AIDSVAX® B/E, conferred 31% efficacy against HIV acquisition compared to placebo [4]. Furthermore, recent data from the RV144 Case-Control Study have identified immune responses that predicted the risk of vaccine recipients to become HIV-1 infected, ie Correlates of Risk. The Case Control Study was performed on 41 infected vaccine recipients, 205 uninfected vaccine recipients and 40 placebo recipients. The primary immunological responses (n=6 responses) measured were predominantly focused on Ab responses (5 of 6 variables measured) and on CD4+ T cell cytokine production. The immune responses identified as Correlates of Risk were measured at the peak of immunogenicity, 2 weeks after the final vaccination in the RV144 trial. Based on these measurements, two correlates of risk significantly associated with different infection rates in vaccine recipients were identified: a) IgG antibodies that bind to scaffolded-V1V2 recombinant protein correlated inversely with infection rate (higher VIV2 antibodies, lower infection rate) and b) plasma Env-specific binding IgA correlated directly with infection rate (higher IgA to Env, higher infection rate). The identification of the V1V2 IgG response as a correlate of protection from HIV infection provides additional support for the veracity of the modest efficacy results observed in the RV144 trial.

The vaccination regimen in the original RV144 trial included ALVAC®HIV vaccine vCP1521 administration at time 0 and Month 1 and ALVAC® HIV vaccine vCP1521+ AIDSVAX® B/E at Months 3 and 6. The modest 31% efficacy against HIV acquisition compared to placebo observed in the RV144 trial was measured at 3 years after the completion of the vaccination regimen. Of interest, the efficacy at 1 year was 60%, thus indicating that the vaccine effect waned over time; this loss of efficacy was associated with a substantial drop in the levels of binding Ab titers including V1V2 antibodies. Based on these results, it has been suggested to include an additional administration of both ALVAC® HIV vaccine vCP1521 and AIDSVAX® B/E at 12 months in future efficacy trials in order to maintain optimal Ab responses. Furthermore, based on the RV144 immunization regimen, protein administration does not occur before Month 3 and higher Ab titers are only achieved after the 2nd protein administration at Month 6. Since higher V1V2 Ab titers appear to correlate with reduced risk of infection, it is important to induce Ab response as early as possible following vaccination. Furthermore, since higher binding Env IgA plasma levels seem to be associated with increased risk for infection, it is also important to monitor the kinetics of vaccine-induced Env IgA plasma levels.

In follow-up to these modest efficacy results and to the identification of correlates of risk in the RV144 efficacy trial, a phase 2b HIV vaccine efficacy trial is being developed that focuses on second-generation vaccine regimens that will provide comparative data with concurrent active arms. This phase 2b trial will not only evaluate promising regimens for efficacy, but it may also provide further validation of the correlates of risk identified in the RV144 Case Control Study, as well as allow for the identification of additional correlates of protection. The products to be evaluated are a trivalent DNA expressing clade C ZM96 Gag, ZM96 gp140 and a CN54 Pol-Nef fusion construct (DNA-HIV-PT123); a bivalent NYVAC (a highly attenuated vaccinia virus) expressing clade C

ZM96 gp140 (NYVAC-HIV-PT1) and ZM96 Gag-CN54 Pol-Nef fusion (NYVAC-HIV-PT4), and two clade C gp120 proteins with MF59 adjuvant.

In summary, data from RV144 have demonstrated that: 1) Vaccine efficacy (VE) waned from 60% 1 year post-trial to 31% 3 years post-trial; 2) Loss of VE is associated with significant drop in binding Ab titers, including V1V2 antibodies; 3) IgG antibodies that bind to scaffolded V1V2 are identified as Correlate of Risk for HIV infection; 4) Higher V1V2 antibodies are correlated with reduced risk of infection [5]. Based on these findings and in preparation of the phase 2b study, the following critical questions need to be addressed in earlier phase 1/2 studies: **1) Will DNA + NYVAC + protein elicit similar Ab responses seen in RV144? 2) Will earlier protein administration result in earlier and more durable Ab responses than seen in RV144? 3) When is the safest and most efficacious time to administer protein in the Research Track phase 2B trial?**

The proposed HVTN096/EV04 clinical trial will address directly these questions:

1. The trial will evaluate a) the safety and immunogenicity of the novel trivalent DNA prime at Months 0 and 1, followed by novel bivalent NYVAC+AIDSVAX® B/E protein boosts at Months 3 and 6; b) the safety and immunogenicity of novel bivalent NYVAC prime at Months 0 and 1 followed by NYVAC+ AIDSVAX® B/E protein boosts at Months 3 and 6; c) the safety and immunogenicity of either trivalent DNA+ AIDSVAX® B/E protein or bivalent NYVAC+ AIDSVAX® B/E protein prime at Months 0 and 1 followed by NYVAC+ AIDSVAX® B/E protein boosts at Months 3 and 6.

2. In addition, this clinical trial will also evaluate whether co-administration of Env protein with DNA/NYVAC or NYVAC will lead to earlier and longer lasting Env-specific IgG responses. It is expected that the vaccine-induced Ab response in the groups with AIDSVAX® B/E co-administration during priming will be of greater magnitude than the Ab response in those groups with NYVAC or DNA administration alone during priming. However, no data are available on the magnitude of the Ab response which will be induced after two priming AIDSVAX® B/E co-administrations. The assessment of the magnitude of the Ab response may provide insights on the levels of vaccine protection that can be induced through the acceleration of vaccine-induced Ab response. Assessment of the durability of the Ab response may provide insights on the waning over time of the vaccine protective effect observed in RV144.

The rationale for investigating earlier administration of Env protein derives from the association between V1V2 Ab responses and reduced risk of infection shown in the RV144 trial. This evaluation of co-administration of protein in both NYVAC alone and DNA/NYVAC in this proposed study will inform the design of a future phase 2b efficacy trial in order to induce earlier and optimal Ab responses.

4.1.1 Rationale for mucosal secretion sampling

Recent data in rhesus macaques demonstrated the ability of intramuscularly administered DNA prime-rAd5 simian immunodeficiency virus (SIV) boost vaccination regimens to elicit SIV-specific cytotoxic T-lymphocytes (CTLs) in mucosal compartments [6,7] and lead to a reduction in the replication of SIVmac251 in gut lymphoid tissue as compared to controls [7]. These data suggest that systemic administration of HIV vaccines designed

to induce HIV-specific T-cells can elicit cells that are effective in reducing HIV replication in the gut.

To address the ability of vaccines given parenterally to elicit mucosal responses, participants enrolled at the Seattle HVTU in HVTN 069 participated in an ongoing study of mucosal responses. Of the nineteen participants enrolled who received the VRC DNA prime and rAd5 boost, potential T-cell epitope (PTE) Env-specific T-cell responses were seen in the blood of 18 (95%) participants. Of the 15 rectal specimens with sufficient cells for analysis at visit 7, 2 (13%) had PTE Env-specific T-cell responses. Although mucosal immune responses appear to be present less frequently than systemic immune responses, these data demonstrate mucosal immune responses to an HIV vaccine.

This study will explore rectal, cervical, salivary, and semen mucosal responses at up to three timepoints to evaluate changes in vaccine-induced immune responses at these mucosal sites and to compare the immune response profiles at these four mucosal sites. Samples collected at Month 0 provide the baseline values of mucosal immunity prior to vaccination. Samples collected at Months 6 1/2 and 12 provide information on the durability of vaccine-induced mucosal immunity.

4.2 DNA-HIV-PT123

The DNA-HIV-PT123 HIV vaccine evaluated in this protocol has a DNA plasmid backbone that was developed at the Dale and Betty Bumpers Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) (Bethesda, MD, USA). The CMV/R promoter consists of the translational enhancer region of the CMV immediate early region 1 enhancer substituted with the 5'-untranslated human T cell leukemia virus type 1 (HTLV-1) R-U5 region of the long terminal repeat (LTR) to optimize gene expression. Other elements of the plasmid include a bovine growth hormone polyadenylation signal termination sequence (Tbgh) and a kanamycin resistance cassette (Kan.). A schematic of the plasmid map is included in Figure 4-1.

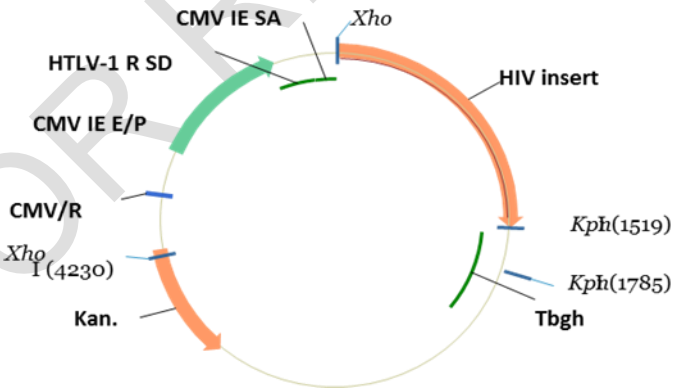


Figure 4-1 Example of the DNA plasmid map

The plasmid and host *E. coli* strain used in the production of the vaccine are characterized in accordance with the relevant sections of the FDA guidances "Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology" (1985), the "Supplement: Nucleic Acid Characterization and Genetic Stability" (1992), "Points to Consider in Human Somatic Cell Therapy and Gene

Therapy" (1991, 1998), and "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications" (1996). Additional information can be found in the Investigator's Brochure (IB).

The IPPOX Foundation DNA-HIV-PT123 HIV vaccine includes 3 DNA plasmids. One encodes clade C ZM96 Gag, another clade C ZM96 Env, and the third encodes CRF07 BC CN54 Pol-Nef. Enhancements made to the inserts include RNA and codon optimization, RNA secondary structure modulation, splice sites removal, TCF binding sites removal, and increasing the GC content. A schematic of the inserts are included in Figure 4-2.

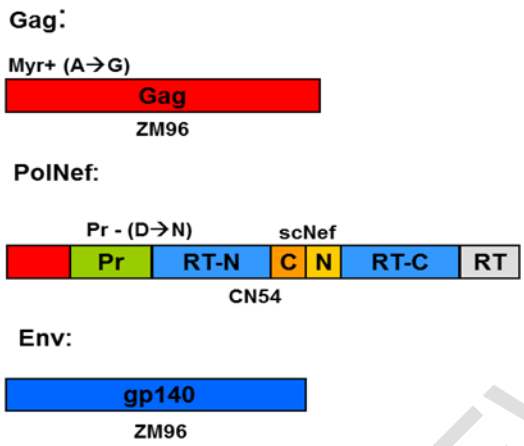


Figure 4-2 DNA-HIV-PT123 plasmid inserts

4.3 NYVAC-HIV-PT1 and NYVAC-HIV-PT4

NYVAC is a highly attenuated vaccinia virus (VV) derived from the VV strain Copenhagen, from which 18 genes, encoding proteins involved in host range and virulence, were deleted (Figure 4-3) [8]. NYVAC is made in chick embryo fibroblasts. NYVAC-derived vectors are able to express antigens from a wide range of species [9-11] and have been used in several clinical trials [12-14].

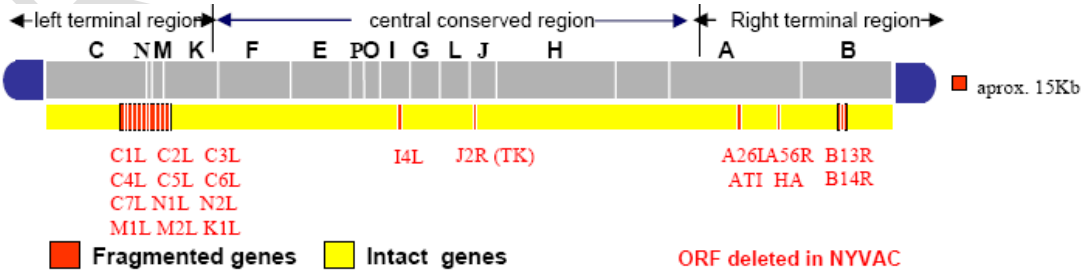


Figure 4-3 NYVAC construct

The NYVAC-HIV-PT1 expresses HIV clade C ZM96 Env gp140, and NYVAC-HIV-PT4 expresses HIV clade C ZM96 Gag and HIV-CRF07 BC CN54 Pol-Nef. These are the same genes as DNA-HIV-PT123. Strategies to enhance insert expression include: 1) RNA and codon optimization; 2) enrichment of the GC content; 3) modification of the

Gag-Pol-Nef readthrough polyprotein, i) reversion of the N terminal Ala-Gly substitution, ii) re-introduction of the natural Gag-Pol frameshift to allow budding and release of Gag-Pol-Nef particles from the transduced cells, and iii) inactivation of the protease. A schematic of the inserts for NYVAC-HIV-PT4 is included in Figure 4-4.

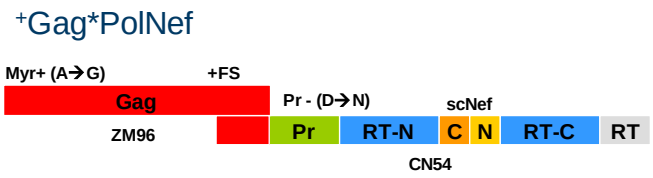


Figure 4-4 NYVAC-HIV-PT4 inserts

4.4 AIDSVAX® B/E

AIDSVAX® B/E is a bivalent HIV gp120 glycoprotein originally developed and manufactured by Genentech Inc. The development and manufacturing rights were subsequently transferred to VaxGen, Inc. and finally transferred to its current developer, Global Solutions for Infectious Diseases (GSID). It is a purified mixture of gp120 proteins produced by recombinant DNA procedures using Chinese hamster ovary (CHO) cell expression. The sequences of MN gp120/HIV-1 and A244 gp120/HIV-1 are expressed as fusion proteins where a 27 amino acid sequence found in the gD protein of herpes simplex virus type I is fused to the amino terminus of each protein. MN and A244 rgp120/HIV-1 are combined to produce the bivalent AIDSVAX® B/E vaccine. AIDSVAX® B/E encompasses both subtype B (MN) and subtype E (A244) proteins that are absorbed onto 600mcg of aluminum hydroxide gel adjuvant.

4.5 Trial design rationale

4.5.1 Dose (amount and number)

As extensively discussed in Section 4.1, based on the importance of eliciting Ab responses shown in the RV144 Case Control Study, it is essential to evaluate immunization regimens with earlier administration of the Env protein vaccine component. The design of the present clinical trial will help inform the immunization regimen that induces earlier and more durable Ab responses. The four immunization regimens are based on a large set of preclinical and clinical data available for both DNA and NYVAC with and/or without protein boosts. The vaccine administration schedule is identical to the RV144 trial. The vaccine doses are based on the large set of immunogenicity data available from previous preclinical and clinical studies of DNA, NYVAC, and AIDSVAX® B/E vaccines. The co-administration at Month 0 and Month 1 of NYVAC plus AIDSVAX® B/E and DNA plus AIDSVAX® B/E in Groups 2 and 4, respectively, will determine whether the earlier administration of protein is associated with more rapid induction and durability of Ab responses as compared to Groups 1 and 3 (without protein administration at Months 0 and 1). The trial design is statistically powered (see Section 6) to detect a difference in the vaccine-induced Ab response after the fourth vaccination and in the durability of the Ab response between Groups 1 and 2 and between Groups 3 and 4.

4.5.2 Schedule

Group 1

Priming with NYVAC will be at Months 0 and 1 followed by NYVAC + AIDSVAX® B/E boosts at Months 3 and 6.

Group 2

Priming with NYVAC + AIDSVAX® B/E will be at Months 0 and 1 followed by NYVAC + AIDSVAX® B/E boosts at Months 3 and 6.

Group 3

Priming with DNA will be at Months 0 and 1 followed by NYVAC + AIDSVAX® B/E boosts at Months 3 and 6.

Group 4

Priming with DNA + AIDSVAX® B/E will be at Months 0 and 1 followed by NYVAC + AIDSVAX® B/E boosts at Months 3 and 6.

4.5.3 Choice of placebo

Sodium Chloride for Injection, 0.9% delivered IM is the placebo for DNA and for NYVAC as well as a placebo to maintain blinding between groups. Aluminum hydroxide adjuvant, 600 mcg delivered IM is the placebo for AIDSVAX® B/E. Placebo injections will be given to equalize the number of injections.

4.5.4 Rationale for Full Protocol Amendment

One participant in study HVTN 092, in which the NYVAC-HIV-PT1 and NYVAC-HIV-PT-4 vaccines under evaluation in HVTN 096 are also being evaluated, was diagnosed with myocarditis that lasted two days. It was considered that this event may be related to the NYVAC study vaccine. All vaccinations in HVTN 096 are now complete and no participants have been reported with myocarditis in HVTN 096. However, as a precautionary measure it has been decided to have a follow-up ECG conducted in all HVTN 096 participants.

4.6 Plans for future product development and testing

Data from this trial is one component of a package of supportive data for a phase 2b trial in South Africa. It is fully integrated in the DNA/NYVAC/Protein phase 2b development plan, which has as a major objective to advance poxvirus/protein vaccination regimens in future phase 2b efficacy trials. The proposed trial will: 1) provide additional safety and immunogenicity data of the novel trivalent DNA and bivalent NYVAC, which are the vaccine candidates planned to be tested in the phase 2b trial development plan; 2) generate safety and immunogenicity data of the novel DNA and NYVAC in combination with proteins; and 3) inform the optimal protein schedule for the induction of earlier Ab responses for the phase 2b trial research track. For future studies with the same NYVAC vaccine, to simplify the vaccine administration, we plan to combine the two NYVAC

components together, so that there would be only one administration of a 2mL volume. Therefore, in HVTN 096, the administering of two 1 mL NYVAC injections in the same deltoid would be informative to the future studies.

It is noted that AIDSVAX® B/E is not the vaccine candidate for the phase 2b trial, which will be a bivalent vaccine of 2 clade C Env proteins. However, the GMP lots of the two Env proteins will not be available before Q3/Q4 2013, which means safety and immunogenicity clinical data for these proteins will only be available by Q2/Q3 2014, at the earliest. As outlined in the rationale section, based on the analysis of the RV144 data, the data to be generated from this study is pivotal for the design of the phase 2b study, and to wait for the selected protein candidate would delay the whole development timeline significantly.

The current plan is to initiate this study in Q2 2012, with the preliminary safety and immunogenicity data available by Q4 2012. These results will provide supportive data for the protocol development of the phase 1/2 trials testing the novel clade C proteins and the phase 2b efficacy trial, which is currently scheduled to start in 2013. It is estimated that this study will accelerate development by 12-18 months.

4.7 Preclinical safety studies

In addition to preclinical repeated-dose toxicology studies with the DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4, there have been several preclinical studies conducted with related products. The DNA-HIV-PT123 product utilizes a plasmid backbone developed by the VRC (p1012 with CMV/R promoter). There are relevant preclinical data on: 1) VRC DNA plasmid HIV vaccines that utilize a very similar DNA backbone, 2) a VRC DNA plasmid Ebola vaccine that utilizes an identical backbone, and 3) a VRC DNA plasmid SARS vaccine that utilizes the identical backbone. These preclinical data have been used to support clinical studies of related VRC DNA plasmid vaccines. A summary of the structures and preclinical studies of the most relevant VRC DNA plasmids that support the DNA-HIV-PT123 product is presented in Table 4-1.

Table 4-1 Summary of the structures and preclinical studies of the relevant VRC DNA plasmids that support the DNA-HIV-PT123 product

Vaccine	Plasmid	Gag	Pol	Nef	Env (A)	Env (B)	Env (C)	Preclinical safety testing
VRC-HIVDNA006-00-VP (6 plasmids)	p1012 w/CMV promoter	Gag-Pol-Nef (A) X Gag-Pol-Nef (B) X Gag-Pol-Nef (C) X			X	X	X	[1]
VRC-EBODNA012-00-VP (3 plasmids)	p1012 w/ CMV/R promoter	Ebola GP's and NP						[2]
VRC-HIVDNA009-00-VP (4 plasmids)	p1012 w/ CMV promoter	Gag-Pol-Nef (B) X			X	X	X	[3]
VRC-HIVDNA016-00-VP (6 plasmids)	p1012 w/ CMV/R promoter	X	X	X	X	X	X	[4]
VRC-SRSDNA015-00-VP (1 plasmid)	p1012 w/ CMV/R promoter	S protein of SARS-CoV						[5]

[1]GLP TherImmune studies for Biodistribution (#1195-102) and repeated-dose toxicity (#1195-103) submitted to BB-IND 10681, SN000). See description below in sections 4.7.1.1 and 4.7.1.2.
[2]GLP TherImmune studies for Biodistribution (#1332-102) and toxicity (#1332-101); submitted to BB-IND 11294, SN000). See description below in section 4.7.2.
[3]Given the high degree of homology of the antigen inserts between the VRC 4 plasmid DNA vaccine (VRC-HIVDNA009-00-VP) and the VRC 6 plasmid DNA vaccine (VRC-HIVDNA006-00-VP), the FDA waived to need for additional preclinical testing for VRC-HIVDNA009-00VP) (BB-IND 11750, SN000, pages 211-226)
[4]The FDA concurred that there is sufficient preclinical safety testing (previously reviewed by the FDA) to support the clinical use of VRC-HIVDNA016-00-VP (BB-IND 11750, SN000, Item 8, page 177). The decision is based on the high degree of homology between the candidate vaccines VRCHIVDNA009-00-VP (BB-IND 10681) and VRC-HIVDNA016-00-VP, and the preclinical data on the VRC Ebola plasmid DNA (VRC-EBODNA012-00-VP) that support the safety of the minor changes in the promoter.
[5]GLP TherImmune studies for Biodistribution and toxicity; submitted to BB-IND 11995, SN000. See description below in Section 4.7.3.

The DNA-HIV-PT123 vaccine contains the same backbone as the VRC DNA vaccines, VRC-HIVDNA016-00-VP, VRC-EBODNA012-00-VP, and VRC-SRSDNA015-00-VP.

VRC-HIVDNA006-00-VP was not used in a clinical trial. However, the preclinical toxicology and biodistribution studies conducted with this vaccine were used to support the clinical testing of a similar 4-plasmid vaccine, VRC-HIVDNA009-00-VP, and subsequently the 6-plasmid vaccine, VRC-HIVDNA016-00-VP. In both cases, the FDA concurred that the preclinical testing performed with VRC-HIVDNA006-00-VP precluded the necessity for further toxicology and biodistribution studies.

4.7.1 Preclinical safety studies conducted with the 6-plasmid DNA vaccine (VRC-HIVDNA006-00-VP)

In this section, a brief summary of preclinical biodistribution and toxicology studies that were performed with the 6-plasmid DNA vaccine (VRC-HIVDNA006-00-VP) is provided. These studies were performed by TherImmune Research Corporation (Gaithersburg, MD) in compliance with Good Laboratory Practices (GLP). The two studies that were performed were a single-dose biodistribution study and a repeated-dose toxicology study using intramuscular injections delivered by a needleless injection system. Both studies were conducted in New Zealand White rabbits.

4.7.1.1 Biodistribution of VRC-HIVDNA006-00-VP

For this evaluation, rabbits (n = 10) received a single intramuscular injection of VRC-HIVDNA006-00-VP (2 mg) delivered by Biojector® 2000 needle-free injection system. The biodistribution of the product was monitored in both blood and tissue samples on study days 8, 30, and 60.

Evaluation on study day 8 showed that the highest signals were found in the tissues at or adjacent to the injection site (muscle and overlying subcutis/skin). The DNA plasmids did not exhibit biodistribution away from the sites of injection. The magnitude of positive signal produced from study day 8 tissues was greatly diminished at each subsequent timepoint (study days 30 and 60), indicating clearance of the test article. All animals survived until the scheduled sacrifice, and no obvious difference in the biodistribution pattern was observed between female and male animals.

4.7.1.2 Repeated-dose toxicology of VRC-HIVDNA006-00-VP

This study was designed to evaluate toxicity of a four-dose regimen in rabbits. A total of 10 rabbits (6 male, 4 female) were immunized on study days 0, 29, 57, and 85 with a total of 8 mg of plasmid DNA per immunization. An equal number of rabbits were immunized with a vehicle control. All animals were sacrificed on study day 99. The following tissues were collected at necropsy for histopathological analysis: adrenals, aorta, bone with marrow, bone with bone marrow, brain, cervix, vagina, uterus, ovaries/testes, prostate, seminal vesicles, epididymides, esophagus, cecum, colon, duodenum, ileum, jejunum, rectum, eyes, lacrimal glands, heart, lungs, kidneys, liver with gallbladder, mammary glands, lymph nodes, parathyroid, nerve, pituitary, injection site muscle, salivary glands, skin, skeletal muscle, spleen, stomach, pancreas, thymus, thyroid, tongue, urinary bladder, spinal cord, and all gross lesions. Blood samples for hematology, serum chemistry, and immunology assays were obtained via puncture of the medial auricular artery prior to the first dose and prior to scheduled sacrifice on study day 99.

Repeated-dose intramuscular administration of VRC-HIVDNA006-00-VP, an HIV-1 DNA plasmid vaccine, using the Biojector® 2000 Needle-Free Injection Management System™ at the dose levels tested induced a mild level of toxicity to New Zealand White rabbits under the study conditions, based on the injection site reactions. The animals receiving the test article showed significant fluctuations in food consumption throughout the study, and a significantly lower weight gain for a very limited period during the study, when compared to the controls. While some organ weight parameters were affected, these alterations could not be verified in the clinical pathology or histology data and therefore would be considered incidental.

4.7.2 Preclinical safety studies conducted with the Ebola plasmid DNA candidate vaccine (VRC-EBODNA012-00-VP)

In addition to the data on the HIV gene inserts in the VRC 1012 backbone, data relevant to the VRC 1012 backbone with the CMV/R promoter were gained in the context of a trivalent product expressing Ebola GP (2 strains) and NP (1 strain) genes. In this section a brief summary of preclinical biodistribution and toxicology studies that were performed with the Ebola plasmid DNA vaccine (VRC-EBODNA012-00-VP) is provided. These studies were performed by TherImmune Research Corporation (Gaithersburg, MD) in compliance with GLP. The two studies that were performed were a single-dose biodistribution study and a repeated-dose toxicology study using intramuscular injections

delivered by a needleless injection system. Both studies were conducted in New Zealand White rabbits.

4.7.2.1 Single-dose biodistribution of VRC-EBODNA012-00-VP in rabbits

Tissue distribution studies were conducted in rabbits in order to evaluate the safety of the DNA plasmid vaccine VRC-EBODNA012-00-VP. The quantification of the plasmid DNA vaccine in blood was expressed per 10µL of blood. Specimens testing below the limit of detection of the assay (10 copies) were identified as less than the limit of detection (LLD). Specimens testing between 10 and 100 copies were below the limit of quantification of the assay and were identified as non-quantifiable. Real-time quantitative PCR (qPCR) sensitivity for detection was tested in a background of 1 mcg of vector-negative genomic DNA at a sensitivity of 100 copies or less per 1 mcg genomic DNA.

These studies were conducted under GLP Regulations. New Zealand White rabbits (3 per sex in Group 1 (phosphate buffered saline [PBS] control) and 15 per sex in Group 2 (VRC-EBODNA012-00-VP) and Group 3 (VRC-4302 control plasmid) received 0.5 mL of PBS or 2.0 mg Ebola vaccine or control plasmid by single intramuscular injection via the Biojector 2000® Needle-Free Injection Management System™ (Biojector) on study day 1. Five animals per sex from Groups 2 and 3 and one animal per sex from Group 1 were necropsied on study days 8, 30 and 60. Parameters measured included mortality, clinical observations, body weights, food consumption and biodistribution of the test article.

Biodistribution qPCR analysis determined that the VRC-EBODNA012-00-VP test article was present in muscle and subcutis at the injection site. For the muscle tissue samples, the frequency of positive tissues and copy number decreased from study day 8 through study day 60. Copy number in the subcutis was greatest at the study day 8 necropsy and decreased progressively through the study day 30 and 60 necropsies, but the frequency of positive findings did not decrease as dramatically as they did in the muscle samples during the study period. Additionally, these muscle and subcutis tissue findings were similar to the Group 3 VRC-4302 control plasmid, suggesting that the mechanism of injection by the Biojector may contribute to the low level persistence of the plasmids in the subcutis of the injection site. Only a few sporadic findings of VRC-EBODNA012-00-VP were evident in other tissues (a single finding in gonad on study day 8, a single finding in brain on study day 8). Other isolated findings include very low level positive results in the lung and the blood of two different PBS control animals at study day 60 and in the lymph nodes of one DNA control animal each at study day 8 and study day 60. Because of the small number of positive results, the lack of a pattern, and their presence in both sets of control animals in addition to test animals, these were possibly the result of false positives or sample contamination. Under these study conditions a single Biojector intramuscular injection of Ebola vaccine VRC-EBODNA012-00-VP in rabbits did not exhibit any obvious signs of toxicity. VRC-EBODNA012-00-VP plasmids distributed into the injection site muscle and subcutis, but decreased in copy number and frequency over time, demonstrating a clearance of the plasmids from the injection site tissues.

4.7.2.2 Repeated-dose studies of VRC-EBODNA012-00-VP in rabbits

These studies were carried out according to GLP Guidelines. The objective of the study was to assess the potential toxicity of repeated-dose intramuscular administration of VRC-EBODNA012-00-VP vaccine following repeated intramuscular injections in rabbits. New Zealand White rabbits received 2 mL of PBS or VRC-EBODNA012-00-VP in PBS by intramuscular injection (4 sites of 0.5 mL per site, 8 mg total per timepoint)

via the Biojector 2000® Needle-Free Injection Management System™ on study days 1, 22, 43 and 64. Animals were then necropsied on study day 66 (acute necropsy) and study day 78 (recovery necropsy). Parameters evaluated included mortality, clinical observations, Draize observations, body weight, food consumption, ophthalmology, clinical pathology, organ weights, gross pathology and histopathology.

No test article-related changes in mortality, clinical observations, absolute organ weights, body weights, food consumption, clinical pathology, bone marrow, or ophthalmology were observed. Treatment with VRC-EBODNA012-00-VP resulted in all animals in the treatment group having increased Ab titers against the Ebola epitopes encoded by the plasmid components of the vaccine, demonstrating immunogenicity of the vaccine components. An increased frequency of recoverable Draize findings (edema and erythema) at the injection site was observed in the VRC-EBODNA012-00-VP-treated animals after the third and fourth doses. This correlated with gross necropsy findings of red discoloration at the injection sites and histopathological findings of inflammation. These findings were likely related to the injection procedure, and to the expected immune response against the transgenes. Control and test animals with increased Draize findings exhibited similar rates of gross necropsy findings of red discoloration at the injection sites and histopathological findings of injection site hemorrhage. Incidence of renal changes identified as nephropathy and mineralization was notably increased in treated females compared to control females but notably decreased in treated males versus control males. These renal changes have been observed in previous TherImmune studies to occur spontaneously in rabbits; hence the relationship to the test article of these reported findings, which involved control and treated rabbits, appears to be equivocal. Support for a lack of test article relation can be found by the lack of gross pathology and organ weight findings in the kidney. Additionally, there were no associated test article-related findings for clinical pathology parameters indicative of kidney function (CREA and BUN) during the study.

4.7.3 Preclinical safety studies conducted with the SARS plasmid DNA candidate vaccine (VRC-SRSDNA015-00-VP)

In this section a brief summary of preclinical biodistribution and toxicology studies that were performed with the SARS plasmid DNA vaccine (VRC-SRSDNA015-00-VP) is provided. The SARS plasmid backbone is identical to the plasmid backbone in the DNA-HIV-PT123 product being used in HVTN 096. The preclinical studies with the SARS plasmid were performed by GeneLogic, Inc. (Gaithersburg, MD, formerly TherImmune) in compliance with GLP. The two studies that were performed were a single-dose biodistribution study and a repeated-dose toxicology study using intramuscular injections delivered by a needleless injection system. Both studies were conducted in New Zealand White rabbits.

4.7.3.1 Single-dose biodistribution of VRC-SRSDNA015-00-VP in rabbits

Gene Logic, Inc. (Gaithersburg, MD, formerly TherImmune) conducted a single-dose biodistribution study of the SARS vaccine, VRC-SRSDNA015-00-VP in New Zealand White rabbits under GLP using intramuscular injections administered via the Biojector 2000® Needle-Free Injection Management System™ (Bioject) on study day 1.

Three groups of New Zealand White rabbits (15 animals/sex/group) received one 0.5 mL intramuscular injection of PBS (Group 1), 2 mg of VRC-SRSDNA015-00-VP (Group 2) or VRC 4302 control plasmid (Group 3) on study day 1 via the Biojector 2000® Needle-Free Injection Management System™. On study days 9, 30, and 61, five

animals/sex/group/timepoint were sacrificed and tissue samples were isolated for biodistribution analysis. Parameters evaluated during the study period included mortality, physical and cageside exams, body weights, body weight changes, food consumption, and biodistribution analysis.

All tissues were shipped to Althea Technologies, Inc. (San Diego, CA) and processed for the presence of the vector in the tissues using a GLP validated TaqMan™ polymerase chain reaction (PCR), developed and qualified to detect a specific target sequence in the vaccine. The lower limit of detection for this assay is 10 copies of the target/mcg of DNA, the lower limit of quantification for the assay is 50 copies of the target/mcg of DNA**.**

Tissues analyzed included: blood, gonads, heart, lung, liver, kidney, adrenal glands, lymph nodes, spleen, thymus, subcutis and thigh muscle (at injection site), bone marrow (from femur on side of injection) and brain.

Results from the 9-, 30-, and 61-Day PCR evaluations showed that the vector is primarily localized to the subcutis at the injection site. No treatment related changes in mortality, physical and cageside exams, body weights, body weight changes, or food consumption were observed. The distribution profile (Table 4-2) consisted of the VRC-SRSDNA015-00-VP test article present at the injection site subcutis (10/10 animals, study day 9; 9/10 animals, study day 30; 3/10 animals, study day 61) and muscle (2/10 animals, study day 9; 5/10 animals, study day 30; 1/10 animals, study day 61), and at low levels (<350 copies each) in the blood (4/10 animals, study day 9). The number of copies of the VRC-SRSDNA015-00-VP test article decreased considerably from study day 9 to study day 61 in all tissues with positive findings and the results were comparable to a plasmid control, VRC 4302 which was tested concurrently.

The subcutis of 6/10 PBS control animals was positive at low levels (range 220-948). This was due to contamination which was traced to the Biojector used for injection.

Table 4-2 Summary of number of test rabbits with positive findings in tissues and range of copies of target/mcg DNA

	Blood	**Subcutis**	**Muscle**
Study Day 9			
Number with positive reactions	4/10	10/10	2/10
Range of copy numbers	119-349	141-513022	109, 958
Study Day 30			
Number with positive reactions	0/10	9/10	5/10
Range of copy numbers	N/A	136-1830	70-227
Study Day 61			
Number with positive reactions	0/10	3/10	1/10
Range of copy numbers	N/A	65-289	130

4.7.3.2 Repeated-dose biodistribution of VRC-SRSDNA015-00-VP in rabbits

This study, conducted by Gene Logic, Inc. (Gaithersburg, MD, formerly TherImmune), was designed to evaluate the potential toxicity of a SARS recombinant DNA vaccine

when administered repeatedly by intramuscular injection to male and female New Zealand White rabbits during a 78-day study period.

Two groups (10 animals/sex/group) of New Zealand White rabbits received 1 mL (2 X 0.5 mL) intramuscular injections of PBS or VRC-SRSDNA015-00-VP on study day 1, 22, 43, and 64. On study day 66, 5 animals/sex/group were necropsied and all surviving animals were necropsied on study day 78. Parameters evaluated during the study period included mortality, clinical and cageside observations, Draize observations, body weights, body weight changes, food consumption, ophthalmologic examinations, clinical pathology, organ weights and ratios, gross pathology, and histopathology.

Under these conditions, repeated intramuscular injection with VRC-SRSDNA015-00-VP resulted in no changes in mortality, clinical and cageside observations, body weights, body weight changes, food consumption, ophthalmologic examinations, clinical pathology, organ weights and ratios, gross pathology, and histopathology. However, VRC-SRSDNA015-00-VP-treated animals generally had a higher frequency of Draize observations, and a longer time required for recovery to normal when compared to their control counterparts. These differences were likely due to the intended immune stimulation of the vaccine inserts.

4.7.4 Summary of preclinical safety studies related to NYVAC-HIV-PT1 and NYVAC-HIV-PT-4

NYVAC-HIV-PT1 and NYVAC-HIV-PT4 contain the same vector backbone as NYVAC-B (vP2009) and NYVAC-HIV-C (vP2010). Extensive toxicity studies have been conducted with NYVAC-B and NYVAC-HIV-C, which are summarized in Sections 4.7.4.1, 4.7.5, and 4.7.6.

4.7.4.1 Repeated-dose toxicity of NYVAC-B in rats

A repeated-dose toxicity study in rats with NYVAC-B (vP2009) administered by IM was performed. Biodistribution of NYVAC-B (vP2009) was also evaluated in cynomolgus macaques chronically infected with the simian immunodeficiency virus SIVmac251.

The test article (NYVAC-B (vP2009) Lot # Z138) was administered IM into the right and left hind limb (0.2 mL into each quadriceps muscle: 0.4 mL total dose on each occasion). Rats receiving treatment were divided into two treatment groups, as shown in Table 4-3:

Table 4-3 Summary of treatment groups in repeated-dose toxicity study in rats

Group number	Group description	Dose volume (mL)	Human equivalent dose	Number of animals/group # Male	Female
1	Control	0.4	-	10	10
2	Test	0.4	0.4xHD	10	10

The five lowest numbered animals in each group were necropsied on study day 30; the five highest numbered animals in each group were necropsied on Day 51.

The animals were administered 0.4 mL (corresponding to 0.4 HD with a HD = 2.5×10^7 PFU) of NYVAC-B (vP2009, lot # Z138) or saline solution (0.9% NaCl) IM on 3 occasions at 14 day intervals (Days 1, 15 and 29). All animals were observed daily for clinical signs of ill health or overt toxicity. In addition, each animal was given a detailed

physical examination at weekly intervals. Body weights were recorded and food consumption was calculated. Ophthalmoscopy investigations were performed on all animals pretreatment, on Day 29 and Day 50. Blood samples from abdominal aorta were collected at necropsy for hematology and chemistry parameters. Immunogenicity parameters were analyzed under the responsibility of the Sponsor. Necropsies were performed after an overnight period without food. Exsanguinations and full macroscopic examination were performed. Organs were weighed before fixation and tissues observed microscopically.

Data from treated animals were compared with control data; statistical analyses were performed where appropriate.

There were no unexpected deaths during the treatment phase of the study. There were no treatment-related clinical signs. There were no apparent effects of treatment on food consumption and body weight. An increase in mean white blood cells, specifically neutrophils, was recorded for males receiving the NYVAC-B vaccine on Day 30. However, this appeared to be due to individual variability and was not considered to be of toxicological significance.

Up to twofold increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were noted on Day 51 in 2 out of 10 males receiving NYVAC-B (vP2009) compared to controls. All other parameters were similar to controls. No abnormalities were observed in the 10 females receiving NYVAC-B. Given that these changes were not seen for the treated females and that all individual values were within historical data ranges, the variations were considered as having no toxicological significance.

No effect of treatment was detected by the ophthalmoscopic examinations. Organ weights, adjusted and unadjusted for body weight, were similar for both treated and control animals.

At the terminal kill, Day 30, there were no macroscopic findings due to effects of the test article. Microscopically, there was a minor increase in inflammatory cell foci/myositis at the injection sites, due to local effects of the test article. There were no microscopic findings due to systemic effects of the test article. At the treatment-free kill, Day 51, there was evidence of partial reversal of the microscopic changes seen at the terminal kill.

Repeated intramuscular administration of 0.4 mL of NYVAC-B vaccine to rats on 3 occasions at 14-day intervals was well tolerated. Macroscopically and microscopically, there were no findings in the treated animals that could be attributed to NYVAC-B vaccine.

4.7.4.2 Biodistribution of NYVAC-B (vP2009) in cynomolgus macaques chronically infected with SIVmac251

The aim of this study was to evaluate in adult cynomolgus macaques, chronically infected with pathogenic SIVmac251, the biodistribution of NYVAC-B (vP2009) vaccine vector expressing clade B proteins of HIV-1.

Eight cynomolgus macaques were injected with 50 animal infectious doses of a pathogenic SIV SIVmac251 (SIV-1 stock). Infection of macaques was followed by: 1) plasma and peripheral blood mononuclear cell (PBMC) associated viral load; 2) presence of anti-SIV antibodies in the serum; 3) percentage and number of circulating CD4+ T-

lymphocytes. Prior to infection with SIV, animals were vaccinated with BCG ($2x10^8$ PFU). Delayed type hypersensitivity and enzyme-linked immunospot (ELISpot) to protein-purified derivative of BCG were tested before and at week 10 after infection with SIV. At week 13 following infection, 4 animals were injected IM with one human dose of NYVAC-B (vP2009) and 4 control animals were injected with saline. The 8 animals were sacrificed 13 (controls) and 14 (NYVAC-B (vP2009)) days later and biodistribution of NYVAC-B (vP2009) was evaluated by PCR in several organs tissues.

All animals were infected with SIVmac251 as evidenced by persistent viremia (plasma and mononuclear blood cells) and seroconversion. Characteristic clinical signs and histopathology of SIV infection were also noticed for all animals: persistent and generalized lymphadenopathy, follicular hyperplasia. Counts of CD4+ T-lymphocytes in blood remained elevated during the study period and no clear sign of immunodeficiency was noticed. NYVAC genome could not be detected in any of the explored samples. Brain and spinal cord could not be tested because of the difficulty of extracting DNA with no contaminants inhibiting the PCR assay. No clear adverse effect associated with NYVAC-B (vP2009) injection could be evidenced although changes in viremia could be observed.

4.7.5 Repeated-dose toxicity and biodistribution study with NYVAC-HIV-C in rats

The objective of this study was to determine the safety, biodistribution and immunogenicity of three doses of NYVAC-HIV-C administered IM to rats. Three doses of NYVAC-HIV-C were administered IM to rats at 14 day intervals (ie at study days 1, 15 and 29).

Each dose of NYVAC-HIV-C contained $10^{7.71}$ 50% cell culture infective dose (CCID50) per 0.4 mL and the volume of each dose was 0.4 mL. The dose level of 0.4 mL is the maximal volume injectable by the IM route. The three doses were administered to maximize the exposure by one additional dose compared to the intended protocol in a subsequent clinical trial (EV01) and the time between two administrations was considered to allow the development of an immune response.

The toxicity parameters recorded included clinical signs, morbidity and mortality, body weights and food consumption. Ophthalmoscopy investigations, hematology and clinical chemistry parameters were evaluated on two occasions at the end of the study. Viral shedding was measured on several occasions after the first administration, on skin, oral and rectal swabs, and in blood and urine samples. Half of the animals were subject to necropsy 24 hours post last dose and the other half at 22 days post last dose.

In addition to the macroscopic and microscopic examination of the organs, the biodistribution of NYVAC-HIV-C in the organs was evaluated by quantitative PCR. The objective of the biodistribution study was to determine whether DNA sequences originating from NYVAC-HIV-C are present in a range of tissues isolated from treated animals.

There have been neither unscheduled deaths nor clinical signs indicative of a reaction to treatment. There has been no effect of treatment on bodyweight and food intake. No ophthalmic lesions or abnormalities were detected. Elevated absolute and relative counts of neutrophils were noted in treated animals 24 hours post last dose. A slight increase in white blood cells associated with a slight decrease in lymphocyte counts was noted in treated females. On study day 51, lower group mean lymphocyte counts were still noted for the treated animals as compared with control animals, whereas the neutrophil counts

were back to normal values. As a result, the total white blood cell counts in treated animals were lower than in control animals. There was no other effect of treatment on haematology parameters or on clinical chemistry parameters. Increased spleen weights were noted for treated females 24 hours post last dose, though this effect had totally reversed 22 days post last dose.

At necropsy, there were no treatment related macroscopic findings. At the 24 hours post last dose necropsy, histopathological examination revealed a minor increase in the severity of inflammatory cell infiltration/myofiber degeneration and fasciitis at the injection site of treated animals compared with control animals. Minor inflammatory cell infiltration was also seen around the sciatic nerves of treated females. No differences were observed between control and treated animals for the same tissues at the second necropsy timepoint, suggesting a reversal of the microscopic changes seen 24 hours post last dose. There were no microscopic findings in treated animals due to systemic effects of the test article.

The only noteworthy findings were increased neutrophil counts (reversible and which could be related to the local inflammation), decreased lymphocyte counts and evidence of inflammation at the vaccine administration sites.

Repeated administrations of NYVAC-HIV-C by IM route revealed some hematologic modifications as increase in neutrophil counts and decrease in lymphocyte counts. These changes were reversible and probably correlated to the local inflammation at the administration site. The reversible increase in spleen weight observed in females was not correlated to any microscopic findings.

Viral particles (between 2 and 1.520 PFU/mL) were detected in 10 out of 15 skin swabs taken immediately after the injection (the other 5 tubes were empty due to technical error), but the quantity was markedly reduced (2 to 12 PFU/mL) in the samples taken 6 hours later. No particle was detected in the skin swabs taken 24 hours, 3 and 10 days after the injection. The 240 particles detected in the rectal sample immediately after the IM injection in the hind limb are unexpected but they may be explained by the proximity of the injection site. No virus was detected in the other samples (including coagulated blood samples).

The analysis of tissues by quantitative PCR revealed the presence of the vector in the injection site samples from seven treated animals and in the spleen of one treated animal 24 hours after the last dose. No localization of the vector to the epididymides (male), ovaries (female), liver, kidneys, lungs, inguinal lymph nodes, mandibular lymph nodes, adrenals, brain or bone marrow was observed at the 24-hour timepoint. At the 22-day timepoint, none of the tissues samples (including injection sites and spleen) were positive.

It was concluded that NYVAC-HIV-C administered three times at 14 day intervals, by the intramuscular route, to the rat at a dose level equivalent to 0.4 times that of the anticipated human clinical dose was well tolerated and immunogenic. The only noteworthy findings were increased neutrophil counts (reversible), increased spleen weights and evidence of inflammation at the administration sites. These effects could result from local inflammation at the injection site and/or from vaccine-induced immune stimulation, rather than from a systemic toxicological effect.

4.7.6 Summary of repeated-dose toxicity study with DNA-C in combination with NYVAC-HIV-C

The objective of this study was to assess the potential toxicity of the anti-HIV DNA vaccine + NYVAC-HIV-C vaccine when administered to BALB/c mice by IM route in a prime/boost regimen over 42 days with recovery from any effects being evaluated over a 20-day period.

A total of 70 BALB/c mice (35 male and 35 female) aged 6-7 weeks were ordered for the study. Of these, 60 animals (30 male and 30 female) were included in treatment groups, while the 10 additional animals were used for any replacements required during initial health screening or treatment.

The 60 mice receiving treatment were divided into two treatment groups, as shown in Table 4-4.

Table 4-4 Summary of treatment groups in toxicity study

Group	Summary of treatment	Number of animals		Dosing			
		Main Study	Recovery phase	Day 1	Day 15	Day 29	Day 43
1	Control	10M + 10F	5M + 5F	Control	Control	Control	Control
2	DNA-C + NYVAC-HIV-C	10M + 10F	5M + 5F	DNA-C vaccine (100 mcg)	DNA-C vaccine (100 mcg)	NYVAC-HIV-C vaccine ($10^{6.7}$ CCID50)	NYVAC-HIV-C vaccine ($10^{6.7}$ CCID50)

Animals (10/group/sex) were euthanized on study day 49 (6 days post last immunization). The remaining animals (5/group/sex) were euthanized on study day 63, 20 days post last vaccination to document safety parameters in the recovery phase.

There were no clinical findings associated with the vaccine, although some mild indications of local irritancy were noted to a similar extent for both groups. While higher eosinophil counts were noted on study day 49 for animals receiving DNA-C/NYVAC-HIV-C when compared with controls, the counts returned to normal levels by study day 63. No effects of treatment on blood chemistry parameters were observed. After the recovery period (study day 63), increased incidences of inflammatory cell infiltrates in the muscle and subcutis at the injection sites were still seen in vaccinated animals, compared to control animals, albeit with a lower frequency and severity compared to the study day 49 timepoint.

The study showed no significant toxic effects, either local or systemic, in BALB/c mice. The following dosing regimen was used: two priming doses of plasmid DNA-C vaccine given by IM injection (100 mcg each, separated by an interval of 14 days), followed by two booster doses of NYVAC-HIV-C vaccine given by IM injection ($10^{6.7}$ $CCID_{50}$ each, separated by an interval of 14 days).

4.7.7 Repeated-dose toxicity study with DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4

The objective of this study is to determine and assess systemic toxicity (short-term and persistent) and local site reactogenicity in New Zealand White rabbits administered a

DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4 prime-boost vaccine regimen via IM injection. The study is in compliance with US FDA GLP (21 CFR 58), excluding test and control analyses and immunogenicity assays. The study is being conducted at Spring Valley Laboratories, Inc. in Sykesville, MD, US.

Table 4-5 Study design of repeated-dose toxicity study with DNA-HIV-PT123 and NYVAC-HIV-PT-1 and NYVAC-HIV-PT-4

Gp	Test/Control Article Days 0, 14, 28 and 42	Dose (mg)	Volume (mL)	Test/Control Article Days 56 and 70[1]	Dose (PFU)	Volume (mL)1	Main Study Necropsy (Day 72)	Recovery Study Necropsy (Day 84)
1	Saline	N/A	1	Saline	N/A	2	5M, 5F	5M, 5F
2	DNA	4	1	NYVAC-HIV-PT1 and NYVAC-HIV-PT4	$\geq 5x10^6$ PFU/ml per NYVAC	2	5M, 5F	5M, 5F

[1]Two separate types of NYVAC (HIV-PT-1 and HIV-PT-4) were administered separately (right and left hind limb) in two separate injections of 1 ml each. Saline control was also be delivered as two separate injections of 1 ml each on Days 56 and 70.

NYVAC-HIV-PT-1 and NYVAC-HIV-PT-4 were administered separately (right and left hind limbs) in two separate injections of 1 ml each. Saline controls were also delivered as two separate injections of 1 mL each on Days 56 and 70.

As shown in Table 4-5, New Zealand White rabbits (20/sex, total 40 rabbits) were randomly assigned to receive either DNA-PT123 on Days 0, 14, 28 and 42 followed by NYVAC- HIV-PT-1 and NYVAC-HIV-PT-4 on Days 56 and 70 or to saline control on the same days. NYVAC-HIV-PT-1 and NYVAC-HIV-PT-4 were administered separately (right and left hind limbs) in two separate injections of 1 ml each on Days 56 and 70. The control group received saline control delivered as a single injection on Days 0, 14, 28 and 42, and delivered as two separate injections of 1 mL each on Days 56 and 70.

Clinical symptoms, body weight, temperature, ophthalmology, injection site reactions and safety laboratory parameters were assessed. Five animals/sex/group were sacrificed two days or two weeks following the final vaccination (Day 72 or 84). Each animal underwent a complete necropsy with organ weights. All tissues collected from animals assigned to the main study (Day 72 necropsy) were examined microscopically. For those animals assigned to the recovery study (Day 84 necropsy) only the injection site with surrounding muscle and gross lesions was microscopically examined.

In accordance with the tox report, all animals survived to scheduled sacrifice. No abnormal findings or statistically significant changes in physical and cageside examinations, ophthalmological examination, body weights, body temperature, administration site, urinalysis and gross necropsy considered to be related to treatment were observed. Some changes were observed in clinical pathology and microscopic observations, most of which could be correlated to an acute inflammatory and immune response that is expected with administration of a test material intended for use as a vaccine. Most changes were temporary and reversible and therefore not considered to constitute a safety concern. Overall, it was concluded that the study results demonstrated that administration of DNA-HIV-PT123 in combination with NYVAC-HIV-PT-1 and NYVAC-HIV-PT-4 was safe and well-tolerated in New Zealand White Rabbits. The

vaccine was immunogenic in all treated animals following the complete immunization schedule.

4.7.8 Single and repeated dose safety and toxicity studies with AIDSVAX IIIB and AIDSVAX MN

Data obtained for AIDSVAX IIIB, the monovalent rgp120 prepared from HIV-1 IIIB, and AIDSVAX MN, the monovalent rgp120 prepared from HIV-1 MN, established the preclinical safety for rgp120/HIV-1 molecules. For example, AIDSVAX IIIB formulation (300 mcg/mL) was tested for acute toxicity and local tolerance in guinea pigs and rabbits and was well tolerated. In baboons, an acute toxicity study using AIDSVAX IIIB showed no evidence of toxicity at dose levels as high as 1500 mcg per injection [15]. In addition, the acute toxicity and local tolerance of the AIDSVAX MN formulation were evaluated in male guinea pigs treated with single IM doses of up to 300 mcg per dose (~750 mcg/kg), and was well tolerated. A 6-month chronic study in rats given seven IM doses (approximately every 4 weeks) of either 300 mcg or 600 mcg of AIDSVAX MN showed no adverse events (AEs) attributable to the vaccine.

The potential for neurotoxic effects was evaluated in rat offspring after exposure in utero and/or during the neonatal period to AIDSVAX MN. Thirty pregnant rats were given AIDSVAX MN, and 30 rats were given vehicle, once every three days from day 1 of presumed gestation until parturition. One pup/sex/litter from treated and control group dams were given a daily subcutaneous injection from day 1 through day 22 postpartum of vehicle AIDSVAX MN (with Alum adjuvant), soluble MN rgp120/HIV-1 (without adjuvant) or MN rgp120/HIV-1 with QS-21 (Aquila Biopharmaceuticals, Framingham, MA). Neurobehavioral and physical development were evaluated (ie preweaning reflex and development, sexual maturation, motor activity, acoustic startle, passive avoidance, functional observational battery, and water maze testing). Tissues were processed for anatomical examination (ie weights of whole and regional brain and neuropathology). Administration of MN rgp120/HIV-1 with or without adjuvant to pups did not cause any persistent effect on any parameter evaluated. Neurohistological examination did not reveal any pathological effects related to treatment. Thus, AIDSVAX MN did not cause neurotoxicity or developmental toxicity in neonatal rats after exposure in utero and/or during the neonatal period.

The A244 rgp120/HIV-1 antigen (manufactured by a similar process as used for AIDSVAX IIIB and AIDSVAX MN) was tested in several safety tests using guinea pigs and mice and was well tolerated.

4.8 Preclinical immunogenicity studies

4.8.1 Immunogenicity of DNA-HIV-PT123 with NYVAC-HIV-PT1 and NYVAC-HIV-PT4 in non-human primates

The immunogenicity of DNA-HIV-PT123 with NYVAC-HIV-PT1 and NYVAC-HIV-PT4 was tested in nonhuman primates (NHPs) as shown in Table 4-6. Also evaluated in this study was the immunogenicity of a replication competent version of NYVAC vector called NYVAC-KC administered either by scarification or IM. Monkeys also received a gp120 protein (TV1 clade C) with an MF59 adjuvant boost. The concentration of the DNA-HIV-PT123 used in the NHP study was 2 mg/mL with 2 injections in the left and right legs. So the total dose was 4 mg. NYVAC-HIV-PT1 and NYVAC-HIV-PT4 were

formulated together with a final dose of $2x10^8$ PFU/mL ($1x10^8$ PFU/mL each). The dose of protein is 100mcg/mL.

Note that the NYVAC doses are at equal molar amount in the NHP studies (Section 4.8.1 and Section 4.8.2), while in the repeated-dose toxicity study (Section 4.7.7) and the proposed clinical trial, the NYVAC doses are not of equal molar amount.

Table 4-6 Summary of study groups for DNA-HIV-PT123 with NYVAC-HIV-PT1 and NYVAC-HIV-PT4 in NHP

Gp	Size	Wk 0	Wk 4	Wk 8	Wk20	Wk28	Wk32
1	8	DNA (IM)	DNA (IM)	DNA (IM)	NYVAC -KC (Scarification)	Protein (IM)	Protein (IM)
2	8	DNA (IM)	DNA (IM)	DNA (IM)	NYVAC -KC (IM)	Protein (IM)	Protein (IM)
3	8	DNA (IM)	DNA (IM)	DNA (IM)	NYVAC (IM)	Protein (IM)	Protein (IM)

The results of the study have demonstrated that DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4 have greatly increased the level of immunogenicity as assessed by both qualitative and quantitative endpoints:

T cell responses

- The novel DNA, DNA-HIV-PT123, was greatly immunogenic with an average of 1500 spot-forming units (SFU)/million PBMC in responders 2 weeks post DNA immunization as measured by IFN-γ ELISpot (Figure 4-5). The increase in the immunogenicity was in the range of 1.5 log as compared to previous HIV DNA vaccines tested by several groups [16-18] and the magnitude of the observed response was comparable to that obtained in NHPs with electroporation [19];
- One NYVAC boost induced a large increase in the percentage of HIV-specific T cells with an average of 4500-5000 SFU/PBMC as measured by IFN-γ ELISpot (Figure 4-5). This is nearly 1 log greater as compared to the first generation DNA/NYVAC vector combination [20];
- The DNA-HIV-PT123/NYVAC regimen induced balanced CD4+ and CD8+ T cell responses as shown by flow cytometry after the 3 DNA immunizations (Figure 4-6). Flow cytometry data have been generated by Dr. Mario Roederer at VRC through the CTC VIMC CAVD program, and flow cytometry data after NYVAC and protein boosts are being performed;
- The DNA-HIV-PT123/NYVAC vaccine regimen induced broad T cell responses as indicated by the large number of targeted peptide pools encompassing Env, Gag, Pol and Nef HIV proteins (Figure 4-6);

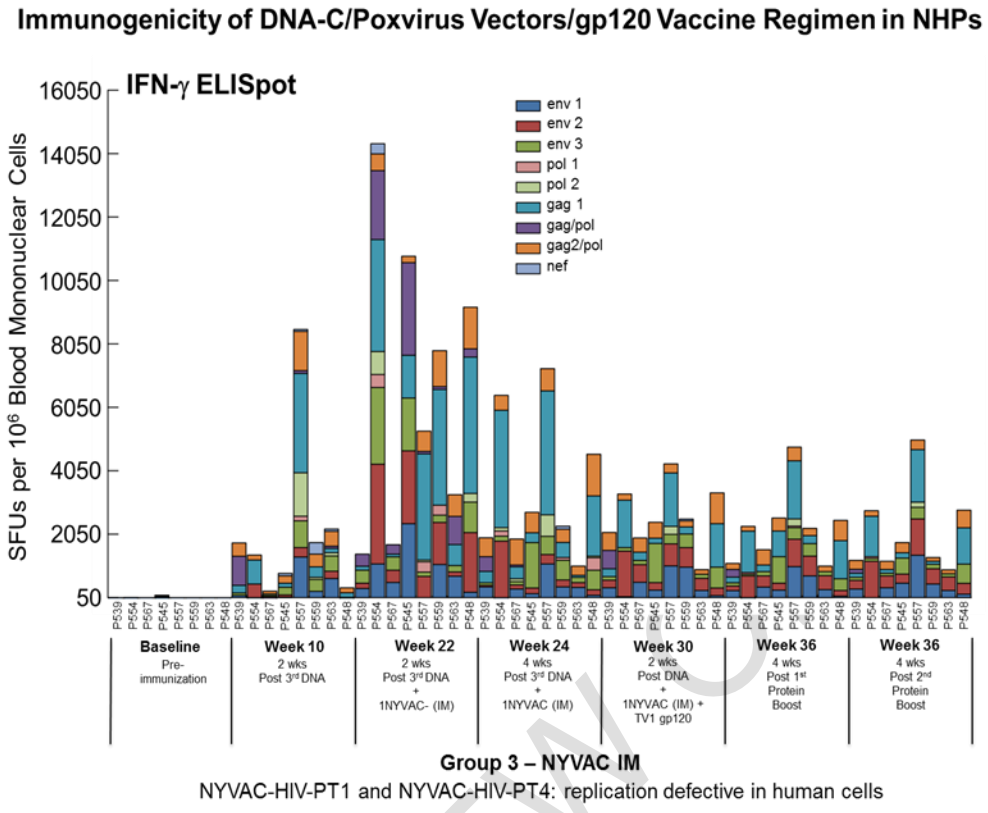


Figure 4-5 Immunogenicity of DNA-C/Poxvirus Vectors/gp120 Vaccine Regimen in NHPs

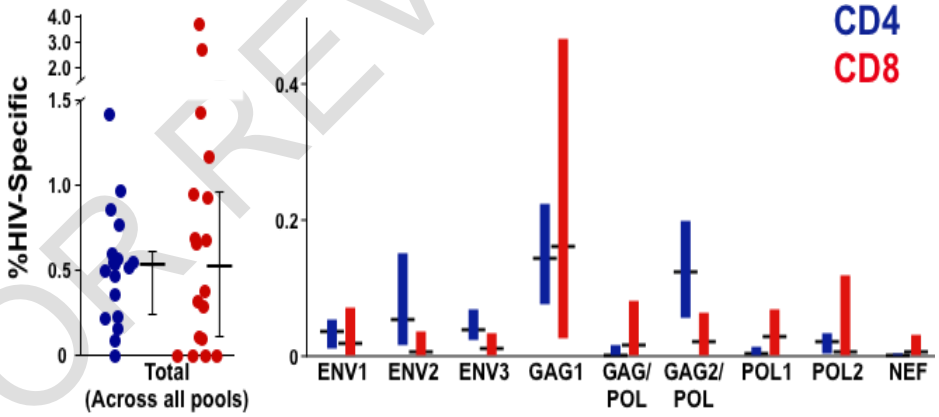


Figure 4-6 Total cytokine response (IFN-γ + TNF-α + IL-2) post 3 DNA immunizations, summed over all peptide pools (left panel) or by individual pool (right panel).

Antibody Responses

Serum and plasma samples have been collected for the analysis of Ab responses. The Ab responses were observed after the protein boost. The Ab responses measured included: a) neutralizing antibody (nAb) responses against Tier 1, 2 and a panel of SHIV viruses, b) Ab-dependent cell-mediated cytotoxicity (ADCC) activity, c) cross clade binding IgG antibodies, d) IgG V1V2-specific antibodies and e) plasma IgA Env-specific Ab. Low level of neutralizing activity and ADCC activity were observed in all 3 groups after

protein boost. The differences in the Ab response levels were not statistically significant ($p>0.05$). Impressive levels of cross clade IgG binding Ab responses and IgG V1V2-specific responses were observed in all 3 groups. Again the differences between the 3 groups were not statistically significant. Similarly, plasma IgA Env-specific Ab responses were observed in all 3 groups after protein boost.

4.8.2 DNA immunization schedule study with DNA-HIV-PT1 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4

The objective of this non-human primate study was to evaluate the effect of 3 different DNA prime schedules on the T-cell and Ab responses following the NYVAC plus protein boost. DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4 were used in this study. The protein is a clade C TV1gp120 and 1086gp120 (mixed at a 1:1 ratio) co-administered with MF59 adjuvant.

The concentration of the DNA-HIV-PT123 used in the NHP study was 2 mg/mL with 2 injections in the left and right legs. So the total dose was 4 mg. NYVAC-HIV-PT1 and NYVAC-HIV-PT4 were formulated together with a final dose of $2x10^8$ PFU/ml ($1x10^8$ PFU/mL each). The total dose of protein is 100mcg/mL.

The study design is provided in Table 4-7.

Table 4-7 Summary of study groups for DNA-HIV-PT123 with NYVAC-HIV-PT1 and NYVAC-HIV-PT4 in NHP

Gp	Size	Wk 0	Wk 2	Wk 4	Wk 8	Wk12	Wk20	Wk24
1	8	DNA	DNA	DNA		NYVAC + protein		NYVAC + protein
2	8	DNA		DNA		NYVAC + protein		NYVAC + protein
3	8	DNA		DNA	DNA		NYVAC + protein	NYVAC + protein

Immunogenicity analyses will be available Q2/2012.

4.8.3 Immunogenicity studies with AIDSVAX MN, AIDSVAX IIIB, and AIDSVAX® B/E

4.8.3.1 Immunogenicity of AIDSVAX MN vs. AIDSVAX IIIB in guinea pigs and rabbits

In initial immunogenicity studies performed in guinea pigs and rabbits [21], AIDSVAX MN was compared with AIDSVAX IIIB for its ability to elicit antibodies that inhibit the binding of rgp120/HIV-1 to recombinant CD4 (rCD4), to react with the PND V3 loop of gp120/HIV-1, and to neutralize a diverse panel of laboratory and clinical isolates of HIV-1. Although both IIIB and MN immunogens elicited similar levels of antibodies to gp120, there were differences in their reactivity. Antisera from animals immunized with AIDSVAX IIIB reacted only with the PND of the homologous strain, whereas antisera to AIDSVAX MN were able to neutralize as many as six of the nine strains tested [21]. In addition, AIDSVAX MN was found to be more effective than AIDSVAX IIIB in eliciting antibodies that inhibit the binding of rgp120/HIV-1 to rCD4. These results demonstrated that certain monovalent vaccines, for example one constructed from HIV-1 MN, can elicit neutralizing antibodies effective against diverse isolates and that the elicited immune response may be effective against antigenically diverse viruses.

4.8.3.2 Immunogenicity of AIDSVAX® B/E in rabbits

While AIDSVAX MN was able to elicit antibodies that neutralized a variety of T-cell tropic viruses, neutralization of macrophage-tropic viruses was more difficult to demonstrate. Preclinical studies with AIDSVAX® B/E showed that rabbit antisera to these vaccines were able to neutralize a variety of T-cell-tropic and macrophage-tropic strains of HIV-1 [22,23]. A rabbit immunization study was conducted to assess if a specific ratio of the two antigens in AIDSVAX® B/E was required to elicit maximum immunogenicity. Varying the subtype B, subtype E antigen ratios from 5:1 to 1:1 to 1:5 had minimal effect on the immune response to either antigen; therefore, VaxGen formulated its bivalent B/E vaccine in a 1:1 ratio of MN rgp120/HIV-1 and A244 rgp120/HIV-1. In laboratory animals, the B/E vaccine was highly immunogenic. In rabbits immunized at day 0, day 14 and day 28, antibodies to gp120 in titers greater than 1:100,000 were elicited after the second immunization and rose slightly after the third immunization. When these sera were mixed with viruses from either the B or E subtypes in a primary isolate neutralization assay used to stimulate primary blood lymphocytes for the target cells, representative viruses were neutralized at titers from 1:20 to 1:1,000 [22,23].

4.8.3.3 Immunogenicity of AIDSVAX IIIB in non-human primates

Chimpanzees, the only laboratory animal susceptible to HIV-1 infection, were used to test the potential ability of AIDSVAX IIIB to protect from HIV-1 infection. In one study [24], two chimpanzees were immunized with 300 mcg of AIDSVAX IIIB and a control chimpanzee received placebo at 0, 1, and 8 months. Three weeks after their final immunization, all animals were challenged with 10 CID_{50} (chimpanzee 50% infective dose) or with 40 $TCID_{50}$ (tissue culture 50% infective dose) of HIV-1 IIIB. The two animals immunized with AIDSVAX IIIB showed no clinical or serologic signs of infection during a 48-month follow-up period, as opposed to the control animal, which had serologic and virologic evidence of infection within 7 weeks of challenge.

4.8.3.4 Immunogenicity of AIDSVAX MN in non-human primates

In a subsequent study [25], three chimpanzees were immunized with 300 mcg of AIDSVAX MN and then challenged with 20 CID_{50} or 748 $TCID_{50}$ of HIV-1 SF2, which has an envelope glycoprotein sequence that differs from MN by over 18%. The single control chimpanzee was infected, whereas the three vaccinated animals showed no sign of infection for over 12 months of follow-up.

No chimpanzee studies were conducted for bivalent AIDSVAX® B/E, as chimpanzee challenge stocks do not exist for the A244 strain of the virus.

4.9 Clinical studies

4.9.1 Clinical data for DNA and NYVAC in EV01, EV02, and EV03

DNA-HIV-PT123, NYVAC-HIV-PT1 and NYVAC-HIV-PT4 have not been administered to humans. However extensive previous clinical experience has been generated with similar vaccines, notably number of NIAID VRC DNA vaccines that have the same DNA plasmid backbone as DNA-HIV-PT123, and different recombinant NYVAC vaccines expressing HIV and antigens from other pathogens that have the same vector backbone as NYVAC-HIV-PT1 and NYVAC-HIV-PT4. Furthermore, the DNA

prime and NYVAC boost regimen has also been extensively studied in humans The EuroVacc Foundation DNA and NYVAC products have been evaluated in various combinations.

In the clinical trial EV01, NYVAC-HIV-C (n=20) or placebo (n=4) was administered at 0 and 1 months. The study indicated that 50% of vaccine recipients had positive interferon gamma (IFN-γ) ELISpot responses [26].

EV02 and EV03 evaluated a DNA with one plasmid expressing a clade C CN54 gp120 and another a CN54 Gag-Pol-Nef polypeptide (DNA-C) followed by a clade C NYVAC boost with the same inserts (NYVAC-HIV-C). Thus far the first generation DNA prime has been evaluated at Months 0, 1 and 0, 1, 2.

In the EV02 clinical trial, it was shown that inclusion of a DNA prime enhanced the immune response. That trial enrolled 40 participants with 35 completing the regimens. Of these 35, 20 received DNA prime at Months 0 and 1 followed by NYVAC boost at Months 5 and 6 and 15 received only 2 doses of NYVAC 4 weeks apart. The DNA-C + NYVAC-HIV-C boost was shown to be a highly immunogenic regimen with T cell responses detected in 90% of vaccine recipients compared to 33% of participants who received NYVAC-HIV-C alone [20].

Volunteers with IFN-γ ELISpot responses in the range of 100 SFU/106 PBMC or above were characterized by flow cytometry, 3 in the NYVAC alone group and 16 in the DNA + NYVAC group. All 19 had HIV-specific CD4+ T cell responses and 50% of the DNA + NYVAC group had detectable HIV-specific CD8+ T cell responses [20]. To evaluate the durability of T cell responses, the protocol was amended to examine T cell responses at week 72 for participants enrolled in Lausanne who had a positive IFN-γ ELISpot response at week 48. Nine of the 11 who were in the DNA + NYVAC group had positive responses at week 72, 1 year after the last injection, while 0 of the 2 in the NYVAC only group had detectable responses.

Vaccine-induced IgG responses against gp140 CN54 were also assessed. Only 25% in the NYVAC only group had positive responses at week 26 (2 weeks post second NYVAC injection), whereas 75% in the DNA + NYVAC group were positive. In sum, the DNA prime enhanced the immune response after the boost by increasing the overall T cell and Ab response rates, increasing the durability of response, and not only inducing a CD4+ T cell response but also increasing the response rate for CD8+ T cells [20].

The EV03 clinical trial showed that priming with 3 DNA injections followed by a NYVAC boost is highly immunogenic, eliciting T cell responses in over 90% of participants. The regimens evaluated in EV03 were DNA administered at Months 0, 1, and 2 followed by a NYVAC boost at Month 6 compared to DNA administered at Months 0 and 1 followed by NYVAC boosts at Months 5 and 6. As shown in Table 4-8, the arm with 3 DNA injections elicited a higher T cell response rate of 94% compared to 81% in the 2 DNA prime arms in a per protocol analysis. The table also illustrates that response rates are strongest to Env, which may prove helpful in a prime-boost regimen that includes an Env protein in the boost [27] [personal communication].

Table 4-8 Proportion of responders by IFN-γ ELISpot at Week 26 or 28 in a per protocol analysis

Antigen	3xDNA + 1xNYVAC n=67	2xDNA + 2xNYVAC n=68
Any	63 (94%)	55 (81%)
Env	62/67 (93%)	53/67 (79%)
Gag/Pol/Nef	26/67 (39%)	17/68 (25%)

The results from EV03 also indicate that the 3 DNA prime injections led to a higher magnitude of T cell response with mean of 774 SFU/10^6 cells compared to 398 for the 2 DNA injection arm as indicated in Table 4-9.

Table 4-9 Magnitude of IFN-γ ELISpot responses at week 26/28 overall (SFUs/106 cells) in positive responders

ITT analysis	Week 26		Week 28	
	3xDNA+1xNYVAC n=58	2xDNA+2xNYVAC n=50	3xDNA+1xNYVAC n=61	2xDNA+2xNYVAC n=54
mean (SD)	774 (622)	398 (318)	597 (519)	357 (319)
Median	545	328	445	235
(IQR; range)	(340-1101; 75-3454)	(178-488; 63-1514)	(170-855; 88-2773)	(123-505; 60-1326)
Mann-Whitney test	p<0.001		p<0.001	

The 3xDNA regimen also elicited durable T cell responses with the majority (72%) of participants still having responses at 72 weeks (1 year after the last injection) as shown in Figure 4-7.

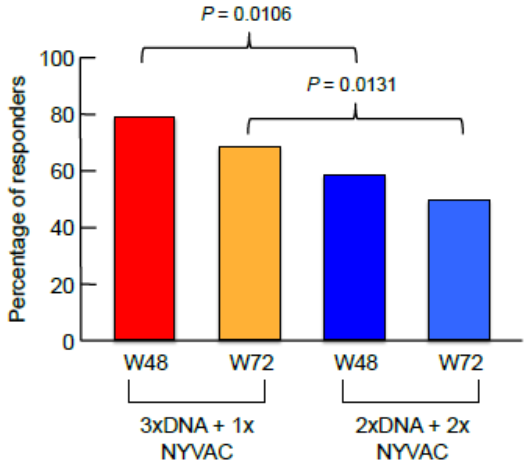


Figure 4-7 Proportion of Env T cell responders at weeks 48 and 72

By IFN-γ ELISpot, the 3xDNA arm elicited broader T cell responses with 37% of responders recognizing Env plus at least one other Gag, Pol, or Nef peptide pool,

compared to 22% in the 2xDNA group. Preliminary flow cytometry data on 27 participants, 14 in the 3xDNA and 11 in the 2xDNA arms also indicates that the 3xDNA regimen confers greater breadth of CD8+ T cell responses as indicated in Figure 4-8.

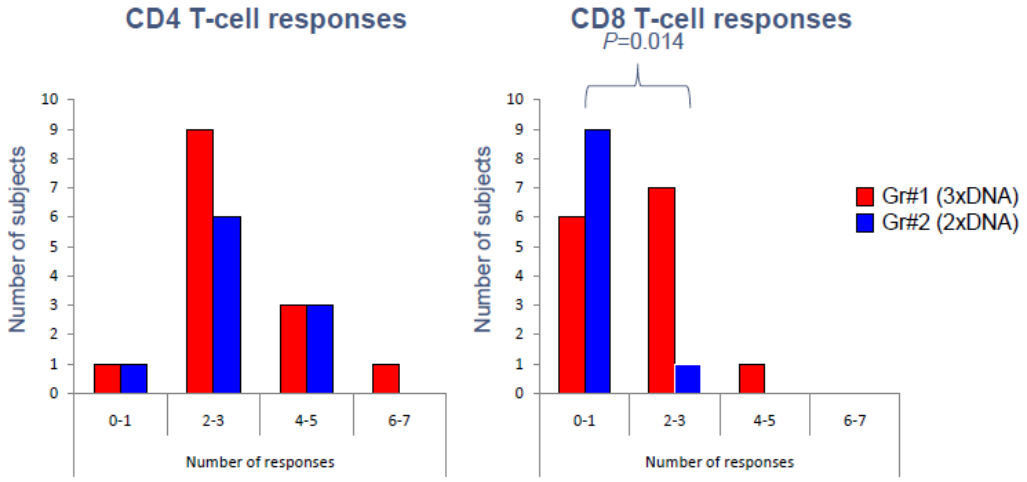


Figure 4-8 CD4+ and CD8+ ICS data on 27 participants, 14 in 3xDNA and 11 participants in 2xDNA (Env/Gag/Pol/Nef pools)

4.9.2 Safety and reactogenicity data in clinical studies of DNA and NYVAC

DNA-HIV-PT123 utilizes a vector backbone developed by Vaccine Research Center, NIAID (p1012 with CMV/R promoter), while NYVAC-HIV-PT1 and NYVAC-HIV-PT4 have the identical vector backbone as NYVAC-HIV-C. As summarized in section 4.9, both the VRC DNA vaccine and NYVAC-HIV-C have been tested extensively in humans.

4.9.2.1 VRC DNA

As outlined in Table 4-10, the VRC DNA plasmids with HIV inserts have been evaluated in 9 clinical studies. Among the local reactogenicity parameters, pain and/or tenderness is the most frequently reported AE for the DNA vaccines. The most frequently reported systemic AEs were headache and malaise/fatigue. To date, there have been no serious adverse events (SAEs) attributable to the DNA vaccine (neither to the rAd5 vaccine nor the combination regimen).

Table 4-10 Clinical Experience with DNA plasmid backbone with HIV gene inserts

Protocol number	clinicaltrials.gov NCT number	Number of ppts received DNA product
VRC 007	NCT00089531	15
VRC 008	NCT00109629	40
VRC 011	NCT00321061	60
VRC 101	NCT00270465	12
HVTN 204	NCT00125970	240
RV 172	NCT00123968	138
IAVI V001	NCT00124007	58
HVTN 073	NCT00574600	40
HVTN 077	NCT00801697	130
HVTN 505	NCT00865566	*1253
Total number of participants who have received DNA HIV product		***1986**
*On April 22, 2013, the NIAID Data Safety Monitoring Board recommended stopping vaccinations in HVTN 505 because preset criteria for declaring efficacy futility were met		

Figure 4-9 below is a summary of reactogenicity of three representative studies.

HVTN 204

A phase 2A multicenter study in the US and South Africa: 480 volunteers were randomized to receive either DNA (4 mg IM by Biojector) at 0, 1 and 2 months followed by rAd5 (10^{10} PFU) at 6 months or placebo [28].

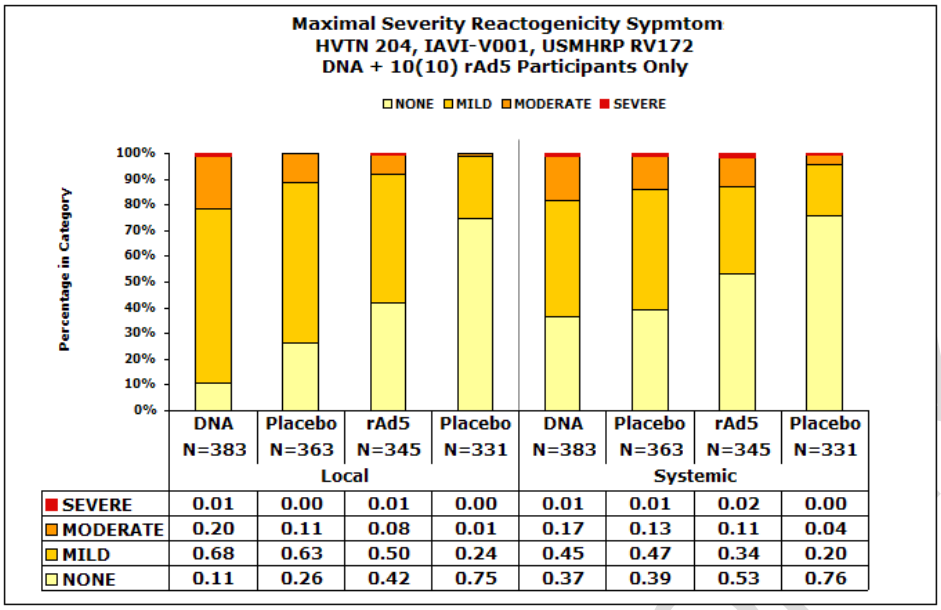
IAVI-V001

A phase 1 study in East Africa: 114 volunteers were randomized to 4 groups receiving the rAd5 vaccine intramuscularly at the dosage level of $1x10^{10}$ or $1x10^{11}$ PFU either alone (single dose at week 0) or as boost (at week 24) following 3 injections of the DNA vaccine (at week 0, 4 and 8) given at 4 mg/dose intramuscularly by needle-free injection using Biojector® [29].

RV172

A phase 1/2 study in East Africa: 324 volunteers were randomized to receive placebo (n=138), a single dose of rAd5 at 10^{10} (n=24) or 10^{11} (n=24) PFU, or DNA at 4 mg at 0, 1 and 2 months followed by rAd5 at either 10^{10} (n=114) or 10^{11} (n=24) PFU boosting at 6 months [30].

Across the diverse population in the triad studies, reactogenicity was reported as none to mild in severity in 70% to 90% of all vaccine recipients. Of note, the DNA in the above studies was delivered **by Biojector**® (a needle free injection device) rather than by **standard needle** as in this protocol.



	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 4-9 Severity of Local and Systemic AEs in the Triad Studies (HVTN 505 Vaccine/Control Regimen Only)

4.9.2.2 NYVAC-HIV-C

NYVAC- C has been tested in three phase 1 and 2 clinical studies: EV01, EV02 and EV03/ANRSVAC20.

EV01 is a small phase 1 study with 20 volunteers who received 2 injections of NYVAC-HIV-C with 4 weeks apart.

EV02 is a phase 1 study with 20 volunteers who received 2 injections of DNA-C (with a ***different*** vector backbone than DNA-HIV-PT123) at weeks 0 and 4 and 2 injections of NYVAC-HIV-C at weeks 20 and 24, and 15 volunteers who received 2 injections of NYVAC-HIV-C with 4 weeks apart.

EV03/ANRSVAC20 is a phase 1/2 multicenter study. Seventy-four (74) volunteers were randomized to the group with 3 injections of DNA-C at weeks 0, 4, 8 and 1 injection of NYVAC-HIV-C at week 24; and seventy-three (73) volunteers were randomized to the group with 2 injections of DNA-C at weeks 0, 4 and 2 injections of NYVAC-HIV-C at weeks 20 and 24.

In all three studies, NYVAC-HIV-C has been shown to be safe and well tolerated. The safety data of EV01 and EV02 have been published [26,31]. Table 4-11 and Table 4-12 below provide a summary of the safety data of NYVAC-HIV-C in the EV03/ANRSVac20, which is also representative of the previous studies EV01 and EV02.

It is important to underscore that the DNA HIV vaccine used in EV02 and EV03 did not have the VRC DNA vector backbone. Therefore, any event reported below related to DNA in EV02 and EV03 does not refer to the DNA which will be used in the present HVTN096/EV04.

Table 4-11 EV03/ANRSVAC20: Number (%) of Participants with Solicited Local Events

Grade of Events	Overall NYVAC	
	Group 1: 3xDNA + 1xNYVAC (n=74)	**Group 2: 2xDNA+2xNYVAC (n=73)**
Grade 1(mild)	39 (53%)	54 (74%)
Grade 2 (moderate)	2 (3%)	7 (10%)
Grade 3 (severe)	2 (3%)	0
Total AEs	*43 (58%)*	*61 (84%)*

Pain was the most common local AE. In Group 1, *pain* was absent in 38 participants (51%), mild in 34 participants (46%) and moderate in 2 participants (3%). In Group 2, pain was reported absent in 17 participants (23%), mild in 51 participants (70%) and moderate in 5 participants (7%). *Itching* in both Group 1 and 2 was absent in 76% of the participants, mild in 23% of the participants and moderate in 1% of the participants. *Erythema* was absent in 58 participants (78%) and mild in 16 participants (22%) in Group 1, and absent in 48 participants (66%) and mild in 25 participants (34%) in Group 2. In Group 1, *induration* was absent in 63 participants (85%), mild in 9 participants (12%) and severe in 2 participants (3%). In Group 2, induration was absent in 52 participants (69%), mild in 20 participants (28%) and moderate in 1 participant (1%).

Table 4-12 EV03/ANRSVAC20: Number (%) of Participants with Solicited Systemic Events

Grade of Events	Overall NYVAC	
	Group 1: 3xDNA + 1xNYVAC (n=74)	**Group 2: 2xDNA+2xNYVAC (n=73)**
Grade 1(mild)	27 (36%)	28 (38%)
Grade 2 (moderate)	4 (5%)	9 (12%)
Grade 3 (severe)	0	1 (1%)
Total AEs	*31 (42%)*	*38 (52%)*

Systemic symptoms (fever, chills/rigors, malaise, myalgia, headache, nausea and vomiting) were absent in 43 participants (58%) in Group 1, and 35 participants (48%) in Group 2. The most common systemic AEs are malaise, myalgia and headache: mild in 15-22% of the participants in Group 1 and 19-26% of the participants in Group 2; moderate in 1-3% of the participants in Group 1 and 3-20% of the participants in Group 2; 1 event was reported as severe in Group 2.

EV03/ANRSVAC20: Laboratory Events

Biochemistry, hematology and microbiology were performed after each vaccination. In total, 11 Grade 2 and 56 Grade 1 abnormalities were reported, and all were considered not clinically significant with the exception of one case of increased ALT after the 1st DNA-C vaccination and resulted in a change of immunization schedule. One Grade 3 raised AST and 1 Grade 3 bilirubin were reported, both occurred after the DNA-C immunization and were considered not related to the vaccine. No changes in routine laboratory parameters were considered clinically significant.

EV03/ANRSVAC20: Serious Adverse Events

There were nine SAEs reported, 8 in Group 1 and 1 in Group 2. Eight were considered to be "unrelated" or "unlikely to be related" to the vaccines. Only one event was considered possibly related to the vaccines, which occurred after the first DNA-C immunization and further immunizations were discontinued with this participant. This event is described below.

Case 1 (EF049007Z): A 48-year-old man suffered a severe vaso-vagal malaise starting within minutes of the first 2 mL DNA-C injection in the thigh, at the time of first vaccination (week 0). Symptoms began with severe local pain at the injection site, followed by loss of consciousness and tonic-clonic spasm. This episode resolved within two minutes. Postictal amnesia was noted. The participant fully recovered within a few minutes without the need of any medications. Additional investigations (ECG, EEG, MRI) were normal, and the investigators concluded that this event was "possibly related to vaccine". In view of the tonic-clonic spam and amnesia, this SAE was considered as unexpected (SUSAR). Following a multi-disciplinary review of the case by the sponsors and medical experts, a decision was made to discontinue immunizations in this individual but there was no indication to change the conduct of the trial.

Table 4-13 Summary of relevant NYVAC titers

Study	Product Name	Titer
Clinical studies		
EV01 (Section 4.9.2.2)	NYVAC-HIV-C (Sanofi lot)	$1x10^{7.7}$ $CCID_{50}$/ml (approximately $3.539x10^{7}$pfu/ml, note this is only indicative)
EV02 (Section 4.9.2.2)	NYVAC-HIV-C (Sanofi lot)	$1x10^{7.7}$ $CCID_{50}$/ml (approximately $3.539x10^{7}$pfu/ml, note this is only indicative)
EV03 (Section 4.9.2.2)	NYVAC-HIV-C (Transgene lot)	$1x10^{7.4}$ pfu/ml
Preclinical studies		
AUP444 (Section 4.8.1)	NYVAC-HIV-PT1 and NYVAC-HIV-PT4	Total dose $2x10^{8}$ pfu/ml ($1x10^{8}$ pfu/ml of each)
AUP485 (Section 4.8.2)	NYVAC-HIV-PT1 and NYVAC-HIV-PT4	Total dose $2x10^{8}$ pfu/ml ($1x10^{8}$ pfu/ml of each)
Toxicity studies		
SVT11-02 (Section 4.7.7)	NYVAC-HIV-PT1 and NYVAC-HIV-PT4	NYVAC-HIV-PT1: $1.2x10^{8}$ pfu/ml, NYVAC-HIV-PT4: $1.13x10^{7}$ pfu/ml
Covance 1651/18 & 19 in rats (Section 4.7.5)	NYVAC-HIV-C (Sanofi lot)	$1x10^{7.7}$ $CCID_{50}$/ml (approximately $3.539x10^{7}$pfu/ml, note this is only indicative), 0.4 ml per injection
RGU/001 in mice (Section 4.7.6)	NYVAC-HIV-C (Sanofi lot)	$1x10^{7.7}$ $CCID_{50}$/ml (approximately $3.539x10^{7}$pfu, note this is only indicative), 100ul per injection
Covance 2405/001 in rats (Section 4.7.4.1)	NYVAC-HIV-B (Transgene lot)	$1x10^{7.4}$ pfu/ml, 0.4 ml per injection
VAC 0301/R (Section 4.7.4.2)	NYVAC-HIV-B (Transgene lot)	$1x10^{7.4}$ pfu/ml

4.9.3 Clinical data in ALVAC-HIV (VCP1521) priming with gp120 AIDSVAX® B/E boost phase 3 trial (RV144)

In the RV144 study, the combination of ALVAC-HIV (Sanofi Pasteur) boosted by AIDSVAX® B/E (at a standard dose of 300 mcg of each antigen in alum adjuvant) was tested for safety and efficacy in a randomized, placebo-controlled double-blinded phase 3 trial in Thailand [4]. Immunizations were administered in a 1:1 ratio of either vaccine or placebo at Weeks 0, 4, 12, and 24; at Weeks 0 and 4 volunteers either received ALVAC or placebo, and at Weeks 12 and 24 volunteers received ALVAC + AIDSVAX® B/E or placebo. The study was designed to evaluate two co-primary endpoints: the prevention of HIV-1 infection and the effect of vaccination on the early viral load after infection. The trial was conducted through facilities of the Thai Ministry of Public Health in Rayong and Chon Buri provinces. From September 2003 through December 2005, a total of 16,402 eligible volunteers between the ages of 18 and 30 years were enrolled. The last volunteer completed study participation in June 2009, and the database was locked in July 2009. The final analyses were completed in September 2009, with the announcement of the results at the AIDS Vaccine Conference 2009, Paris, France.

Both intention-to-treat (ITT) and per-protocol (PP) analyses were conducted. The ITT analysis included all subjects who underwent randomization. Seven persons who were enrolled and vaccinated were found to be positive for HIV-1 RNA at baseline. The PP analysis included a subgroup of subjects in the ITT analysis who received the entire series of vaccinations within the defined time period, who remained eligible to participate in the study, and who did not have HIV infection at the time of the fourth vaccination. A separate subgroup analysis called the modified ITT (mITT) analysis was used for the

interim and final analyses and excluded the seven volunteers who were found to have HIV infection at baseline.

There were 132 infections (56 in the vaccine group and 76 in the placebo group) in the ITT analysis involving 16,402 subjects; 86 infections (36 in the vaccine group and 50 in the placebo group) in the PP analysis involving 12,452 subjects; and 125 infections (51 in the vaccine group and 74 in the placebo group) in the mITT analysis involving 16,395 subjects. The observed vaccine efficacy was 26.4% in the ITT analysis (see Figure 4-10); 26.2% in the PP analysis (see Figure 4-11); and 31.2% in the mITT analysis (see Figure 4-12). There was no significant difference in the mean viral load among subjects who were found to have HIV infection in the vaccine group, as compared with those in the placebo group (see Figure 4-13).

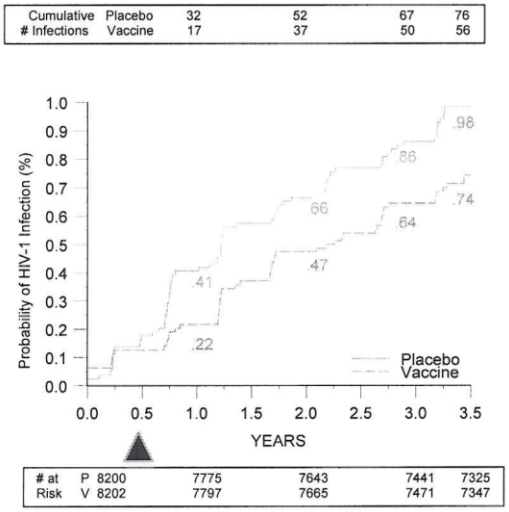


Figure 4-10 RV144 Intention-to-Treat Analysis

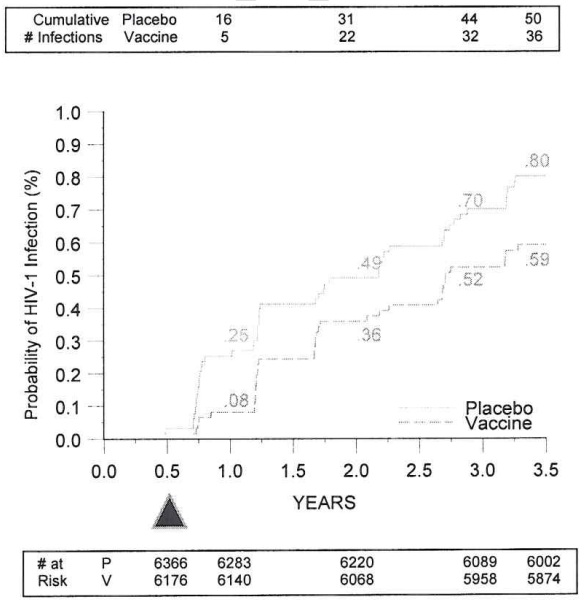


Figure 4-11 RV144 Per-Protocol Analysis

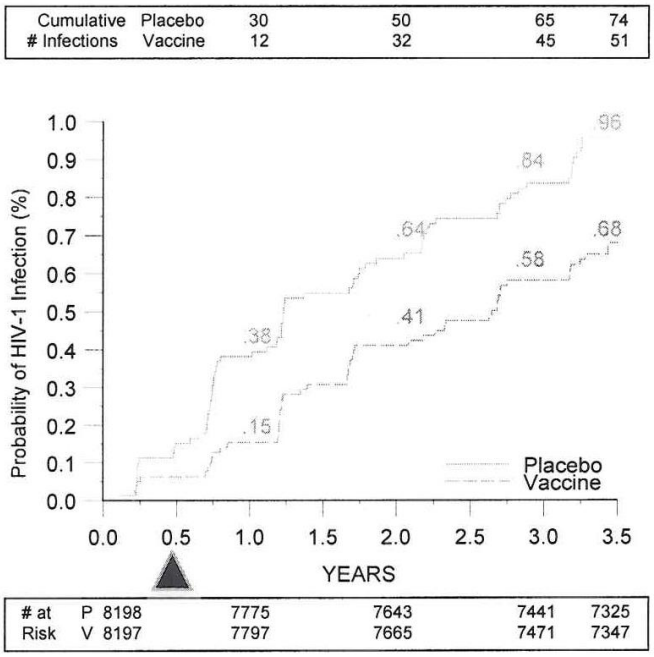


Figure 4-12 RV144 Modified Intention-to-Treat Analysis

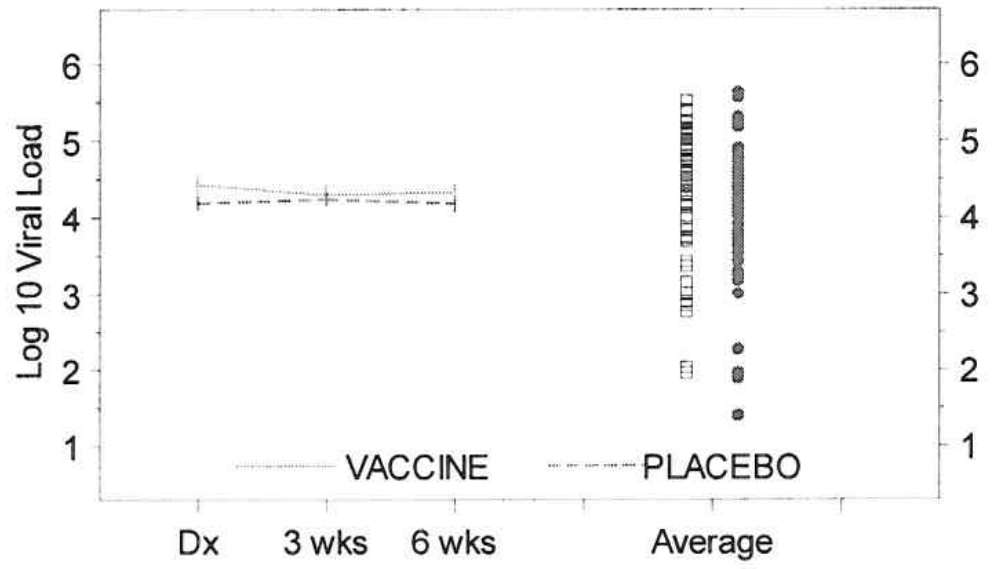


Figure 4-13 RV144 Viral Load in Subjects with Early HIV-1 Infection

The phase 3 efficacy trial of the prime-boost of ALVAC-HIV and AIDSVAX® B/E was conducted in Thailand (RV144). The immunization schedule was Day 0, Weeks 4, 12, and 24 for ALVAC-HIV. Boosting with AIDSVAX® B/E occurred at Weeks 12 and 24.

To evaluate immunogenicity, plasma and cells from volunteers who did not have HIV infection were analyzed at various timepoints after vaccination. After removal of a small subgroup of samples for future matched case-control studies, random samples were identified and provided in a blinded fashion to the Armed Forces Research Institute of Medical Sciences Laboratory at a ratio of samples from the vaccine group to samples from the placebo group of approximately 4:1. The immunogenicity of the vaccine

regimen was measured with the use of the following validated assays: IFN-γ ELISpot and CD4+ and CD8+ intracellular cytokine staining (ICS) for interferon-γ and interleukin-2 to Gag and Env; binding Ab to gp120 in the MN strain, gp120 in the A244 strain (CM244), and p24 Gag; and lymphoproliferation to gp120 MN, gp120 A244, and p24. Both humoral and cellular immunogenicity assays have been employed to understand the correlates of protection in RV144.

4.9.4 Safety in ALVAC-HIV priming with gp120 AIDSVAX® B/E boost phase 3 trial (RV144)

In the Thai RV144 study, AEs were monitored every 6 months for 3.5 years, and pregnancy outcomes were recorded [32]. Of the 16,402 volunteers, 69% of the participants reported an AE at any time on study after the first dose of administration. Only 32.9% of events occurred within 30 days post any vaccination, 3.2% attributed to vaccine and overall AE rates did not differ between groups. The frequency of SAEs was similar in vaccine (14.3%) and placebo (14.9%) recipients (p=0.33). None of the reported SAEs were related to vaccine. Female participants experienced a higher frequency of AEs and SAEs in both vaccine and placebo groups. The reasons for this difference are unclear.

None of the 160 deaths (85 in vaccines, 75 in placebo, p=0.43) were assessed as related to vaccine and were mostly related to trauma and cardiovascular causes.

Approximately 30% of female participants reported a pregnancy during the study. Overall, the prime-boost regimen did not result in more abnormal pregnancy outcomes in vaccine than in placebo female recipients. Abnormal pregnancy outcomes were experienced in 17.1% and 14.6% (p=0.13) of vaccine and placebo recipients with pregnancies, respectively. Among pregnancies with estimated dates of conception within three months of a vaccination, the large majority of these abnormal outcomes were spontaneous or elective induced abortions reported in 22.2% and 15.3% of vaccine and placebo pregnant recipients, respectively (p=0.08).

Reactogenicity was recorded for three days following vaccination. Most of the vaccine recipients experienced some type (either local or systemic) of reaction, significantly more frequently than in placebo recipients; local reactions were more frequently observed than systemic reactions; pain and tenderness and headache, fatigue, arthralgia, and myalgia were the most frequently reported local and systemic reactions, respectively, while fever was rarely reported; ALVAC-HIV is more reactogenic than AIDSVAX® B/E compared to placebo; the frequency of the reactions gradually declined with subsequent vaccine administrations; all local and systemic reactogenicity symptoms were mild to moderate in nature, resolving rapidly and spontaneously in the vast majority of cases within three days. Local reactions occurred in 88.0% of vaccine and 61.0% of placebo recipients (p<.001) and were more frequent after ALVAC-HIV than AIDSVAX® B/E vaccination. Systemic reactions were more frequent in vaccine than placebo recipients (77.2% vs. 59.8%, p<.001).

The ALVAC-HIV and AIDSVAX® B/E vaccine regimen was found to be safe, well-tolerated and may be suitable for large-scale public use in Thailand.

4.10 Potential risks of study products and administration

Table 4-14 Summary of potential risks of study products and administration

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 antibody test result
Less common	• 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, other transient lesions, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis
Unknown frequency	• Myopericarditis (NYVAC)
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

5 Objectives and endpoints

5.1 Primary objectives and endpoints

Primary objective 1:

To evaluate the safety and tolerability of i) NYVAC prime plus NYVAC + AIDSVAX® B/E boosts; ii) NYVAC + AIDSVAX® B/E prime plus NYVAC + AIDSVAX® B/E boosts; iii) DNA prime plus NYVAC + AIDSVAX® B/E boosts; iv) DNA + AIDSVAX® B/E prime plus NYVAC + AIDSVAX® B/E boosts in HIV-1-uninfected healthy adults

Primary endpoint 1:

Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and adverse and SAEs

Primary objective 2:

To evaluate and compare the immunogenicity of 4 prime-boost regimens, comparing between:

- NYVAC and NYVAC/AIDSVAX® B/E primes (Group 1 versus 2)
- DNA and DNA/AIDSVAX® B/E primes (Group 3 versus 4)

Primary endpoint 2:

HIV-specific cross-clade binding IgG Env Ab response as assessed by multiplex assay 2 weeks after the fourth vaccination

Primary objective 3:

To evaluate and compare the durability of the vaccine-induced Ab response for different priming regimens when followed by two doses of NYVAC/AIDSVAX® B/E, comparing between:

- NYVAC and NYVAC/AIDSVAX® B/E primes (Group 1 versus 2)
- DNA and DNA/AIDSVAX® B/E primes (Group 3 versus 4)

Primary endpoint 3:

HIV-specific cross-clade binding IgG Env Ab response as assessed by multiplex assay at multiple timepoints between 2 weeks and 12 months after the last NYVAC/AIDSVAX® B/E boost

5.2 Secondary objectives and endpoints

Secondary objective 1:

To evaluate and compare the immunogenicity of priming regimens, comparing between:

- NYVAC and NYVAC/AIDSVAX® B/E primes (Group 1 versus 2)
- DNA and DNA/AIDSVAX® B/E primes (Group 3 versus 4)

Secondary endpoint 1:

HIV-specific cross-clade binding IgG Env Ab response as assessed by multiplex assay 2 weeks after the second vaccination

Secondary objective 2:

To evaluate the immunogenicity of different priming regimens when followed by two doses of NYVAC/AIDSVAX® B/E, comparing between:

- NYVAC and NYVAC/AIDSVAX® B/E primes (Group 1 versus 2)
- DNA and DNA/AIDSVAX® B/E primes (Group 3 versus 4)
- NYVAC and DNA primes (Group 1 versus 3)
- NYVAC/AIDSVAX® B/E and DNA/AIDSVAX® B/E primes (Group 2 versus 4)

Secondary endpoints 2:

- HIV-specific total binding IgG and IgA binding Ab responses as assessed by multiplex assay 2 weeks after the third and fourth vaccinations
- Anti-V2 IgG binding Ab responses assessed by binding to gp70-V1V2 2 weeks after the third and fourth vaccinations
- nAb magnitude and breadth against tier 1 and tier 2 HIV-1 isolates as assessed by area under the magnitude-breadth curves 2 weeks after the third and fourth vaccinations
- Magnitude and frequency of HIV-specific T cell responses as assessed by flow cytometry and/or multiplex bead array 2 weeks after the third and fourth vaccinations

Secondary objective 3

To evaluate the durability of vaccine induced immune responses in the four vaccination regimens

Secondary endpoints 3:

- HIV-specific total binding IgG and IgA binding Ab responses as assessed by multiplex assay 6 months after the last vaccination
- Anti-V2 IgG binding Ab responses assessed by binding to gp70-V1V2 6 months after the last vaccination

- nAb magnitude and breadth against tier 1 and tier 2 HIV-1 isolates as assessed by area under the magnitude-breadth curves 6 months after the last vaccination
- Magnitude and frequency of HIV-specific T cell responses as assessed by flow cytometry and/or multiplex bead array 6 months after the last vaccination

Secondary objective 4:

To evaluate the immunogenicity of the 4 priming regimens

Secondary endpoints 4:

- HIV-specific total binding IgG and IgA binding Ab responses as assessed by multiplex assay 2 weeks after the second vaccination
- Anti-V2 IgG binding Ab responses assessed by binding to gp70-V1V2 2 weeks after the second vaccination
- nAb magnitude and breadth against tier 1 and tier 2 HIV-1 isolates as assessed by area under the magnitude-breadth curves 2 weeks after the second vaccination
- Magnitude and frequency of HIV-specific T cell responses as assessed by flow cytometry and/or multiplex bead array 2 weeks after the second vaccination

5.3 Exploratory objectives

Exploratory objective 1

To evaluate HIV-specific mucosal IgA, IgG, and V2 Ab responses elicited by the study vaccine regimens

Exploratory endpoint 1

HIV-specific total IgG, IgA, and V2 binding Ab responses in cervical, rectal, semen, and/or saliva secretions, as assessed by binding Ab multiplex assay

Exploratory objective 2

To further evaluate HIV-specific immune responses to the vaccine regimens in HIV-1-uninfected healthy adults

Exploratory endpoints 2 (may be conducted)

- Titers of Ab-dependent cellular cytotoxicity antibodies
- HIV-specific IgG subclass (IgG1-IgG4) characterization as determined by HIV-1 multiplex Ab assay
- Avidity indices for Env-specific Ab
- HIV-specific IgG epitope mapping by peptide array
- B-cell ELISpot
- HIV-capturing non-neutralizing antibodies as assessed by competitive virus capture assay

- Expression of cytokines (eg IL-10, IL-13) by multiplex bead array following antigen-specific stimulation of PBMC

Exploratory objective 3

To compare characteristics of the immune responses induced by this regimen with that of future regimens utilizing DNA/NYVAC/Env protein +MF59.

Exploratory endpoint 3

Humoral and cellular immunogenicity data obtained from state of the art, immunologically relevant assays will be compared.

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target 96 healthy, HIV-uninfected adult participants. There will be four groups, 20 vaccine recipients and 4 placebo recipients in each group.

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. Hence, sample size calculations for safety in the section below are based on the target sample sizes. It is possible, however, for immunogenicity data to be missing; previous HVTN and AIDS Vaccine Evaluation Group (AVEG) studies suggest 10% is a reasonable estimate for the rate of missing data. For this reason, the sample size calculations for immunogenicity in Sections 6.1.2 through 6.1.6 account for 10% of enrolled participants having missing data for the primary immunogenicity endpoints.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. Sample size calculations for safety are expressed in terms of the ability to detect AEs requiring expedited reporting.

Probabilities of observing 0, 1 or more, and 2 or more events among arms of size 20 and among the combined vaccine arms (n=80) are presented in Table 6-1 for a range of possible true AE rates. These calculations provide a complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine. In previous AVEG HIV vaccine trials, 3.5% of control participants experienced an SAE; in HVTN vaccine trials (as of January 2006) about 1% of control participants experienced such an event. Based on Table 6.1 we see that if the true rate of AEs is 1%, there is an 82% chance that no events will be observed in an individual vaccine arm, and a 45% chance that no events will be observed in any of the 4 vaccine arms.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 20 or all vaccine arms combined (n=80), for different true event rate

True event rate (%)	Pr(0/20)	Pr(1+/20)	Pr(2+/20)	Pr(0/80)	Pr(1+/80)	Pr(2+/80)
1	0.818	0.182	0.017	0.448	0.552	0.191
3.5	0.490	0.510	0.154	0.058	0.942	0.774
5	0.358	0.642	0.264	0.017	0.983	0.914
10	0.122	0.878	0.608	0.000	1.000	0.998
20	0.012	0.988	0.931	0.000	1.000	1.000
30	0.001	0.999	0.992	0.000	1.000	1.000
40	0.000	1.000	0.999	0.000	1.000	1.000

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval (CI) for the true rate of an AE based on the observed data. Table 6-2 shows the 2-sided 95% CIs for the probability of an event based on a particular observed rate. Calculations are done using the score test method [33]. If none of the 80 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population

is 5.5%. For each individual vaccine arm (n = 20), the 2-sided upper confidence bound for this rate is 18.9%.

Table 6-2 Two-sided 95% CIs based on observing a particular rate of safety endpoints for individual arms of size 20 and all vaccine arms combined (n = 80)

Observed event rate	CI (%)
0/20	(0, 18.9)
1/20	(0, 25.4)
2/20	(1.56, 31.3)
0/80	(0, 5.5)
1/80	(0, 7.4)
2/80	(0.16, 9.2)

6.1.2 Sample size calculations for immunogenicity

The first component of the analysis will entail comparing the rate of the IgG binding Ab response after the fourth vaccination (Month 6.5) between each of the vaccine arms and the pooled placebo group using a binomial test. The Holm method [34] will be used to control the family-wise error rate at 5%. The arms that are significant at the adjusted $p < 0.05$ level are said to pass the "Month 6.5 placebo screen."

To address primary objective 2, the analysis will compare the regimens that pass the Month 6.5 placebo screen with respect to magnitude of the IgG binding Ab response after the fourth vaccination (Month 6.5). Groups 1 and 2 will be compared, and groups 3 and 4 will be compared, each time using a two-sided Wilcoxon rank sum test with 5% type-I error rate.

Before comparing vaccine regimens with respect to durability of the IgG binding Ab response (primary objective 3), a second screening will take place. The rate of the IgG binding Ab response at the last immunogenicity timepoint (Month 18) will be compared between each of the vaccine arms and the pooled placebo group. Again a binomial test and the Holm multiplicity correction will be employed. The arms that are significant at the adjusted $p < 0.05$ level are said to pass the "Month 18 placebo screen." Only arms that pass the Month 18 placebo screen will be investigated with respect to durability of response.

To address primary objective 3, durability of the IgG binding Ab response will be assessed for regimens that pass the Month 18 placebo screen. Vaccine groups 1 and 2 and between groups 3 and 4 will be compared. A single exponential decay model will be fit to the response data from Months 6.5, 7, 9, 12, 15, and 18 for each vaccine arm using generalized estimating equations (GEE). This model will be used to estimate the half-life of the response for each arm, which will then be compared between vaccine groups 1 and 2 (and 3 and 4) using a two-sided generalized Wald test with 5% type-I error rate. Bootstrapping will be used to obtain CIs and to compute a p-value.

6.1.3 Power for detecting vaccine arms superior to placebo at Month 6.5

The trial has nearly 100% power to detect clear superiority of the vaccine arms versus placebo with respect to rate of IgG binding Ab response. This result is based on pilot data for the IgG binding Ab response in the Thai RV144 trial, where the placebo group

consisted entirely of zero responses and the vaccine group had 98% non-zero responses. For our calculations, binomial response data were simulated, where placebo recipients uniformly had zero responses and each of the 4 vaccine arms had 70%, 85% or 98% non-zero responses. Calculations were based on 14 subjects in the placebo group and 18 in each vaccine arm to allow for 10% missing immunogenicity data. 100% of 5,000 simulated datasets found all vaccine arms to be significantly better than placebo at the Holm-adjusted $p < 0.05$ level based on a two-sided binomial test. We conclude that the trial is exceptionally well-powered to detect vaccine regimens with the same or substantially lower IgG binding response rates as observed in the RV144 trial.

6.1.4 Power for comparing groups 1 vs 2 and 3 vs 4 with respect to IgG binding Ab response at Month 6.5 (primary objective 2)

Power was estimated assuming that all four vaccine regimens passed the Month 6.5 placebo screen, with 18 subjects per arm to allow for 10% missing immunogenicity data. Results are shown for comparing groups 1 and 2, and are the same for comparing groups 3 and 4.

Data were simulated assuming that the proportion of non-zero responses was either 0.85 or 0.98 in both groups. Non-zero responses, modeled on the natural log scale, were drawn from a normal distribution. The mean non-zero response in group 1 was set to 5.9. This was the mean non-zero response in the RV144 vaccine arm. The mean non-zero response in group 2 was varied between 6.4 and 8.4. The standard deviation (SD) of the non-zero response was set to 1.3 for both groups; this was the SD of the non-zero response in the RV144 vaccine arm.

Table 6-3 shows the power for comparing groups 1 and 2 (or groups 3 and 4) with respect to IgG binding Ab response at Month 6.5 using a two-sided Wilcoxon rank sum test with 5% type-I error. Note that if 98% of individuals have non-zero responses in both groups and 5.9 and 7.9 are the mean non-zero responses in the lower and higher groups, the overall mean responses are 5.8 and 7.7, respectively. The power for comparing such groups is 98%. However if only 85% of subjects have non-zero responses the power is 79%. We conclude that the trial is well-powered to compare groups that differ in mean non-zero response by at least 1 SD under a 98% non-zero response rate, or by 1.75 SD under an 85% response rate.

Table 6-3 Power for comparing two vaccine groups with respect to IgG binding Ab response at Month 6.5 using a two-sided Wilcoxon rank sum test with type-I error 0.05, as a function of the proportion of non-zero responses in both groups and the mean non-zero response in group 2. The mean non-zero response in group 1 was set to 5.9. The SD of the non-zero response was 1.3 for both groups. Calculations assume 18 participants per group. Power was estimated using 5,000 simulations.

Mean of non-zero response in group 2	85% non-zero responses in both groups	98% non-zero responses in both groups
6.4	0.13	0.17
6.9	0.34	0.53
7.4	0.61	0.87
7.9	0.79	0.98
8.4	0.90	1.00

6.1.5 Power for detecting vaccine arms superior to placebo at Month 18

The trial is well-powered to compare response rates between the vaccine arms and placebo at Month 18. This result is based on simulating binomial response data, where placebo recipients uniformly had zero responses and each of the 4 vaccine arms had a 55, 57, 60, 62, or 65% non-zero response rate. For comparison, we note that in the RV144 trial, 99% of vaccine recipients had positive IgG binding Ab responses 6 months after the last vaccination. Our calculations were based on 14 subjects in the placebo group and 18 in each vaccine arm to allow for 10% missing immunogenicity data. Vaccine and placebo groups were compared using two-sided binomial tests with Holm multiplicity correction. As shown in Table 6-4 , the power for comparing vaccine groups with 55% response rates to placebo is 78%; with a 60% non-zero response rate the power is 91%. We conclude that the trial is well-powered to detect vaccine regimens with substantially lower response rates than observed in the RV144 trial.

Table 6-4 Power for comparing IgG binding Ab response rates at Month 18 between each vaccine group and the pooled placebo group using a binomial test, as a function of the proportion of non-zero responses in the worst-performing vaccine group. Holm-adjusted p-values are used to control the family-wise error rate at 0.05. Calculations assume 18 participants per vaccine group and 14 participants in the placebo group. Power was estimated using 5,000 simulations.

Response rate in worst-performing vaccine group (%)	Power
55	0.78
57	0.85
60	0.91
62	0.95
65	0.98

6.1.6 Power for comparing the durability of IgG binding Ab response between groups 1 vs 2 and 3 vs 4 (primary objective 3)

Power was estimated assuming that all four vaccine regimens passed the Month 18 placebo screen, with 18 subjects per arm to allow for 10% missing immunogenicity data. Results are shown for comparing groups 1 and 2, and are the same for comparing groups 3 and 4.

Data were simulated assuming that peak IgG binding Ab responses are observed at Month 6.5 (after the last boost). The proportion of non-zero responses at the peak timepoint was set to either 0.85 or 0.98 in both groups. Non-zero responses at the peak timepoint were assumed to be normally distributed on the natural log scale. Responses at subsequent timepoints (Months 8.5 through 18) were generated from a single exponential decay model, whereby the log response at a given timepoint (t) is normally distributed with mean equal to the log peak response minus a decay parameter (k) multiplied by the time elapsed between peak timepoint and t. The half-life corresponding to such a model is log(2)/k. In group 1, the mean and SD of the non-zero peak log response were set to 5.9 and 1.3, respectively, corresponding to the estimates of these values in RV144. The SD in the log response at the subsequent timepoints was also assumed to be 1.3. The half-life was set to 45 days; this is an estimate of the half-life of the IgG binding Ab response in RV144. For group 2, the mean and SD of the positive peak log response were also set to 5.9 and 1.3; power is invariant to this mean parameter. The SD in the log response at the subsequent timepoints was again assumed to be 1.3. The half-life for group 2 was varied between 50 and 65 days, representing 1.1 to 1.4 times the half-life in the group 1.

The analysis of durability was restricted to subjects with non-zero responses at Month 6.5, the peak timepoint. Ab half-life was estimated by fitting a GEE model to the data for each group, and the half-lives compared using a two-sided generalized Wald test with 5% type-I error rate. Bootstrapping was used to compute a p-value.

Table 6-5shows the power for comparing groups 1 and 2 (or groups 3 and 4) with respect to IgG binding Ab half-life, based on the subset of subjects with non-zero binding Ab responses at Month 6.5. Power is reported as a function of the proportion of non-zero responses at Month 6.5, assumed to be the same in the two groups, and of the half-life in the second group. We conclude that if the response rates at Month 6.5 are 98%, the trial has adequate power to compare one group to another provided the half-life is increased by a factor of 1.2 or more. If the response rates are 85%, a 1.3-fold increase in the half-life is required for adequate power.

Table 6-5 Power for comparing two vaccine groups with respect to half-life of the IgG binding Ab response, based on the subset of subjects with non-zero binding Ab responses at Month 6.5. Power is reported as a function of the proportion of non-zero responses at Month 6.5, assumed to be the same in the two groups, and of the half-life in the second group. Responses follow an exponential decay model. In group 1 the half-life is 45 days. The groups are compared using a two-sided generalized Wald test with 5% type-I error. Calculations assume 18 participants per group. Power was estimated using 2,000 simulations.

Half life in group 2	85% non-zero response rate at Month 6.5 for both groups	98% non-zero response rate at Month 6.5 for both groups
50	0.33	0.36
55	0.74	0.80
60	0.94	0.96
65	0.99	0.99

6.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through the SDMC's Web-based randomization system. The randomization will be done in blocks to ensure balance across arms. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments. Randomization of the first 20 participants will reflect the enrollment restriction that no two participants enrolled on the same day are enrolled to group 1 or 2 or to group 3 or 4, that five participants are enrolled in each group, and that of these five, four are vaccine recipients and one is a placebo recipient. Randomization of the remaining 76 participants will be done without these restrictions.

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm assignments (eg, vaccine or placebo). Study product assignments are accessible to those HVTN CRS pharmacists, DAIDS protocol pharmacists and contract monitors, and SDMC staff who are required to know this information in order to ensure proper trial conduct. Any discussion of study product assignment between pharmacy staff and any other HVTN CRS staff is prohibited. The HVTN SMB members also are unblinded to treatment assignment in order to conduct review of trial safety.

When a participant leaves the trial prior to study completion, the participant will be told he or she must wait until all participants are unblinded to learn his or her treatment assignment.

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 096/EV04 PSRT should be consulted before emergency unblinding occurs. The study is double-blinded in that neither subjects nor staff have knowledge of the exact group they were assigned to or whether they were assigned vaccine or placebo.

6.4 Statistical analysis

All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. An important secondary analysis of immunogenicity will be restricted to the per-protocol population, defined as participants who receive all vaccinations. Since enrollment is concurrent with receiving the first vaccination, all participants will have received at least 1 vaccination and therefore will provide some safety data. The analysis is a modified intent-to-treat analysis in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

Statistical analyses will be performed using SAS, StatXact, S-Plus, and/or R statistical software.

6.4.1 Analysis variables

The analysis variables consist of baseline participant characteristics, safety, and immunogenicity variables for primary- and secondary-objective analyses.

6.4.2 Baseline comparability

Groups will be compared for baseline participant characteristics using descriptive statistics.

6.4.3 Safety analysis

Since enrollment is concurrent with receiving the first vaccination, all participants will have received at least 1 vaccination and therefore will provide some safety data.

6.4.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits.

6.4.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment arm the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing arms is not planned since interpretation of differences must rely heavily upon clinical judgment.

A listing of SAEs requiring expedited reporting will provide details of the events including severity, relationship to study product, time between onset and last vaccination, number of vaccinations received, and a summary of the events.

6.4.3.3 Local laboratory values

Boxplots of local laboratory values will be generated for baseline values and for values measured during the course of the study by treatment arm and visit. Each boxplot will show the first quartile, the median, and the third quartile. Outliers (values outside the boxplot) will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

6.4.3.4 Reasons for vaccination discontinuation and early study termination

The number and percentage of participants who discontinue vaccination and who terminate the study early will be tabulated by reason and treatment arm.

6.4.4 Immunogenicity analysis

6.4.4.1 Statistical analysis to address primary objectives

The first component of the analysis will entail comparing the rate of the IgG binding Ab response after the fourth vaccination (Month 6.5) between each of the vaccine regimens and the pooled placebo group using a binomial test. The Holm method will be used to control the family-wise error rate at 5%. The arms that are significant at the adjusted $p < 0.05$ level are said to pass the "Month 6.5 placebo screen".

To address primary objective 2, the analysis will compare the regimens that pass the Month 6.5 placebo screen with respect to magnitude of the IgG binding Ab response after the fourth vaccination (Month 6.5). Groups 1 and 2 will be compared, and groups 3 and 4 will be compared, each time using a two-sided Wilcoxon rank sum test with 5% type-I error rate.

To address primary objective 3, a first step will be to compare the rate of the IgG binding Ab response at the last immunogenicity timepoint (Month 18) between each of the vaccine regimens and the pooled placebo group. Again a binomial test and the Holm multiplicity correction will be employed. The arms that are significant at the adjusted $p < 0.05$ level are said to pass the "Month 18 placebo screen", and these arms will be further investigated with respect to durability of response.

Durability of the IgG binding Ab response will be assessed for regimens that pass the Month 18 placebo screen. Vaccine groups 1 and 2 and groups 3 and 4 will be compared. An exponential decay model will be fit to the response data from Months 6.5, 7, 9, 12, 15, and 18 for each vaccine arm using GEE. This model will be used to estimate the half-life of the response for each vaccine group, which will then be compared between groups 1 and 2 (3 and 4) using a two-sided generalized Wald test with 5% type-I error rate.

6.4.4.2 General approach for secondary and exploratory analyses

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Assay results that are unreliable, from specimens collected outside of the visit window, or from HIV-infected participants postinfection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

Additional analyses may be performed for participants who received all scheduled injections per protocol.

For secondary and exploratory endpoints that are qualitative (ie, positive or negative) analyses will be performed by tabulating the frequency of positive responses for each assay by arm at each time-point that an assessment is performed. For vaccine arms, crude and net response rates will be presented with their corresponding exact 95% CI estimates, as well as the Fisher's exact test p-value comparing vaccine to pooled placebo group. For the pooled placebo group, crude response rates and exact 95% CI estimates will be presented.

To compare the response rates among the 4 vaccine arms, first an overall test for any difference in crude response rate among any of the 4 vaccine arms will be conducted, using Fisher's exact test. If this test is significant at the 2-sided 0.05 level, then the Agresti-Coull method will be used to construct 2-sided (1-0.05/6) ×100% CIs about the differences in response rates for each of the pair-wise comparisons of vaccine arms. Significance of the differences will be based on whether the CI excludes zero. If assays are run from samples taken at multiple timepoints, the probability of observing at least 1 positive response by a given timepoint and the probability of observing more than 1 positive response by a given timepoint will be estimated, with corresponding CIs, for each vaccine arm using maximum likelihood-based methods [35].

For continuous assay variables, the difference between vaccine arms at a specific time-point will be tested with a nonparametric Wilcoxon rank sum test if the data are not normally distributed and with a 2-sample t-test if the data appear to be normally distributed. An appropriate data transformation (eg, log10 transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance). Similar to the comparison of response rates between vaccine arms, first an overall test will be done to evaluate any differences among the 4 vaccine arms, using the Kruskal-Wallis rank test or an F-test (depending on the normality assumption). Secondly, if the overall test is significant at the 2-sided 0.05 level, then individual tests comparing the 6 pairs of vaccine arms will be done. If rank-based tests are used then the tests will be inverted to construct 2-sided (1-0.05/6) ×100% CIs about the differences in location centers of the 6 pair-wise comparisons of vaccine arms, and if actual-value tests are used then Fisher's least significant differences procedure will be used to construct simultaneous 95% CIs about the 6 pairs of mean differences in outcomes.

More sophisticated analyses of continuous assay variables employing repeated measures methodology (for example, repeated measures analysis of variance [ANOVA] or GEEs) may be utilized to incorporate immune responses over several time-points. However, inference from such analyses would be limited by the small sample size of this study. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05/K$, where K is the number of pairwise vaccine arms compared head-to-head. Graphical representations of the longitudinal immune responses will also be given.

Some immunologic assays have underlying continuous or count-type readout that is often dichotomized into responder/non-responder categories. For these assays, graphical and tabular summaries of the underlying distributions will be made. If arm comparisons in these underlying distributions reveal that differences are best summarized as a shift in the location of the distribution, then results will be presented in the form of arm means (or medians) with associated CIs and statistical tests for differences between arms as described above. If arm comparisons in these underlying distributions reveal that differences are best summarized by a mixture model (ie, responder and non-responder

subgroups are clearly identifiable), then results will be presented in the form of response rates with associated CIs and statistical tests as described above. In addition, Lachenbruch's test statistic [36] will be used for evaluating the composite null hypothesis of equal response rates in the 2 arms and equal response distributions among responders in the 2 such arms. This test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure will be used to obtain a 2-sided p-value.

6.4.4.3 Missing Data

Based upon previous AVEG and HVTN trials, missing 10% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed PBMCs. To achieve unbiased statistical estimation and inferences with nonparametric tests and generalized linear models fit by GEE methods, missing data are assumed to be missing completely at random (MCAR). MCAR assumes that the probability of an observation being missing does not depend upon the observed responses or upon any unobserved covariates but may depend upon observed covariates (eg, missing more among whites than nonwhites). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then nonparametric tests and GEE methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests. These models will include as covariates all available baseline predictors of the missing outcomes.

If a substantial amount of immunogenicity data are missing (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific time-point will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing depends upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right- censored, then the linear mixed effects models of Hughes [37] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements will be done using weighted GEE [38] methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

6.4.5 Analyses prior to end of scheduled follow-up visits

Any analyses conducted prior to the end of the scheduled follow-up visits should not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments. In particular, early unblinded analyses by treatment assignment require careful consideration and should be made available on a need to know basis only.

6.4.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 096/EV04 PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity

An unblinded statistical analysis by treatment assignment of an immunogenicity endpoint may be performed when the Laboratory Program has completed testing at least 80% of samples from the primary immunogenicity visit and all participants have completed the visit. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developers, and other key HVTN members and investigators. Distribution will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the study. Any analyses conducted prior to the end of the study should not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments.

7 Selection and withdrawal of participants

Participants will be healthy, HIV-1-uninfected (seronegative) adults who comprehend the purpose of the study and have provided written informed consent. Volunteers will be recruited and screened; those determined to be eligible, based on the inclusion and exclusion criteria, will be enrolled in the study. Final eligibility determination will depend on results of laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions.

Investigators should always use good clinical judgment in considering a volunteer's overall fitness for trial participation. Some volunteers may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria. Medical, psychiatric, occupational, or other conditions may make evaluation of safety and/or immunogenicity difficult, and some volunteers may be poor candidates for retention.

Determination of eligibility, taking into account all inclusion and exclusion criteria, must be made within 56 days prior to enrollment unless otherwise noted in sections 7.1 and 7.2.

7.1 Inclusion criteria

General and Demographic Criteria

1. **Age** of 18 to 50 years

2. **Access to a participating HVTN CRS** and willingness to be followed for the planned duration of the study

3. Ability and willingness to provide **informed consent**

4. **Assessment of understanding**: volunteer demonstrates understanding of this study; completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly

5. **Willing to be contacted annually** after completion of scheduled clinic visits for a total of 5 years following initial study injection.

6. **Agrees not to enroll in another study** of an investigational research agent

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

HIV-Related Criteria

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

9. Willingness to discuss **HIV infection risks**, amenable to **HIV risk reduction counseling**, and committed to maintaining behavior consistent with low risk of HIV exposure through the last required protocol clinic visit

10. Assessed by the clinic staff as being at **"low risk" for HIV infection**

Laboratory Inclusion Values

Hemogram/Complete Blood Count (CBC)

11. **Hemoglobin** ≥ 11.0 g/dL for volunteers who were born female, ≥ 13.0 g/dL for volunteers who were born male

12. **White blood cell count** = 3,300 to 12,000 cells/mm^3

13. **Total lymphocyte count** ≥ 800 cells/mm^3

14. **Remaining differential** either within institutional normal range or with site physician approval

15. **Platelets** = 125,000 to 550,000/mm^3

Chemistry

16. **Chemistry panel**: ALT, AST, and alkaline phosphatase < 1.25 times the institutional upper limit of normal; creatinine <1.1x institutional upper limit of normal.

Virology

17. **Negative HIV-1 and -2 blood test**: Non-US sites may use locally available assays that have been approved by HVTN Laboratory Operations.

18. **Negative Hepatitis B surface antigen (HBsAg)**

19. **Negative anti-Hepatitis C virus antibodies (anti-HCV)**, or negative HCV polymerase chain reaction (PCR) if the anti-HCV is positive

Urine

20. **Normal urine**:

    - Negative urine glucose, and
    - Negative or trace urine protein, and
    - Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis with red blood cells levels within institutional normal range).

Reproductive Status

21. **Volunteers who were born female**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination

22. **Reproductive status**: A volunteer who was born female must:

- Agree to consistently use effective contraception (see Appendix B) for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment through the last required protocol clinic visit. Effective contraception is defined as using any of the following methods:
  - Condoms (male or female) with or without a spermicide,
  - Diaphragm or cervical cap with spermicide,
  - Intrauterine device (IUD),
  - Hormonal contraception, or
  - Successful vasectomy in the male partner (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite unprotected sexual activity postvasectomy);
- Or not be of reproductive potential, such as having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation;
- Or be sexually abstinent.

23. **Volunteers who were born female must also agree not to seek pregnancy through alternative methods**, such as artificial insemination or in vitro fertilization until after the last required protocol clinic visit

7.2 Exclusion criteria

General

1. **Blood products** received within 120 days before first vaccination
2. **Investigational research agents** received within 30 days before first vaccination
3. **Intent to participate in another study** of an investigational research agent during the planned duration of the HVTN 096/EV04 study
4. **Pregnant or breastfeeding**

Vaccines and other Injections

5. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 096/EV04 PSRT will determine eligibility on a case-by-case basis.
6. **Non-HIV experimental vaccine(s) received within the last 5 yea**rs in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure by the FDA. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 096/EV04 PSRT will determine eligibility on a case-by-case basis. For volunteers who have received an experimental vaccine(s) greater than 5 years ago, eligibility for enrollment will be determined by the HVTN 096/EV04 PSRT on a case-by-case basis.

7. **Live attenuated vaccines** other than influenza vaccine received 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)

8. **Influenza vaccine or any vaccines that are not live attenuated vaccines** and were received within 14 days prior to first vaccination (eg, tetanus, pneumococcal, Hepatitis A or B)

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

Immune System

10. **Immunosuppressive medications** received within 168 days before first vaccination. (Not excluded: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatitis; or [4] a single course of oral/parenteral corticosteroids at doses < 2 mg/kg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment.

11. **Serious adverse reactions to vaccines** including anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain. (Not excluded: a volunteer who had a nonanaphylactic adverse reaction to pertussis vaccine as a child.)

12. **Immunoglobulin** received within 60 days before first vaccination

13. **Autoimmune disease**

14. **Immunodeficiency**

Clinically significant medical conditions

15. **Untreated or incompletely treated syphilis infection**

16. **Clinically significant medical condition**, physical examination findings, clinically significant abnormal laboratory results, or past medical history with clinically significant implications for current health. A clinically significant condition or process includes but is not limited to:

    - A process that would affect the immune response,
    - A process that would require medication that affects the immune response,
    - Any contraindication to repeated injections or blood draws,
    - A condition that requires active medical intervention or monitoring to avert grave danger to the volunteer's health or well-being during the study period,
    - A condition or process for which signs or symptoms could be confused with reactions to vaccine, or
    - Any condition specifically listed among the exclusion criteria below.

17. **Any medical, psychiatric, 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 volunteer's ability to give informed consent

18. **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.

19. **Current anti-tuberculosis (TB) prophylaxis or therapy**

20. **Asthma exclusion criteria:**

    Asthma other than mild, well-controlled asthma. (Symptoms of asthma severity as defined in the most recent National Asthma Education and Prevention Program (NAEPP) Expert Panel report).

    Exclude a volunteer who:

    - Uses a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
    - Uses moderate/high dose inhaled corticosteroids, or
    - In the past year has either of the following:
    - Greater than 1 exacerbation of symptoms treated with oral/parenteral corticosteroids;
    - Needed emergency care, urgent care, hospitalization, or intubation for asthma.

21. **Diabetes mellitus** type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

22. **Thyroidectomy, or thyroid disease** requiring medication during the last 12 months

23. **Hypertension**:

    - If a person has been found to have elevated blood pressure or hypertension during screening or previously, exclude for blood pressure that is not well controlled. Well-controlled blood pressure is defined as consistently ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm Hg systolic and ≤ 100 mm Hg diastolic. For these volunteers, blood pressure must be ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic at enrollment.

    - If a person has NOT been found to have elevated blood pressure or hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 100 mm Hg at enrollment.

24. **BMI** ≥ 40; ≤ 18; or BMI ≥ 35 with 2 or more of the following: age > 45, systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, current smoker, known hyperlipidemia

25. **Bleeding disorder** diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

26. **Malignancy** (Not excluded: Volunteer who has had malignancy excised surgically and who, in the investigator's estimation, has a reasonable assurance of sustained cure, or who is unlikely to experience recurrence of malignancy during the period of the study)

27. **Seizure disorder:** History of seizure(s) within past three years. Also exclude if volunteer has used medications in order to prevent or treat seizure(s) at any time within the past 3 years.

28. **Asplenia**: any condition resulting in the absence of a functional spleen

29. History of hereditary **angioedema**, acquired angioedema, or idiopathic angioedema.

30. **Hypersensitivity to eggs or egg products**

31. Subjects who have **2 or more of the following cardiac risk factors:**
    - participant report of history of elevated blood cholesterol defined as fasting LDL > 160 mg/dL;
    - first degree relative (eg, mother, father, brother, or sister) who had coronary artery disease before the age of 50 years);
    - current smoker; or
    - body mass index (BMI) ≥ 35

32. **Electrocardiogram (ECG) with clinically significant findings**, or features that would interfere with the assessment of myo/pericarditis, as determined by the contract ECG Lab, cardiologist, or study clinician including any of the following:
    - conduction disturbance (complete left or complete right bundle branch block or nonspecific intraventricular conduction disturbance with QRS ≥120 ms, any 2nd or 3rd AV block, or QTc prolongation [> 450 ms]);
    - significant repolarization (ST segment or T wave) abnormality;
    - significant atrial or ventricular arrhythmia;
    - frequent atrial or ventricular ectopy (eg, frequent premature atrial contractions, 2 premature ventricular contractions in a row);
    - ST elevation consistent with ischemia; or
    - evidence of past or evolving myocardial infarction

33. **History of myocarditis, pericarditis, cardiomyopathy, congestive heart failure with permanent sequelae, clinically significant arrhythmia (including arrhythmia requiring medication, treatment, or clinical follow-up**

7.3 Participant departure from vaccination schedule or withdrawal

This section concerns an individual participant's departure from the vaccination schedule. Pause rules for the trial as a whole are described in section 11.4.

7.3.1 Delaying vaccinations for a participant

Under certain circumstances, a participant's scheduled vaccination will be delayed. The factors to be considered in such a decision include but are not limited to the following:

- Within 45 days prior to any study injection
  - Receipt of blood products or immunoglobulin
- Within 30 days prior to any study injection
  - Receipt of live attenuated vaccines other than influenza vaccine
  - Receipt of allergy treatment with antigen injections
- Within 14 days prior to any study injection
  - Receipt of influenza vaccine or any vaccines that are not live attenuated vaccines (eg, pneumococcal)
- Prevaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.

Vaccinations should not be administered outside the visit window period specified in the HVTN 096/EV04 Study Specific Procedures.

In order to avoid vaccination delays and missed vaccinations, participants who plan to receive licensed vaccines and/or allergy treatments should be counseled to schedule receipt of these substances, when possible, outside the intervals indicated above. The effects of these substances on safety and immunogenicity assessments and their interactions with study vaccines are unknown. Therefore, if circumstances allow, these substances should also be avoided in the 2-week interval between a study vaccination and completion of the 2-week postvaccination follow-up visit.

7.3.2 Participant departure from vaccination schedule

Every effort should be made to follow the vaccination schedule per the protocol. If a participant misses a vaccination and the visit window period for the vaccination has passed, that vaccination cannot be given. The participant should be asked to continue study visits. The participant should resume the vaccination schedule with the next vaccination unless there are circumstances that require further delay or permanent discontinuation of vaccination (see Sections 7.3.1 and 7.3.3).

7.3.3 Discontinuing vaccination for a participant

Under certain circumstances, an individual participant's vaccinations will be permanently discontinued. Specific events that will result in stopping a participant's vaccination schedule include:

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 096/EV04 PSRT).

- Clinically significant condition (ie, a condition that affects the immune system or for which continued vaccinations and/or blood draws may pose additional risk), including but not limited to the following:
  - Pregnancy (regardless of outcome);
  - Any grade 4 local or systemic reactogenicity symptom, lab abnormality, or AE that is subsequently considered to be related to vaccination;
  - Any grade 3 lab abnormality or other clinical AE (exception: fever or vomiting and subjective local and systemic symptoms) that is subsequently considered to be related to vaccination; or
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 096/EV04 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination; or
- Investigator determination in consultation with Protocol Team leadership (eg, for repeated nonadherence to study staff instructions).
- Participant misses more than 2 vaccinations(s) (see Section 7.3.2).

Such participants should be counseled on the importance of continuing with the study and strongly encouraged to participate in follow-up visits and protocol-related procedures per the protocol for the remainder of the trial, unless medically contraindicated or termination from the study is required by applicable regulations.

In addition, vaccinations will be stopped for participants diagnosed with HIV infection. HIV-infected participants will not continue in the trial (see Sections 7.3.4 and 9.8.1).

7.3.4 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,
- Participant becomes HIV-infected, or
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff).
- Any condition where termination from the study is required by applicable regulations.

8 Study product preparation and administration

CRS pharmacist should consult the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for standard pharmacy operations. The protocol schema is shown in Table 3-1. See the IBs for further information about study products.

8.1 Vaccine regimen

The schedule of vaccination is shown in section 3 and additional information is given below.

*Sodium Chloride for Injection, 0.9% has been added to groups 1, 3, and 4 to equalize the number of injections among groups.

Group 1

Treatment 1 (T1): NYVAC-HIV-PT1 at greater than or equal to 5 x 10^6 PFU/ml and NYVAC-HIV-PT4 at greater than or equal to 5 x 10^6 PFU/ml; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

Sodium Chloride for Injection, 0.9%* to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 1;

THEN

NYVAC-HIV-PT1 at greater than or equal to 5 x 10^6 PFU/ml and NYVAC-HIV-PT4 at greater than or equal to 5 x 10^6 PFU/ml each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 3 and 6;

AND

AIDSVAX® B/E (600mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Control 1 (C1): Placebo for NYVAC-HIV-PT1 and Placebo for NYVAC-HIV-PT4; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

Sodium Chloride for Injection, 0.9%* to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 1;

THEN

Placebo for NYVAC-HIV-PT1 and Placebo for NYVAC-HIV-PT4; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 3 and 6;

AND

Placebo for AIDSVAX® B/E to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Group 2

Treatment 2 (T2): NYVAC-HIV-PT1 at greater than or equal to 5 x 10^6 PFU/ml and NYVAC-HIV-PT4 at greater than or equal to 5 x 10^6 PFU/mL; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0, 1, 3 and 6;

AND

AIDSVAX® B/E (600 mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0, 1, 3, and 6.

Control 2 (C2): Placebo for NYVAC-HIV-PT1 and Placebo for NYVAC-HIV-PT4; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0, 1, 3 and 6;

AND

Placebo for AIDSVAX® B/E to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0, 1, 3, and 6.

Group 3

Treatment 3 (T3): DNA-HIV-PT123 (4 mg/mL) and Sodium Chloride for Injection, 0.9%*; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

Sodium Chloride for Injection, 0.9%* to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 1;

THEN

NYVAC-HIV-PT1 at greater than or equal to 5 x 10^6 PFU/ml and NYVAC-HIV-PT4 at greater than or equal to 5 x 10^6 PFU/ml each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 3 and 6;

AND

AIDSVAX® B/E (600 mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3and 6.

Control 3 (C3): Placebo for DNA-HIV-PT123 and Sodium Chloride for Injection, 0.9%*; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

Sodium Chloride for Injection, 0.9%* to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 1;

THEN

Placebo for NYVAC-HIV-PT1 and Placebo for NYVAC-HIV-PT4; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 3 and 6;

AND

Placebo for AIDSVAX® B/E to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Group 4

Treatment 4 (T4): DNA-HIV-PT123 (4 mg/mL) and Sodium Chloride for Injection, 0.9%*; each to be administered as 1 mL in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

AIDSVAX® B/E (600 mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 1;

THEN

NYVAC-HIV-PT1 at greater than or equal to 5×10^6 PFU/mL and NYVAC-HIV-PT4 at greater than or equal to 5×10^6 PFU/mL; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 3 and 6;

AND

AIDSVAX® B/E (600 mcg/mL) to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Control 4 (C4): Placebo for DNA-HIV-PT123 and Sodium Chloride for Injection, 0.9%*; each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

Placebo for AIDSVAX® B/E to be administered as 1 mL IM in the RIGHT deltoid where DNA injections are given (unless medically contraindicated) at Months 0 and 1;

THEN

Placebo for NYVAC-HIV-PT1 and Placebo for NYVAC-HIV-PT4 each to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Month 3 and 6;

AND

Placebo for AIDSVAX® B/E to be administered as 1 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

8.2 Study product formulation

See the IBs for additional information about study products.

DNA-HIV-PT123 (labeled as DNA-HIV-PT123 [4 mg/mL]) is supplied as a 4 mg/mL DNA solution in a 2 mL sterile glass vial containing a volume to deliver 1 mL of a clear, colorless, sterile isotonic solution. The product must be stored at -20^{0}C or colder.

NYVAC-HIV-PT1 (labeled as NYVAC-HIV-PT1 [≥5x10^6 PFU/mL]) is supplied as a slightly cloudy, white to rose sterile suspension in a 2 mL sterile glass vial with silver/yellow aluminum capping containing a volume to deliver 1mL of ≥5x10^6 PFU/ml NYVAC. The product must be stored at -70^{0}C or colder.

NYVAC-HIV-PT4 (labeled as NYVAC-HIV-PT4 [≥5x10^6 PFU/mL]) is supplied as a slightly cloudy, white to rose sterile suspension in a 2 mL sterile glass vial with silver/red aluminum capping containing a volume to deliver 1 mL of ≥5x10^6 PFU/ml NYVAC. The product must be stored at -70^{0}C or colder.

AIDSVAX® B/E (labeled as AIDSVAX® B/E active (MN/A244 rgp 120/HIV-1) is supplied as a sterile suspension in single-use glass vials with silver/blue aluminum capping containing a volume to deliver 1 mL (300mcg/mL) of each rgp120/HIV-1 protein absorbed onto a total of 600 mcg aluminum hydroxide gel adjuvant. The product must be stored at 2^0 to 8^{0}C.

Sodium Chloride for Injection, 0.9%*

Sodium Chloride for Injection, 0.9%, will be used in Groups 1, 3 and 4 to equalize the number of injections among all four groups. Product must be stored as directed by the manufacturer.

Placebo for DNA-HIV-PT123 (Sodium Chloride for Injection, 0.9%)

Sodium Chloride for Injection, 0.9%, will be used as the placebo for DNA-HIV-PT123. Product must be stored as directed by the manufacturer.

Placebo for NYVAC-HIV-PT1 (Sodium Chloride for Injection, 0.9%)

Sodium Chloride for Injection, 0.9% will be used for the NYVAC-HIV-PT1. Product must be stored as directed by the manufacturer.

Placebo for NYVAC-HIV-PT4 (Sodium Chloride for Injection, 0.9%)

Sodium Chloride for Injection, 0.9% will be used for the NYVAC-HIV-PT4. Product must be stored as directed by the manufacturer.

Placebo for AIDSVAX® B/E (600 mcg/mL of aluminum hydroxide gel adjuvant)

The placebo for AIDSVAX® B/E (labeled as AIDSVAX® Placebo) is supplied as a sterile suspension in single-use glass vials with silver/blue aluminum capping containing a volume to deliver 1 mL (600 mcg alum/mL) of aluminum hydroxide gel adjuvant. The product must be stored at 2° to 8°C.

8.3 Preparation of study products

8.3.1 DNA-HIV-PT123

One vial of DNA-HIV-PT123 (labeled as DNA-HIV-PT123 [4 mg/mL]) will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the study product from the freezer and allow it to thaw at room temperature.

Once thawed, the pharmacist, using aseptic technique, will gently swirl the vial and then withdraw 1 mL into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in the LEFT deltoid. The study product should be administered as soon as possible after preparation.

Any partial vials or expired filled syringes should be autoclaved immediately prior to disposal and disposed of in accordance with institutional or pharmacy policy.

8.3.2 NYVAC-HIV-PT1

One vial of NYVAC-HIV-PT1 will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the study product from the freezer and allow it to thaw at room temperature.

Once thawed, the pharmacist will gently swirl the vial and then using aseptic technique withdraw 1 mL into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. The study product should be administered as soon as possible after preparation.

Any unused portion of entered vials or expired prefilled syringes should be autoclaved immediately prior to disposal and disposed of in accordance with institutional or pharmacy policy. If they will be incinerated, they do not need to be autoclaved.

8.3.3 NYVAC-HIV-PT4

One vial of NYVAC-HIV-PT4 will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the study product from the freezer and allow it to thaw at room temperature.

Once thawed, the pharmacist will gently swirl the vial and then using aseptic technique withdraw 1 mL into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. The study product should be administered as soon as possible after preparation.

Any unused portion of entered vials or expired prefilled syringes should be autoclaved immediately prior to disposal and disposed of in accordance with institutional or pharmacy policy. If they will be incinerated, they do not need to be autoclaved.

8.3.4 AIDSVAX® B/E

One vial of AIDSVAX® B/E will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the study product from the refrigerator to allow the vial to equilibrate to room temperature. Vaccine that is stored at 2°C to 8°C can form an appearance of a cloudy ring or 'halo' on the vial or vial neck. If such a condition is observed, the vial can be rolled gently. If the condition persists once equilibrated to room temperature and after rolling, or if inhomogeneous particulates or material is observed, DO NOT use the contents for preparation.

The pharmacist will then gently roll the mixture in the vial (do not shake), and withdraw 1 mL into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid. The study product should be administered within 2 hours of being drawn into the syringe.

Any unused portion of entered vials or expired prefilled syringes should be autoclaved immediately prior to disposal and disposed of in accordance with institutional or pharmacy policy. If they will be incinerated, they do not need to be autoclaved.

8.3.5 Sodium Chloride for Injection, 0.9%* (Group 1)

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid. The study product should be administered within 2 hours of being drawn into the syringe.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.6 Sodium Chloride for Injection, 0.9%* (Group 3)

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a 3 or 5 mL syringe. This process will be repeated a second time for a total of two syringes.

One syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. This study product should be administered as soon as possible after preparation.

The other syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid. This study product should be administered within 2 hours of being drawn into the syringe.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.7 Sodium Chloride for Injection, 0.9%* (Group 4)

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. This study product should be administered as soon as possible after preparation.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.8 Placebo for DNA-HIV-PT123 (Sodium Chloride for Injection, 0.9%)

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. This study product should be administered as soon as possible after preparation.

Any unused portion of reconstituted vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.9 Placebo for NYVAC-HIV-PT1 (Sodium Chloride for Injection, 0.9%)

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. This study product should be administered as soon as possible after preparation.

Any unused portion of reconstituted vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.10 Placebo for NYVAC-HIV-PT4 (Sodium Chloride for Injection, 0.9%)

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. This study product should be administered as soon as possible after preparation.

Any unused portion of reconstituted vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.3.11 Placebo for AIDSVAX® B/E (Aluminum Hydroxide Gel Adjuvant)

One vial of AIDSVAX® Placebo will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the study product from the refrigerator to allow the vial to equilibrate to room temperature. Vaccine that is stored at 2°C - 8°C can form an appearance of a cloudy ring or 'halo' on the vial or vial neck. If such a condition is observed, the vial can be rolled gently. If the condition persists once equilibrated to room temperature and after rolling, or if inhomogeneous particulates or material is observed, DO NOT use the contents for preparation.

The pharmacist will then gently roll the mixture in the vial (do not shake), and withdraw 1 mL into a 3 or 5 mL syringe.

The syringe should be labeled as "HVTN 096 Study Product" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid. The study product should be administered within 2 hours of being drawn into the syringe.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.4 Administration

When preparing a dose in a syringe and administering the dose, consideration should be given to the volume of solution in the needle before and after the dose is administered. Particularly, if the needle used to withdraw the product is replaced prior to vaccine administration, consideration should be given to conserving the full dose of product. The pharmacy and clinic staff members are encouraged to work together to administer the dose specified in the protocol.

All injections are to be given using standard IM injection technique.

For all injections administered in the left deltoid (unless medically contraindicated), a plaster will be placed over the injection site 10 minutes after vaccination, for at least 24 hours. The plaster has to be transparent, so that the injection sites can be observed, and impermeable and well attached, so that the spread of the NYVAC vaccine virus (if administered) will be prevented and the blinding will be maintained (if product other than NYVAC). Two injections administered into the same arm should be at least 2.4 cm apart. If the two injections are administered in the contralateral deltoid due to medical contraindication, the plaster will still need to be applied.

For all injections administered in the right deltoid (unless medically contraindicated), the person administering the injection should gently roll the syringe prior to administration of the study product.

If an injection is administered in the contralateral deltoid due to a medical contraindication, the appropriate study staff should document this clearly. Under this circumstance, this is NOT a protocol violation.

8.5 Acquisition of study products

DNA-HIV-PT123 will be provided by IPPOX Foundation.

NYVAC-HIV-PT1 will be provided by EuroVacc Foundation.

NYVAC-HIV-PT4 will be provided by EuroVacc Foundation.

AIDSVAX® B/E will be provided by USMHRP.

Sodium Chloride for Injection, 0.9%* will not be provided through the protocol and must be obtained by the site.

Placebo for DNA-HIV-PT123 (Sodium Chloride for Injection, 0.9%) will not be provided through the protocol and must be obtained by the site.

Placebo for NYVAC-HIV-PT1 (Sodium Chloride for Injection, 0.9%) will not be provided through the protocol and must be obtained by the site.

Placebo for NYVAC-HIV-PT4 (Sodium Chloride for Injection, 0.9%) will not be provided through the protocol and must be obtained by the site.

Placebo for AIDSVAX® B/E (Aluminum Hydroxide Gel Adjuvant) will be provided by GSID.

Once all regulatory requirements have been met by the site for shipment of study product, the HVTN Regulatory Affairs Unit will notify EuroVacc so that study products can be shipped. Each foundation and GSID will have study product shipped to the CRS Pharmacist of Record (PoR).

8.6 Pharmacy records

The CHUV HVTN CRS pharmacist is required to maintain complete records of all study products. The pharmacist of record is responsible for maintaining randomization codes and randomization confirmation notices for each participant in a secure manner.

8.7 Final disposition of study products

All unused study products must be returned to the original source after the study is completed or terminated unless otherwise instructed by the original source in writing.

9 Clinical procedures

The schedule of clinical procedures is shown in Appendix F.

9.1 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 CRS staff provide to the participant, before and during the trial. HVTN CRS 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, HVTN CRS 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.

An HVTN CRS 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. HVTN CRSs must submit recruitment and prescreening materials to IRB/EC and any applicable Regulatory Entity (RE) for human subjects protection review and approval.

Note: As defined in the DAIDS Protocol Registration Manual, an RE is "Any group other than the local IRB/EC responsible for reviewing and/or approving a clinical research protocol and site-specific ICFs prior to implementation at a site." CRSs are responsible for knowing the requirements of their applicable REs.

9.1.1 Screening consent form

Some HVTN CRSs have approval from their IRB/EC and any applicable RE to use a general screening consent form that allows screening for an unspecified HIV vaccine trial. In this way, HVTN CRS staff can continually screen potential participants and, when needed, proceed quickly to obtain protocol-specific enrollment consent. Sites conducting general screening or prescreening approved by their IRB/EC and any applicable RE may use the results from this screening to determine eligibility for this protocol, provided the tests are conducted within the time periods specified in the eligibility criteria. Without a general screening consent, screening for a specific study cannot take place until the site is activated by HVTN Regulatory Affairs.

9.1.2 Protocol-specific consent forms

The protocol-specific consent forms describe the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. A sample protocol-specific consent form for the main study is located in Appendix A.

Each HVTN CRS is responsible for developing a protocol-specific consent form for local use, based on the sample protocol-specific consent form in Appendix A. The consent form must be developed in accordance with requirements of the following:

- CRS's IRB/EC,
- CRS's institution and any applicable REs, and
- Elements of informed consent as described in Title 45, CFR Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonisation (ICH) E6, Good Clinical Practice: Consolidated Guidance 4.8.

Study sites are strongly encouraged to have their local CABs review their sites-specific consent forms. 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 instructions throughout the document for developing specific content.

Sites should follow the instructions in the Protocol-specific Official Memo distributed along with this protocol regarding when they may begin using their site-specific protocol consent forms.

9.1.3 VISP registry consent form

Experimental HIV vaccines may induce Ab production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP) (see Section 9.8.1). In order to provide poststudy HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of poststudy testing and counseling services to HIV vaccine recipients.

The VISP registry consent form describes the purpose of the VISP registry, the participant information to be included in the registry, confidentiality protections, and risks and benefits associated with inclusion in the registry. The VISP registry consent form is contained in Appendix C.

The VISP Registry consent form will be presented to all participants. It is recommended to be presented no later than the last scheduled vaccination visit.

9.1.4 Assessment of Understanding

Study staff are responsible for ensuring that participants fully understand the study before enrolling them. This process involves reviewing the informed consent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely.

An Assessment of Understanding is used to document the participant's understanding of key concepts in this HIV vaccine trial. The participant must complete the Assessment of Understanding before enrollment. Staff may provide assistance in reading and understanding the questions and responses, if necessary. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

IRB/EC and any applicable RE may require that a participant has signed either a screening or protocol-specific consent document prior to administering the Assessment of Understanding. The consent process (including the use of the Assessment of Understanding) should be explained thoroughly to the IRB/EC and any applicable RE, whose recommendations should be followed.

9.2 Pre-enrollment procedures

Screening may occur over the course of several contacts/visits, up to and including before vaccination on day 0. All inclusion and exclusion criteria must be assessed within 56 days before enrollment, unless otherwise specified in the eligibility criteria (or below in this section).

After the appropriate informed consent has been obtained and before enrollment, the following procedures are performed:

- Medical history, documented in the case history record;
- Assessment of whether the volunteer is at low risk for HIV infection;
- Complete physical examination, including height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots (record the complete generic name for all medications);
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - HBsAg,
  - Anti-HCV antibodies,
  - Syphilis test,

- CBC with differential and platelets,
- Chemistry panel (ALT, AST, alkaline phosphatase, and creatinine),
- Urine dipstick (urinalysis if indicated; see Section 9.10),
- Urine or serum pregnancy test (volunteers who were born female),
- 12-lead ECG with interpretation;

- Administration of behavioral risk assessment questionnaire;

- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html);

- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in section 9.8; and

- Discussion of pregnancy prevention. A pregnant or breastfeeding person may not be enrolled in this trial. Specific criteria and assessment of contraception and pregnancy status are described in study inclusion criteria. Discussion of pregnancy prevention includes advising a participant who was born female and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.

9.2.1 Use of screening results from another HVTN study

If a participant screens for an HVTN study at the same HVTN CRS but then does not join that study, screening results from that effort may be applied to the screening for this protocol, as long as the screening was done under participant consent, the participant has signed a consent form to begin screening for this study, and the tests were conducted within the time periods specified in the eligibility criteria (see Sections 7.1 and 7.2).

9.3 Enrollment and vaccination visits

Enrollment is simultaneous with first vaccination. The time interval between randomization and enrollment should not exceed 4 working days. The HVTN CRS registers the participant by scheduling the day 0 visit (enrollment) via the Web-based randomization system, and requests the randomization assignment. Circumstances may require a participant's enrollment visit to be changed. This may exceed the 4-day randomization time limit.

At all vaccination visits, the following procedures are performed before vaccination:

- 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;

- Assessment of baseline reactogenicity parameters;
- Assessment of concomitant medications (as described in section 9.2);
- Assessment of any new or unresolved AEs/intercurrent illnesses; and
- Urine or serum pregnancy test (for participants who were born female).

At the enrollment visit on Day 0 (Visit 2), the following procedures are performed before vaccination:

- Mucosal secretion specimen collection and STI testing from study volunteers who agree to provide mucosal secretion samples. (If participant agrees to provide only saliva samples, STI testing is not required.)

NOTE: If a participant does not enroll in this study, the site will review any positive STI results and make referrals to therapy as needed.

Following completion of all procedures in the preceding list and results indicate that vaccination may proceed, vaccination is prepared and administered (see Sections 8.3 and 8.4).

Immediately following vaccination, the participant remains in the clinic for observation. An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes. Before leaving the clinic, the participant is given the postvaccination symptom log and is instructed on how to complete it. The site will make arrangements to obtain daily reports of reactogenicity events from the participant during the reactogenicity period (as described in section 9.11).

The following procedures will be performed at all vaccination visits. These procedures may be performed prior to or following vaccination:

- Risk reduction counseling (as described in section 9.8);
- Pregnancy prevention assessment (as described in section 9.2 and 9.9); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation).

Additional procedures will be performed at scheduled visits as specified in Appendix F .

- Administration of behavioral risk assessment questionnaire;
- Administration of social impact assessment questionnaire (types of impacts assessed involve personal relationships, medical insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the placebo.

- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate; and
- Cardiac symptoms assessment (as described in Section 9.5); and
- Specimen collection (should be completed prior to vaccination)

9.4 Follow-up visits

The following procedures are performed at all scheduled follow-up visits:

- Risk reduction counseling (as described in section 9.8);
- Pregnancy prevention assessment (as described in section 9.2 and 9.9); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation);
- Assessment of new or continuing concomitant medications (as described in section 9.2); and
- Assessment of new or unresolved AEs/intercurrent illnesses.

The following procedures will be performed at scheduled follow-up visits as specified in Appendix F:

- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;
- HIV infection assessment including pretest counseling. A subsequent follow-up contact is conducted 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 appropriate;
- 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;
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Cardiac symptoms assessment (as described in section 9.5);

- 12-lead ECG with interpretation;
- Specimen collection; and
- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - Chemistry panel (see Section 9.2), and
  - Urine testing for gonorrhea and chlamydia, and serology for syphilis (see Section 9.6) for those participants who agree to mucosal secretion collection, and
  - Urine dipstick (urinalysis if appropriate; see Section 9.10); and
  - Urine or serum pregnancy test (for participants who were born female).

9.5 Cardiac monitoring

Myo/pericarditis has been observed in recipients of vaccinia vaccinations used to protect against smallpox. It has been an *uncommon* occurrence in vaccinia recipients. Approximately 280 volunteers have received NYVAC vaccinations similar to this NYVAC construct and there have been no cases of myo/pericarditis associated with those NYVAC vaccinations. Approximately 100 volunteers have received the NYVAC-HIV-PT1 and PT-4 vaccine under evaluation in this study and there has been one case of myocarditis in another study, which is considered possibly related. Screening ECGs were conducted on all HVTN 096 participants; and one follow-up ECG has been added to the protocol. The eligibility criteria exclude potential participants with pre-existing cardiac risk factors and/or cardiac conditions, or certain ECG findings which could compromise the detection of myo/pericarditis. ECG interpretation will be provided by a dedicated cardiologist or other study physician certified in the interpretation of ECGs.

9.5.1 ECG testing

A 12-lead ECG is required at screening. A follow-up 12-lead ECG will be performed at the next regularly scheduled clinic visit or at an interim visit. ECG equipment will be provided by the HVTN or accessed locally by the site. ECG interpretation will be provided by a local cardiologist from the CHUV Department of Cardiology.

9.5.2 Evaluation of suspected myo/pericarditis

The classic presentation of myocarditis may not always be apparent with very early involvement. Since apparently benign symptoms may be suggestions of or mimic myo/pericarditis, there should be a low threshold for additional investigation of chest sensation or symptoms referable to the chest. As with evaluation for all potentially serious health problems in study participants, the protocol team recommends that the clinic physician be involved in the evaluation and clinical decision making associated with cardiac symptoms.

Any participant who develops symptoms or findings suggestive of possible myo/pericarditis (such as chest pain, dyspnea, palpitations, congestive heart failure) following vaccination will be evaluated with an electrocardiogram (ECG), cardiac Troponin I or T test (cTnI or cTnT), and creatine kinase-MB test (CK-MB) by study staff

as long as performing these tests in the research setting does not interfere with prompt medical care of the participant. Symptoms or findings that lead to a cardiac evaluation or referral for suspected myo/pericarditis should be reported by phone or email to the SDMC Clinical Affairs staff within 24 hours (contact information listed in *HVTN 096/EV04 Study Specific Procedures, Key Resource Guide*).

The participant with symptoms and cardiac enzyme findings and/or ECG findings consistent with suspected or probable myo/pericarditis will be referred to a cardiologist for consultation and care. Asymptomatic participants with objective findings of myo/pericarditis such as ECG and cardiac enzyme abnormalities should also be evaluated and referred appropriately. The site will communicate a request to the cardiologist that the initial evaluation include any of the following tests that have not been done previously for evaluation of that specific cardiac event: an ECG, cTnI or cTnT, CK-MB, and echocardiography. The site will request permission from the participant for access to medical records related to the evaluation. An AE of myo/pericarditis related to vaccine would be followed by study staff until resolution and the participant will be contacted 1 year after the event to complete follow-up of the AE.

Any episode of myo/pericarditis at any grade must be reported to SDMC Clinical Affairs immediately and reported as an SAE requiring expedited reporting, as described in Section11. Study staff will follow any AE of myo/pericarditis until resolution.

9.5.3 Cardiac symptoms assessment

At study visits up to 3 months after the last vaccination (see Appendix F), participants will be questioned specifically about symptoms and signs suggestive of myo/pericarditis or other cardiovascular complications as listed below:

- Shortness of breath
- Chest pain/discomfort
- Palpitations
- Unexplained fatigue
- Combination of fever, chills, or myalgias/arthralgias

If cardiopulmonary symptoms are reported, the study staff will perform a cardiopulmonary evaluation (as part of the abbreviated physical examination). Any report of these or any other signs and symptoms suggestive of any new cardiovascular condition will prompt an appropriate diagnostic evaluation as medically indicated.

9.6 Mucosal secretion sampling

Mucosal secretion samples will be collected from all study participants who agree to these procedures at timepoints indicated in Appendix E and Appendix F. These samples include salivary, semen (males only), rectal, or cervical secretions (females only).

Participants will be tested for the following infections at the mucosal sampling visits: gonorrhea, chlamydia, and syphilis. Test results will be provided to participants and all participants who test positive for one or more of these infections will receive counseling as well as treatment or referral for treatment as appropriate.

Participants who were born female must report having had a pap smear within the 3 years prior to enrollment, with the latest result reported as normal or ASCUS (atypical squamous cells of undetermined significance), in order to participate in cervical mucosal sampling. For these participants, a pregnancy test must also be performed prior to any cervical mucosal sampling. Cervical mucosal sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, within the visit window. In addition, cervical and rectal sampling will not be performed (or may be deferred to a later date within the visit window) if a participant is known to have an active STI at the scheduled timepoint. Participants who are eligible and who agree to provide cervical and/or rectal secretion samples should be advised not to have unprotected sex (ie, receptive vaginal or anal sex without a condom) for 24 hours prior to sample collection.

9.7 Annual health contacts

Participants will be contacted annually for a total of 5 years following initial study injection (see Appendix G). At these contacts, CRS staff will collect the information listed below. Clinic visits will only be required if HIV confirmatory testing is necessary (see Section 9.8.1); however, a clinic visit may be arranged for other reasons.

- Confirmation of vital status; if deceased, attempt to learn cause and date 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 more than 30 days of medical intervention or medication;
  - New diagnosis of HIV infection; and
  - Pregnancies and outcomes, including congenital anomalies/birth defects.

All such events will be recorded, and AEs will be assessed for relationship to study product(s). A safety monitoring team reviews reports from these contacts quarterly. This monitoring team comprises a DAIDS Medical Officer, Core medical monitor, and an SDMC Clinical Affairs Safety Associate.

9.7.1 Interim contacts

CRSs may report safety information obtained at a contact other than the annual contact. These contacts are reported as interim visits.

9.8 HIV counseling and testing

HIV counseling will be performed in compliance with the CDC's guidelines or other local guidelines for HIV counseling and referral. HIV testing will be performed in accordance with the current HVTN HIV testing algorithm following enrollment.

Participants will be counseled routinely during the trial on the avoidance of HIV infection and 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 HVTN CRSs and will be discouraged from doing so during study participation and/or during any period of vaccine-induced positive serology.

Study staff will take particular care to inform study participants of the likelihood of routine HIV testing being offered or performed outside the study CRS 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. CRS staff should inform participants of their right to opt out of HIV testing outside the study site. CRS 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 CRS staff should advise study participants that they may decline testing preemptively. CRS staff should also inform participants if positive results must be reported to local public health authorities. CRS staff should also inform participants of the need to maintain study blinding by getting HIV testing only at the study CRS. CRS staff should provide participants with CRS contact information and should encourage participants to ask medical providers to contact the CRS. The CRS can verify that the participant is in an HIV vaccine clinical trial and should only be tested at the study CRS.

Potential participants identified as being HIV infected during screening are not enrolled. All participants who become HIV infected during the study will be terminated from this study. Potential and enrolled participants identified as HIV infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.8.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

The study product may elicit an Ab response to HIV proteins. Therefore, vaccine-induced positive serology may occur in this study. Several precautionary measures will be taken to distinguish intercurrent HIV infection from vaccine-induced positive serology. These precautionary measures include:

- Participants will have physical examinations at visits specified in Appendix F. Signs or symptoms of an acute HIV infection syndrome, an intercurrent illness consistent with HIV-1 infection, or probable HIV exposure would prompt a diagnostic workup per the HVTN algorithm for Recent Exposure/Acute Infection Testing to determine HIV infection.

- HIV testing will be performed at multiple timepoints throughout the study (see Appendix E). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the HVTN Site Lab Reference Manual), which is able to distinguish vaccine-induced Ab responses from actual HIV infections.

- All participants can receive HIV-1 diagnostic testing from the site following their last scheduled visit until they are told that they did not receive an HIV vaccine or that they do not have vaccine-induced seropositivity.

- All participants who received vaccine product and who have vaccine-induced positive or indeterminate HIV-1 serology (as measured by the standard anti-HIV Ab screening tests) at or after the study is unblinded will be offered poststudy HIV-1 diagnostic testing (per the HVTN poststudy HIV-1 testing algorithm) periodically and free of charge as medically/socially indicated (approximately every 6 months).

9.9 Contraception status

Contraception status is assessed and documented at every scheduled clinic visit for a participant who was born female and who is sexually active in a way that could cause that participant to become pregnant. Prior to enrollment and throughout the study, staff will ask participants to verbally confirm their use of adequate contraceptive methods. A participant who was born female and is sexually active in a way that could cause that participant to become pregnant should be reminded at all scheduled clinic visits of the importance of using contraception and should be referred to specific counseling, information, and advice as needed. (Specific contraception requirements are listed in section 7.1). This reminder should be documented in the participant's study record.

Self-reported infertility—including having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation—must be documented in the participant's study record.

9.10 Urinalysis

Dipstick testing may be performed in the clinic or the lab, as long as the required elements (glucose, protein, and hemoglobin) are tested. The examination is performed on urine obtained by clean catch.

If the screening dipstick is transiently abnormal due to menses or infection, document this issue in the participant's source documentation. For infection, provide appropriate treatment and/or referral. Following resolution, repeat the dipstick and, if within the eligibility limits specified in the protocol, the participant may be enrolled.

Follow-up urinalysis should be deferred if a participant is menstruating, but should be performed as soon as possible. If a follow-up dipstick is abnormal due to a participant's menstrual period, document in the comment section of the case report form (CRF) and repeat the dipstick once the participant is no longer menstruating. A micro-urinalysis is not required.

9.11 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are followed until resolution and 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 August 2009).

The reactogenicity assessment period is 7 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record symptoms using a postvaccination symptom log and to contact the site daily during the assessment period. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 to resolution. Participants are instructed to contact the clinic for events that arise during the period between vaccination and the next scheduled visit. In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, vaccine-related lesions, and lymph nodes. Events not listed on a CRF, or with an onset after the reactogenicity assessment period (day of vaccination and 7 full days after), or those meeting SAE/AEs requiring expedited reporting to DAIDS criteria, are recorded on an adverse experience log form.

Table 9-1 Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Baseline: before vaccination	HVTN CRS staff
	Early: 25-60 minutes after vaccination	HVTN CRS staff
	Between early assessment and 11:59pm day 0	HVTN CRS staff or participant
1	Between 12:00am and 11:59pm day 1	HVTN CRS staff or participant
2	Between 12:00am and 11:59pm day 2	HVTN CRS staff or participant
3	Between 12:00am and 11:59pm day 3	HVTN CRS staff or participant
4	Between 12:00am and 11:59pm day 4	HVTN CRS staff or participant
5	Between 12:00am and 11:59pm day 5	HVTN CRS staff or participant
6	Between 12:00am and 11:59pm day 6	HVTN CRS staff or participant
7[b]	Between 12:00am and 11:59pm day 7	HVTN CRS staff or participant

[a] Day of vaccination

[b] New or unresolved reactogenicity symptoms present on day 7 are followed until resolution

9.11.1 Assessment of systemic and local symptoms

Systemic symptoms include increased body temperature, malaise and/or fatigue, myalgia, headache, chills, arthralgia, nausea, and vomiting. Local symptoms include pain and/or tenderness proximal to the injection site. The daily maximum severity reached for each symptom during the assessment period is reported.

Body temperature is measured by oral or infrared thermometry and reported in degrees Celsius. If temperature is measured in Fahrenheit, the conversion to Celsius should be documented in the participant's chart note. A measurement is taken once daily during the assessment period and should be repeated if participant is feeling feverish.

9.11.2 Assessment of injection site

Typical injection site reactions are erythema/induration/swelling/edema. The maximum horizontal and maximum vertical measurements for all injection site reactions are recorded.

All injection site reactions are monitored until resolution. Areas greater than 25 cm^2 are followed daily; otherwise, the frequency of follow-up is based on clinician judgment.

9.11.3 Assessment of lymph nodes

This assessment is required only when reactogenicity assessments are performed by HVTN CRS staff, not by the participant.

Only the proximally draining lymph nodes are assessed (eg, axillary nodes on the same side of the body for injections given in the deltoid). Lymph nodes are first evaluated for enlargement and tenderness. If they are found to be enlarged, measurements are taken to determine the size (widest diameter) of the enlarged node(s).

9.12 Visit windows and missed visits

Visit windows are defined in HVTN 096/EV04 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, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

Procedures performed at an interim visit are usually 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.

If a missed visit required vaccination, please refer to section 7.3.2 and section 7.3.3 for resolution.

9.13 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, platelet count, and chemistry panel), pregnancy testing, social impact assessment, and HIV test.

9.14 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given, but remaining visits and study procedures should be completed unless medically contraindicated or applicable regulations require termination from the study. In case of required termination, enrollment in an observational study should be offered to the participant. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome.

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN Site Lab Reference Manual provides further guidelines for operational issues concerning the clinical and processing laboratories. The manual includes guidelines for general specimen collection, special considerations for phlebotomy; specimen labeling; whole blood processing; HIV screening/diagnostic testing; and general screening and safety testing.

Tube types for blood collection are specified in Appendix E. For tests performed locally, the local lab may assign appropriate tube types.

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

Adjust volumes as appropriate for specific site(s).

Required blood volumes per visit are shown in Appendix E. The FHCRC laboratory will further specify the tube type and collection volumes in special instructions posted to the protocol-specific section of the HVTN website. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated, or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 500 mL in any 56-day (8-week) period.

10.3 Primary immunogenicity timepoint

The primary immunogenicity timepoint in this study is at visit 9 (day 182) (ie, 2 weeks after the second and fourth vaccination visit). Endpoint assays for humoral and cellular responses are performed on all participants at the primary immunogenicity timepoint and may be performed at baseline. Depending on the number of responders observed, assays for humoral and cellular responses may be performed on all participants at other timepoints; the schedule is shown in Appendix E.

10.4 Endpoint assays: humoral

10.4.1 HIV-1 multiplex antibody assay

Total binding IgG and IgA antibodies to clade B, C and AE isolates will be assessed on plasma samples from all study participants using a validated binding Ab multiplex assay (BAMA).

10.4.2 Neutralizing antibody assay

HIV-1-specific nAb assays will be performed on serum samples from all study participants taken at the primary immunogenicity timepoint. Specimens from the baseline and other timepoints may also be analyzed, contingent on the results of the primary immunogenicity timepoint. Tier 1 assays will test neutralization of HIV-1 strains represented in the highly neutralization-sensitive tier 1 clades B, C and AE isolates and pseudovirions matched to the vaccine constructs. Tier 2 assays may be conducted to test neutralization of a panel of primary clades B, C and AE isolates. Neutralization of additional isolates may be assessed.

10.5 Endpoint assays: cellular

10.5.1 Flow cytometry

Flow cytometry will be used to examine vaccine-specific CD4+ and CD8+ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine construct. ICS parameters will include cytokines such as IFN-γ, IL-2, and TNF-α, and may include other cytokines to identify T cells of specific functionality (such as Th2 and Th17). Markers of cytotoxic potential (Granzyme B, perforin and CD57) may also be included. Data will be reported as percentages of CD4+ or CD8+ T cells responding to a specific peptide pool. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

10.5.2 Multiplex cytokine bead array

Luminex assays may be performed to examine cytokines, chemokines, and other immunomodulatory factors that emerge following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine construct. Panel selection for makers will be based on the markers' established or potential importance as immune correlates or biomarkers of protection from HIV acquisition or disease progression. Such analytes may include, but are not limited to: IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, MIP-1β, TNF-β and GM-CSF.

10.6 Genotyping

Molecular human leukocyte antigen (HLA) typing may be performed on enrolled participants using cryopreserved PBMC collected at baseline, initially on specimens from participants who demonstrate vaccine-induced T-cell responses at postvaccination timepoints. Other participants (including placebo recipients) may be HLA-typed to support future studies of immunological interest at the discretion of the HVTN Laboratory Program. Other markers, such as genes associated with immune responses or HIV-1 disease progression, may also be assessed.

10.7 Exploratory studies

Samples may be used for other testing and research related to furthering the understanding of HIV immunology or vaccines. In addition, cryopreserved samples may be used to perform additional assays to support standardization and validation of existing or newly developed methods.

10.7.1 Antibody-dependent cellular cytotoxicity (ADCC) assay

As an exploratory analysis, the induction of antibodies capable of mediating Ab-dependent cellular cytotoxicity (ADCC) may be investigated. The ADCC assay will use either gp120-coated cells or HIV-1 -nfected cells as targets (eg, CEM.NK.CCR5 cells) and either cryopreserved PBMC or a suitable cell line as effector cells. Percent specific ADCC activity in plasma will be based on either degranulation or lysis, and will be quantified by either flow cytometry or luminescence, respectively. If reactivity is detected against viruses of the same clade as the vaccine strain, the breadth of responses will also be evaluated.

10.7.2 IgG subtyping

Data obtained from the HIV-1 multiplex Ab assay will be analyzed to determine the distribution of isotypes (IgG1, IgG2, IgG3, IgG4) of vaccine-induced antibodies.

10.7.3 Antibody avidity

Antigen-specific IgG avidity may be determined using a BIAcore instrument. Binding responses (steady-state), dissociation constant (Kd) and dissociation rate constant (Kd, off-rate) as measures of Ab affinity will be determined for Ab responses in participants' plasma samples. Higher avidity Ab responses will be defined as those with relatively lower Kd (~nM range) and slower off-rates (~10^{-3} s^{-1}) for a specific antigen. To monitor changes in Ab avidity, surface plasmon resonance (SPR)-based assays provide the capability to detect lower avidity Ab with faster off-rates (Kd < 0.1 s^{-1}).

10.7.4 Antibody epitope mapping by peptide array

Linear Ab epitopes may be mapped using peptide array technology. The array will consist of 15mer peptides overlapping by 12 amino acids covering multiple full length Env consensus sequences consisting of ConA, ConB, ConC, ConD, ConAG, ConAE, and ConM.

10.7.5 Virion capture assay

The virion capture assay will use participants' plasma samples to measure the ability of HIV specific binding antibodies (purified IgG and/or purified IgA) to bind to intact virions.

10.7.6 Mucosal antibodies

As an exploratory endpoint, total binding IgG and IgA antibodies to HIV-1 may be assessed on cervical, rectal, semen, and/or saliva secretions using the binding Ab multiplex assay.

10.7.7 B-cell ELISpot

B-cell ELISpot assays may be conducted on PBMCs to enumerate B cells that secrete HIV-specific antibodies. The total number of B cells secreting IgG and/or IgA will be compared to the number of B cells secreting HIV-specific antibodies using Env proteins as antigens. Responses will be reported as the percentage of antigen-specific B cells among total Ab-secreting cells.

10.8 Other use of stored specimens

The HVTN aims not only to test vaccine candidates but also to continue to explore the correlates of immunity to HIV. In order to do so, the HVTN intends to store blood and fluid samples from participants. These samples will be used for other testing and research related to furthering the understanding of virology, immunology, or vaccinology 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/EC and any applicable regulatory bodies 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 he or she signs 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 decides against allowing other research using his or her samples, or at any time rescinds prior approval for such other use, the study site investigator or designee must notify HVTN Regulatory Affairs in writing. In either case, after database lock, the HVTN Laboratory Program will request that the repository destroy all specimens with the participant identification numbers (PTIDs) 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 Principal Investigators (PIs).

Study site must notify HVTN Regulatory Affairs if applicable requirements pose a conflict with or impose restrictions on the use of stored specimens.

10.9 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 applicable 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 safety review

11.1 Safety monitoring and oversight

This trial is sponsored by EuroVacc and will be conducted in Switzerland: hence safety reporting will be based on the SwissMedic guidelines.

11.1.1 HVTN 096/EV04 PSRT

The HVTN 096/EV04 PSRT is composed of the following members:

- EuroVacc medical officer representative,
- Protocol chair and cochair,
- Protocol Team leader,
- Core medical monitor,
- SDMC Clinical Affairs safety associate, and
- DAIDS medical officer representative

The clinician members of HVTN 096/EV04 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, project manager, vaccine developer representatives, clinical trial manager, and others may also be included in HVTN 096/EV04 PSRT meetings.

11.1.2 HVTN Safety Monitoring Board

The SMB is a multidisciplinary group consisting of biostatisticians, clinicians, and experts in HIV vaccine research that, collectively, has experience in the conduct and monitoring of vaccine trials. Members of the SMB are not directly affiliated with the protocols under review.

The SMB reviews safety data, unblinded as to treatment arm, approximately every 4 months during the main study, as defined in Section 3 (for safety reviews during the *Annual health contacts* period, please see Section 9.7). The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting. To increase the sensitivity for detecting potential safety problems, the SMB will review safety data aggregated across multiple protocols that use the same or similar vaccine candidates. The SMB conducts additional special reviews at the request of the HVTN 096/EV04 PSRT.

Study site will receive SMB summary minutes and are responsible for forwarding them to their IRB/EC and any applicable RE.

11.1.3 SDMC roles and responsibilities in safety monitoring

The roles and responsibilities of the SDMC in relation to safety monitoring include:

- Maintaining a central database management system for HVTN clinical data;
- Providing reports of clinical data to appropriate groups such as the HVTN 096/EV04 PSRT and HVTN SMB (see Section 11.1.2);
- Daily monitoring of clinical data for events that meet the safety pause and HVTN 096/EV04 PSRT AE review criteria (see Section 11.4);
- Notifying HVTN CRSs and other groups when safety pauses are instituted and lifted (see Section 11.4);
- Querying HVTN CRSs for additional information regarding reported clinical data; and
- Providing support to the HVTN 096/EV04 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

Site must submit all safety forms (eg, reactogenicity, adverse experience, urinalysis, local lab results and concomitant medications) before the end of the next business day after receiving the information. The forms should not be held in anticipation of additional information at a later date. If additional information is received at a later date, the forms should be updated and refaxed before the end of the next business day after receiving the new information.

11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs 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 on the DAIDS Regulatory Support Center website at http://rsc.tech-res.com/safetyandpharmacovigilance/, except:

- Unintentional weight loss of less than 5% loss in body weight from baseline is not required to be reported as an AE;
- PR interval $\leq$ 0.219 sec will not be reported as an AE;
- Sinus arrhythmia will not be reported as an AE; and
- Asymptomatic increase in QTc interval < 0.06 sec above baseline, with a QTc $\leq$ 0.45 sec, will not be reported as an AE.

The definition of Grade 1 mild prolonged PR interval (Adult > 16 years) that will be used is:

- 0.22 - 0.25 sec.

The criteria for prolonged QTc interval that will be used are:

- Grade 1, mild: asymptomatic, QTc interval 0.45–0.47 sec;
- Grade 2, moderate: asymptomatic, QTc interval 0.48–0.49 sec;
- Grade 3 severe: asymptomatic, QTc interval ≥ 0.50 sec OR increase in interval ≥ 0.06 sec above baseline; and
- Grade 4, potentially life-threatening: life-threatening consequences (eg, Torsade de pointes or other associated serious ventricular dysrhythmia).

All AEs are reported to the SDMC on the appropriate CRF. Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting (using SUSAR reporting category) to SwissMedic (section 11.2) and (2) if the AE meets the criteria for a safety pause/prompt AE review (section 11.4).

The study site is expected to notify SDMC Clinical Affairs staff of any serious safety concern requiring their attention (see Table 11-1). Telephone numbers and email addresses are listed in the Key Resource Guide of the HVTN 096/EV04 Study Specific Procedures. Concerns requiring immediate attention should be communicated by calling the SDMC Clinical Affairs safety phone.

In the case of email notification, SDMC Clinical Affairs staff will reply during working hours (US Pacific Time) to confirm that the email has been received and reviewed. If email service is not available, the HVTN CRS should notify SDMC Clinical Affairs of the event by telephone, and then submit CRFs.

In addition, site investigators are required to submit AE information in accordance with SwissMedic's requirements.

11.2.3 Serious Adverse Events and Reporting to SwissMedic

An AE is considered to be an "SAE" by ICH GCP criteria if it results in the following:

- death,
- a threat to life,
- requires in-patient hospitalisation or prolongs existing hospitalisation (hospitalisation for elective treatment of a pre-existing condition is not included),
- results in persistent or significant disability or incapacity,
- is a congenital anomaly (ie, the outcome of pregnancy involving a participant), or
- is any other important medical condition*.

*Examples of conditions regarded as "any other important medical condition" include myo/pericarditis, allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias which did not result in hospitalisation or development of drug dependency.

Any SAE that is considered related to study product and unexpected will qualify for expedited reporting to SwissMedic. The expedited reporting period for this study comprises the entire study period for each individual participant (from study enrolment until study completion or discontinuation from the study).

Site staff should report any SAE requiring expedited reporting to Clinical Affairs at SCHARP within 24 hours of the site's awareness of the event. Clinical Affairs staff notifies the HVTN 096 PSRT as soon as possible during working hours (US Pacific Time) – or, if the information was received during off hours, by the morning of the next working day – that a prompt PSRT AE review is needed. The PSRT review will take place within 48 hours.

When PSRT confirms that the event qualifies in relationship and expectedness as an SAE requiring expedited reporting, the Sponsor or designee(s) prepares and files expedited reports to appropriate regulatory authorities and ECs within the timelines required by SwissMedic.

The study products for which expedited reporting are required are:

- DNA-HIV-PT123 or placebo **(Sodium Chloride for Injection, 0.9%)**
- NYVAC-HIV-PT1 and NYVAC-HIV-PT4 or placebo **(Sodium Chloride for Injection, 0.9%)**
- AIDSVAX® B/E with alum or placebo **(Aluminum Hydroxide Gel Adjuvant)**

While the participant is in the main study reporting period (see Section 3), the SAE / SUSAR Reporting Category will be used. After completion of the main study, only SUSAR (the same rule as in the main study period) will be reported on an expedited basis.

If the PSRT believes unblinding of the site PI to treatment assignment will assist with the clinical management of the SAE, the PSRT will consult the independent HVTN SMB for a recommendation. In the event the HVTN SMB determines that unblinding is indicated, the SMB will inform the site physician of the participant's treatment assignment in such a manner as to maintain the study blind of the PSRT and study team. For additional impact and management of SAEs on the study, refer to section 11.4.

11.3 Safety reviews

11.3.1 Initial safety evaluation

Enrollment across all participating HVTN CRSs will be restricted to a maximum of 2 participants per day until 20 participants have been enrolled with 5 in each group. The HVTN 096 PSRT will review the cumulative safety data including at minimum local and systemic reactogenicity data reported for the first 168 hours postvaccination on each of these 20 participants, and will determine whether it is safe to proceed with full enrollment of the study.

11.4 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccinations with the product related to the event that triggered the pause will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 096/EV04 PSRT AE review are summarized in Table 11-1. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the HVTN 096/EV04 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in section 7.3.

Table 11-1 AE notification and safety pause/AE review rules

Event and relationship to study products	Severity	HVTN CRS action	SDMC action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and fax forms immediately[a]	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and fax forms immediately	Immediate HVTN 096/EV04 PSRT notification
SAE, related	Grade 3	Email and fax forms immediately	Prompt HVTN 096/EV04 PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and fax forms immediately	Prompt HVTN 096/EV04 PSRT AE review to consider pause

For all safety pauses, the SDMC Clinical Affairs staff notifies the HVTN 096/EV04 PSRT, HVTN Regulatory Affairs, EuroVacc and participating HVTN CRS. When an immediate safety pause is triggered, the SDMC Clinical Affairs staff also notifies the HVTN SMB.

Once a trial is paused, the HVTN 096/EV04 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the SMB if necessary. SDMC Clinical Affairs staff notifies the participating HVTN CRS, HVTN Regulatory Affairs, and EuroVacc of the decision regarding resumption or discontinuation of study vaccinations.

If an immediate HVTN 096/EV04 PSRT notification or prompt HVTN 096/EV04 PSRT AE review is triggered, the SDMC Clinical Affairs staff notifies the HVTN 096/EV04 PSRT as soon as possible during working hours (US Pacific Time)—or, if the information was received during off hours, by the morning of the next work day. If a prompt HVTN 096/EV04 PSRT AE review cannot be completed within 72 hours of SDMC notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

The HVTN CRS is responsible for submitting to its IRB/IEC and any local regulatory authority protocol-related safety information (such as safety reports, notification of vaccine holds due to the pause rules, etc), as required.

In addition, all other AEs are reviewed routinely by the HVTN 096/EV04 PSRT (see Section 11.5.2).

11.5 Review of cumulative safety data

Routine safety review occurs at the start of enrollment and then throughout the study.

[a] Phone numbers and email addresses are listed in HVTN 096 Study Specific Procedures, Key Resource Guide.

[b] Does not include subjective reactogenicity symptoms (injection site pain, tenderness, fatigue/malaise, myalgia, arthralgia, chills, headache, and nausea).

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.

11.5.1 Daily review

Blinded daily safety reviews are routinely conducted by the SDMC Clinical Affairs staff for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 096/EV04 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the SDMC Clinical Affairs staff and the HVTN 096/EV04 PSRT review clinical safety reports on a weekly basis and conduct calls to review the data as appropriate. After the injections and the final 2-week safety visits are completed, less frequent reporting and safety reviews may be conducted at the discretion of the HVTN 096/EV04 PSRT. The SDMC Clinical Affairs staff reviews reports of clinical and laboratory AEs. Events identified during the review that are considered questionable, inconsistent, or unexplained are referred to the HVTN CRS clinic coordinator for verification.

11.6 Study termination

This study may be terminated early by the determination of the HVTN 096/EV04 PSRT, HVTN SMB, SwissMedic, DAIDS, EuroVacc, or other vaccine developers. In addition, the conduct of this study at an individual HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

12 Protocol conduct

This protocol and all actions and activities connected with it will be conducted in compliance with the principles of GCP (ICHE6), and according to DAIDS and HVTN policies and procedures as specified in the *HVTN Manual of Operations*, DAIDS Clinical Research Policies and Standard Procedures Documents including procedures for the following:

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

Any policies or procedures that vary from SwissMedic/DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 096/EV04 *Study Specific Procedures.*

12.1 Social impacts

Participants in this study risk experiencing discrimination or other personal problems, resulting from the study participation itself or from the development of VISP. The HVTN CRS is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding ways to assist a participant dealing with a social impact, a designated NIAID or HVTN Core representative can be contacted.

Social harms are tabulated by the SDMC and are subjected to descriptive analysis. The goal is to reduce their incidence and enhance 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

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

Because this study is evaluating products containing recombinant DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant DNA Molecules.* Information about the study must be submitted to site Institutional Biosafety Committees (IBC) and must be approved before participants are enrolled at the site. 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. If this protocol is amended, investigators should follow the requirements of their IBC.

12.3 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 to report study findings that may otherwise concern their health or welfare.

When such communication is needed, the CRS will request that its IRB/EC and any applicable RE expedite review of the message. If this review cannot be completed in a timeframe consistent with the urgency of the required communication, the site should contact the participant first, and then notify the IRB/EC and any applicable RE of the matter as soon as possible.

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 096/EV04.

Protocol history and modifications

Date	Protocol version	Protocol modification	Comment	
December 12, 2013	Version 2.0	Full Protocol Amendment 1	Item 1	Added new section 4.5.4, *Rationale for Full protocol Amendment*: discussion of diagnosis of myocarditis possibly related to a NYVAC vaccination in a participant in HVTN 092
			Item 2	Modified in Section 4.10, *Potential risks of study products and administration*, Table 4-14: myopericarditis (NYVAC) moved to separate category of Unknown frequency
			Item 3	Revised in Section 9.4, *Follow-up visits:* 12-lead ECG with interpretation added
			Item 4	Added to Section 9.5, *Cardiac monitoring*: clarification regarding the occurrence of myopericarditis in one participant who received a NYVAC vaccination in HVTN 092
			Item 5	Added to Section 9.5.1, *ECG testing*: a follow-up 12-lead ECG
			Item 6	Added to Appendix D, *Table of procedures (for sample informed consent form):* new footnote keyed to the line for Electrocardiogram (ECG)
			Item 7	Added to Appendix F, *Clinical procedures:* new text in the footnote associated with the ECG at screening
			Item 8	Added new Appendix H, *Addendum to informed consent*
			Item 9	Revised in Section 4.9.2.1, *VRC DNA*, Table 4-10 to reflect HVTN 505 vaccinations stopped for futility
March 8, 2013	Version 1.02	Amendment 2	Item 1	Added to Section 9.7, *Annual health contacts*: updated template language regarding frequency and responsibility for reviewing safety reports when study participants are being followed with annual health contacts
			Item 2	Added to Section 11.1.2, *HVTN Safety Monitoring Board*: a change in template language regarding the frequency of Safety Monitoring Board review of safety data during the main study and during annual health contacts
August 23, 2012	Version 1.01	Amendment 1	Item 1	Revised in Section 11.2.2, *AE reporting*: the percentage of unintentional weight loss that would require an adverse event (AE) report
June 6, 2012	Version 1.0	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:

- SwissMedic Clinical Trial Regulations and Guidelines. Available at *http://www.swissmedic.ch/index.html?lang=en*
- 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 Protocol Registration Manual. Available at http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/prmanual.pdf
- 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
- HVTN 096/EV04 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 096/EV04 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN Site Lab Reference Manual. Accessible through the HVTN website.
- HVTN Manual of Operations. Accessible through the HVTN website.
- HVTN algorithm for diagnosis of HIV infections. Part of the HVTN Site Lab Reference Manual (see above).
- International Conference on Harmonisation (ICH) E6 (R1), Guideline for Good Clinical Practice: section 4.8, Informed consent of trial subjects. Available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf
- Participants' Bill of Rights and Responsibilities. Accessible through the HVTN website.

- NIH Guidelines for Research Involving Recombinant DNA Molecules. Available at http://oba.od.nih.gov/rdna/nih_guidelines_oba.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.

- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://phacs.nichdclinicalstudies.org/publicDocs/DAIDS_SourceDocPolicy.pdf

- Title 21, Code of Federal Regulations, Part 50. Available at http://www.access.gpo.gov/nara/cfr/waisidx_08/21cfr50_08.html.

- Title 45, Code of Federal Regulations, Part 46. Available at http://www.access.gpo.gov/nara/cfr/waisidx_07/45cfr46_07.html.

- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at http://www.iata.org/ps/publications/dgr/Pages/index.aspx

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

15 Acronyms and abbreviations

Ab	antibody
AE	adverse event
ALT	alanine aminotransferase
ART	antiretroviral therapy
AST	aspartate aminotransferase
AVEG	AIDS Vaccine Evaluation Group
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	confidence intervals
CRF	case report form
CRS*	clinical research site
CTL	cytotoxic T lymphocyte
DSMB	NIAID Data and Safety Monitoring Board
EC	Ethics Committee
ELISpot	enzyme-linked immunospot
FDA	US Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IFN-γ	interferon gamma
IND	Investigational New Drug
IRB	Institutional Review Board
MAR	missing at random
MCAR	missing completely at random
nAb	neutralizing antibody
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NIH	US National Institutes of Health
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PI	Principal Investigator
PSRT	Protocol Safety Review Team

PTE	potential T-cell epitope
RE	regulatory entity
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SFU	spot-forming unit
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
VISP	Vaccine induced seropositivity
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.

16 Literature cited

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Appendix A Sample informed consent form

Title: A phase 1 double blind placebo-controlled clinical trial to evaluate the safety and to compare the priming ability of NYVAC alone versus NYVAC + AIDSVAX® B/E, and DNA alone versus DNA + AIDSVAX® B/E when followed by NYVAC + AIDSVAX® B/E boosts in healthy, HIV-1-uninfected adult participants

HVTN protocol number: HVTN 096/EV04

Site: [Insert site name]

Thank you for your interest in our research study. Please read this consent form or ask someone to read it to you. If you decide to join the study, we will ask you to sign this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test HIV vaccines. HIV is the virus that causes AIDS.

About 96 people will take part in this study at the Lausanne site. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

1. **We are doing this study to answer several questions.**

    - Are the study vaccines safe to give to people?
    - Are people able to take the study vaccines without becoming too uncomfortable?
    - How do people's immune systems respond to the study vaccines? (Your immune system protects you from disease.)

2. **The study vaccines cannot give you HIV.**

The study vaccines are not made from actual HIV. It is impossible for the study vaccines to give you HIV. Also, they cannot cause you to give HIV to someone else.

3. **We do not know if the study vaccines will decrease, increase, or not change your chance of becoming infected with HIV if you are exposed to the virus.**

Site: Any change to the language in this section requires approval from HVTN Regulatory Affairs.

Several studies have tested whether HIV vaccines can reduce the risk of getting HIV from another person. In some studies, people who got the vaccine seemed to have the *same* risk of getting HIV as people who did not get the vaccine. In one study, people who got the vaccine seemed to have a *lower* risk of getting HIV than people who did not get

the vaccine. In another study, some men who got the vaccine had a *higher* risk of getting HIV than men who did not get the vaccine.

This study differs from the studies in which people who got the vaccine had a higher or lower risk of getting HIV. The study staff can tell you about the differences.

We do not know whether the vaccines in this study will affect your risk of getting HIV from another person. The risk could be higher, lower, or unchanged. It's very important to avoid exposure to HIV during and after the study. We will tell you how to avoid HIV.

4. These study vaccines are experimental.

There are three vaccines in this study. They are: DNA-HIV-PT123 (which we will call "DNA"), NYVAC-HIV-PT-1/NYVAC-HIV-PT4 (which we will call "NYVAC ") and AIDSVAX® B/E (which we will call "AIDSVAX"). From here on, we will call them the study vaccines. They are experimental HIV vaccines. That means we do not know whether the vaccines will be safe to use in people, or whether they will work to prevent HIV infection. These vaccines are used only in research studies.

The NYVAC and DNA vaccines were developed by the EuroVacc Foundation and the IPPOX Foundation, respectively. The AIDSVAX vaccine was originally developed by Genentech, Inc., and is now being developed by Global Solutions for Infectious Diseases (GSID). It is being provided for this study by the United States Military HIV Research Program (USMHRP).

The DNA and NYVAC study vaccines contain pieces of man-made HIV DNA. DNA is a natural substance found in all living things, including people and some viruses. DNA instructs the body to make proteins. When these study vaccines are injected, the DNA will tell the body to make small amounts of proteins that look like the ones found in HIV. This study aims to evaluate if your immune system is able to develop a response to these proteins.

The DNA study vaccine is made only of DNA. The NYVAC study vaccine is made out of a virus called vaccinia. It is similar to the smallpox vaccine that has been used worldwide. Neither the smallpox vaccine nor the vaccine used in this study can give you smallpox.

The AIDSVAX study vaccine is made of man-made proteins which are similar to proteins from the outer surface of HIV. Your body's immune system may respond to this study vaccine by making antibodies that recognize and fight against HIV proteins. Antibodies are special proteins made by the body that can recognize and prevent infections.

Sometimes the body responds better when the vaccine is combined with another substance that helps to alert the immune system. These substances are called adjuvants. The AIDSVAX study vaccine includes an adjuvant called Aluminum hydroxide (Alum). The placebo for AIDSVAX is also Alum. (See #11 below for the description of a placebo.) The exact DNA and NYVAC vaccines for this study have not been given to people before. A similar DNA vaccine has been given to about 1300 people in other studies. A similar NYVAC vaccine has been given to about 287 people in other studies. Combinations of similar DNA and NYVAC study vaccines have been given to about 170 people. In all these studies, people were able to take the vaccines without becoming too uncomfortable. Also, these vaccines did not cause health problems. AIDSVAX has been

previously used in thousands of study volunteers in Thailand and has an excellent safety profile. AIDSVAX has not been given before in combination with these DNA or NYVAC vaccines.

Joining the study

5. It is completely up to you whether or not to join the study.

Take your time in deciding. If it helps, talk to people you trust, such as your doctor, friends or family. If you decide not to join this study, or if you leave it after you have joined, your other care at this clinic and the benefits or rights you would normally have will not be affected.

If you join this study, you may not be allowed to join other HIV vaccine or HIV prevention studies now or in the future. Also during the study, you should not donate blood or tissue.

If you choose not to join this study, you may be able to join another study.

Site: Remove item 6 if you use a separate screening consent that covers these procedures.

6. If you decide to join the study, we will screen you to see if you are eligible.

Screening involves a physical exam, HIV test and health history. A physical exam may include, but is not limited to:

- Checking your weight, temperature and blood pressure
- Looking in your mouth and throat
- Listening to your heart and lungs
- Feeling your abdomen (stomach and liver)

We will also do blood and urine tests. These tests tell us about some key aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for syphilis, hepatitis B, and hepatitis C. We will ask you about medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were born female, we will test you for pregnancy.

We will do an electrocardiogram (ECG or EKG) to see if you can join the study. The ECG tells us about your heart rate, rhythm, and blood flow. For the ECG, we will place leads on your chest, arms and legs using suction cups with gel or stickers, and you will need to lie still for several minutes.

We will review the screening results with you, and offer you counseling and referral if you need medical care. We will not pay for this medical care. The screening results may show you are not eligible to join the study, even if you want to. You cannot be in this study while you are in another study where you receive a study product.

7. **If you were born female and could become pregnant, you must agree to use birth control to join this study.**

   Site: List approved birth control methods here if you do not want to hand out the separate Approved Birth Control Methods sheet.

   You must agree to use effective birth control from three weeks before your first injection through completion of the last required study clinic visit. We will talk to you about effective birth control methods. They are listed on a handout that we will give to you. *Site: Delete the preceding sentence if you list approved birth control methods in this consent form.* If you join the study, we will test you for pregnancy at some visits, including before each study injection.

Being in the study

If you meet the study requirements and want to join, here is what will happen:

8. **You will come to the clinic for scheduled visits about 14 times over 18 months.**

   Site: Insert range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)

   Visits can last from [#] to [#] hours.

   You may have to come for more visits if you have a lab or health issue.

   We may contact you after the main study ends (for example, to tell you about the study results).

9. **After you finish your clinic visits, we will contact you annually to ask about your health.**

   After the clinic visits are completed, we will contact you once each year to check on your health. These annual health contacts will continue until 5 years after you received your first study injection.

   We will talk more about this part of the study in section 26 of this form.

10. **We will give you [Site: Insert compensation] for each study visit you complete.**

    This amount is to cover the costs of [Site: Insert text]

    Site: Insert any costs to participants (eg, birth control costs for female participants who could become pregnant).

    You do not have to pay anything to be in this study.

11. **We will give you either the study vaccines or a placebo.**

    Not everyone in this study will get the study vaccines. Some people will get the placebo, injections that do not contain vaccine. We will compare the results from people who got the placebo with results from people who got the study vaccines. The placebo for two of

the vaccines is sterile salt water (saline) and the placebo for the third vaccine is called Alum.

You have a 5-in-6 chance of receiving the study vaccines.

Site: Modify the randomization metaphor in the below paragraph as appropriate to your local culture.

Whether you get the study vaccines or the placebo is completely random, like flipping a coin.

The reason we are testing the study vaccines is because we do not know whether they work or are safe. That means we do not know whether it is better to get the vaccine or to get the placebo. In either case, you need to take steps to protect yourself from HIV infection.

The clinic staff has no say in whether you get the study vaccines or the placebo. They will not know which one you are getting, and neither will you. Only the pharmacist at your site will have this information while the study is going on, and he or she will keep it a secret.

You will have to wait until everyone completes their final study visits to find out whether you got the study vaccines or the placebo. This could be several years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

12. We will give you the study products on a schedule.

You will be in one of 4 groups. Everyone in the study will get 3 injections at each injection visit. Two injections will be given with a needle into the upper Left arm, and one into the upper Right arm.

Injection Schedule

Group	First injection	1 month later	3 months later	6 months later
1	NYVAC	NYVAC	NYVAC + AIDSVAX® B/E	NYVAC + AIDSVAX® B/E
	or	or	or	or
	placebo	placebo	placebo	placebo
2	NYVAC + AIDSVAX® B/E	NYVAC + AIDSVAX® B/E	NYVAC + AIDSVAX® B/E	NYVAC + AIDSVAX® B/E
	or	or	or	or
	placebo	placebo	placebo	placebo
3	DNA	DNA	NYVAC + AIDSVAX® B/E	NYVAC + AIDSVAX® B/E
	or	or	or	or
	placebo	placebo	placebo	placebo

4	DNA + AIDSVAX® B/E	DNA + AIDSVAX® B/E	NYVAC + AIDSVAX® B/E	NYVAC + AIDSVAX® B/E
	or	or	or	or
	placebo	placebo	placebo	placebo

You will have to wait in the clinic for about a half hour after each injection to see if there are any problems. Then for that night and for seven more days, you will need to write down how you are feeling and if you have any symptoms. Contact the clinic staff if you have any issues or concerns after receiving an injection. If you have a problem, we will continue to check on you until it goes away.

13. In addition to giving you the study products, we will perform these procedures:

- Regular HIV testing, as well as counseling on your results and on how to avoid getting HIV;
- Physical exams;
- Taking blood and urine samples;
- Collection of saliva (this is optional);
- Collection of rectal fluids (this is optional);
- Collection of cervical fluids if you were born female (this is optional);
- Collection of semen if you were born male (this is optional),
- Testing for certain infections that may be sexually transmitted (if you agree to the optional collection of rectal, semen, and/or cervical fluids). If you do not enroll in the study, we will review your STI results with you and refer you to care, if needed.
- Pregnancy tests if you were born female;
- Questions about your health, including medications you may be taking;
- Personal questions about your HIV risk, including sexual behavior and drug use;
- Questions about any personal problems or benefits you may have from being in the study; and
- ECG.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 210 mL (less than 2 tablespoons to about 1 cup). Your body will make new blood to replace the blood we take out.

Site: You may want to add a sentence to the end of the previous paragraph contextualizing the blood volumes described (eg, "To compare, people who donate blood

in the US can give a total of about 500 mL in an 8-week period.”). Modify the example for cultural relevance and alter blood volumes as necessary.

Site: Paste table of procedures in this section or distribute it as a separate sheet if it is helpful to your study participants.

We will review the results of these procedures and tests with you at your next visit, or sooner if necessary. We will also offer you counseling and referral for needed care.

14. If you agree, we will collect salivary, rectal, semen, and cervical fluids.

Because most people are exposed to HIV on their penis, vagina, or rectum, it is important to learn more about vaccine effects in these locations and in other similar locations. For this reason, we want to collect cervical, rectal, semen, and salivary fluids before you receive your first injection and at your clinic visits at Months 6 1/2 and 12. We would only do this if you agree and are able to provide the samples.

If you agree and are able to provide the cervical and/or rectal samples, you must not have unprotected vaginal or anal sex for 24 hours before providing these samples. This will help make sure the samples you provide give accurate lab readings. If you have sex during that time, please ask your partner to use a condom.

We will collect salivary fluid by asking you to spit into a collection container. If necessary, we can provide you with chewing wax, which helps produce saliva.

For participants who were born female and who agree, we will collect cervical fluids by inserting a speculum into your vagina and placing a special piece of filter paper in the opening of the cervix.

For participants who were born male and who agree to give semen samples, we will ask you to masturbate and collect your semen in a container. You can collect your semen at the study site or you can do it at home and bring it in.

We will collect rectal fluids by wiping the lining of your rectum with a cotton swab, sponge, or brush or we may place a special strip of absorbing paper inside the rectum for about 5 minutes. An anoscope, a plastic viewing tube 2-3 inches long and ½ inch wide, may be inserted into the rectum so that the clinician can see better when doing this procedure.

At the end of this consent form, we will ask you if you allow us to collect these samples. You can decide not to give these samples and still be in the study. You can decide to provide some of these samples and not others. If you agree to provide these samples, you can change your mind at any time during the study.

15. We will test your samples for reactions to the study products.

We will be looking for side effects. If any of the results are important to your health, we will tell you.

We will send your samples (without your name) to a lab to see how your immune system responds to the study products. This may include limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. Limited genetic testing involves only some of your genes (your genome). The

researchers will not look at all of your genes, only the genes related to the immune system and diseases. These tests are for research purposes only. The lab will not give the results to you or this clinic, and the results will not become part of your study record. Your samples may be sent to the HVTN lab in the United States.

16. When we take samples from you for this study, we take extra samples in case we have to repeat tests. If the samples are no longer needed for this study, the HVTN wants to keep them for use in other studies.

These other studies are likely to be about HIV and the immune system. However, they could also help researchers understand other diseases.

At the end of this form we will ask if you agree to donate your extra samples combined with limited information. It is your decision. What you decide will not affect your study participation or any care you receive here. If you do not agree, the HVTN will make sure that your samples are destroyed when they are no longer needed for this study. If you agree, there is no limit on how long your samples will be stored. You can change your mind at any time and your samples will be destroyed.

The HVTN will not sell your samples or information.

What information might be shared with the samples? We will not share any information that would make it easy for anyone to identify you. However, some information may be personal, such as your race, ethnicity, sex, and health, including HIV status. Other information may be what product you received and how your body responded to the product.

What type of studies might be done with the extra samples and information? We cannot guess exactly how your extra samples and information will be used. To use them, any researcher must have his or her institutional review board (IRB) or ethics committee (EC) review the use of the samples. The IRB/EC protects the rights and well-being of people in research.

The studies may include limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. Limited genetic testing involves only some of your genes, not all of your genes (your genome). The researchers will not look at all of your genes, only the genes related to the immune system and diseases.

If you agree, your extra samples and information could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases.

The researchers may put the information from the genome wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. The risk of this is very small.

Will I see the results of the studies? The researchers will not report their results to you, this clinic, or your doctor. The results will not be in your medical record. These other

studies will not benefit you personally and they are not necessary for your medical care. Instead, the studies might help the public through new scientific discoveries.

17. We will do our best to protect your private information.

Your study records and samples will be kept in a secure location. We will label all of your samples and most of your records with a code number, not your name or other personal information. However, it is possible to identify you, if necessary. We will not share your name with the lab that does the tests on your samples, or with anyone else who does not need to know your name.

Clinic staff will have access to your study records. Your records may also be reviewed by groups who watch over this study to see that we are protecting your rights, keeping you safe, and following the study plan. These groups include:

- The US National Institutes of Health and its study monitors,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] , (this group protects the rights and well-being of people in research),
- SwissMedic,
- IPPOX Foundation and people who work for them,
- EuroVacc Foundation and people who work for them,
- Global Solutions for Infectious Diseases (GSID) and the people who work for them,
- United States Military HIV Research Program (USMHRP) and the people who work for them,
- The HIV Vaccine Trials Network (HVTN) and people who work for them,
- The HVTN Safety Monitoring Board or the National Institute of Allergy & Infectious Diseases (NIAID) Data and Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. At this clinic, we have to report the following information:

Site: Include any public health or legal reporting requirements. Bulleted examples should include all appropriate cases (reportable communicable disease, risk of harm to self or others, etc.).

- [Item 1]

- [Item 2]
- [Item 3]

Researchers who use your stored samples and limited information for other research will also do their best to protect your private information. The samples and limited information they receive will be labeled with a code number. They will not have your name or any personal information. Any reviewers of those studies will take steps to keep your records private.

The results of this study, and other studies that use the samples or information you agree to donate, may be published. No publication will use your name or identify you personally.

We may share information from the study with other researchers. We will not share your name or personal information.

18. We may stop your injections or take you out of the study at any time. We may do this even if you want to stay in the study and even if you were scheduled for additional injections.

This may happen if:

- you do not follow instructions,
- the researcher thinks that staying in the study might harm you,
- you get HIV,
- you enroll in a different research study where you receive another study product, or
- the study is stopped for any reason.

If we stop your injections, we may ask you to stay in the study to complete other study procedures.

19. If you become pregnant during the study, we will continue with some procedures but not injections.

We will do this for as long as it is safe for you and your developing baby.

If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery.

20. If you get infected with HIV during the study, we will help you get care and support.

You will not be able to stay in this study. We will counsel you about your HIV infection and about telling your partner(s). We will tell you where you can get support and medical care, and about other studies you may want to join. We will not provide or pay for any of your HIV care directly. *Site: Modify the preceding sentence as appropriate*

Risks

21. There are risks to being in this study.

This section describes the risks and restrictions that we are aware of. Unknown risks, that might be serious, can exist. We will tell you if we learn anything new that may affect your willingness to stay in the study.

Risks of routine medical procedures:

In this study, we will do some routine medical procedures. These are taking blood and giving injections. These procedures can cause bruising, pain, fainting, soreness, redness, swelling, itching, muscle damage, and (rarely) infection where the needle was inserted. Taking blood can cause a low blood cell count (anemia), making you feel tired.

General risks of vaccines:

Rarely, a vaccine can cause an allergic reaction, including a rash, hives, or difficulty breathing. Allergic reactions can be life-threatening. You should tell us if you have ever had a bad reaction to any injection or vaccine.

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Most people can still do their planned activities after getting a vaccine. Rarely, people experience side effects that limit their normal activities or make them go to the doctor.

Very rarely, a vaccine causes an autoimmune disease in a person, or makes an autoimmune disease worse. An autoimmune disease happens when your immune system attacks your own body, instead of attacking an infection.

Scientists think it may be possible that a vaccine could cause cancer, but we have never seen this happen with any HIV study vaccines.

Risks of the study vaccines:

This exact DNA study vaccine has not been given to people before. In studies with similar DNA vaccines, the most common complaints were pain or itching at the injection site, headache, and feeling tired.

This NYVAC study vaccine has not been given to people before. In studies with similar NYVAC vaccines, the most common complaints after an injection were pain at the injection site, headache, and feeling tired. Other symptoms may be muscle aches, chills, and swelling. Some people have redness at the injection site.

The NYVAC study vaccine is similar to the smallpox vaccine that has been used worldwide to protect against smallpox. The smallpox vaccine may cause certain heart problems in some people. This result has not been seen in any study using a NYVAC vaccine in people. While we do not expect any heart problems to happen in this study, we want to be careful. If you join the study, we will ask you about symptoms that could suggest heart problems. You will need to let us know promptly if you are having any problems that could be related to your heart, such as extreme tiredness, chest pain, or difficulty breathing.

This AIDSVAX® B/E study vaccine has been given to thousands of people without causing serious problems. The most common symptoms at the injection site have been tenderness or pain resulting in some limited arm movement, and, less often, injection site swelling. The most common reactions in the body have been headache, tiredness, muscle or joint aches, and a mild increase in body temperature.

Personal problems/discrimination/testing HIV antibody positive:

About 10 to 20% of people who join HVTN studies report personal problems or discrimination because of joining an HIV vaccine study. Family or friends may worry, get upset or angry, or assume that you are infected with HIV or at high risk and treat you unfairly as a result. Rarely, a person has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

The study vaccines are likely to cause you to test positive on some types of HIV tests. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccines, a routine HIV test done outside this clinic may say you have HIV, even if you don't. For this reason, you should plan to get HIV tests only at this clinic during the study. Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccines.

If you receive a positive test result caused by the study vaccines at any time, we can provide you with free HIV testing for as long as you need it. If this happens, we do not know how long you will test positive due to the study vaccines. If you receive a positive HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

It is unlikely, but you could test negative at the end of the study and positive some time later, even though you don't have HIV. This could happen if different HIV tests come into use. We will give you a phone number to call for more information.

If someone believes you are infected with HIV even if you are not, you could face discrimination and other problems. For example, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If you do have a positive HIV antibody test caused by the study vaccines, you will not be able to donate blood or organs. Your family and friends may treat you differently. We will give you a brochure that tells you more about testing HIV positive because of an HIV vaccine, and how you can avoid some of these problems.

Site: Modify the preceding paragraph if applicable.

Site: Delete the following paragraph if local HIV testing of newborns is done via nucleic acid test.

If you become pregnant and have VISP, we don't know if the antibodies could be passed to your baby. We know that this happens with other vaccines, like tetanus vaccine. But, the antibodies from the mother go away over time. We will arrange for the baby to have a test that can tell the difference between true HIV infection and a VISP result. We can do this testing for free for as long as it is needed.

Embarrassment/anxiety:

You may feel embarrassed when we ask about your HIV risks, such as having sex and using drugs. You may also feel embarrassed when we ask you about semen sample collection. Also, waiting for your HIV test results or other health test results could make you feel anxious. You could feel worried if your test results show that you are infected with HIV. If you feel embarrassed or anxious, please tell us and we will try to help you.

Risks of ECG/EKG

For the ECG, we will place leads on your chest, arms and legs using suction cups with gel or stickers. Some people may develop a rash or irritation where the leads were placed on the body.

Risks of collecting rectal and cervical fluids

Collection of cervical fluids may cause some discomfort. This discomfort is similar to what happens during a routine Pap smear. It does not usually last very long.

Collection of rectal fluids may involve use of an anoscope. The anoscope is a plastic viewing tube, 2-3 inches long and ½ inch wide, which may be inserted into the rectum so that the clinician can see better when doing this procedure. This may cause temporary discomfort.

During any of these procedures, you may feel anxious or embarrassed. If you feel uncomfortable in any way, please tell us and we will try to help you.

Risks of disclosure of your personal information:

We will take several steps to protect your personal information. Although the risk is very low, it is possible that your personal information could be given to someone who should not have it. If that happened, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information if you would like it.

Unknown risks:

We do not know if the study vaccines will increase, decrease, or not change your risk of becoming infected with HIV if exposed. If you get infected with HIV, we do not know how the study vaccines might affect your HIV infection or how long it takes to develop AIDS.

We do not know if getting these study vaccines will affect how you respond to any future approved HIV vaccine. It could be that a future HIV vaccine may not work as well for you because you got the study vaccines. Currently, no HIV vaccine has been approved for use.

We do not know how the study vaccines will affect a pregnant participant or a developing baby.

Benefits

22. The study may not benefit you.

We do not know whether getting the study vaccines might benefit you in any way. However, being in the study might still help you in some ways. The counseling that you get as part of the study may help you avoid getting HIV. The lab tests and physical exams that you get while in this study might detect health problems you don't yet know about.

This study may help in the search for a vaccine to prevent HIV. However, if the study vaccines later become approved and sold, there are no plans to share any money with you. You will also not receive any money if you decide to donate your extra samples and limited information for other research, even if this research leads to a new product or discovery.

Your rights and responsibilities

23. If you join the study, you have rights and responsibilities.

You have many rights that we will respect. You also have responsibilities. We list these in the Participant's Bill of Rights and Responsibilities. We will give you a copy of it.

Leaving the study

24. Tell us if you decide to leave the study.

You are free to leave the study at any time and for any reason. Your care at this clinic and your legal rights will not be affected, but it is important for you to let us know.

We will ask you to come back to the clinic one last time for a physical exam, and we may ask to take some blood and urine samples. We will also ask about any personal problems or benefits you have experienced from being in the study. We believe these steps are important to protecting your health, but it is up to you whether to complete them.

Injuries

25. If you get sick or injured during the study, contact us immediately.

If, during the clinical study or for up to five years after the end of the study, you have any physical, psychological or social problem resulting from your participation in this study, or if you have an unexpected side effect related to the vaccine, you must immediately inform the medical team of the Center for Vaccinology and Immunotherapy (VIC). The VIC staff will do as much as possible to assist you medically and will ensure the appropriate follow-up for any other problem.

In case of an emergency, a doctor from the clinical study must be informed as soon as possible, by telephoning the number that appears on the card that will be provided to you upon your enrollment.

There are compensatory arrangements, in case you suffer an injury related to your participation in this study. We have subscribed to an insurance policy with the Gerling Company for any potential damages related to the experimental vaccines. In such an

event, the principal investigators for the study, Professor G. Pantaleo and Dr. Bart, will be immediately informed by the medical team and will make the necessary arrangements for your compensation. The NIH will not compensate you for study-related injuries.

If you have a complaint concerning the personnel, you may also direct it to Professor G. Pantaleo or to the competent authorities at Centre Hospitalier Universitaire Vaudois (CHUV). CHUV will respond to any possible injuries that you may be subject to due to medical negligence on behalf of one of its employees, as part of the clinical study in which you are participating.

Annual health contacts

26. After your clinic visits end, we will contact you once a year until 5 years after your first injection.

We will contact you by phone or email *[Site: Modify mode of contact as appropriate]* once a year to ask questions about your health. If you prefer to answer these questions in person, you can come to the clinic to do this.

If we have any concerns about your health, we may need to have more contact with you. You are also welcome to contact us at any time if you have concerns about your health related to being in the study.

If we ask you to come to the clinic, we will give you [Site: Insert compensation amount] for each visit. This amount is to cover the costs of [Site: Insert text].

If someone outside this study clinic told you that you are infected with HIV, we will ask you to come back to the clinic for another HIV test. We will draw about 15 mL (1 tablespoon) of blood. We may ask you to come back more than once for this testing.

Because we will want to contact you once a year, please tell us if your address or phone number changes, if you are moving away, or if you do not want us to contact you anymore.

You can tell us at any time that you don't want any more annual health contacts. If you do so, you will not lose any benefits or rights you would normally have.

All other information that is discussed earlier in this consent also applies to the annual health contacts.

Questions

27. If you have questions or problems at any time during your participation in this study, use the following important contacts.

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].

Your permissions and signature

28. In section 14 of this form, we told you about collection of salivary, cervical, semen, and rectal fluid samples, which is optional. Please write your initials or make your mark in the boxes next to the options you choose.

☐ I agree to provide salivary fluid samples.

☐ I do not agree to provide salivary fluid samples.

☐ I agree to provide cervical fluid samples.

☐ I do not agree to provide cervical fluid samples.

☐ I agree to provide rectal fluid samples.

☐ I do not agree to provide rectal fluid samples.

☐ I agree to provide semen samples.

☐ I do not agree to provide semen samples.

29. In Section 16 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please write your initials or make your mark in the box next to the option you choose.

☐ I agree to donate my extra samples combined with limited information for other studies related to HIV, the immune system and other diseases. This may include limited genetic testing.

OR

☐ I agree to the option above and also to donate my extra samples combined with limited information for use in genome wide studies. I understand my genome and limited information may be put into a protected genome wide database.

OR

☐ I do not agree to donate my extra samples combined with limited information for use in other studies.

30. If you agree to join this study, you will need to sign below. Before you sign this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should 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 Approved birth control methods (for sample informed consent form)

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby.

If you were born female and are sexually active in a way that could lead you to get pregnant, you must agree to use effective birth control, from 3 weeks before your first injection until completion of your last required study clinic visit.

Effective birth control means using any of the following methods every time you have sex:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Male or female condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for one year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your "tubes tied") or confirmed successful placement of a product that blocks the fallopian tubes;
- You are having sex only with a female partner or partners;
- You only have oral sex; or,
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, you need to use male or female condoms to protect yourself from HIV infection.

Appendix C HVTN VISP registry consent

The HIV Vaccine Trials Network (HVTN) would like your permission to enter your name and link it to information about you in a computer registry (the "VISP registry"). By having your name and vaccine study information in the VISP registry, trained staff can quickly help you if you have problems with VISP.

About VISP

The body makes antibodies to prevent infection. Most vaccines cause the body to make antibodies as a way of preventing infection. Your body may make antibodies to HIV because you received an HIV vaccine. Some HIV test results could come back positive even if you are not infected with HIV. This is called a VISP (vaccine-induced seropositive) test result. We do not know who will have VISP test results or how long these test results may last.

People with VISP test results need specific HIV tests. These tests can tell whether a test result is VISP or a real HIV infection. These people may need help explaining their VISP situation if someone outside the study wants to test them for HIV. VISP test results may cause problems in several areas like insurance, job applications, the military, prison, visa applications, emigration/immigration, and blood and tissue donation.

We are asking you for your permission to enter your name in the registry now in case you have VISP test results later. The registry will not be used for any other purpose.

What are the benefits of the registry?

Your study site will help you with problems related to VISP test results. The site staff will need to verify your study participation and if you received an HIV vaccine. The registry gives the site staff quick access to this information.

If you choose not to have your name entered in the registry, site staff still will do their best to help you. However, it will take longer to get that information. If your study site is no longer doing HIV vaccine studies, your records may be stored securely off site. It is possible your records may not be found.

What information does the registry contain and how is it protected?

The registry contains the following information:

- Your participant ID (the code used for you instead of your name at your study site)
- The study network and study you were in
- The site where you began the study
- The date you began the study

- Your date of birth or age
- If you received an HIV vaccine that may cause you to test VISP

We are asking for your permission to enter your name into the registry and link it to the information above.

The registry will NOT contain:

- Your HIV test results
- Your phone number or any other way to contact you

Any other personal information that you discuss with the site staff will be kept separate from the registry. We will keep your name in the registry until you tell us you want it removed.

All people who work with your registry information sign agreements to keep the information confidential.

The registry is a secured computer database. It can only be accessed with a password.

What are the risks?

The only risk to having your name entered and linked to the other pieces of information in the registry is that someone who is not authorized might see your information. The risk of this happening is low because of the security protections in place. However, we cannot guarantee this will never happen.

What if I have more questions about the registry?

Please talk to your study site if you have any questions about the registry now or in the future.

If I agree now, can I change my mind later?

Yes. You can contact your study site to tell them that you would like your name to be deleted from the registry. Your decision will not affect your participation in the main HIV vaccine study.

By signing this form, you do not give up any legal rights.

Please write your initials or make your mark in the box next to the option you choose.

☐ I **AGREE** to allow my name to be entered and linked to the information in the HVTN VISP registry.

☐ I **DO NOT AGREE** to allow my name to be entered and linked to the information in the HVTN VISP registry.

Please sign or make your mark 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, a witness should 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 D Table of procedures (for sample informed consent form)

		Time after first injection visit (in months)												
Procedure	Screening visit(s)	First injection visit	½	1	1½	3	3½	6	6½	7	9	12	15	18
Injection		√		√		√		√						
Medical history	√													
Complete physical	√													√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	
Electrocardiogram (ECG)**	√													
Cardiac symptom assessment	√	√	√	√	√	√	√	√	√	√	√			
Urine test	√		√				√							
Blood drawn	√	√	√		√		√		√	√	√	√	√	√
Pregnancy test (participants born female)	√	√		√		√		√	√†		√	√†		
HIV testing	√						√		√		√	√	√	√
Mucosal secretion samples (salivary, rectal, semen, cervical)*		√							√			√		
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√

*Participation in the collection of mucosal secretion samples is optional.
**A follow-up ECG will be conducted at a regularly scheduled clinic visit or an interim visit as soon as possible.
†Pregnancy tests apply only to participants providing mucosal samples (Months 6.5 and 12)

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

Appendix E Laboratory procedures

				Tube volume (mL)														
			Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	
			Day:	Screening visit[4]	D0	D14	D28	D42	D84	D98	D168	D182	D196	D273	D364	D455	D545	
			Month:		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M7	M9	M12	M15	M18	
Description	**Ship to[1,2]**	**Assay location[2]**	**Tube[3]**	**Tube size (vol capacity)[3]**		**VAC1**		**VAC2**		**VAC3**		**VAC4**						**Total**
BLOOD COLLECTION																		
Screening or diagnostic assays																		
Screening HIV test	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	**5**
HBsAg/anti-HCV/Syphilis	Local lab	Local lab	SST	5mL or 8.5mL	8.5	5 [13]	—	—	—	—	—	—	5 [13]	—	—	5 [13]	—	—	**23.5**
HIV in-study diagnostic test[9]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	—	—	10	—	10	—	10	10	10	20	**70**
Safety labs																			
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	5	—	5	—	5	—	5	—	—	—	5	—	**30**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	5	—	5	—	5	—	5	—	—	—	5	—	**30**
Cardiac monitoring[14]	Local lab	Local lab	Hep	5mL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**0**
Immunogenicity assays[6]																			
HLA typing[7]	CSR	FHCRC	ACD	8.5mL	—	17	—	—	—	—	—	—	—	—	—	—	—	—	**17**
Humoral Assays																			
HIV binding Ab assay[10]	CSR	Duke	EDTA	10mL	—	***10***	—	—	***10***	—	***10***	—	***10***	10	10	***10***	10	10	**90**
HIV neut Ab assay[11]	CSR	Duke	SST	8.5mL	—	8.5	—	—	***8.5***	—	***8.5***	—	***8.5***	—	—	***8.5***	—	—	**42.5**
Cellular Assays																			
ICS	CSR	FHCRC	ACD	8.5mL	—	34	—	—	***34***	—	***34***	—	***34***	—	—	***34***	—	—	**170**
PBMC multiplex bead array	CSR	FHCRC	ACD	8.5mL	—	34	—	—	34	—	34	—	***34***	—	—	34	—	—	**170**
B-cell ELISpot	CSR	FHCRC	ACD	8.5mL	—	17	—	—	17	—	17	—	17	—	—	17	—	—	**85**
Storage (includes blood for exploratory endpoint assays)																			
Serum	CSR	—	SST	8.5mL	—	8.5	—	—	8.5	—	8.5	—	8.5	—	—	8.5	—	—	**42.5**
PBMC	CSR	—	ACD	8.5mL	—	59.5	—	—	59.5	—	59.5	—	59.5	—	—	59.5	—	—	**297.5**
Plasma	CSR	—	ACD	—	—	y	—	—	y	—	y	—	y	—	—	y	—	—	**0**
Plasma	CSR	—	EDTA	5mL or 10mL	—	10	—	—	10	—	10	—	10	—	—	10	—	—	**50**
Maximum Total					**23.5**	**203.5**	**10**	**0**	**191.5**	**0**	**201.5**	**0**	**206.5**	**10**	**20**	**196.5**	**30**	**30**	**1123**
Maximum 56-Day total					**23.5**	**227**	**237**	**237**	**428.5**	**191.5**	**393**	**0**	**206.5**	**216.5**	**20**	**196.5**	**30**	**30**	
URINE COLLECTION																			
Urinalysis	Local lab	Local lab	—		X	—	X	—	—	—	X	—	—	—	—	—	—	—	
Pregnancy test[8]	Local lab	Local lab	—		X	X	—	X	—	X	—	X	X[12]	—	X	X[12]	—	—	
MUCOSAL SPECIMEN COLLECTION (optional)																			
Cervical Secretions	CSR	Duke	—		—	X	—	—	—	—	—	—	X	—	—	X	—	—	
Rectal Secretions	CSR	Duke	—		—	X	—	—	—	—	—	—	X	—	—	X	—	—	
Saliva	CSR	Duke	—		—	X	—	—	—	—	—	—	X	—	—	X	—	—	
Semen	CSR	Duke	—		—	X	—	—	—	—	—	—	X	—	—	X	—	—	
STI Testing																			
GC/Chlamydia	Local lab	Local lab	—		—	X	—	—	—	—	—	—	X	—	—	X	—	—	

y= up to 5mL of ACD plasma will be harvested during PBMC processing. No separate blood draw is needed.

[1] CSR= Central Specimen Repository

[2] HVTN Laboratory Program includes laboratories at UW-VSL, Duke, and FHCRC. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA); Duke = Duke University Medical Center (Durham, North Carolina, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA)

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

[4] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.
[5] Chemistry panels are defined in Sections 9.2 (pre-enrollment) and Section 9.3 (postenrollment).
[6] Immunogenicity assays will be performed at visits 2 (only for binding Ab assay), 5, 9, and 12. Based on the number of responders observed at these timepoints, lab assays may be performed on all participants for humoral and/or cellular responses at other timepoints as shown.
[7] Genotyping may be performed on enrolled participants using cryopreserved PBMC collected at baseline, initially in participants who demonstrate vaccine-induced T cell responses at post-vaccination timepoints.
[8] Pregnancy test may be performed on blood specimens.
[9] At an early termination visit for a withdrawn or terminated participant (see Section 9.13), blood should be drawn for HIV diagnostic testing, as shown for visit 11 above.
[10] 10mL EDTA blood collected for the HIV binding Ab assay will also cover specimen needs for the Ab avidity, Ab peptide array, and the virus capture assays.
[11] 8.5mL of SST blood collected for the HIV nAb assay will also cover specimen needs for the ADCC assay.
[12] Pregnancy tests at these visits only for participants providing cervical and/or rectal secretion samples.
[13] Syphilis testing required only for those providing mucosal samples. However, no STI testing is required if participant agrees to provide only saliva samples
[14].Cardiac monitoring will be performed as outlined in section 9.5.2 of the protocol and associated tests will be performed only when clinically indicated.

Appendix F Clinical Procedures

Visit:	01[a]	02	03	04	05	06	07	08	09	10	11	12	13	14	Post
Day:		D0	D14	D28	D42	D84	D98	D168	D182	D196	D273	D364	D455	D545	
Month:		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M7	M9	M12	M15	M18	
Procedure	Scr.	VAC1		VAC2		VAC3		VAC4							
Study procedures[b]															
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Signed protocol consent, VISP consent, and annual health contact consent	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	X	X	—	—
ECG*	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cardiac symptom assessment	X	X	X	X	X	X	X	X	X	X	X	—	—	—	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Pregnancy prevention assessment[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Behavioral risk assessment	X	—	—	—	—	—	—	—	—	X	—	—	—	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Outside testing and belief questionnaire	—	—	—	—	—	—	—	—	—	X	—	—	—	—	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[d]	X	—	—	—	—	—	X	—	X	—	X	X	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	X	—	X	—	X	X	X	X
Local lab assessment															
Urine dipstick	X	—	X	—	—	—	X	—	—	—	—	—	—	—	—
Pregnancy (urine or serum HCG)[e]	X	X	—	X	—	X	—	X	X**	—	X	X**	—	—	—
CBC, differential, platelet	X	—	X	—	X	—	X	—	X	—	—	—	X	—	—
Chemistry panel (see Section 9.2)	X	—	X	—	X	—	X	—	X	—	—	—	X	—	—
Gonorrhea and chlamydia (see Section 9.6)	—	X	—	—	—	—	—	—	X	—	—	X	—	—	—
Syphilis, Hepatitis B, Hepatitis C	X	X†	—	—	—	—	—	—	X†	—	—	X†	—	—	—
Mucosal secretion collection (optional)															
Salivary, rectal, cervical, and/or semen	—	X	—	—	—	—	—	—	X	—	—	X	—	—	—
Vaccination procedures															
Vaccination[f]	—	X	—	X	—	X	—	X	—	—	—	—	—	—	—

[a] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

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

[c] Pregnancy prevention compliance occurs only with participants who were born female and are capable of becoming pregnant.

[d] Includes pretest counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.

[e] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination. Pregnancy test to determine eligibility may be performed at screening or on day 0 prior to first vaccination. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[f] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration. Lab tests may be drawn within the 3 days prior to vaccination.

Reactogenicity assessments[g]	—	X	—	X	—	X	—	X	—	—	—	—	—	—	—
Poststudy															
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

[g] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.11).

*ECG is required at screening. This procedure should also be performed if clinically indicated. An ECG will also be performed at a regularly scheduled clinic visit or interim visit as soon as possible.

**Pregnancy tests at these visits only for participants providing cervical and/or rectal secretion samples.

†Only Syphilis testing will occur at this timepoint.

Appendix G Procedures at CRS for annual health contacts

Contact[a] Day	728	1092	1446	1820
Month	24	36	48	60
Procedures				
Vital status and health events[b]	X	X	X	X

[a] Clinic visits are not required, except that any participant reporting a diagnosis of HIV infection will be asked to come to the clinic so that HIV status can be confirmed.

[b] See Section 9.7.

Appendix H Addendum to Informed Consent

For Protocol HVTN 096/EV04 (DAIDS ID 11889), Version 2.0: A phase 1 double blind placebo-controlled clinical trial to evaluate the safety and to compare the priming ability of NYVAC alone versus NYVAC + AIDSVAX® B/E, and DNA alone versus DNA + AIDSVAX® B/E when followed by NYVAC + AIDSVAX® B/E boosts in healthy, HIV-1-uninfected adult participants

Review of study information

You are a participant in a study called HVTN 096/EV04, which tests safety and immune responses to 3 experimental HIV vaccines. We have new information to share with you.

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

After you have reviewed this form and had all of your questions answered, you will be asked to sign this form. You will get a copy to keep.

New information about the vaccine trial

We have some news we want to share with you about HVTN 096. The NYVAC vaccines being tested in HVTN 096 are also being tested in another HIV vaccine study called HVTN 092. On October 26, 2013 a participant in that study had some chest discomfort and difficulty taking deep breaths. That participant was diagnosed with myocarditis, which is an inflammation of the muscle of the heart. The symptoms appeared within a couple days after getting an injection of the NYVAC vaccine and went away two days later.

The most common cause of myocarditis is infection by viruses. However, some types of vaccines also have been linked with this kind of heart inflammation. This includes smallpox vaccines that are similar to the NYVAC vaccine used in HVTN 096.

Participant safety is our highest concern. Since you received the NYVAC vaccine or placebo, we are asking you to have an electrocardiogram (ECG or EKG). An ECG tells us about your heart rate and rhythm. You had an ECG at one of your first clinic visits. You do not have to pay for the ECG.

We will tell you if we learn anything more about the NYVAC vaccine. In the meantime, the important thing for you to know is that we want to check your heart since you received the NYVAC vaccine or placebo.

What if I choose to not have an ECG?

You can choose not to have an ECG and still be in the study. If you do not have an ECG, you will not lose any benefits or rights you would normally have.

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 form that you signed earlier, about the study purpose, the risks and benefits of participation, your responsibility, 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 you are encouraged to read it again.

↦ I agree to have an ECG. Initial_____

↦ I do not agree to have an ECG. Initial_____

If you have read this addendum to the consent form (or had it explained to you) and had your questions answered, 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.