PROTOCOL

HVTN 105

A phase 1b clinical trial to evaluate the safety and immunogenicity of different combinations of DNA-HIV-PT123 and AIDSVAX® B/E in healthy, HIV uninfected adult participants

DAIDS DOCUMENT ID 11986

BB IND *TBD* HELD BY DAIDS

CLINICAL TRIAL SPONSORED BY

Division of AIDS (DAIDS)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)
Department of Health and Human Services (DHHS)
Bethesda, Maryland, USA

STUDY PRODUCTS PROVIDED BY

IPPOX Foundation (Lausanne, Switzerland)
Global Solutions for Infectious Diseases (South San Francisco, California, USA)

April 7, 2014
105v1 FOR REVIEW ONLY

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 (CAB) are required by 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.

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

- The HVTN provides training so that all participating sites similarly ensure fair participant selection, protect the privacy of research participants, and obtain meaningful informed consent. 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 & 5 and 21 CFR 56.111 (a) 4 & 5: Informed consent is sought from each prospective subject or the subject's legally authorized representative as required by 45 CFR 46.116 and 21 CFR Part 50; informed consent is appropriately documented as required by 45 CFR 46.117 and 21 CFR 50.27

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 HVTN Core and routinely by the HVTN 105 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 the United States, research participants in HVTN protocols are protected by a Certificate of Confidentiality from the US NIH, which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form. In addition, each staff member at each study site in this protocol signs a Confidentiality Agreement with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A phase 1b clinical trial to evaluate the safety and immunogenicity of different combinations of DNA-HIV-PT123 and AIDSVAX® B/E in healthy, HIV uninfected adult participants

Primary objectives

Primary objective 1:

To evaluate the safety and tolerability of the combination of AIDSVAX® B/E and DNA-HIV-PT123 administered in different sequences or simultaneously in HIV-uninfected healthy adults

Primary objective 2:

To evaluate the binding antibody (Ab) and T-cell responses of the combination of AIDSVAX® B/E and DNA-HIV-PT123 administered in different sequences or simultaneously in HIV-uninfected healthy adults

Study products and routes of administration

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

- DNA-HIV-PT123: containing a mixture of 3 DNA plasmids in a 1:1:1 ratio, each at 1.33 mg: 1) clade C ZM96 *gag*, 2) clade C ZM96 *gp140*, and 3) clade C CN54 *pol-nef*, delivered at a total dose of 4 mg, administered IM.

- Placebo: Sodium Chloride for Injection USP, 0.9%, administered IM.

Table 3-1 Schema

Group	N	Deltoid	Injection schedule in months (days)			
			0 (0)	1 (28)	3 (84)	6 (168)
1	26	Left	Placebo	Placebo	DNA-HIV-PT123	DNA-HIV-PT123
		Right	AIDSVAX® B/E	AIDSVAX® B/E	Placebo	Placebo
2	26	Left	DNA-HIV-PT123	DNA-HIV-PT123	Placebo	Placebo
		Right	Placebo	Placebo	AIDSVAX® B/E	AIDSVAX® B/E
3	26	Left	DNA-HIV-PT123	DNA-HIV-PT123	DNA-HIV-PT123	DNA-HIV-PT123
		Right	Placebo	Placebo	AIDSVAX® B/E	AIDSVAX® B/E
4	26	Left	DNA-HIV-PT123	DNA-HIV-PT123	DNA-HIV-PT123	DNA-HIV-PT123
		Right	AIDSVAX® B/E	AIDSVAX® B/E	AIDSVAX® B/E	AIDSVAX® B/E
Total	104					

Participants

104 healthy, HIV-uninfected volunteers aged 18 to 50 years at low-risk for HIV infection

Design

Multicenter, randomized, double-blind trial

Duration per participant

12 months of scheduled clinic visits

Estimated total study duration

16 months (includes enrollment and follow up)

Investigational New Drug (IND) sponsor

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

Study product providers

- DNA-HIV-PT123: IPPOX Foundation (Lausanne, Switzerland)
- 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 (UW-VSL) (Seattle, Washington, USA)

Endpoint assay laboratories

- Duke Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA)
- Neutralizing Antibody Assay Laboratory (Duke-NAB), Duke University Medical Center (Durham, North Carolina, USA)
- FHCRC/University of Washington (Seattle, Washington, USA)

Study sites

HVTN Clinical Research Sites (HVTN CRSs) to be specified in the Site Announcement Memo

Safety monitoring

HVTN 105 PSRT; HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

Protocol leadership

Chair	Michael Keefer University of Rochester	*Statistician*	Sue Li SCHARP, FHCRC
Cochair	Nadine Rouphael Hope Clinic at Emory University	*Medical officer*	G. Laissa Ouedraogo DAIDS, NIAID
Protocol Team leader	Cecilia Morgan HVTN Core, FHCRC		

Other contributors to the original protocol

Core medical monitor	Shelly Karuna HVTN Core, FHCRC	*Clinical trials manager*	Carissa Karg HVTN Core, FHCRC
Vaccine developer representative	Song Ding IPPOX Foundation	*SDMC project manager*	Lisa Sunner SCHARP, FHCRC
Vaccine developer representative	Giuseppe Pantaleo IPPOX Foundation	*HVTN Laboratory program representative*	Eva Chung HVTN Laboratory Program, FHCRC
GSID representative	Carter Lee GSID	*SDMC HVTN program manager*	Gina Escamilla SCHARP, FHCRC
GSID representative	Vineeta Gulati GSID	*Protocol development coordinator*	Ryan Jensen HVTN Core, FHCRC
USMHRP representative	Charla Andrews U.S. Military HIV Research Program	*Community engagement unit representative*	Jim Maynard HVTN Core, FHCRC
Regulatory affairs	Renee Rivers HVTN Core, FHCRC	*Community educator/recruiter*	Robert Bucklew Cleveland CRS
Clinic coordinator	Catherine Bunce University of Rochester	*Technical editor*	Adi Ferrara HVTN Core, FHCRC
Community Advisory Board (CAB) members	Angela Broad SFDDPH CRS	*Clinical safety specialist*	Jill Zeller HVTN Core, FHCRC
	Sheila Bello-Irizarry Rochester CRS	*DAIDS protocol pharmacist*	Scharla Estep DAIDS, NIAID
		Statistical Research Associate	Hongwei Wang SCHARP, FHCRC

4 Background

The RV144 efficacy trial demonstrated a 31% reduction in HIV infection in vaccinees who received ALVAC®-HIV vaccine vCP1521 expressing Env, Gag, and Pro (at 0, 1, 3, and 6 months) and boosted with a gp120 protein, AIDSVAX® B/E (at 3 and 6 months), compared to placebo and provided valuable insights into the potential importance of binding Ab responses in preventing acquisition [4]. Furthermore, recent data from the RV144 Case-Control Study have identified immune responses to the V1V2 region of HIV *env* that predicted the risk of vaccine recipients to become HIV-1 infected, ie, 'correlates of risk' [5,6]. These results are in contrast to those from two earlier phase 3 trials of AIDSVAX® candidate vaccines that failed to show efficacy. These studies were conducted by VaxGen and examined gp120 administered in alum x 7 over 2.5 years to individuals at high-risk for HIV acquisition; injection drug users (IDU) in Thailand (VAX003; AIDSVAX® B/E) and men who have sex with men (MSM) in clade B regions of the world (VAX004; AIDSVAX® B/B). Although these discordant results could have been influenced by differences in study populations and predominant transmission routes, a recent study comparing Ab responses in RV144 and VAX003 (both trials carried out in Thailand) showed that IgG3 binding to HIV Env V1V2 was induced more frequently and at a higher magnitude in RV144 than in VAX003 [7]. This finding suggests that the combination of recombinant envelope protein with an immunogen presenting antigen by an alternative pathway (i.e., 'prime-boost regimen') may provide important contributions to a functional immune response. In addition, the results from RV144 showing a waning of efficacy with time underscore the need to develop vaccine regimens that will provide durable immune protection, which likely will entail the induction of long-lived B- and T-cell memory.

The need to reproduce and improve upon the immune responses seen in RV144 clearly calls for further evaluation of recombinant envelope protein vaccines administered in prime-boost regimens with pox-vectored candidates; however, it is important to explore alternative combination regimens that include recombinant envelope proteins. DNA vaccines have been extensively studied in combination with other attenuated live vector vaccines such as modified vaccinia Ankara, NYVAC and adenovirus serotype 5, and combinations of attenuated live vector vaccines have also been investigated [8]. However, there is a relative paucity of data on the combination of DNA and recombinant envelope protein vaccines and essentially no human data exists on the use of envelope protein candidates as a priming immunogen. HVTN 105 will help to address this knowledge gap by systematically examining the combination of DNA and envelope protein candidates sequentially (with DNA both as a prime and a boost) and, simultaneously, using the RV144 vaccination schedule, as well as an arm matching the RV144 regimen with DNA replacing ALVAC. In addition, the candidate vaccines proposed here are being used in a series of studies being conducted by the HVTN in collaboration with the IPPOX Foundation and EuroVacc Foundation that are also building upon RV144's pox-vector priming/rgp120 boosting schedule. Thus, HVTN 105 may be able to provide additional insight into a broader range of combination vaccine regimens currently in clinical trials.

4.1 Rationale for trial concept

4.1.1 Overview of prime-boost/combination vaccination regimens

The original rationale for boosting with envelope protein established in the early 1990's was to overcome anti-vector immunity that limited boosting responses to vaccinia vectored-gp160 (HIVAC-1e; Bristol-Myers Squibb) [9]. By the mid-1990's the canarypox (ALVAC) vector replaced replication-competent vaccinia as the poxvirus vector of choice primarily due to the added margin of safety it provided and the AIDS Vaccine Evaluation Group (AVEG) protocol, AVEG 022, which enrolled participants in 1995, was the first study to provide simultaneous administration of ALVAC and protein as a boost at 9 and 12 months [10]. Subsequently, AVEG 022A (enrolled during 1996-7) was the first study to investigate simultaneous administration of ALVAC and protein from the initial vaccination timepoint and included an arm that was ultimately administered in RV144 (ALVAC alone priming at months 0 and 1 along with ALVAC + protein combined boosting at months 3 and 6) [11]. Of note, all but one of these AVEG studies administered the poxvirus as the priming vaccination; the only study that examined protein priming one study conducted by the AVEG (Protocol 002A; enrolled in 1991) examined protein priming with a vaccinia (HIVAC-1e) boost vs vaccinia priming with a protein boost, but this was conducted in vaccinia-immune participants, the experimental groups had only 10 participants each, and all AVEG studies predated the establishment of the current menu of standardized/validated cellular and humoral assays available to the HVTN.

In total, the HVTN (or its preceding network, AVEG) has conducted 12 protocols of poxvirus/recombinant protein combinations, but only 2 studies with a DNA plasmid/recombinant envelope protein prime-boost regimen and in one of these the DNA vaccine was boosted with a pox-vectored candidate (NYVAC) plus protein (HVTN 096/EV04). Finally, as indicated above, only one prime-boost protocol was conducted by the AVEG in which envelope protein was the prime, but this was long before the existence of the HVTN's powerful new multicolor flow cytometry-based assays and newly implemented panel of serologic assays. Given that highly protective immune responses have not yet been induced through active immunization, we feel that establishing the immune profile of additional vaccine combination regimens is indicated.

4.1.2 DNA prime, protein boost

While RV144 has established a benchmark for the design of clinical trials of recombinant envelope proteins primed by pox-vectored candidates, attenuated live vector vaccines can present a number of challenges in terms of manufacture and vector-related safety issues. DNA vaccines are relatively straightforward to construct and modify and once made are stable at room temperature and thus easy to store and deploy globally. In preclinical studies, DNA vaccines elicit strong and sometimes protective immune responses against viruses, bacteria, parasites, autoimmune diseases, and cancer [12]. DNA vaccines have been licensed for use against West Nile virus in horses, melanoma in dogs, and infectious hematopoietic necrosis virus in Salmonid fish. However, no DNA vaccine has yet been licensed for human use due in part to its generally poor immunogenicity. However, advances in constructing DNA plasmids for optimal expression and alternative delivery methods such as electroporation may appreciably improve these responses. As mentioned earlier, a number of studies of DNA candidates in combination with attenuated live vector immunogens have been conducted or are underway. Whether plasmid DNA

vaccines will prove to be a part of a combination HIV vaccine regimen in the future is still an open question.

DNA-protein combination vaccine regimens have only more recently been investigated in clinical trials. Two studies conducted by the NIAID Vaccine Research Center (VRC) in 2008-2010 assessed influenza H5 DNA priming followed by boosting with H5/N1 monovalent inactivated vaccine (MIV) and showed that the combination approach enhanced the magnitude of hemagglutination inhibition antibodies to protective levels in 81% of vaccine recipients with geometric mean titers 4-fold higher than MIV given twice [13]. In addition, this effect was most evident when the booster vaccination was given at least 12-24 weeks after the prime as compared to 4-8 week intervals [14].

Only three clinical trials have been conducted using the DNA + recombinant envelope protein prime-boost platform with HIV immunogens; in each the envelope protein/boost was administered subsequent to the time of DNA priming. The first study was conducted by Lu and colleagues at the University of Massachusetts Medical School. In this study, participants who received 2 dose levels of a multi-clade HIV DNA prime followed by a multi-clade HIV gp120 protein with QS-21 adjuvant boost demonstrated env-specific interferon gamma (IFN-γ) ELISpot responses in over 90% of participants 2 weeks after the final vaccination, which persisted at 1 year (5 months after the final vaccination) in over 80% of participants. Also, env-specific binding antibodies were induced at a higher magnitude than what has been seen from recombinant envelope vaccine regimens alone and persisted in most participants at 1 year with only a modest decrease in titer [15]. Furthermore, the DNA + protein platform elicited a different Ab profile than that from recombinant HIV envelope protein alone or a pox-vector prime + protein boost, which also supports further evaluation of this platform [16].

HVTN 088 evaluated an HIV gp140 clade C protein in MF59 adjuvant administered to participants who had received HIV DNA boosted by HIV gp140 clade B protein in MF59® as a part of HVTN 049 conducted 5-7 years earlier. Even before administration of the clade C gp140 boost, a significant portion of the HVTN 049 participants still had detectable T-cell and binding Ab responses, suggesting remarkably durable memory responses induced by the DNA + protein combination. This impression was further confirmed by the finding of strong and rapid boosting responses after the administration of the clade C gp140 protein [17]. In addition, the original findings from HVTN 049 indicated that the DNA + gp140 protein prime-boost regimen induced, 1) significantly higher titers of homologous neutralizing antibodies (nAbs), and 2) CD4+ responses of significantly greater magnitude (among the group that was primed with 1gm of DNA), in comparison with the group that was immunized with gp140 protein alone [18].

The findings from these clinical trials provide intriguing clues as to an approach that may facilitate the induction of nAbs and long-lasting memory B and T cell responses and provides justification to examine the DNA-HIV-PT123 + protein combination regimen in HVTN 105.

4.1.3 Simultaneous administration of DNA and protein vaccines

Beyond the potential benefits of priming with DNA or protein, another approach that has been of interest to the HVTN is the co-administration of protein with another vaccine type. Co-administration of a protein from the initial vaccination timepoint has the potential benefits of more rapidly eliciting both Ab and T-cell responses from the first vaccination and this approach has been taken in past HVTN trials with ALVAC-HIV

(AVEG protocols -022A [11], -026 [19] -202 [20] and -203 [21]). In addition, protein co-administered with NYVAC, as well as protein co-administered with DNA is currently being evaluated in HVTN 096/EV04, and this is the only clinical trial that we are aware of that has co-administered DNA and protein (DNA-HIV-PT123 and AIDSVAX® B/E have been given simultaneously at months 0 and 1, followed by simultaneous boosting with a NYVAC-HIV and AIDSVAX® B/E). This regimen has been well-tolerated although the study is still blinded and final results are pending (see Section 4.6.1).

The bulk of the justification for simultaneous administration of DNA + protein comes from studies in preclinical studies using different vaccine candidates than those proposed in HVTN 105. The Pavlakis group demonstrated the potential immunologic advantages of co-administration of protein with DNA in NHPs. In this 4-arm study rhesus macaques received either DNA alone, DNA co-administered with protein, 2 DNA priming injections followed by 2 protein boosts, or sham DNA vaccination. The DNA vaccine plasmids expressed SIV Env, Gag, Pol, Nef, Tat, and Vif, were given with rhesus IL-12 DNA adjuvant administered IM with electroporation. The protein vaccine consisted of inactivated SIVmac239 particles. Vaccinations were given at month 0, 2, 4 and 9, and followed by repeated low-dose mucosal challenge with heterologous SIVsmE660. The 2 groups that received DNA vaccine (DNA alone, or DNA co-administered with protein) at all 4 timepoints had higher levels of vaccine-induced SIV-specific IFN-γ^+ T cells. However, the group that received co-administered DNA and protein had the highest levels of Env binding Abs and higher avidity that persisted at a higher rate than the other groups. In addition, Env binding Abs and avidity correlated with slower SIV acquisition and cytotoxic CD4+ effector memory inversely correlated with peak viral load [22,23]. In addition, the Haigwood group recently demonstrated in rabbits that DNA + protein co-administration was superior to protein administration alone in terms of antibody kinetics, magnitude, avidity and neutralization potency [24].

These studies suggest that a qualitative difference in humoral and T-cell responses may result from co-administration of protein with DNA, as compared to the more common approach of priming with DNA or viral vector immunogens and provides justification for the systematic investigation of protein/DNA co-administration proposed in HVTN 105.

4.1.4 Protein as a prime

There is some recent evidence from preclinical studies in the literature suggesting that a protein prime followed by DNA or viral vector boosting can elicit robust, durable IgG Ab and/or T-cell responses that would be desirable for a vaccination regimen intended to benefit public health [25-29]. Of the 5 studies identified, 2 were done in the mouse model [26,28], 3 utilized the nonhuman primate (NHP) model [25,27,29], and 3 reported direct comparisons of the opposite vaccination sequence of prime/boost (protein prime followed by DNA or vector boost vs. DNA or vector prime followed by protein boost) [26,28,29]. Of note, 4 of the studies that looked at protein priming examined the effect of novel adjuvants given with the protein prime and their ability to affect subsequent post-boost immune responses [25-27,29], but only 2 of these included comparator arms with protein adjuvanted in alum alone.

4.1.5 Prime-boost studies of DNA and protein vaccines

In one study, NHPs were immunized with DNA vs HIV protein with AS02 adjuvant (each containing matching HIV env and tat, plus SIV nef inserts) x 4 as DNA alone, protein alone, DNA x 2 followed by protein x 2, and protein x 2 followed by DNA x 2

and challenged with SHIV-89.6p. Although prechallenge immune responses did not differ appreciably between the groups (aside from earlier Ab responses among recipients of protein priming), the protein prime/DNA group was the only group to show a statistically significant improvement in control of postchallenge viral load [29].

4.2 Study product descriptions

The DNA vaccine being evaluated in HVTN 105 is DNA-HIV-PT123, developed by the IPPOX Foundation. The construct utilizes a backbone developed by the Dale and Betty Bumpers Vaccine Research Center (VRC), NIAID, NIH (Bethesda, MD, USA) and expresses ZM96gp140 Env, ZM96 Gag and CN54 Pol-Nef, each in a separate plasmid.

The AIDSVAX® B/E protein has been studied in the RV144 efficacy trial in Thailand and is currently being studied in a trial using the RV144 vaccine regimen in South Africa (HVTN 097). In addition, AIDSVAX® B/E in combination with the DNA-HIV-PT123 vaccine (and NYVAC-HIV-PT1/ NYVAC-HIV-PT4) is being evaluated in Switzerland (HVTN 096/EV04, see Section 4.6.1).

4.2.1 DNA-HIV-PT123

The investigational DNA-HIV-PT123 vaccine to be evaluated in this protocol has a DNA plasmid backbone that was developed by the Dale and Betty Bumpers Vaccine Research Center (VRC), NIAID, 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 includes a bovine growth hormone polyadenylation signal termination sequence (Tbgh) and a kanamycin resistance cassette (Kan.). A schematic of the plasmid map is shown in Figure 4-1.

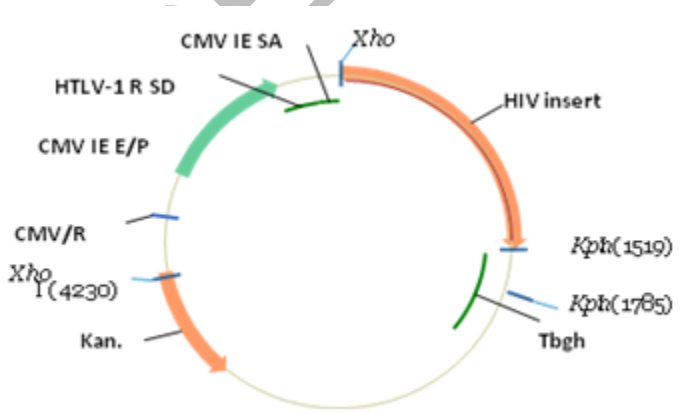

Figure 4-1 Example DNA plasmid map

The DNA-HIV-PT123 vaccine contains a mixture of 3 DNA plasmids in a 1:1:1 ratio, each at 1.33 mg: 1) a plasmid encoding clade C ZM96 Gag, 2) a plasmid encoding clade C ZM96 gp140 Env, and 3) a plasmid encoding clade C CN54 Pol-Nef polypeptide, delivered at a total dose of 4 mg, administered IM. A schematic of the inserts is included in Figure 4-2.

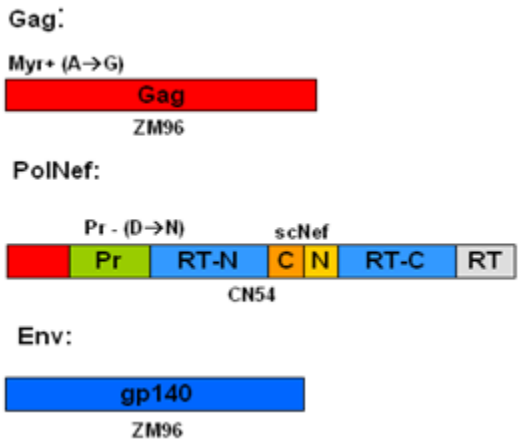

Figure 4-2 DNA-HIV-PT123 plasmid inserts schematics

Additional information on the construction of these plasmids is provided in the Investigator's Brochure (IB).

4.2.2 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 1 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 adsorbed onto 600mcg of aluminum hydroxide gel adjuvant.

4.3 Trial design rationale

Primary objective 1 in HVTN 105 is designed to assess the safety and tolerability of AIDSVAX® B/E with DNA-HIV-PT123, in different temporal sequences or co-administered at the same time. In addition, HVTN 105 is designed to evaluate the immunogenicity of co-administration of DNA-HIV-PT123 with AIDSVAX® B/E protein at all vaccination timepoints with arms that assess sequential administration of protein and DNA-HIV-PT123 and a combination of these two approaches matching the RV144 prime-boost strategy (Primary objective 2). These study objectives are novel having not been rigorously investigated in clinical trials and will contribute to our knowledge base of optimizing prime-boost vaccination regimens.

The two immunogens proposed for this trial are being assessed in several other collaborative HVTN/EuroVacc protocols and the design of this trial is complementary to HVTN 096/EV04. Thus, HVTN 105 will be able to give further insight into prime-boost strategies as illustrated in Table 4-1 and Table 4-2.

Potential cross-protocol comparisons:

HVTN 105 Group 2 vs HVTN 096/EV04 Group 3. Assess the contribution of NYVAC co-administration with protein at months 3 and 6 after priming with DNA-HIV-PT123 alone.

HVTN 105 Group 3 vs HVTN 096/EV04 Group 1. Assess the contribution of NYVAC vs. DNA-HIV-PT123 given in combination with protein boost at 3 and 6 months (both also reflect the ALVAC/gp120 combination regimen used in RV144).

HVTN 105 Group 4 vs HVTN 096/EV04 Group 2. Assess the relative contribution of co-administration of DNA-HIV-PT123 vs NYVAC with protein at all vaccinations.

Table 4-1 Study design of HVTN 105

HVTN 105				
Group	0	1 mo	3 mos	6 mos
1	P	P	D	D
2	D	D	P	P
3	D	D	D+P	D+P
4	D+P	D+P	D+P	D+P

P= AIDSVAX® B/E
D= DNA-HIV-PT123

Table 4-2 Study design of HVTN 096/EV04

HVTN 096/EV04				
Group	0	1 mo	3 mos	6 mos
1	N	N	N+P	N+P
2	N+P	N+P	N+P	N+P
3	D	D	N+P	N+P
4	D+P	D+P	N+P	N+P

P= AIDSVAX® B/E
N= NYVAC-HIV-PT1 and NYVAC-HIV-PT4
D= DNA-HIV-PT123

As for HVTN 096/EV04 (and other protocols with these immunogens), immunogenicity readouts in this study will be HIV-specific humoral and cellular immune responses at 2 weeks post-final vaccination as well as a duration timepoint at the last scheduled clinic visit.

In summary, the design of the HVTN 105 combination vaccination regimen is based upon the vaccination schedule used in RV144 and includes the same rgp120 B/E protein vaccine, but replaces the canarypox (ALVAC) vector immunogen with a DNA plasmid construct. HVTN 105 will provide safety and immunogenicity data for the sequential administration of this vaccine combination (in both orders), co-administration of the 2 immunogens at all 4 timepoints, as well an arm reflecting the RV144 prime-boost vaccination strategy. Importantly, this will be the first study conducted by the HVTN to evaluate co-administration of DNA-HIV-PT123 and gp120 protein at all vaccination timepoints (HVTN 096/EV04 administered DNA-HIV-PT123 and gp120 protein simultaneously at only the first two vaccination timepoints) and the first to evaluate priming with rgp120 protein (in alum) alone. The data from this study could inform the

design of future combination vaccination strategies via sophisticated immunologic read-outs that can provide a greater depth of understanding than has been possible before.

4.3.1 Dose (amount and number) and schedule

Participants will receive 1 of 4 vaccine regimens.

The first regimen (Group 1) comprises 1 injection (1 mL) of 600mcg of AIDSVAX® B/E administered IM in the right deltoid and 1 injection (1 mL) of placebo administered IM in the left deltoid at months 0 and 1 along with 1 injection (1 mL) of 4 mg of DNA-HIV-PT123 (containing clade C ZM96 *gag* and *env*, along with CN54 *pol-nef*) administered IM in the left deltoid and 1 injection (1 mL) of placebo administered IM in the right deltoid at months 3 and 6.

The second regimen (Group 2) comprises 1 injection (1 mL) of 4 mg of DNA-HIV-PT123 administered IM in the left deltoid and 1 injection (1 mL) of placebo administered IM in the right deltoid at months 0 and 1 along with 1 injection (1 mL) of 600mcg of AIDSVAX® B/E administered IM in the right deltoid and 1 injection (1 mL) of placebo administered IM in the left deltoid at months 3 and 6.

The third regimen (Group 3) comprises 1 injection (1 mL) of 4 mg of DNA-HIV-PT123 administered IM in the left deltoid and 1 injection (1 mL) of placebo administered IM in the right deltoid at months 0 and 1 along with 1 injection (1 mL) of 600mcg of AIDSVAX® B/E administered IM in the right deltoid and 1 injection (1 mL) of 4 mg of DNA-HIV-PT123 administered IM in the left deltoid at months 3 and 6.

The fourth regimen (Group 4) comprises 1 injection (1 mL) of 4 mg of DNA-HIV-PT123 administered IM in the left deltoid and 1 injection (1 mL) of 600mcg of AIDSVAX® B/E administered IM in the right deltoid at months 0, 1, 3, and 6.

4.3.2 Choice of placebo

Sodium Chloride for Injection USP, 0.9% (US), serves as the vaccine placebo. Sodium chloride for injection is nonreactogenic and is well tolerated.

4.4 Summary of preclinical safety studies

4.4.1 Preclinical safety study of DNA-HIV-PT123 in combination with NYVAC-HIV-PT1 and NYVAC-HIV-PT4

A toxicity study (SVT11-02) in rabbits was conducted in 2011-2012 to determine and assess systemic (short-term and persistent) and local site reactogenicity of DNA-HIV-PT123 followed by a boost with attenuated vaccinia, NYVAC-HIV-PT1 and NYVAC-HIV-PT4 (see Table 4-3). The study was conducted at Spring Valley Laboratories, Inc. in Sykesville, MD, US.

Table 4-3 Study design of the toxicity study with DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4

Group	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)	Total # Animals
1	Saline	N/A	1	Saline	N/A	2	5M, 5F	5M, 5F	10M/10 F
2	DNA-HIV-PT123	1	1	NYVAC-HIV-PT1 and NYVAC-HIV-PT4[1]	$\geq 5 \times 10^6$ PFU per NYVAC	2	5M, 5F	5M, 5F	10M/10 F

[1] Two separate types of NYVAC (HIV-PT-1 and HIV-PT-4) were administered separately in 2 separate injections of 1 mL each. Saline control was delivered as 2 separate injections of 1 mL each.

New Zealand White rabbits (20/sex, total 40 rabbits) were randomly assigned to receive IM injections of either DNA-HIV-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-PT1 and NYVAC-HIV-PT4 were administered separately (right and left hind limbs) in 2 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 2 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 2 days or 2 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 toxicity report, all animals survived to scheduled sacrifice. No abnormal findings or statistically significant changes in physical and cageside examinations, ophthalmological examination, body weights, administration site, urinalysis and gross necropsy considered to be related to treatment were observed. Body temperatures were briefly elevated following the NYVAC administration. 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-PT1 and NYVAC-HIV-PT4 was safe and well-tolerated in New Zealand White Rabbits. The vaccine was immunogenic in all treated animals following the complete immunization schedule.

4.4.2 Preclinical safety studies of AIDSVAX® B/E

As this product is well characterize preclinically and clinically (see Section 4.6.2), please refer to the AIDSVAX® B/E IB for preclinical data.

4.5 Summary of preclinical immunogenicity studies in nonhuman primates

The immunogenicity of the DNA-HIV-PT123 product has been tested in NHP studies in combination with NYVAC and/or HIV protein. The vaccine used in these studies is the research grade material equivalent to DNA-HIV-PT123. The gp120 protein was formulated with MF59® adjuvant. Results from the following 2 studies summarized below demonstrate that the DNA-HIV-PT123 is immunogenic and also primes the immune response when combined with a boost. Further information regarding the preclinical immunogenicity studies; please refer to the DNA-HIV-PT123 IB.

4.5.1 AUP444: Immunogenicity study comparing the priming ability of NYVAC or DNA-HIV-PT123 followed by NYVAC and/or protein boost in NHP

The priming ability of NYVAC or DNA-HIV-PT123+NYVAC for protein boost was evaluated in this NHP study (see Table 4-4).

The study was initially designed with 6 immunizations for the DNA-HIV-PT123/NYVAC/protein group (last immunization at week 32) and 4 immunizations for the NYVAC/protein group (last immunization at week 24). These vaccination series are also referred to as "primary immunization" in this study. Based on the initial immunogenicity data, a late boost at week 49 was added.

Table 4-4 AUP444 study design

Size	Wk 0	Wk 4	Wk 8	Wk 12	Wk20	Wk 24	Wk28	Wk32	Wk 49
8	DNA-HIV-PT123[1] (IM)	DNA-HIV-PT123 (IM)	DNA-HIV-PT123 (IM)		NYVAC[2] (IM)		Protein[3] (IM)	protein (IM)	protein (IM)
8	NYVAC (IM)	NYVAC (IM)		NYVAC + protein (IM)		NYVAC + protein (IM)			NYVAC + protein (IM)

[1]DNA: DNA-HIV-PT123 used in the study was 2mg/mL in 2 injections (total dose 4mg)
[2]NYVAC: NYVAC-HIV-PT1 and NYVAC-HIV-PT4 were formulated together at the concentration of 2×10^8 PFU/mL (1×108 PFU/mL each). Note that, while the NYVAC doses are mixed equimolar in these NHP immunogenicity studies, this is not the case in the repeated-dose toxicity study (Section 2.7.3) and in this clinical trial.
[3]Protein is monovalent gp120 protein (TV1 clade C) formulated with MF59. The dose is 100 mcg.

The results of the study have demonstrated that the DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4 elicit HIV-specific CD4+ and CD8+ T-cell responses as assessed by both qualitative and quantitative endpoints. The results relative to the DNA-HIV-PT123 indicated:

- Given alone, DNA-HIV-PT123 was highly immunogenic with an average of 1500 spot forming units (SFUs) per 10^6 peripheral blood mononuclear cells (PBMC) after 3x DNA-HIV-PT123 immunization, as measured at week 10 by IFN-γ enzyme-linked immunospot (ELISpot) (Figure 4-3). The increase in the immunogenicity was in the range of 1.5 logs as compared to previous HIV DNA vaccines tested by several groups [30-32] and the magnitude of the observed response was comparable to that obtained in NHPs with electroporation [33];

- DNA-HIV-PT123 prime induced broad T-cell responses as indicated by the large number of targeted peptide pools encompassing Env, Gag, Pol and Nef HIV proteins (Figure 4-4

- The group with the DNA-HIV-PT123/NYVAC/Protein regimen showed a substantial increase in Ab responses after the 3rd protein late boost (Figure 4-5) [34];

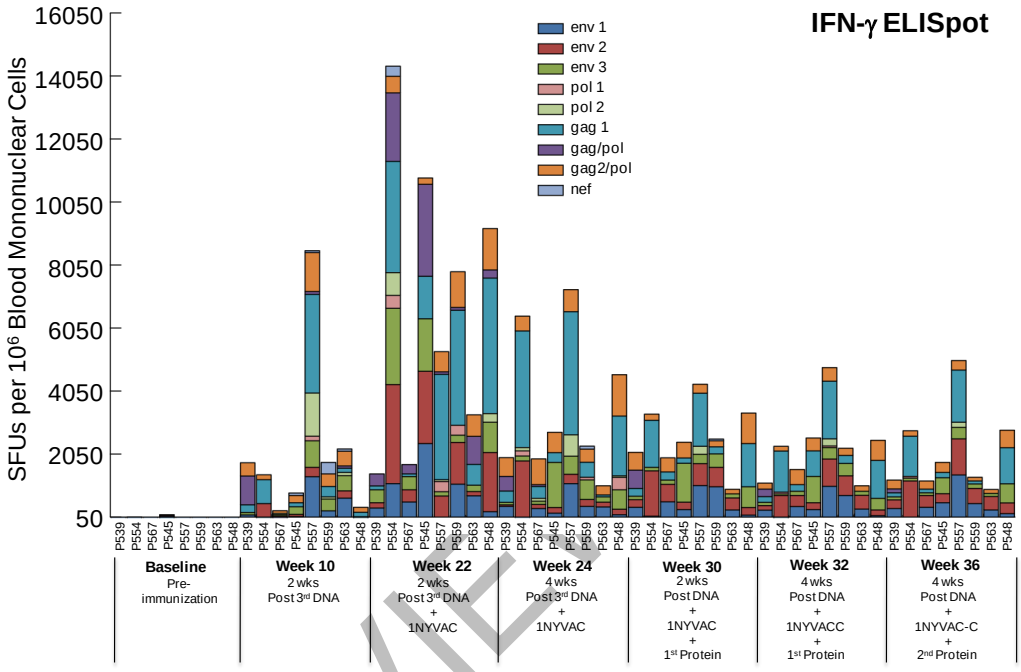

Figure 4-3 AUP444 - vaccine induced T-cell responses following DNA-HIV-PT123/NYVAC/protein immunization

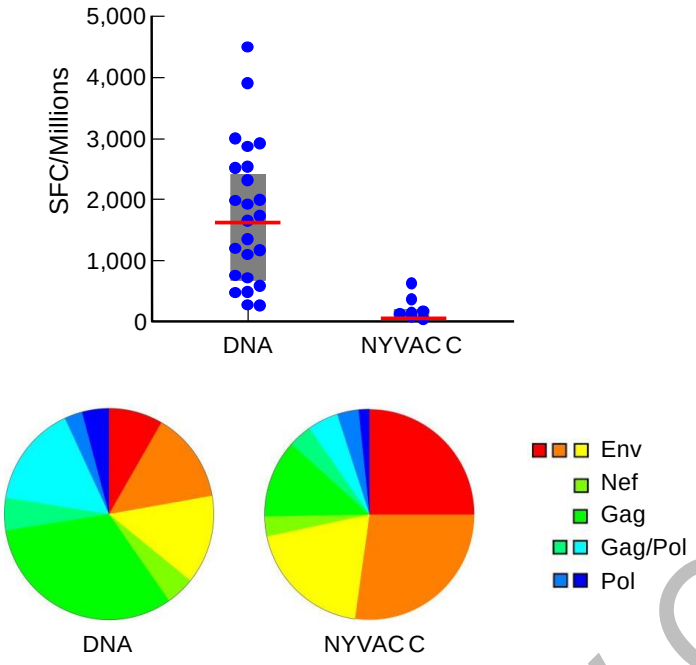

Figure 4-4 Breadth of responses comparing DNA-HIV-PT123/NYVAC/protein regimen (post 3 DNA-HIV-PT123 injections) vs. NYVAC/protein regimen (post 2 NYVAC injections)

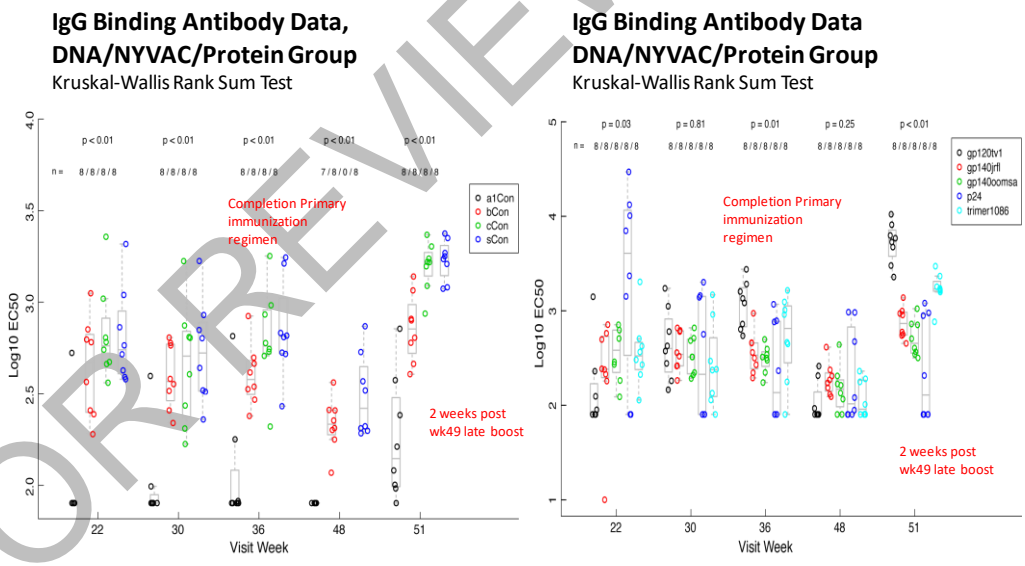

Figure 4-5 IgG Ab responses after completion of primary immunization regimens and post week 49 late boost for the DNA-HIV-PT123/NYVAC/protein group

4.5.2 AUP512: protein schedule vaccination study

The objective of this non-human primate study is to evaluate the effect of 3 different DNA-HIV-PT123 prime schedules on the T-cell and Ab responses following the NYVAC plus protein boost. This study also includes an arm with DNA-HIV-PT123 + protein co-administered at all timepoints.

The study design is provided in Table 4-5.

Table 4-5 Study design of AUP512

Gp	Size	Wk 0	Wk 4	Wk12	Wk 24
1	12	NYVAC	NYVAC	NYVAC + protein	NYVAC + protein
2	12	NYVAC + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein
3	12	DNA-HIV-PT123 + protein	DNA-HIV-PT123 + protein	NYVAC + protein	NYVAC + protein
4	8	DNA-HIV-PT123	DNA-HIV-PT123	NYVAC + protein	NYVAC + protein
5	8	DNA-HIV-PT123 + protein	DNA-HIV-PT123 + protein	DNA-HIV-PT123 +protein	DNA-HIV-PT123 + protein
Total	52				

DNA: DNA-HIV-PT123 used in the study was 2mg/mL in 2 injections (total dose 4mg)
NYVAC: NYVAC-HIV-PT1 and NYVAC-HIV-PT4 were formulated together at the concentration of 2×10^8 PFU/mL (1×10^8 PFU/mL each).
Protein is bivalent gp120 protein (clade C TV1 and 1086) formulated with MF59. The total dose of 100 mcg (50 mcg of each protein).

Preliminary immunogenicity data demonstrated that:

- IgG binding Ab responses against Env are detectable in Groups #2, #3 and #5 at week 6 (after two immunizations and co-administration with gp120 proteins). These responses are further boosted at week 14 after the 3rd immunization. IgG binding Ab responses against Env are detectable in Groups #1 and #4 only at week 14 (after the first protein co-administration);

- Peptide microarray for mapping of linear epitope has been completed on a subset of 15 AUP512 animals (3 from each of the 5 groups) that are among the top binders for Env and/or gp70-V1V2 binding in the Luminex assay. For each animal we tested wk0 and wk26 samples. The results obtained indicate:
  - Animals in AUP 512 developed binding Abs targeting multiple epitopes:
    - C1, C1-V1, V2, C3, V3, C5 of gp120.
  - No binding was seen in gp41.
  - V3-response dominates in most animals, followed by C5, C3, and C1.
  - No significant difference was seen among the groups for binding patterns.

- Appearance of nAbs follows the same kinetics of the IgG binding antibodies;

T-cell responses were greater in the DNA-HIV-PT123 prime/NYVAC boost groups.

4.5.3 Preclinical immunogenicity of the AIDSVAX® product

As this product is well characterize preclinically and clinically (see Section 4.6.2), please refer to the AIDSVAX® B/E IB for preclinical data.

4.6 Clinical studies

4.6.1 Clinical studies with DNA-HIV-PT123

HVTN 096/EV04: DNA-HIV-PT123, NYVAC (NYVAC-HIV-PT1 and NYVAC-HIV-PT4), and AIDSVAX® B/E are currently being tested in an early phase clinical trial.

HVTN 096/EV04 is being conducted exclusively in Lausanne, Switzerland under Swissmedic regulatory authority and entitled "*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*." HVTN096/EV04 completed accrual of 96 participants in April, 2013. As of March 27th, 2014 there have been no SAEs reported that were deemed related to the study products. There was one death by cranial trauma in a motorcycle accident. In studies where these vaccines and other similar vaccines were given, the most common complaints have been injection site pain/itching, myalgia/arthralgia, headache, and malaise/fatigue. Most of these reactions have been mild to moderate. By October, 2013, all participants had completed the vaccine regimens in HVTN 096/EV04, and the majority of reported AEs have been mild or moderate. The most common AE deemed by the site investigator to be related to study product was lymphadenopathy (n=7, 7.3%).

HVTN 092 (BB-IND 15315): DNA-HIV-PT123 in combination with bivalent NYVAC (NYVAC-HIV-PT1 and NYVAC-HIV-PT4) vaccines will provide additional safety and immunogenicity data. The study opened in April, 2013 and is being conducted in the US and Lausanne, Switzerland. As of November 2013, 143 participants had been enrolled. The DNA-HIV-PT123 vaccine has been well-tolerated. The most common complaints have been mild or moderate injection site pain/tenderness, myalgia, headache, and malaise/fatigue. There have been no SAEs related to the DNA-HIV-PT123 vaccine. There was 1 SAE -- a case of myocarditis deemed related to NYVAC vaccination that prompted a hold on vaccinations.

4.6.2 AIDSVAX® clinical experience

Recombinant sub-unit vaccines consisting of HIV-1 envelope glycoprotein, specifically glycoprotein 120 (gp120) have been developed as a strategy to be used in an effective HIV vaccine regimen, as most Abs that neutralize HIV-1 are directed to the envelope protein of the virus. Both monovalent and bivalent vaccines based on the gp120 protein have been developed. Recombinant gp120 (rgp120) produced in genetically engineered mammalian cell lines, and isolated in a highly purified form, has demonstrated ability to elicit nAbs in rodents [35,36], non-human primates [37], and humans [38-40].

A monovalent recombinant gp120 vaccine from the MN strain of the HIV-1 virus, a subtype B antigen, AIDSVAX® MN (MN rgp120/HIV-1), derived from a T cell tropic sub-type B virus) was selected for vaccine development because it elicited nAbs against diverse viral isolates beyond sub-type B [35,36]. AIDSVAX® MN immunization has protected chimpanzees from infection by heterologous SF-2 virus [41]. There has been extensive testing of 2 monovalent vaccines, the AIDSVAX® MN and AIDSVAX® IIIB, in 19 phase 1 and 2 trials, both in HIV infected and uninfected individuals. Over 400 HIV-1 uninfected men, women and infants born to HIV-1 infected women have received the monovalent vaccine in both the US and Thailand. Six hundred HIV infected children, adolescents, pregnant women, and adult men and women have also received this vaccine.

A 2nd recombinant gp120 antigen made from the A244 strain (a sub-type E envelope antigen) was produced from macrophage-tropic virus and combined with the AIDSVAX® MN in a 1:1 ratio (300 mcg of each) to create a bivalent formulation called AIDSVAX® B/E, in order to broaden the potential effect of the vaccine (T-cell and macrophage-tropic products). AIDSVAX® B/E has been tested for safety and immunogenicity in phase 1/2 trials in both the US and Thailand using 3 different doses, administered 4 times (0, 1, 6

and 12 months). The bivalent vaccines have also been tested in phase 3 trials with AIDSVAX® B/B in VAX004 in a broad range of individuals at high-risk for HIV acquisition (but mostly MSM) in North America and the Netherlands and AIDSVAX® B/E in VAX 003 enrolling injection drug users in Thailand and neither were shown to be efficacious.

Table 4-6 summarizes the clinical experience of AIDSVAX® in human trials.

Table 4-6 Summary of AIDSVAX® trials

Candidate vaccine	# receiving AIDSVAX®	Protocol	Status
AIDSVAX® MN or AIDSVAX® IIIB	1000	19 phase 1 and 2 trials	Completed
AIDSVAX® B/E	92	VAX001	Completed
AIDSVAX® B/B AIDSVAX® B/E	92 30	VAX002	Completed
AIDSVAX® B/E	1,250	VAX003	Completed
AIDSVAX® B/B	3,600	VAX004	Completed
AIDSVAX® B/E	97	RV135	Completed
AIDSVAX® B/E	8,197	RV144	Completed
Total	**14, 358**		

4.6.2.1 Safety of AIDSVAX® alone

In the VAX001 participants, amongst 92 low risk volunteers and intravenous drug users, the most common vaccine related events were reactogenicity signs and symptoms within the first 3 days of immunization. Among those, the most common were pain and tenderness at the injection site, which was reported at least once by 75% of the volunteers. Other reactogenicity included malaise, myalgia, fever, injection site erythema, induration, headache, nausea and lymphadenopathy. The majority of the reactogenicity was of a mild severity. There were 11 SAEs reported by 10 volunteers, including hospitalization for cellulitis of the right foot, bronchitis, uncomplicated spontaneous abortion, pulmonary tuberculosis (TB), skull fracture, anal-fistula, substance induced psychosis, hypokalemia, high alanine aminotransferase (ALT;due to Hepatitis C and alcohol abuse). None of these SAEs were attributed to study product. The same reactogenicity profile was seen in VAX 002, the AIDSVAX® B/E trial conducted in 30 subjects in the US.

In the phase 3 trial conducted in Thailand (VAX003), pain and tenderness at the injection site was the most common reported reactogenicity event, reported within 3 days of immunization by 68% of volunteers injected with AIDSVAX® B/E or placebo (66% placebo; 71% vaccinees). Other common reactogenicity events included fatigue reported in 54% of volunteers (53% placebo; 56% vaccinees), malaise in 51% of volunteers (49% placebo; 52% vaccinees), limited arm movement in 48% of volunteers (46% placebo; 50% vaccinees), myalgia in 47% of volunteers (45% placebo; 49% vaccinees), headache in 43% of volunteers (43% placebo; 44% vaccines), and injection site edema in 29% of volunteers (25% placebo; 33% vaccinees). No more than 3.2% of volunteers (2.9% placebo; 3.5% vaccinees) reported these as severe. A total of 414 SAEs were reported, all of which were considered unrelated to the vaccine. 116 deaths occurred in this study, of which 102 occurred in HIV uninfected individuals, (48 placebo, 54 vaccinees), none of

which were attributable to study vaccine. Most common deaths were due to overdose, sepsis, accidental injury, suicide, cirrhosis of the liver and pneumonia.

4.6.2.2 Immunogenicity AIDSVAX® alone

The addition of envelope glycoproteins from different strains of HIV-1 has been found to expand the breadth of the immune response. In the Thai study, VAX 001, 100% seroconversion to both MN rgp120 and A244 rgp120 was observed in 3 different dose groups at months 1.5, 6.5, 12.5, and 18. Percentage seroconversion to each V2 peptide was high, with 100% of participants in VAX001 seroconverted to nAbs to homologous MN HIV-1 isolates at month 6.5, month 12.5 and at month18. Similarly, in VAX002, a phase 1/2 study in the US, 100% seroconversion to MN rgp120 was observed at the same time-points as seen in VAX001. In the phase 3 VAX 003 study, both antigens were well tolerated and immunogenic, producing both MN neutralizing and CD4 binding Abs. Of the volunteers tested in VAX003, 100% had an immune response, with substantial increases in Ab titers after each dose given (see AIDSVAX® IB).

4.7 Potential risks of study products and administration

Table 4-7 summarizes the potential risks associated with administration of the study products.

Table 4-7 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 Ab 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 or theoretical risks	• Muscle damage at the injection site • Autoimmune disease or cancer • Effects on a participant's response to an approved HIV vaccine administered in the future • Effects on susceptibility to HIV, if the participant is exposed to HIV • Effects on the course of HIV infection/disease, if the participant is infected with HIV • Effects on the fetus and on pregnancy

5 Objectives and endpoints

5.1 Primary objectives and endpoints

Primary objective 1:

- To evaluate the safety and tolerability of the combination of AIDSVAX® B/E and DNA-HIV-PT123 administered in different sequences or simultaneously in HIV-uninfected healthy adults

Primary endpoint 1:

- Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and adverse events (AEs) and serious adverse events (SAEs)

Primary objective 2:

- To evaluate the binding Ab and T-cell responses of the combination of AIDSVAX® B/E and DNA-HIV-PT123 administered in different sequences or simultaneouslyin HIV-uninfected healthy adults

Primary endpoints 2:

- HIV-specific binding Ab response as assessed by binding Ab multiplex assay 2 weeks after the 4th vaccination

- Response rate and magnitude of CD4+ and CD8+ T-cell responses as assessed by intracellular cytokine staining assays (ICS) 2 weeks after the 4th vaccination

5.2 Secondary objectives and endpoints

Secondary objective 1:

- To compare the binding Ab and T-cell responses between groups; comparing:
    - AIDSVAX® B/E as a prime followed by a DNA-HIV-PT123 boost and a DNA-HIV-PT123 prime followed by AIDSVAX® B/E boost (Group 1 vs. Group 2)
    - DNA-HIV-PT123 as a prime followed by co-administered DNA-HIV-PT123 and AIDSVAX® B/E boost at 3 and 6 months and a DNA-HIV-PT123 prime followed by AIDSVAX® B/E boost (Group 3 vs. Group 2).
    - DNA-HIV-PT123 as a prime followed by co-administered DNA-HIV-PT123 and AIDSVAX® B/E boost at 3 and 6 months and AIDSVAX® B/E as a prime followed by a DNA-HIV-PT123 boost (Group 3 vs. Group 1).

- Co-administration of DNA-HIV-PT123 and AIDSVAX® B/E and AIDSVAX® B/E as a prime followed by DNA-HIV-PT123 boost (Group 4 vs. Group 1)

- Co-administration of DNA-HIV-PT123 and AIDSVAX® B/E and DNA-HIV-PT123 as a prime followed by AIDSVAX® B/E boost (Group 4 vs. Group 2)

- Co-administration of DNA-HIV-PT123 and AIDSVAX® B/E and DNA-HIV-PT123 as a prime followed by co-administered DNA-HIV-PT123 and AIDSVAX® B/E boost at 3 and 6 months (Group 4 vs. Group 3).

Secondary endpoints 1:

- HIV-specific binding Ab response as assessed by binding Ab multiplex assay 2 weeks after the 4th vaccination

- Response rate and magnitude of CD4+ and CD8+ T-cell responses as assessed by intracellular cytokine staining assays (ICS) 2 weeks after the 4th vaccination

Secondary objective 2:

- To evaluate the ability of the different regimens to elicit HIV nAbs

Secondary endpoint 2:

- 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 4th vaccination

Secondary objective 3:

- To evaluate the durability of the HIV-specific immune responses

Secondary endpoints 3:

- HIV-specific Ab and T-cell responses 6 months after the 4th vaccination

5.3 Exploratory objectives

Exploratory objective 1:

- To further evaluate the immunogenicity of the vaccine regimens

Exploratory endpoint 1:

- Contingent upon results from the primary and/or secondary immunogenicity objectives described above, additional immunogenicity assays may be performed based on the HVTN Laboratory Assay Algorithm

Exploratory objective 2:

- To conduct analyses related to furthering the understanding of HIV, immunology, vaccines, and clinical trial conduct

Exploratory endpoint 2 (may include, but not limited to):

- To assess Ab and/or T-cell response(s) after the prime or compare the response after the prime to the response(s) after the boost

- Comparing immune responses in this study with responses from other trials.

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 104 healthy, HIV-uninfected adult participants, aged 18 to 50 years old, at low risk of HIV infection. Each participant will be randomly assigned to receive 1 of 4 vaccine regimens (Groups 1-4).

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. However, for immunogenicity analyses, it is possible that data may be missing for various reasons, such as participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). Immunogenicity data from 9 phase 1 and 1 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of June 2011), indicate that 15% is a reasonable estimate for the rate of missing data for 2 weeks after the 4th vaccination (week 26). For this reason, the sample size calculations in Section 6.1.2 account for enrolled participants having 15% 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. The ability of the study to detect SAEs (See Section 11.2.2) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each vaccine arm of the study (n =26), there is a 90% chance of observing at least 1 event if the true rate of such an event is 8.5% or more; and there is a 90% chance of observing no events if the true rate is 0.4% or less. For Groups 1-4 with the combination of AIDSVAX® and DNA-HIV-PT123 (n =104), there is a 90% chance of observing at least 1 event if the true rate of such an event is 2.2% or more; and there is a 90% chance of observing no events if the true rate is 0.1% or less. As a reference, in HVTN vaccine trials from December 2000 through December 2012, about 4% of participants who received placebos experienced an SAE.

Probabilities of observing 0, 1 or more, and 2 or more events among arms of size 26 for each of Groups 1-4 and size 104 for Groups 1-4 combined are presented in Table 6-1 for a range of possible true AE rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 26 for each Group and size 104 for Groups 1-4 combined, for different true event rates

True event rate (%)	Pr(0/26)	Pr(1+/26)	Pr(2+/26)	Pr(0/104)	Pr(1+/104)	Pr(2+/104)
1	77.0	23.0	2.8	35.2	64.8	27.9
4	34.6	65.4	27.9	1.4	98.6	92.4
5	26.4	73.6	37.6	0.5	99.5	96.9
10	6.5	93.5	74.9	0	100	100
20	0.3	99.7	97.7	0	100	100
30	0	100	99.9	0	100	100
40	0	100	100	0	100	100

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an AE based on the observed data. Table 6-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method [42]. If none of the 104 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 (n=104) is 3.6%. For each individual vaccine arm (n =26), the 2-sided upper confidence bound for this rate is 12.9%.

Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 26 and 104

Observed event rate	Confidence interval (%)
0/26	[0, 12.9]
1/26	[0.7, 18.9]
2/26	[2.1, 24.1]
0/104	[0, 3.6]
1/104	[0.2, 5.2]
2/104	[0.5, 6.7]

6.1.2 Sample size calculations for immunogenicity

The main goals of this trial regarding immunogenicity outcomes involve a preliminary estimation of response rates and magnitudes based on data from the HIV-specific binding Ab response as assessed by multiplex assay and peptide pools for CD4+ and CD8+ responses separately as assessed by ICS assays 2 weeks after the 4th vaccination among vaccinees in each vaccine arm. No adjustment for multiple comparisons will be made for the use of multiple assays. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% confidence intervals for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-3. Calculations are done using the score test method [42]. The sample size of 22 is used in the power calculations for each arm (n=26) (Groups 1-4) assuming there is 15% loss of data.

Table 6-3 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the vaccinees (n = 22)

No. of responses	Observed response rate (%)	Confidence interval
3/22	13.6	[4.7, 33.3]
6/22	27.3	[13.2, 48.2]
9/22	40.9	[23.3, 61.3]
12/22	54.5	[34.7, 73.1]
15/22	68.2	[47.3, 83.6]
18/22	81.8	[61.5, 92.7]
21/22	95.5	[78.2, 99.2]

Fisher exact statistics is used to test the null hypothesis of equal response rates between 2 arms. The numbers presented in Table 6-4 are the minimum true response rates in one arm (say Group 2) given the possible true response rates in other arm (say Group 1) such that the study has 80% or 90% statistical power to distinguish the different response rates between the two arms. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05. Based on Table 6-4, the study is adequately powered ($\geq$ 80%) to distinguish the response rate difference between two arms if the true response rate difference between the two arms is 37-46% (depending on the true response rate in Arm 1).

Table 6-4 Power for comparison of response rates between two arms (n1=22, n2=22)

True response rate Arm 1 (%)	Minimum true response rate in Arm 2 (%) (n1=22, n2=22)	
	80% power	90% power
10	52	59
20	65	72
30	76	82
40	85	89
50	92	95
60	97	100

As shown in Table 6-4, there is limited power for a formal comparison of immunogenicity response levels between vaccine arms of size n = 26 and, hence, formal comparisons are not listed in the study primary objectives. For 80% or 90% power, the sizes of differences that the trial is powered to detect are fairly large.

An alternative to formal comparisons of arms is to rank the arms by their response rates. For arms of size n=26, we can assess the reliability of this study to select the best arm with respect to the magnitude of response rates. Table 6-5 shows various true response rates for which this study will correctly select the arm with the highest response rate with 0.8 or 0.9 probability. Each line in Table 6-5 shows the results based on 40,000 simulated datasets of response rates for 4 arms of size n=26 generated using 2 different binomial probabilities, with the best response probability used to generate data for one arm and the second best response probability used to generate data for the remaining 3 arms [43]. If the difference in response rate between the best and second best arms is smaller than the assumed difference, the chance of correctly selecting the arm with the true highest response rate will be less than 80% (90%).

Table 6-5 True immunogenicity response rates for which the regimen with the highest response probability will be correctly selected with 0.8 (0.9) probability

Second best response probability	Best response probability	Difference
10%	25% (31%)	15% (21%)
20%	39% (44%)	19% (24%)
30%	50% (56%)	20% (26%)
40%	60% (66%)	20% (26%)
50%	70% (76%)	20% (26%)
60%	80% (84%)	20% (24%)
70%	87% (91%)	17% (21%)
80%	94% (97%)	14% (17%)

When the response rates of two arms are comparable, the response magnitudes are compared. The response magnitudes are measured by the intensities of IgG binding Ab assessed by multiplex assay or percentages of CD4+ T cells or CD8+ T cells assessed by ICS assay. Means of the log-transformed response magnitudes among positive responders are compared between two arms using t-test.

The numbers presented in Table 6-6 are the minimum differences in mean (in standard deviation [SD]) between two arms that can be distinguished with a statistical power of 80% or 90% given a range of possible true response rates in both arms. These calculations use 2-sided t-test with a Type I error rate of 0.05 assuming the log-transformed response levels are normally distributed with equal variances between two arms. The minimum detectable difference in mean depends on the sample size, the true response rate, and SD among the positive responders. For an example, according to the data of the vaccine recipients who received 4 doses of ALVAC-HIV (cCP1521) vaccine as prime and 2 doses of AIDSVAX® B/E as boost co-administrated with the last 2 doses of prime vaccine in RV144, non-zero IgG binding Ab response was 98%; mean and SD of IgG binding Abs among positive responders were 5.9 and 1.3 in log-scale, respectively. Based on the estimations presented in Table 6-6, this study has 80% (90%) power to detect 3.0log (3.6log) difference in mean or 2.5-fold (2.9-fold) change in geometric mean between two arms. For another example, according to the data of the 70 recipients who received 3 does of DNA-HIV-PT123 HIV vaccine as prime and a single dose of NYVAC-C HIV vaccine as boost in EV03, the response rate (based on ELISpot assay) was 91%; mean and SD of CD4+ and CD8+ T cell response magnitudes (based on ICS assay) from 14 of the EV03 vaccine recipients were 0.23 (0.59) and 0.269 (0.276) in $\log_{10}$-scale, respectively. Based the estimations presented in Table 6-6, this study has 80% (90%) of power to detect $0.25\log_{10}$ ($0.29\log_{10}$) difference in mean or 1.8-fold (1.9-fold) change in geometric means between two arms.

Table 6-6 Minimum mean difference (in log scale) with 80% or 90% power to distinguish the response magnitude between 2 arms among positive responders

Common true response rate (%)	Minimum mean difference in SDs (n1=22, n2=22)	
	Power=80%	Power=90%
50	1.19	1.38
60	1.09	1.26
70	1.01	1.17
80	0.94	1.09
90	0.89	1.03
100	0.84	0.98

An alternative to formal comparisons of arms is to rank the arms by their mean response magnitudes among the positive responders. For arms of size n=26, we can assess the reliability of this study to select the best arm with respect to the magnitude of response means among the responders. Our assessment depends on the assumptions for percentage of positive responders and SD of the response magnitude among the positive responders. Table 6-7 and Table 6-8 shows the true response means among the positive responders, for which this study will correctly select the arm with the highest response mean with 0.8 or 0.9 probability based on the estimations of the percentage of positive responders and SD from RV144 data and EV03 data for IgG binding Ab and T cell responses, respectively. Each line in Table 6-7 and Table 6-8 shows the results based on 40,000 simulated datasets of response means for 4 arms of size n=26 generated using 2 different normal distributions, with the best (highest) response mean used to generate data for one arm and the second best (lower) response mean used to generate data for the remaining 3 arms [43]. If the difference in response magnitudes between the best and second best arms is smaller than the assumed difference, the chance of correctly selecting the arm with the true highest response will be less than 80% (90%).

Table 6-7 True immunogenicity mean response levels for which the regimen with the highest response probability will be correctly selected with 0.8 (0.9) probability for IgG binding Ab

Second best response mean	Best response mean	Difference (in log-scale)	Fold Difference
2.0	2.53 (2.68)	0.53 (0.68)	1.7 (2.0)
3.0	3.53 (3.69)	0.53 (0.69)	1.7 (2.0)
4.0	4.52 (4.67)	0.52 (0.67)	1.7 (2.0)
5.0	5.53 (5.68)	0.53 (0.68)	1.7 (2.0)
6.0	6.54 (6.69)	0.54 (0.69)	1.7 (2.0)

Table 6-8 True immunogenicity mean response levels for which the regimen with the highest response probability will be correctly selected with 0.8 (0.9) probability for CD4+ (CD8+) T cell responses

Second best response mean	Best response mean	Difference (in log10-scale)	Fold Difference
0.1	0.22 (0.26)	0.12 (0.16)	1.3 (1.4)
0.2	0.32 (0.36)	0.12 (0.16)	1.3 (1.4)
0.3	0.43 (0.46)	0.13 (0.16)	1.3 (1.4)
0.4	0.52 (0.56)	0.12 (0.16)	1.3 (1.4)
0.5	0.62 (0.66)	0.12 (0.16)	1.3 (1.4)

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

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm assignments. 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 105 PSRT should be consulted before emergency unblinding occurs.

6.4 Statistical analysis

This section describes the final study analysis, unblinded as to treatment arm assignment. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. 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.

Analyses for primary endpoints will be performed using SAS and R. All other descriptive and inferential statistical analyses will be performed using SAS, StatXact, or R statistical software.

No formal multiple comparison adjustments will be employed for multiple safety endpoints, multiple primary immunogenicity endpoints, or secondary endpoints. However, multiplicity adjustments will be made for certain immunogenicity assays, as discussed below, when the assay endpoint is viewed as a collection of hypotheses (eg, testing multiple peptide pools to determine a positive response).

6.4.1 Analysis variables

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

6.4.2 Baseline comparability

Treatment arms will be compared for baseline participant characteristics using descriptive statistics.

6.4.3 Safety/tolerability 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 and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and systemic symptoms will be calculated. Kruskal-Wallis tests will be used to test for differences in severity between arms.

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 reported to the DAIDS Regulatory Support Center (RSC) Safety Office will provide details of the events including severity, relationship to study product, time between onset and last vaccination, and number of vaccinations received.

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.

For each local laboratory measure, summary statistics will be presented by treatment arm and timepoint, as well as changes from baseline for postenrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the DAIDS AE Grading Table (see section 11.2.2) will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will be included in the tabulation of AEs described above.

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 General approach

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. Additional analyses may be performed, limited to participants who received all scheduled injections per protocol. 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.

Qualitative assay data (eg, IgG binding Ab response from the multiplex assay or CD4+/CD8+ T cell response from the ICS assay) will analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method [42]. Fisher's exact tests will be used to compare the response rates of any 2 vaccine arms, with a significant difference declared if the 2-sided p-value is ≤ 0.05.

In addition to response rate estimates for each timepoint, 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 confidence intervals, for each vaccine arm using maximum likelihood-based methods [44].

For continuous assay data (eg, IgG binding Ab intensity from the multiplex assay or percentage of positive cells from the ICS assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. The difference between arms at a specific timepoint 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, log or $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance).

Some immunologic assays have underlying continuous or count-type readout that is dichotomized into responder/nonresponder categories (eg, IgG binding Ab intensity or percentage of CD4+ and CD8+ T cell response). If treatment arm differences for these assays are best summarized by a mixture model, then Lachenbruch's test statistic [45] will be used to evaluate 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 is used to obtain a 2-sided p-value. For

estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

More sophisticated analyses employing repeated measures methodology (for example, repeated measures analysis of variance [ANOVA] or generalized estimating equations) may be utilized to incorporate immune responses over several timepoints and to test for differences over time. 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 $p \leq 0.05$.

Based upon previous AVEG and HVTN trials, missing 10-15% of immunogenicity results for a specific assay is common due to study participants terminating from the study early or missing visits, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with nonparametric tests and generalized linear models fit by generalized estimating equation (GEE) methods, missing data need 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 covariates included in the model (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 timepoint 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 may depend 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 [46] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [47] methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

6.4.4.2 Analysis of CD4+ and CD8+ T-cell response as measured by the ICS assay

The analysis of CD4+ and CD8+ T-cell response rates as measured by the ICS assay will be evaluated and compared as described under the general approach. In determining the positivity of response, no multiple comparison adjustment will be made for the analysis of the two T-cell subsets. However, within each T-cell subset, the positivity call for each peptide pool will adjust for the number of peptide pools used in the assay. The magnitude of response will be analyzed as described for continuous data in the general approach

section. For each T-cell subset, graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint, with a box plot of data from positive responders superimposed on the individual data values. Statistical testing comparing the magnitudes will be based on positive responders only.

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 during the study, for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 105 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 a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after at least one of the primary immunogenicity endpoints of the same class (humoral or cell-mediated) if no primary endpoint of the same class, at least one of the primary immunogenicity endpoints reaches the aforementioned threshold. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key HVTN members and investigators. Distribution of reports 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 scheduled follow-up visits.

7 Selection and withdrawal of participants

Participants will be healthy, HIV 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. **Agrees not to enroll in another study** of an investigational research agent before the last required protocol clinic visit.

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

HIV-Related Criteria:

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

8. Willingness to discuss HIV infection risks and amenable to HIV risk reduction counseling.

9. Assessed by the clinic staff as being at **"low risk" for HIV infection** and committed to maintaining behavior consistent with low risk of HIV exposure through the last required protocol clinic visit.

Laboratory Inclusion Values

Hemogram/CBC

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

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

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

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

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

Chemistry

15. **Chemistry panel**: ALT < 1.25 times the institutional upper limit of normal (IULN) and creatinine ≤ IULN.

Virology

16. **Negative HIV-1 and -2 blood test**: Volunteers must have a negative FDA-approved enzyme immunoassay (EIA).

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

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

Urine

19. **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

20. **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. Persons who are NOT of reproductive potential due to having undergone

total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

21. **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
        - Any other contraceptive method approved by the HVTN 105 PSRT
        - 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 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.

22. **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. **Body mass index (BMI)** ≥ 40; or BMI ≥ 35 with 2 or more of the following: age > 45, systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, known hyperlipidemia
4. **Intent to participate in another study** of an investigational research agent before the last required protocol clinic visit
5. **Pregnant or breastfeeding**

Vaccines and other Injections

6. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 105 PSRT will determine eligibility on a case-by-case basis.

7. **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 105 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 105 PSRT on a case-by-case basis.

8. **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)

9. **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)

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

Immune System

11. **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.

12. **Serious adverse reactions to vaccines** including history of 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.)

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

14. **Autoimmune disease** (Not excluded: mild, well-controlled psoriasis)

15. **Immunodeficiency**

Clinically significant medical conditions

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

17. **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.

18. **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

19. **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.

20. **Current anti-TB prophylaxis or therapy**

21. **Asthma** other than mild or moderate, 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 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.

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

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

24. **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.

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.

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 11.3.

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 105 Study Specific Procedures.

In order to avoid vaccination delays and missed vaccinations, participants who plan to receive licensed vaccines, 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 105 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 105 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).

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.

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 9.5.1).

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 pharmacists 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 Investigator's Brochures 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.

Group 1

Treatment 1 (T1): Placebo for DNA-HIV-PT123 to be administered as 1 mL in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

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

THEN

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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): DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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 (unless medically contraindicated) at Months 0 and 1.

THEN

Placebo for DNA-HIV-PT123 to be administered as 1 mL 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.

Group 3

Treatment 3 (T3): DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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 (unless medically contraindicated) at Months 0 and 1.

THEN

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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.

Group 4

Treatment 4 (T4): DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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.

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 presented as a solution for injection at a total DNA concentration of approximately 4 mg/mL in PBS buffer. The DNA-HIV-PT123 vaccine contains an equi-mass mixture of 3 different recombinant DNA plasmids in a 1:1:1 ratio. DNA-HIV-PT123 4 mg/mL is supplied in a 2 mL sterile glass vial containing 1.2 mL (extractable volume 1 mL) of a clear, colorless, sterile solution. The product must be stored at -20^{0}C (± 5 ^{0}C).

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 containing a volume to deliver 1 mL (300mcg/mL) of each rgp120/HIV-1 protein adsorbed onto a total of 600 mcg aluminum hydroxide gel adjuvant. The product must be kept refrigerated (2^0 to 8^{0}C).

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

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

Placebo for AIDSVAX® B/E (Sodium Chloride for Injection USP, 0.9%)

Sodium Chloride for Injection USP, 0.9% will be used as the placebo for the AIDSVAX® B/E. Product must be stored as directed by the manufacturer.

8.3 Preparation of study products

8.3.1 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.

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

The syringe should be labeled as "AIDSVAX B/E 600 mcg or Placebo 1 mL" and must 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.

8.3.2 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 "DNA 4 mg or Placebo 1 mL". 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.

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

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

The syringe should be labeled as "DNA 4 mg or Placebo 1 mL". 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.

8.3.4 Placebo for AIDSVAX® B/E (Sodium Chloride for Injection USP, 0.9%)

Sodium Chloride for Injection USP, 0.9% will be needed to prepare the dose. The pharmacist using aseptic technique, will withdraw 1 mL of Sodium Chloride for Injection USP, 0.9% into a 3 or 5 mL syringe. An overlay must be applied to the syringe.

The syringe should be labeled as "AIDSVAX B/E 600 mcg or Placebo 1 mL" and must 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 AIDSVAX® B/E or Placebo (for AIDSVAX® B/E) injections, 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.

At sites where registered pharmacists are legally authorized to administer injections, the HVTN CRS may choose to have the HVTN CRS pharmacist administer vaccinations.

8.5 Acquisition of study products

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

AIDSVAX® B/E will be provided by GSID.

Placebo for all study products (DNA-HIV-PT123 and AIDSVAX® B/E (Sodium Chloride for Injection USP, 0.9%)) will not be provided through the protocol and must be obtained by the site.

Once an HVTN CRS is protocol registered, the pharmacist can obtain study products from the NIAID Clinical Research Products Management Center (CRPMC) by following the ordering procedures given in Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

8.6 Pharmacy records

The 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 CRPMC after the study is completed or terminated unless otherwise instructed by the CRPMC. The procedures and relevant form are included in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

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

Without a general screening consent, screening for a specific study cannot take place until the site receives protocol registration from the DAIDS Protocol Registration Office's Regulatory Support Center (RSC).

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.

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. A separate sample consent form for other uses of specimens is located in Appendix C.

Each HVTN CRS is responsible for developing a protocol-specific consent form(s) for local use, based on the sample protocol-specific consent forms in Appendix A and Appendix C. The consent form(s) 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.

Regarding protocol registration, sites should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

9.1.3 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 Abs,
  - Syphilis test,
  - Complete blood count (CBC) with differential and platelets,
  - Chemistry panel (ALT and creatinine),
  - Urine dipstick (urinalysis if indicated; see Section 9.7),
  - Urine or serum pregnancy test (volunteers who were born female); Persons who are not of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing;
- 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 9.5; 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 (volunteers who were born female); Persons who are not of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

Following completion of all procedures in the preceding list and results indicate that vaccination may proceed, vaccination is prepared and administered (see Sections 0 and 8.3.1).

Administration of all injections during a vaccination visit must be accomplished within 1 calendar day.

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 reports of reactogenicity events from the participant (see section 9.8).

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 9.5);
- Pregnancy prevention assessment (as described in section 9.2 and 9.6);
- 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 some vaccination visits as specified in Appendix F:

- 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);
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Administration of behavioral risk assessment questionnaire;
- 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; 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 9.5);
- Pregnancy prevention assessment (as described in section 9.2 and 9.6);
- 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.

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

- Administration of behavioral risk assessment questionnaire;

- HIV infection assessment including pretest counseling. A subsequent follow-up contact is conducted to provide posttest 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;

- Urine or serum pregnancy test (volunteers who were born female); Persons who are not of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing;

- Specimen collection;

- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - Chemistry panel (see Section 9.2),
  - Urine dipstick (urinalysis if appropriate; see Section 9.7),

9.5 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.

9.5.1 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 F). 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).

9.5.2 VISP registry

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 9.5.1). 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 registry contains the names of all study participants, unless they request that their names be removed.

9.6 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.

9.7 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.

9.8 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 3 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 3 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 3 full days after), or those meeting SAE/AE 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[b]	Between 12:00am and 11:59pm day 3	HVTN CRS staff or participant

[a] Day of vaccination

[b] New or unresolved reactogenicity symptoms present on day 3 are followed until resolution

9.8.1 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.

9.8.2 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.

9.8.3 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).

9.9 Visit windows and missed visits

Visit windows are defined in HVTN 105 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.

9.10 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.

9.11 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 may be redirected to another laboratory or may 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

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 fourth vaccination visit). Endpoint assays for humoral and cellular responses are performed on 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 participants at other timepoints; the schedule is shown in Appendix E.

10.4 Endpoint assays: cellular

10.4.1 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 included in the vaccine regimen. 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 Endpoint assays: humoral

10.5.1 HIV-1 multiplex Ab assay

Total binding IgG (potentially IgG3 and other subclasses based on immunogenicity) and IgA Abs to HIV-specific antigens will be assessed on plasma/serum samples from study participants taken at the primary immunogenicity timepoint and baseline. Specimens from other timepoints as well as other HIV antigens may also be assayed based on the results of the initial assay.

10.5.2 Neutralizing Ab 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 at the discretion of the HVTN Laboratory Program, which may be 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 viruses. The tier 2 assays will test neutralization of a panel of heterologous primary isolates [48].

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 control 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-1disease progression may also be assessed.

10.7 Lab assay algorithm

The Lab Assay Algorithm lists assays to characterize cellular, humoral, and innate immune responses as well as host genetics that may be conducted to determine endpoints in HVTN vaccine trials. The type of assay(s) employed will be dependent on the response obtained by the primary immunogenicity assays at relevant timepoints. Please note that the Lab Assay Algorithm will be updated periodically to include new assays.

10.8 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.

10.9 Other use of stored specimens

The HVTN stores specimens from all study participants indefinitely, unless a participant requests that specimens be destroyed or if required by IRB/EC, or RE.

Other use of specimens is defined as studies not described in the protocol.

This research may relate to HIV, vaccines, the immune system, and other diseases. This could include limited genetic testing and, potentially, genome-wide studies. This research is done only to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other testing on specimens will occur only after review and approval by the HVTN, the IRB/EC of the researcher requesting the specimens, and the CRS's IRBs/ECs if required.

The protocol sample informed consent form is written so that the participant either explicitly allows or does not allow their samples to be used in other research when they sign the form. Participants who initially agree to other use of their samples may rescind their approval once they enter the study; such participants will remain in this study and their samples will only be used for the studies described in this protocol. 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, HVTN Regulatory Affairs directs the HVTN Lab Program not to use samples from these participants for such other uses.

CRSs must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on other use of specimens.

10.10 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

11.1.1 HVTN 105 PSRT

The HVTN 105 PSRT is composed of the following members:

- DAIDS medical officer representative,
- Protocol chair and cochair,
- Protocol Team leader,
- Core medical monitor, and
- Clinical safety specialist.

The clinician members of HVTN 105 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, project manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 105 PSRT meetings.

11.1.2 HVTN SMB

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 study. The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of AEs requiring expedited reporting to DAIDS. 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 105 PSRT.

Study sites 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 105 PSRT and HVTN SMB (see Section 11.1.2).

11.1.4 HVTN Core roles and responsibilities in safety monitoring

The roles and responsibilities of HVTN Core in relation to safety monitoring include:

- Daily monitoring of clinical data for events that meet the safety pause and HVTN 105 PSRT AE review criteria (see Section 11.3);
- Notifying HVTN CRSs and other groups when safety pauses or planned holds are instituted and lifted (see Section 11.3);
- Querying HVTN CRSs for additional information regarding reported clinical data; and
- Providing support to the HVTN 105 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

Sites 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 RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/, except that unintentional weight loss of less than 10% loss in body weight from baseline is not required to be reported as an AE.

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 to DAIDS (Section 11.2.3) and (2) if the AE meets the criteria for a safety pause/prompt AE review (Section 11.3).

Sites are expected to notify the CSS 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 105 Study Specific Procedures. Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

In the case of email notification, the CSS 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 the CSS of the event by telephone, then submit CRFs.

In addition, site investigators are required to submit AE information in accordance with IRB/EC and any applicable RE requirements.

11.2.3 Expedited reporting of adverse events to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 (January 2010) of the *Manual for Expedited Reporting of Adverse Events* to DAIDS (DAIDS EAE Manual), which is available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/. The SAE Reporting Category will be used for this study.

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

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

The study products for which expedited reporting are required are:

- DNA-HIV-PT123
- AIDSVAX® B/E
- Placebo: Sodium Chloride for Injection USP, 0.9%

While the participant is in the study reporting period (See Section 3), the SAE Reporting Category will be used.

After the protocol-defined AE reporting period for the study, unless otherwise noted, only Suspected, Unexpected Serious Adverse Reactions as defined in Version 2.0 of the DAIDS EAE Manual must be reported to DAIDS, if the study staff become aware of the events.

The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the FDA in accordance with 21 CFR 312.32 (IND Safety Reports). However, because safety is a primary study endpoint, the Sponsor Medical Officer will not be unblinded to study treatment assignment when there is an assessment

of relatedness of the SAE with the study product(s); and the safety report will be sent to the FDA based on the blinded attribution assessment.

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 11.3.

11.3 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccination 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 105 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 105 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	HVTN Core 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 105 PSRT notification
SAE, related	Grade 3	Email and fax forms immediately	Prompt HVTN 105 PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and fax forms immediately	Prompt HVTN 105 PSRT AE review to consider pause

[a] Phone numbers and email addresses are listed in HVTN 105 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).

For all safety pauses, HVTN Core notifies the HVTN 105 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and participating HVTN CRSs. When an immediate safety pause is triggered, HVTN Core also notifies the HVTN SMB.

Once a trial is paused, the HVTN 105 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the SMB if necessary. HVTN Core notifies the participating HVTN CRSs, HVTN Regulatory Affairs, DAIDS PAB, DAIDS RAB, and DAIDS SPT of the decision regarding resumption or discontinuation of study vaccinations. Based on the HVTN 105 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 105 PSRT notification or prompt HVTN 105 PSRT AE review is triggered, HVTN Core notifies the HVTN 105 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 105 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 requires that each CRS submit to its IRB/EC protocol-related safety information (such as IND safety reports, notification of vaccine holds due to the pause rules, and notification of other unplanned safety pauses). CRSs must also follow all applicable RE reporting requirements.

In addition, all other AEs are reviewed routinely by the HVTN 105 PSRT (see Section 11.4.2).

11.4 Review of cumulative safety data

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

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.

11.4.1 Daily review

Blinded daily safety reviews are routinely conducted by HVTN Core for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 105 PSRT AE review criteria.

11.4.2 Weekly review

During the injection phase of the trial, the HVTN 105 PSRT reviews 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 105 PSRT. HVTN Core 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.

11.5 Study termination

This study may be terminated early by the determination of the HVTN 105 PSRT, HVTN SMB, FDA, NIH, Office for Human Research Protections (OHRP), or vaccine developer. 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;
- Specimen collection, processing, and analysis;
- Ancillary studies, and
- Destruction of specimens.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 105 *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 version history of, and modifications to, Protocol HVTN 105 are described below.

Protocol history and modifications

Date: April 7, 2014

Protocol version: 1.0
Protocol modification: Original protocol

14 Document references (other than literature citations)

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

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines. Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf.
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/
- Division of AIDS 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/gradingtables.aspx
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/safetyandpharmacovigilance/manualforexpeditedreporting.aspx
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 105 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 105 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.
- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at http://www.iata.org/ps/publications/dgr/Pages/index.aspx.
- HVTN Lab assay algorithm
- 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

- Investigator's Brochure. AIDSVAX® B/E Vaccine. Dated 05 March 2013.

- Investigator's Brochure. DNA-HIV-PT123 vaccine. Dated 12 February 2014.

- 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.

- Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks, July 2008.

- 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://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=21:1.0.1.1.19&idno=21

- Title 45, Code of Federal Regulations, Part 46. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=45:1.0.1.1.25&idno=45

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
AVEG	AIDS Vaccine Evaluation Group
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
DAERS	DAIDS Adverse Event Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
ELISpot	enzyme-linked immunospot
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
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
IUD	intrauterine device
IULN	institutional upper limit of normal
MAR	missing at random
MCAR	missing completely at random
MMR	measles, mumps, and rubella
nAb	neutralizing antibody

NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NIH	US National Institutes of Health
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PFU	plaque forming unit
PI	Principal Investigator
PSRT	Protocol Safety Review Team
RAB	DAIDS Regulatory Affairs Branch
RE	regulatory entity
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SFC	spot-forming cell
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
TB	tuberculosis
UW-VSL	University of Washington Virology Specialty Laboratory
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

1. UNAIDS. Ethical considerations in biomedical HIV prevention trials. **2007**.

2. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research. **1979**.

3. Council for International Organizations of Medical Sciences (CIOMS). International ethical guidelines for biomedical research involving human subjects. Bull Med Ethics **2002**;17-23.

4. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med **2009**;361:2209-20.

5. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med **2012**;366:1275-86.

6. Rolland M, Edlefsen PT, Larsen BB, Tovanabutra S, Sanders-Buell E, Hertz T, Decamp AC, Carrico C, Menis S, Magaret CA, Ahmed H, Juraska M, Chen L, Konopa P, Nariya S, Stoddard JN, Wong K, Zhao H, Deng W, Maust BS, Bose M, Howell S, Bates A, Lazzaro M, O'Sullivan A, Lei E, Bradfield A, Ibitamuno G, Assawadarachai V, O'Connell RJ, DeSouza MS, Nitayaphan S, Rerks-Ngarm S, Robb ML, McLellan JS, Georgiev I, Kwong PD, Carlson JM, Michael NL, Schief WR, Gilbert PB, Mullins JI, Kim JH. Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. Nature **2012**;490:417-20.

7. Yates NL, Liao HX, Fong Y, DeCamp A, Vandergrift NA, Alam SM, Ferrari G, Berman PW, Francis D, Sinangil F. HIV-1 Env V1V2 responses correlate with decreased transmission risk in the RV144 trial and distinguish vaccine-elicited humoral responses from the Vax003 vaccine trial. Science Translational Medicine. In press **2013**.

8. Morgan C, Jin X, Yu X, DeRosa S, Kublin J, Metch B, Keefer M, The NIAID HIV Vaccine Trials Network. DNA Plasmid HIV Vaccine Design, Number of Doses, Participant Gender, And Body Mass Index Affect T-Cell Responses Across HIV Vaccine Clinical Trials. 2012.

9. Cooney EL, McElrath MJ, Corey L, Hu SL, Collier AC, Arditti D, Hoffman M, Coombs RW, Smith GE, Greenberg PD. Enhanced immunity to human immunodeficiency virus (HIV) envelope elicited by a combined vaccine regimen consisting of priming with a vaccinia recombinant expressing HIV envelope and boosting with gp160 protein. Proc Natl Acad Sci U S A **1993**;90:1882-6.

10. AIDS Vaccine Evaluation Group 022 Protocol Team. Cellular and humoral immune responses to a canarypox vaccine containing human immunodeficiency virus type 1 Env, Gag, and Pro in combination with rgp120. J Infect Dis **2001**;183:563-70.

11. Gupta K, Hudgens M, Corey L, McElrath MJ, Weinhold K, Montefiori DC, Gorse GJ, Frey SE, Keefer MC, Evans TG, Dolin R, Schwartz DH, Harro C, Graham B, Spearman PW, Mulligan M, Goepfert P. Safety and immunogenicity of a high-titered canarypox vaccine in combination with rgp120 in a diverse population of HIV-1-uninfected adults: AIDS Vaccine Evaluation Group Protocol 022A. J Acquir Immune Defic Syndr **2002**;29:254-61.

12. Liu MA. DNA vaccines: an historical perspective and view to the future. Immunol Rev **2011**;239:62-84.

13. Ledgerwood JE, Wei CJ, Hu Z, Gordon IJ, Enama ME, Hendel CS, McTamney PM, Pearce MB, Yassine HM, Boyington JC, Bailer R, Tumpey TM, Koup RA, Mascola JR, Nabel GJ, Graham BS. DNA priming and influenza vaccine immunogenicity: two phase 1 open label randomised clinical trials. Lancet Infect Dis **2011**;11:916-24.

14. Ledgerwood JE, Zephir K, Hu Z, Wei CJ, Chang L, Enama ME, Hendel CS, Sitar S, Bailer RT, Koup RA, Mascola JR, Nabel GJ, Graham BS. Prime-boost interval matters: a randomized phase 1 study to identify the minimum interval necessary to observe the H5 DNA influenza vaccine priming effect. J Infect Dis **2013**;208:418-22.

15. Wang S, Kennedy JS, West K, Montefiori DC, Coley S, Lawrence J, Shen S, Green S, Rothman AL, Ennis FA, Arthos J, Pal R, Markham P, Lu S. Cross-subtype antibody and cellular immune responses induced by a polyvalent DNA prime-protein boost HIV-1 vaccine in healthy human volunteers. Vaccine **2008**;26:1098-110.

16. Vaine M, Wang S, Liu Q, Arthos J, Montefiori D, Goepfert P, McElrath MJ, Lu S. Profiles of human serum antibody responses elicited by three leading HIV vaccines focusing on the induction of Env-specific antibodies. PLoS ONE **2010**;5:e13916.

17. Day T, Morgan C. Probing Scientific Questions to Advance HIV Vaccine Development. HVTN News 5[1], 1-11. 2013.

18. Spearman P, Lally MA, Elizaga M, Montefiori D, Tomaras GD, McElrath MJ, Hural J, De Rosa SC, Sato A, Huang Y, Frey SE, Sato P, Donnelly J, Barnett S, Corey LJ. A trimeric, V2-deleted HIV-1 envelope glycoprotein

vaccine elicits potent neutralizing antibodies but limited breadth of neutralization in human volunteers. J Infect Dis **2011**;203:1165-73.

19. Evans TG, Keefer MC, Weinhold KJ, Wolff M, Montefiori D, Gorse GJ, Graham BS, McElrath MJ, Clements-Mann ML, Mulligan MJ, Fast P, Walker MC, Excler JL, Duliege AM, Tartaglia J. A canarypox vaccine expressing multiple human immunodeficiency virus type 1 genes given alone or with rgp120 elicits broad and durable CD8+ cytotoxic T lymphocyte responses in seronegative volunteers. J Infect Dis **1999**;180:290-8.

20. Belshe RB, Stevens C, Gorse GJ, Buchbinder S, Weinhold K, Sheppard H, Stablein D, Self S, McNamara J, Frey S, Flores J, Excler JL, Klein M, Habib RE, Duliege AM, Harro C, Corey L, Keefer M, Mulligan M, Wright P, Celum C, Judson F, Mayer K, McKirnan D, Marmor M, Woody G. Safety and immunogenicity of a canarypox-vectored human immunodeficiency virus Type 1 vaccine with or without gp120: a phase 2 study in higher- and lower-risk volunteers. J Infect Dis **2001**;183:1343-52.

21. Russell ND, Graham BS, Keefer MC, McElrath MJ, Self SG, Weinhold KJ, Montefiori DC, Ferrari G, Horton H, Tomaras GD, Gurunathan S, Baglyos L, Frey SE, Mulligan MJ, Harro CD, Buchbinder SP, Baden LR, Blattner WA, Koblin BA, Corey L. Phase 2 study of an HIV-1 canarypox vaccine (vCP1452) alone and in combination with rgp120: negative results fail to trigger a phase 3 correlates trial. J Acquir Immune Defic Syndr **2007**;44:203-12.

22. Patel V, Jalah R, Kulkarni V, Valentin A, Rosati M, Alicea C, von Gegerfelt A, Huang W, Guan Y, Keele BF, Bess J, Jr., Piatak M, Jr., Lifson JD, Williams WT, Shen X, Tomaras GD, Amara RR, Robinson HL, Johnson WE, Broderick KE, Sardesai NY, Venzon DJ, Hirsch VM, Felber BK, Pavlakis GN. DNA and virus particle vaccination protects against acquisition and confers control of viremia upon heterologous simian immunodeficiency virus challenge. Proc Natl Acad Sci USA **2013**;110:2975-80.

23. Li J, Valentin A, Kulkarni V, Rosati M, Beach RK, Alicea C, Hannaman D, Reed SG, Felber BK, Pavlakis GN. HIV/SIV DNA vaccine combined with protein in a co-immunization protocol elicits highest humoral responses to envelope in mice and macaques. Vaccine **2013**;31:3747-55.

24. Pissani F, Malherbe DC, Schuman JT, Robins H, Park BS, Krebs SJ, Barnett SW, Haigwood NL. Improvement of antibody responses by HIV envelope DNA and protein co-immunization. Vaccine **2014**;32:507-13.

25. Wille-Reece U, Flynn BJ, Lore K, Koup RA, Miles AP, Saul A, Kedl RM, Mattapallil JJ, Weiss WR, Roederer M, Seder RA. Toll-like receptor agonists influence the magnitude and quality of memory T cell responses after prime-boost immunization in nonhuman primates. J Exp Med **2006**;203:1249-58.

26. Pattacini L, Mize GJ, Graham JB, Fluharty TR, Graham TM, Lingnau K, Wizel B, Perdiguero B, Esteban M, Pantaleo G, Shen M, Spies GA, McElrath MJ,

Lund JM. A novel HIV vaccine adjuvanted by IC31 induces robust and persistent humoral and cellular immunity. PLoS ONE **2012**;7:e42163.

27. Flynn BJ, Kastenmuller K, Wille-Reece U, Tomaras GD, Alam M, Lindsay RW, Salazar AM, Perdiguero B, Gomez CE, Wagner R, Esteban M, Park CG, Trumpfheller C, Keler T, Pantaleo G, Steinman RM, Seder R. Immunization with HIV Gag targeted to dendritic cells followed by recombinant New York vaccinia virus induces robust T-cell immunity in nonhuman primates. Proc Natl Acad Sci U S A **2011**;108:7131-6.

28. Chen H, Chuai X, Deng Y, Wen B, Wang W, Xiong S, Ruan L, Tan W. Optimisation of prime-boost immunization in mice using novel protein-based and recombinant vaccinia (Tiantan)-based HBV vaccine. PLoS ONE **2012**;7:e43730.

29. Koopman G, Mortier D, Hofman S, Mathy N, Koutsoukos M, Ertl P, Overend P, van WC, Thomsen LL, Wahren B, Voss G, Heeney JL. Immune-response profiles induced by human immunodeficiency virus type 1 vaccine DNA, protein or mixed-modality immunization: increased protection from pathogenic simian-human immunodeficiency virus viraemia with protein/DNA combination. J Gen Virol **2008**;89:540-53.

30. Amara RR, Ibegbu C, Villinger F, Montefiori DC, Sharma S, Nigam P, Xu Y, McClure HM, Robinson HL. Studies using a viral challenge and CD8 T cell depletions on the roles of cellular and humoral immunity in the control of an SHIV-89.6P challenge in DNA/MVA-vaccinated macaques. Virology **2005**;343:246-55.

31. Robinson HL, Sharma S, Zhao J, Kannanganat S, Lai L, Chennareddi L, Yu T, Montefiori DC, Amara RR, Wyatt LS, Moss B. Immunogenicity in Macaques of the Clinical Product for a Clade B DNA/MVA HIV Vaccine: Elicitation of IFN-gamma, IL-2, and TNF-alpha Coproducing CD4 and CD8 T Cells. AIDS Res Hum Retroviruses **2007**;23:1555-62.

32. Mooij P, Balla-Jhagjhoorsingh SS, Beenhakker N, van Haaften P, Baak I, Nieuwenhuis IG, Heidari S, Wolf H, Frachette MJ, Bieler K, Sheppard N, Harari A, Bart PA, Liljestrom P, Wagner R, Pantaleo G, Heeney JL. Comparison of human and rhesus macaque T-cell responses elicited by boosting with NYVAC encoding human immunodeficiency virus type 1 clade C immunogens. J Virol **2009**;83:5881-9.

33. Rosati M, Valentin A, Jalah R, Patel V, von Gegerfelt A, Bergamaschi C, Alicea C, Weiss D, Treece J, Pal R, Markham PD, Marques ET, August JT, Khan A, Draghia-Akli R, Felber BK, Pavlakis GN. Increased immune responses in rhesus macaques by DNA vaccination combined with electroporation. Vaccine **2008**;26:5223-9.

34. Pantaleo G. Second Generation Poxvirus Vector Platforms: NYVAC +/- DNA. AIDS Vaccine 2012, Boston, MA, 9-12 September, **2012**.

35. Lasky LA, Groopman JE, Fennie CW, Benz PM, Capon DJ, Dowbenko DJ, Nakamura GR, Nunes WM, Renz ME, Berman PW. Neutralization of the

AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science **1986**;233:209-12.

36. Berman PW, Matthews TJ, Riddle L, Champe M, Hobbs MR, Nakamura GR, Mercer J, Eastman DJ, Lucas C, Langlois AJ, . Neutralization of multiple laboratory and clinical isolates of human immunodeficiency virus type 1 (HIV-1) by antisera raised against gp120 from the MN isolate of HIV-1. J Virol **1992**;66:4464-9.

37. Berman PW, Gregory TJ, Riddle L, Nakamura GR, Champe MA, Porter JP, Wurm FM, Hershberg RD, Cobb EK, Eichberg JW. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature **1990**;345:622-5.

38. Schwartz DH, Gorse G, Clements ML, Belshe R, Izu A, Duliege AM, Berman P, Twaddell T, Stablein D, Sposto R, . Induction of HIV-1-neutralising and syncytium-inhibiting antibodies in uninfected recipients of HIV-1IIIB rgp120 subunit vaccine. Lancet **1993**;342:69-73.

39. Belshe RB, Graham BS, Keefer MC, Gorse GJ, Wright P, Dolin R, Matthews T, Weinhold K, Bolognesi DP, Sposto R, . Neutralizing antibodies to HIV-1 in seronegative volunteers immunized with recombinant gp120 from the MN strain of HIV-1. NIAID AIDS Vaccine Clinical Trials Network. JAMA **1994**;272:475-80.

40. McElrath MJ, Corey L, Montefiori D, Wolff M, Schwartz D, Keefer M, Belshe R, Graham BS, Matthews T, Wright P, Gorse G, Dolin R, Berman P, Francis D, Duliege AM, Bolognesi D, Stablein D, Ketter N, Fast P. A phase II study of two HIV type 1 envelope vaccines, comparing their immunogenicity in populations at risk for acquiring HIV type 1 infection. AIDS Vaccine Evaluation Group. AIDS Res Hum Retroviruses **2000**;16:907-19.

41. Berman PW, Murthy KK, Wrin T, Vennari JC, Cobb EK, Eastman DJ, Champe M, Nakamura GR, Davison D, Powell MF, Bussiere J, Francis DP, Matthews T, Gregory TJ, Obijeski JF. Protection of MN-rgp120-immunized chimpanzees from heterologous infection with a primary isolate of human immunodeficiency virus type 1. J Infect Dis **1996**;173:52-9.

42. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat **1998**;52:119-26.

43. Bechhofer RE, Santner TJ, Goldsman DM. Design and analysis of experiments for statistical selection, screening, and multiple comparisons. New York: John Wiley & Sons, **1995**.

44. Hudgens MG. Estimating cumulative probabilities from incomplete longitudinal binary responses with application to HIV vaccine trials. Statistics in Medicine **2003**;22:463-79.

45. Lachenbruch PA. Comparisons of two-part models with competitors. Stat Med **2001**;20:1215-34.

46. Hughes JP. Mixed effects models with censored data with application to HIV RNA levels. Biometrics **1999**;55:625-9.

47. Rotnitzky A, Robins J. Analysis of semi-parametric regression models with non-ignorable non-response. Stat Med **1997**;16:81-102.

48. Seaman M, Janes H, Hawkins N, randpre L, Devoy C, Giri A, Coffey R, Harris L, Wood B, Daniels M, Bhattacharya T, Lapedes A, Polonis V, McCutchan F, Gilbert P, Self S, Korber B, Montefiori D, Mascola J. Tiered Categorization of a Diverse Panel of HIV-1 Env Pseudoviruses for Assessment of Neutralizing Antibodies. J Virol **2010**;84:1439-52.

Appendix A Sample informed consent form

Title: A phase 1b clinical trial to evaluate the safety and immunogenicity of different combinations of DNA-HIV-PT123 and AIDSVAX® B/E in healthy, HIV uninfected adult participants

HVTN protocol number: HVTN 105

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 or make your mark on 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 different types of HIV vaccines. HIV is the virus that causes AIDS.

About 104 people will take part in this study at multiple sites. 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.)
   - Does the order in which the study vaccines are given affect the immune responses to the study vaccines?

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.

Sites: 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 2 study vaccines. The study vaccines are called DNA-HIV-PT123 and AIDSVAX® B/E. From here on, we will call them the DNA vaccine and the AIDSVAX vaccine or the study vaccines. They are experimental HIV vaccines. That means we do not know whether the vaccines are safe to use in people, or whether they will work to prevent HIV infection. These study vaccines are used only in research studies.

One of the study vaccines contains 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 the DNA vaccine is injected, it will tell your body to make small amounts of proteins that look like the ones found in HIV. This study will see if your body's immune system responds to the proteins.

The AIDSVAX vaccine:

The AIDSVAX vaccine is managed by Global Solutions for Infectious Diseases (GSID).

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

The AIDSVAX vaccine has been tested in over 10,000 people. This study vaccine has not made people too uncomfortable and the vaccine has not caused serious health problems

Sometimes the body responds better when a vaccine is combined with another substance that helps to alert the immune system. These substances are called adjuvants. The AIDSVAX vaccine is made with an adjuvant called Aluminum hydroxide (alum). Alum is used in many licensed vaccines.

The DNA vaccine:

The DNA vaccine was developed by the IPPOX Foundation (Lausanne, Switzerland).

The DNA vaccine is currently being tested in people in 2 studies in the US and in Switzerland. These studies are testing the DNA in combination with other vaccines. As of November 2013, over 180 people have received the DNA vaccine without becoming too uncomfortable and the vaccine has not caused serious health problems. However, studies with a small number of people do not tell us everything about the safety of the study vaccines.

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.

Risks of the study vaccines:

The AIDSVAX vaccine:

This AIDSVAX 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. Less often, some people had injection site swelling or hardness. The most common reactions in the body have been headache, tiredness, swollen glands, muscle or joint aches, and a mild increase in body temperature.

A few people had changes in blood and urine test results following injections. The changes did not cause health problems. We do not know if these changes were caused by the study vaccines.

We do not know if participants in this study will have similar side effects to those seen in earlier studies.

The DNA vaccine:

In studies with this and similar DNA vaccines, the most common complaints were pain or itching at the injection site, headache, and feeling tired. Scientists think it may be possible that a DNA vaccine could cause cancer, but we have never seen this happen with any HIV study vaccines.

The placebo for the study vaccines:

Some people in this study will also get a placebo. A placebo does not contain any vaccine. Sterile salt water is being used as the placebo for this study. We do not expect it to cause any health problems in people.

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. You cannot be in this study while you are in another study where you receive a study product. 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 aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for: hepatitis B, hepatitis C, and syphilis. 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. People who have had a complete hysterectomy (removal of the uterus and ovaries, verified by medical records), are not required to have a pregnancy test.

   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.

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 should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 3 weeks before your first injection until 6 months after your last study injection. 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 include the birth control sheet 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 10 times over about 1 year.**

   Site: Insert number of visits and 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 study ends (for example, to tell you about the study results).

9. **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).

   Payments you receive for being in the study may be taxable. This happens if we pay you more than $600 between January 1 and December 31 of the same year. The clinic staff may need to ask you for your Social Security number for tax reasons.

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

10. **Everyone in this study will get both study vaccines.**

    Everyone in this study will get both of the study vaccines. Some people will also get a placebo. Which study vaccines you will get and whether you will get any placebo is completely random, like flipping a coin.

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

The clinic staff have no say in choosing which study vaccines you will receive. They will not know which study vaccines you are getting, and neither will you. Only the pharmacist at your site will have this information while the study is going on.

You will have to wait until everyone completes their final study visits to find out which study vaccines you received. 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.

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

You will be in 1 of 4 groups. You will get a total of 8 injections during the study. All injections are given with a needle into the upper arm.

Injection Schedule				
Group	First Injection	1 month later	3 months later	6 months later
1	 Left Arm Right Arm Placebo AIDSVAX	 Left Arm Right Arm Placebo AIDSVAX	 Left Arm Right Arm DNA Placebo	 Left Arm Right Arm DNA Placebo
2	 Left Arm Right Arm DNA Placebo	 Left Arm Right Arm DNA Placebo	 Left Arm Right Arm Placebo AIDSVAX	 Left Arm Right Arm Placebo AIDSVAX
3	 Left Arm Right Arm DNA Placebo	 Left Arm Right Arm DNA Placebo	 Left Arm Right Arm DNA AIDSVAX	 Left Arm Right Arm DNA AIDSVAX
4	 Left Arm Right Arm DNA AIDSVAX	 Left Arm Right Arm DNA AIDSVAX	 Left Arm Right Arm DNA AIDSVAX	 Left Arm Right Arm DNA AIDSVAX

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 three 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.

12. In addition to giving you the study products, we will:

- Do regular HIV testing, as well as counseling on your results and on how to avoid getting HIV;
- Do physical exams;
- Take blood and urine samples;
- Do pregnancy tests if you were born female; persons who have had a complete hysterectomy (removal of the uterus and ovaries, verified by medical records), are not required to have pregnancy tests;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study; and
- Ask questions about whether you have had any HIV tests outside the study.

When we take blood, the amount will depend on the lab tests we need to do. It will be an amount between 10 mL and 230 mL (a little less than 1 tablespoon to a little less than 1 cup). Your body will make new blood to replace the blood we take out.

We will be looking for side effects. We will review the results of these procedures and tests with you at your next visit, or sooner if necessary. If any of the results are important to your health, we will tell you. We will also offer you counseling and referral for needed care.

13. We will counsel you on avoiding HIV infection.

We will ask you personal questions about your HIV risk factors such as sexual behavior and drug use. We will talk with you about ways of lowering your risk of getting HIV. If you become infected with HIV, we will talk with you about ways to avoid giving the virus to someone else. We will help you develop a risk reduction plan. Some topics we may discuss include:

- What you think causes risky behavior for you.
- Methods to avoid getting HIV or giving it to someone else.

These may include not having sex, using condoms, or other behavior changes, such as cutting down on alcohol. We will talk about new methods of HIV prevention, and can give you information on how to access them.

14. We will test your samples for this study.

We will send your samples (without your name) to a lab to see how your immune system responds to the study products. The researchers may:

- Take cells from your samples and grow more of them. We may grow more of your cells over time, so that they can continue to contribute to this study.

- Do limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. 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.

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.

Site: Delete next section if using separate consent for use of samples and information in other studies

15. When we take samples from you for this study, we take extra samples in case we have to repeat tests. When samples are no longer needed for this study, the HVTN wants to keep them for use in other studies. We will call these "extra samples."

This section gives you information so you can decide if you want your extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

Do I have to agree? No. You are free to say yes or no, or to change your mind after you sign this form. At your request, we will destroy all extra samples that we have. Your decision will not affect your being in this study or have any negative consequences here.

Where are the samples stored? Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* The central repositories for the HVTN are located in the United States.

How long will the samples be stored? There is no limit on how long your extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

Will I be paid for the use of my samples? No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

Will I benefit from allowing my samples to be used in other studies? Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not needed for your medical care. They are not part of your medical record. The studies are only being done for research purposes.

Will the HVTN sell my samples and information? No, but the HVTN may share your samples with other researchers. Once we share your samples and information, we will not be able to get them back.

How do other researchers get my samples and information? When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will

review their plan. *[Site: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN keeps track of your decision about how your samples and information can be used.

What information is shared with other researchers? The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

What kind of studies might be done with my extra samples and information? The studies will be related to HIV, vaccines, the immune system and other diseases. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.
- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

If you agree, your samples 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. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

Who will have access to my information in studies using my extra samples?

People who may see your information are:

- Researchers who use your stored samples and limited information for other research
- Government agencies that fund or monitor the research using your samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples or information may be published. No publication will use your name or identify you personally.

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

Site: Check HIPAA authorization for conflicts with this section.

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.

We do need to share your name with the HVTN in case you need proof in the future that you participated in an HIV vaccine study. The HVTN will keep your name in a secure file with these items:

- The name of your study
- Your age or date of birth
- Your study ID number
- What study product(s) you received

There are no HIV test results kept in this file. The HVTN will not share any information that could identify you without your agreement. The HVTN will remove your name from the file if you do not want it there.

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,
- The US Food and Drug Administration,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- IPPOX Foundation, Global Solutions for Infectious Diseases and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board,
- 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]

We have a Certificate of Confidentiality from the US government, to help protect your privacy. With the certificate, we do not have to release information about you to someone who is not connected to the study, such as the courts or police. Sometimes we can't use the certificate. Since the US government funds this research, we cannot withhold information from it. Also, you can still release information about yourself and your study participation to others.

The results of this study 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 information that can identify you.

When the study is done, we may share the information from the study with others so they can see it and use it. We will not share any information that will let someone identify you.

17. **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.

18. **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.

19. 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. *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

Other Risks

20. There are other risks to being in this study.

This section describes the other risks and restrictions we know about. There may also be unknown risks, even serious ones. 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.

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 body makes antibodies to fight or 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 HIV study vaccines. The study vaccines are likely to cause you to test positive on some types of HIV tests, even if you are not infected with HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccines, a routine HIV test done outside this clinic is likely to 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.

Site: Modify the following paragraph if applicable. 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.

If you become pregnant during or after the study 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. These antibodies from the mother are not a danger to the baby, and they go away over time. If the baby continues to have VISP, we can do this testing for free for as long as it is needed. For most babies antibodies from the mother last for about six months.

You should always tell the delivery staff if you have VISP. However, you may still be tested for HIV using the antibody test when you deliver your baby. If your test is positive and the delivery staff believes you have an HIV infection, your baby may be started on antiretroviral treatment when it is not needed. If this happens, we can arrange for you and the baby to have a test that can tell the difference between true HIV infection and a VISP result.

Embarrassment/anxiety:

You may feel embarrassed when we ask about your HIV risks, such as having sex and using drugs. 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 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.

Risks of genetic testing:

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

In the very unlikely event that your genetic information becomes linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work. GINA does not help or protect you against discrimination by companies that sell life, disability or long-term care insurance.

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

21. 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.

Your rights and responsibilities

22. 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

23. 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

24. If you get sick or injured during the study, contact us immediately.

Your health is important to us. We will help you get the medical care you need.

If someone gets sick or injured in an HVTN study, the HVTN decides whether the injury is probably related to the study products and/or procedures. If the HVTN decides it was more likely due to the study products or procedures than any other cause, then the HVTN will use their funds to pay for treatment. If the HVTN decides otherwise, then you and your health insurance *(Sites: insert locale- appropriate medical insurance language in the preceding sentence)* would be responsible for treatment costs. You may disagree with the decision the HVTN makes about your injuries. At your request the HVTN will ask experts who are not connected with the HVTN to review its decision.

In this study, the IPPOX Foundation and Global Solutions for Infectious Diseases have put insurance in place to cover the cost of medical expenses that arise from injuries caused by their respective study products.

For injuries caused by study procedures, the HVTN has limited funds to cover the cost of medical treatment.

No matter what, you still have the right to use the court system to address payment for your injuries if you are not satisfied.

Some injuries are not physical. For example, someone might be harmed psychologically or emotionally by being in an HIV vaccine study. Or they might lose wages from injuries because they could not go to work. No funds have been set aside to pay for nonphysical injuries, even if they are related to participation in the study.

Questions

25. 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

Site: Delete this section if using a separate consent for use of samples and information in other studies

26. In Section 15 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 allow 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 and keeping my cells growing over time.

OR

☐ I agree to the option above and also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing limited genetic testing, growing more of my cells, or genome wide studies.

27. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on 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 6 months after your last study injection.

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 Sample consent form for use of samples and information in other studies

Title: Title: A phase 1b clinical trial to evaluate the safety and immunogenicity of different combinations of DNA-HIV-PT123 and AIDSVAX® B/E in healthy, HIV uninfected adult participants

HVTN protocol number: HVTN 105

Site: [Insert site name]

When samples are no longer needed for this study, the HVTN wants to keep them for use in other studies. We will call these "extra samples."

This form gives you information so you can decide if you want your extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

1. Do I have to agree?

No. You are free to say yes or no, or to change your mind after you sign this form. At your request, we will destroy all extra samples that we have. Your decision will not affect your being in this study or have any negative consequences here.

2. Where are the samples stored?

Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* The central repositories for the HVTN are located in the United States.

3. How long will the samples be stored?

There is no limit on how long your extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

4. Will I be paid for the use of my samples?

No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

5. Will I benefit from allowing my samples to be used in other studies?

Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not needed for your medical care. They are not part of your medical record. The studies are only being done for research purposes.

6. Will the HVTN sell my samples and information?

No, but the HVTN may share your samples with other researchers. Once we share your samples and information, we will not be able to get them back.

7. How do other researchers get my samples and information?

When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN keeps track of your decision about how your samples and information can be used.

8. What information is shared with other researchers?

The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

9. What kind of studies might be done with my extra samples and information?

The studies will be related to HIV, vaccines, the immune system and other diseases. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.
- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

If you agree, your samples 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. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

10. What are the risks of genetic testing?

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

Site: include the following paragraph

In the very unlikely event that your genetic information becomes linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work.

GINA does not help or protect you against discrimination by companies that sell life, disability or long-term care insurance.

11. Who will have access to my information in studies using my extra samples?

People who may see your information are:

- Researchers who use your stored samples and limited information for other research
- Government agencies that fund or monitor the research using your samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples or information may be published. No publication will use your name or identify you personally.

Questions

12. If you have questions or problems about allowing your samples and information to be used in other studies, use the following important contacts.

If you have questions about the use of your samples or information or if you want to change your mind about their use, contact [name and telephone number of the investigator or other study staff].

If you think you may have been harmed because of studies using your samples or information, contact [name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact [name/title/phone of person on IRB or other appropriate organization].

13. Please write your initials or make your mark in the box next to the option you choose.

☐ I allow 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 and keeping my cells growing over time.

OR

☐ I agree to the option above and also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing limited genetic testing, growing more of my cells, or genome wide studies.

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 D Table of procedures (for sample informed consent form)

Procedure	Screening visit(s)	First injection visit	Time after 1st injection visit (in months)								
			½	1	1½	3	3½	6	6½	9	12
Injection		√		√		√		√			
Medical history	√										
Complete physical	√										√
Brief physical		√	√	√	√	√	√	√	√	√	
Urine test	√		√						√		
Blood drawn	√	√	√		√		√		√	√	√
Pregnancy test (participants born female)[a]	√	√		√		√		√		√	
HIV testing and counseling	√						√		√	√	√
HIV risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√
Interview and/or questionnaire	√	√	√	√	√	√	√	√	√	√	√

[a] Persons who had a complete hysterectomy (removal of the uterus and ovaries, verified by medical records), are not required to have a pregnancy test

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

				Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	
				Day:	Screening visit[3]	D0	D14	D28	D42	D84	D98	D168	*D182*	D273	D364	
				Month:		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M9	M12	
						VAC1		**VAC2**		**VAC3**		**VAC4**				
Description	**Ship to[1,2]**	**Assay location[2]**	**Tube[4]**	**Tube size (vol capacity)[4]**		**AIDSVAX B/E OR DNA OR DNA+AIDSVAX B/E**		**AIDSVAX B/E OR DNA OR DNA+AIDSVAX B/E**		**DNA OR AIDSVAX B/E OR DNA+AIDSVAX B/E**		**AIDSVAX B/E OR DNA+AIDSVAX B/E**				**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	5	-	-	-	-	-	-	-	-	-	-	**5**
HIV diagnostic algorithm[9]	UW-VSL	UW-VSL	EDTA	10mL	-	-	-	-	-	-	10	-	10	10	20	**50**
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**
Immunogenicity assays[6]																
HLA Typing[7]	CSR	FHCRC	ACD	8.5mL	-	17	-	-	-	-	-	-	-	-	-	**17**
Cellular Assays																
ICS	CSR	FHCRC	ACD	8.5mL	-	59.5	-	-	59.5	-	59.5	-	***59.5***	-	59.5	**297.5**
Humoral Assays																
HIV-1 binding Ab multiplex assay	CSR	Duke-DHVI	SST	8.5ml	-	8.5	-	-	8.5	-	8.5	-	***8.5***	-	8.5	**42.5**
HIV-1 Neutralizing Ab assay	CSR	Duke-NAB	SST	8.5ml	-	8.5	-	-	8.5	-	8.5	-	***8.5***	-	8.5	**42.5**
Storage																
Serum storage	CSR	—	SST	8.5mL	-	17	-	-	17	-	17	-	17	-	17	**85**
PBMC storage	CSR	—	ACD	8.5mL	-	42.5	-	-	42.5	-	42.5	-	93.5	-	42.5	**263.5**
Maximum Total					**20**	**153**	**10**	**0**	**146**	**0**	**156**	**0**	**207**	**20**	**156**	**868**
Maximum 56-Day Total					**20**	**173**	**183**	**183**	**329**	**146**	**302**	**0**	**207**	**20**	**156**	
URINE COLLECTION																
Urinalysis	Local lab	Local lab			X	-	X	-	-	-	-	-	X	-	-	
Pregnancy Test[8]	Local lab	Local lab			X	X	-	X	-	X	-	X	-	X	-	

[1] CSR = central specimen repository.

[2] HVTN Laboratory Program includes laboratories at UW-VSL, Duke-DHVI, Duke-NAB, and FHCRC. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA); Duke-DHVI = Duke Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA); Duke-NAB = Duke Neutralizing Antibody Laboratory (Durham, North Carolina, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA).

[3] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.3 (postenrollment).

[6] Immunogenicity assays will be performed at M0 (for binding Ab assay) and M6.5. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other timepoints.

[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 postvaccination timepoints.

[8] Pregnancy tests may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[9] At an early termination visit for a withdrawn or terminated participant (see Section 9.10), blood should be drawn for HIV diagnostic testing, as shown for visit 11 above.

Appendix F Procedures at HVTN CRS

Visit:	01[a]	02	03	04	05	06	07	08	09	10	11	Post
Day:		D0	D14	D28	D42	D84	D98	D168	D182	D273	D364	
Month:		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M9	M12	
Procedure	Scr.	VAC1		VAC2		VAC3		VAC4				
Study procedures[b]												
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—
Signed protocol consent	X	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	X	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	—	—
Risk reduction counseling	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	—
Behavioral risk assessment	X	—	—	—	—	X	—	X	—	—	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	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	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[d]	—	—	—	—	—	—	X	—	X	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	X	—	X	X	X
Local lab assessment												
Screening HIV test	X	—	—	—	—	—	—	—	—	—	—	—
Urine dipstick	X	—	X	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[e]	X	X	—	X	—	X	—	X	—	X	—	—
CBC, differential, platelet	X	—	X	—	X	—	X	—	X	X	—	—
Chemistry panel	X	—	X	—	X	—	X	—	X	X	—	—
Syphilis, Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—
Vaccination procedures												
Vaccination[f]	—	X	—	X	—	X	—	X	—	—	—	—
Reactogenicity assessments[g]	—	X	—	X	—	X	—	X	—	—	—	—
Poststudy												
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	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. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. 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.

[g] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.8).