HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 124

A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A,B,C,A/E) / *gag* (C) DNA and gp120 (A,B,C,A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1–uninfected adult participants

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CLINICAL TRIAL SPONSORED BY

Division of AIDS (DAIDS)
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Bethesda, Maryland, USA

STUDY PRODUCT(S) PROVIDED BY

University of Massachusetts Medical School (UMMS)
Worcester, Massachusetts, USA
Infectious Disease Research Institute (IDRI)
Seattle, Washington, USA

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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. If a program for antiretroviral therapy (ART) provision is not available at a site and ART is needed, a privately established fund will be used to pay for access to treatment to the fullest extent possible.

- The HVTN provides training so that all participating sites similarly ensure fair participant selection, protect the privacy of research participants, and obtain meaningful informed consent. During the study, participants will have their wellbeing monitored, and to the fullest extent possible, their privacy protected. Participants may withdraw from the study at any time.

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

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

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

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

- The HVTN values the role of in-country Institutional Review Boards (IRBs), Ethics Committees (ECs), and other Regulatory Entities (REs) 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/REs 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/RE 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 persons assigned female at birth); and (f) providing safety monitoring.

2.2 Reasonable risk/benefit balance

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

In 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 participant selection

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

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

2.4 Appropriate informed consent

45 CFR 46.111 (a) 4 and 5 and 21 CFR 56.111 (a) 4 and 5: Informed consent is sought from each prospective subject or the subject's legally authorized representative as required by 45 CFR 46.116 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 124 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) 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 and Appendix B). 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 an Agreement on Confidentiality and Use of Data and Specimens with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A,B,C,A/E) / *gag* (C) DNA and gp120 (A,B,C,A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1–uninfected adult participants

Primary objective

To evaluate the safety and tolerability of Env (A, B, C, A/E) / Gag (C)-expressing DNA plasmids and recombinant gp120 (A, B, C, A/E) protein/GLA-SE adjuvant, given individually, in combination, or as a prime-boost series in healthy HIV-1 uninfected adults

Study products, doses, and routes of administration

- DNA vaccine: Env (A, B, C, A/E) / Gag (C) DNA plasmids. A total dose of 2 mg of the 5 DNA plasmids (0.4 mg each) will be given as a 0.8 ml injection IM into the deltoid.

- Protein vaccine: Recombinant gp120 (A, B, C, A/E) proteins/GLA-SE adjuvant. GLA-SE is a stable oil-in-water emulsion (SE) containing glucopyranosyl Lipid A (GLA), a synthetic analog of monophosphoryl Lipid A (MPL®). A total dose of 400 microgram (mcg) of the 4 recombinant proteins (100 mcg each) and 5 mcg of GLA-SE will be given as a 1 ml injection IM into the deltoid.

- Placebo for DNA vaccine: Sodium Chloride for Injection, USP 0.9% given as a 0.8 ml injection IM into the deltoid.

- Placebo for protein vaccine: Sodium Chloride for Injection, USP 0.9% given as a 1 ml injection IM into the deltoid.

Table 3-1 Schema

Part A	Group	N	Month 0 (Day 0)	Month 2 (Day 56)			
	1	10	Protein/ GLA-SE	Protein/ GLA-SE			
		2	Placebo	Placebo			
		12					
Part B	Group	N	Month 0 (Day 0)	Month 1 (Day 28)	Month 3 (Day 84)	Month 6 (Day 168)	Month 8 (Day 224)
	2	21	DNA + Placebo	DNA + Placebo	DNA + Placebo	Protein/ GLA-SE + Placebo	Protein/ GLA-SE + Placebo
		3	Placebo + Placebo	Placebo + Placebo	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo
	3	21	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE
		3	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo
		48					
	Total	60 (52/8)					

Notes

DNA/placebo and protein/GLA-SE and placebo study products will be given in separate arms, such that participants in Part B will receive 1 injection in each arm at each vaccination timepoint (unless medically contraindicated).

Enrollment will proceed in stages to optimize safety. Groups 2 and 3 will enroll simultaneously. See Section 11.3 for details.

Participants

60 healthy, HIV-1–uninfected volunteers aged 18 to 50 years; 52 vaccinees, 8 placebo recipients

Design

Multicenter, randomized, placebo-controlled, double-blind trial

Duration per participant

Part A: 8 months of scheduled clinic visits (main study) followed by a health contact 12 months after the last vaccination

Part B: 14 months of scheduled clinic visits (main study) followed by a health contact 12 months after the last vaccination

Estimated total study duration

32 months (includes enrollment, planned safety holds, follow-up, and health contact)

Investigational New Drug (IND) sponsor

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

Study product providers

- Env (A,B,C,A/E) / Gag (C) DNA plasmids: University of Massachusetts Medical School (UMMS) (Worcester, Massachusetts, USA)
- Recombinant gp120 (A,B,C,A/E) protein: University of Massachusetts Medical School (UMMS) (Worcester, Massachusetts, USA)
- GLA-SE adjuvant: Infectious Disease Research Institute (IDRI) (Seattle, Washington, USA)

Core operations

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

Statistical and data management center (SDMC)

Statistical Center for HIV/AIDS Research and Prevention (SCHARP), Fred Hutch (Seattle, Washington, USA)

HIV diagnostic laboratory

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

Endpoint assay laboratories

- Duke University Medical Center (Durham, North Carolina, USA)
- Fred Hutch/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 124 PSRT; HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

Protocol leadership

Chair	Ian Frank University of Pennsylvania 215-662-7419 franki@mail.med.upenn.edu	*Statistician*	Sue Li SCHARP, Fred Hutch 206-667-7066 sli@scharp.org
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Other contributors to the original protocol

Core medical monitor	Marnie Elizaga HVTN Core, Fred Hutch	*Clinical safety specialist*	Jill Zeller HVTN Core, Fred Hutch
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	Zachary Sagawa Infectious Disease Research Institute ((IDRI)	*Statistical research associate*	Daryl Morris SCHARP, Fred Hutch
		SDMC Associate director of Lab Science	April Randhawa SCHARP, Fred Hutch
Laboratory protocol operations manager	On Ho HVTN Laboratory Program, Fred Hutch	*Clinical data manager*	Ingrid Durenberger SCHARP, Fred Hutch
Laboratory program representative	Nicole Frahm HVTN Laboratory Program, Fred Hutch	*Senior clinical data manager*	Gina Escamilla SCHARP, Fred Hutch
Regulatory affairs associate	Denelle Reilly HVTN Core, FHCRC	*DAIDS Project Officer*	Vijay Mehra DAIDS, NIAID
Clinic coordinator	Pamela Cunningham University of Alabama Birmingham	*Protocol development manager*	Ramey Fair HVTN Core, Fred Hutch
Community Advisory Board (CAB) members	Dennis Hodtwalker Univ of Alabama Birmingham CAB	*Technical editor*	Erik Schwab HVTN Core, Fred Hutch
	Ja'Nae Tyler Univ of Pennsylvania CAB	*Community engagement unit representative*	Gail Broder HVTN Core, Fred Hutch
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4 Background

4.1 Rationale for trial concept

A successful HIV vaccine may require generation of broad immune responses against viruses with highly diverse sequences. Thus, it is critical to develop new candidate HIV vaccines that can achieve balanced immune responses including both antibody and T cell immune responses and, in particular, induce broadly cross reactive, high titer and high quality antibody responses.

The Polyvalent DNA-Protein HIV-1 Vaccine-201401 (PDPHV-201401) regimen has the potential to achieve this goal. The DNA prime-protein boost approach has been shown to reliably generate both high level humoral and cellular immune responses in animals and humans (4). In addition, this strategy has been successful at eliciting low titers of neutralizing antibodies (nAb) against Tier 1B or Tier 2-like HIV-1 isolates across multiple subtypes in a previous phase 1 clinical trial (DP6-001; see Section 4.8.1) (5).

Other investigators are pursuing the development of other strategies to elicit broadly neutralizing antibodies (bnAbs) to prevent HIV-1 infection. One strategy aims to guide B cells along an accelerated path of B cell selection and antibody maturation, similar to that which occurs over years in HIV-infected people (6, 7). 'Minimal' Env immunogens are focused on eliciting antibodies against one of the 5 sites on the HIV Env that are targets for bnAbs (8, 9). Another strategy uses stable soluble cleaved gp140 trimers (SOSIP gp140 trimers) mimicking the Env protein spike, to present multiple bnAb epitopes (10, 11). Bivalent gp120s have been evaluated for immunogenicity and efficacy as protein only (Vax003, Vax004) (12, 13) and as viral vector prime - protein boost protocols (RV144) (14). Only sporadic, low titers of Tier 2 virus nAbs were seen in Vax004 (15, 16), which were not protective (15).

Other vaccine strategies, such as various combinations of DNA-HIV-PT123, ALVAC-HIV (vCP2438), and a bivalent subtype C gp120 with adjuvant, are attempting to improve upon the RV144 trial regimen and the non-neutralizing antibody functions identified as correlates of risk in that study, including IgG binding antibody to scaffolded gp70 V1V2 envelope protein, which correlated inversely with risk (17-19), whereas Env-specific binding IgA correlated directly with risk (17). Results of subsequent investigations suggest that FcR-mediated antibody effector functions may have contributed to modest efficacy in RV144 (20-23).

The PDPHV-201401 protocol differs from the above approaches by using gp120 from 4 different major subtypes of HIV-1, to expand the breadth of the immune responses. A DNA prime is used instead of a viral vector prime, to avoid vector-specific immune responses and focus immune responses against the Env immunogen inserts.

The vaccine regimens to be tested are multigenic, polyvalent, and heterologous – three important characteristics to use in HIV vaccine strategy. As it is unlikely that antigens from any one gene would be sufficient for an effective HIV regimen, the vaccine regimens include both Env and Gag for their respective abilities to elicit antibody and T cell responses. For a frequently mutating virus with multiple subtypes such as HIV-1, it is important to have multiple variants of a protective immunogen (envelope protein, Env) to produce a polyvalent formulation. This vaccine regimen includes four Env antigens from subtypes A, B, C, and A/E. Notably, IgG to subtype A, B, C and CRF01_AE V1V2-scaffold antigens were also identified as inverse correlates of risk in the RV144 trial. Additional correlates were IgG to V3 CRF01_AE and plasma levels of antibody-dependent cell-mediated cytotoxicity (ADCC) activity against Env V2, in the setting of low Env-specific binding IgA (19, 21, 24). This polyvalent vaccine regimen has the potential to elicit those responses. It has been demonstrated in a previous phase 1 trial (DP6-001) (25) that the sequential administration of two types of vaccines (DNA and protein) delivering the same subunit gp120 antigens was more effective than either type of vaccine alone . Therefore, HVTN 124 will compare the relative immunogenicity between sequential administration and co-administration of the DNA vaccine and the matching recombinant gp120 (A, B, C, A/E) proteins in GLA-SE adjuvant. The potential advantages of co-administration are earlier induction of antibody, which if effective, would lead to earlier protection, and higher cumulative doses of both DNA and protein vaccine products, in comparison with the sequential DNA–protein immunization approach. An important objective is to determine which approach will achieve the highest response rate, magnitude, and durability of antibody responses, and to characterize any qualitative differences in immune responses to the two regimens.

A first-generation version of the DNA prime-protein boost regimen developed by the University of Massachusetts Medical School (UMMS) team generated significant Env-specific antibodies, low titer but cross-clade neutralizing antibodies, and cell-mediated immune (CMI) responses (both CD4 and CD8 T cells) in participants in the DP6-001 study (5, 26) (see Section 4.8.1). In HVTN 124, CD4+ and CD8+ T cell responses will also be analyzed.

To improve upon the immunogenicity and safety profile observed in the DP6-001study, several modifications have been made to the vaccine formulations for HVTN 124. One change is to further enhance the immunogenicity of Env antigens by carefully selecting optimal gene inserts from four different HIV-1 subtypes. Also, the adjuvant to be administered with the protein vaccine, GLA-SE, differs from the QS-21 adjuvant that was used in the DP6-001 study. QS-21 has been associated with an increased risk of adverse events of the skin in other clinical studies (27). To further minimize risk, this study will enroll participants in stages, to monitor for any adverse reactions (Section 11.3).

In summary, the primary goal of this study is to evaluate the safety and immunogenicity of the polyvalent DNA - protein HIV-1 vaccine regimen with newly optimized inserts for Envs from subtypes A, B, C, and A/E, and for subtype C Gag (DNA only), building on results from the DP6-001 study which

demonstrated balanced humoral and cellular immune responses, including cross-clade neutralization of pseudoviruses expressing Env antigens.

4.1.1 Preclinical monovalent DNA prime-protein boost HIV vaccine studies

The DNA prime-protein boost approach is not a novel concept but it is understudied in human trials. Reports of this approach have been published since the mid-1990s (28, 29). Testing a wide range of primary HIV-1 Env antigens for their immunogenicity in mice, rabbits and non-human primates has demonstrated that there is a qualitative difference between antibodies induced by the gp120-based DNA plus protein approach versus the gp120 protein alone approach (30). After DNA prime-protein boost, increased antibody titers, antibody persistence and neutralizing activities against a wide range of HIV-1 isolates were significantly improved over DNA or protein immunization alone. The quality of antibody responses, as measured by antibody avidity, was also improved by the DNA prime-protein boost approach compared to the protein alone approach (29, 31).

Studies conducted with JR-FL *env* DNA vaccine in rabbits indicated that DNA prime followed by protein boost was more effective than either DNA or protein alone in eliciting high level anti-Env IgG responses with no difference between the gp120 or gp140 DNA primes (32). The endpoint titers for anti-gp120 IgG in DNA prime-protein boosted rabbits reached above 1:500,000, while the protein alone approach was only able to elicit titers of ~1:50,000. Only the DNA prime-protein boost approach elicited consistent JR-FL-specific neutralizing activities. At least two subsequent studies from other research groups confirmed the value of this DNA prime-protein boost approach (33, 34).

4.1.2 Preclinical polyvalent DNA prime-protein boost HIV vaccine studies

Recent studies from the UMMS team have shown that a polyvalent DNA prime, protein boost series was more effective than the polyvalent gp120 recombinant protein vaccines alone in eliciting germinal center (GC) B cell responses in mice as well as anti-gp120 titers and avidity of gp120-specific antibodies [35]. In addition, more recent data from UMMS further demonstrated that DNA immunization can activate innate immunity signaling pathways involving absent in melanoma 2 (AIM2) and stimulator of interferon genes (STING), for better antigen-specific antibody responses [36,37].

Studies performed in rabbits demonstrated that the DNA prime-protein boost approach was able to elicit nAb responses against primary viral isolates across several subtypes, and that the polyvalent Env formulation was more effective than the monovalent formulation in generating broader nAbs (35). Analyses on the breadth of neutralizing activities revealed a clear advantage of the polyvalent Env formulations: polyvalent sera were able to neutralize 68.9% of viruses included in the assay while the monovalent group only neutralized 38.4% of the viruses ($p < .001$). Among non-subtype B viruses only, the polyvalent sera were able to neutralize 56.3% of the 8 viruses from subtypes A, C, D and A/E as compared to

the monovalent group that only neutralized 14.1% of the same set of non-B viruses (35).

The polyvalent DNA prime-protein boost approach was also tested in non-human primate studies (36, 37). After protein boosting, regardless of whether DNA had initially been delivered via gene gun, intra-muscular (IM), or intra-dermal (ID) injection, there was a significant increase of antibody titers among groups receiving different routes of DNA priming immunization without significant differences for peak level anti-gp120 IgG responses, indicating a DNA prime can be efficiently administered via different delivery approaches.

Macaques immunized with a polyvalent DNA prime-protein boost immunization regimen were then rectally challenged with a homologous SHIV expressing an R5 Env (BaL). Four out of the six immunized macaques had sterilizing immunity with an undetectable viral load. The remaining 2 had reduced peak levels of viral load, and the seven immunization-naïve macaques showed high levels of viremia (36).

4.1.3 Optimizing inserts and the choice of adjuvant for PDPHV-201401

A large study has been conducted by UMMS to differentiate the potential of individual HIV-1 Env antigens to elicit nAbs that could neutralize a diverse panel of viruses. A total of 62 primary gp120 antigens have been screened through this process. Sera from rabbits that received a gp120-expressing DNA vaccine three times, and were then boosted twice with a fixed number of five gp120 proteins, were analyzed at Monogram Biosciences, using its high throughput assay against a panel of 16 pseudotyped viruses. These included three TCLA or sensitive Env antigens and 13 primary Env antigens from subtypes A, B, C, D, and A/E. Among this group of 62 gp120 antigens, 10 (16%) elicited nAb against more than 50% of this panel of viruses and another 11 (18%) elicited nAb against 26-50% of viruses. The remaining 41 gp120 antigens (66%) elicited nAb which neutralized between 6-25% of viruses in this panel (unpublished data). The immunogens selected for PDPHV-201401 were those that were able to elicit nAbs against the largest percentage of the pseudotyped viruses for their respective subtypes.

A bioinformatics study was done using the four gp120 sequences from the PDPHV-201401 formulation to determine levels of coverage against worldwide HIV-1 Env viral sequences that can be achieved by increasing the valency, ie, the number of Env immunogens represented in the vaccine (monovalent, bivalent, trivalent, or quadrivalent).

As shown in Figure 4-1, showing the study results using a heat map approach (colder colors mean less of a match and warmer colors mean a better match), it is clear that with increasing valency, the coverage of high-match subtypes/circulating recombinant forms was gradually increased, across subtypes/circulating recombinant forms. With the quadrivalent formulation, no subtype is matched less than 90% for 11 major subtypes (Figure 4-1).

While this is purely a modeling analysis, it shows the power of polyvalency – a key principle in modern vaccinology.

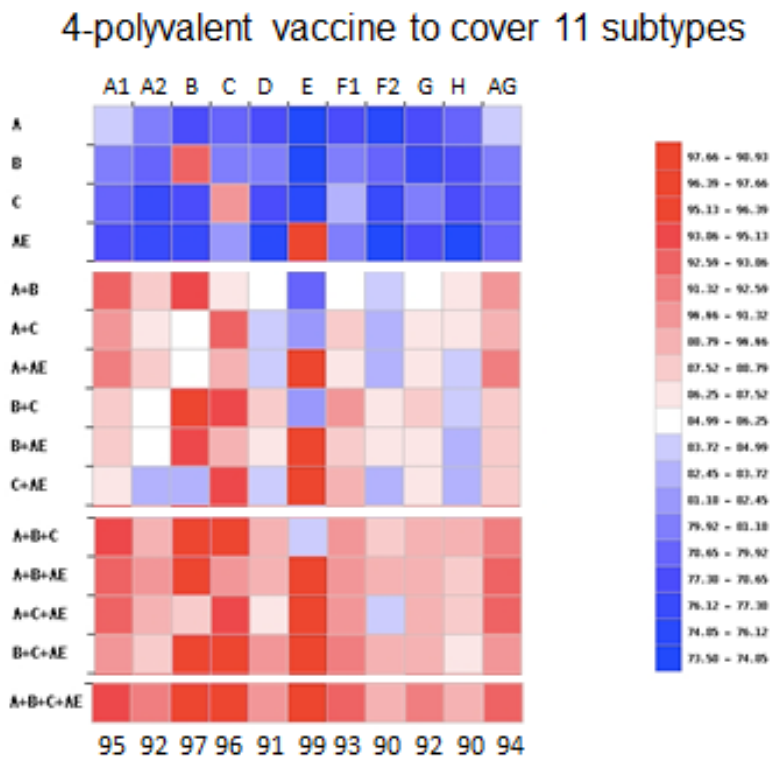

Figure 4-1. Bioinformatics analysis on the potential coverage of HIV-1 viruses by 1-, 2-, 3-, or 4- valent gp120 vaccines (A, B, C, and A/E). The heat map shows the percent of coverage: cold/blue (low match) to hot/red (high match) colors representing the levels of coverage.

Gag-mediated CMI responses, more than any other HIV-1 antigen-specific CMI responses, have been shown to play an important role in the control and containment of virus replication in HIV-1 infected patients. While previous small animal and non-human primate model studies have validated the immunogenicity of *gag* DNA vaccines included in this multiple-gene HIV vaccine design, the UMMS team conducted an additional mouse study as part of the IPCAVD program to confirm the relative immunogenicity of various *gag* DNA vaccine designs.

Balb/C mice (10 mice/group) were immunized with wild type *gag*-C8 (*gag*-C8.wt) or codon optimized *gag*-C8 (*gag*-C8.opt) DNA vaccine. At 1 week after the 4th DNA immunization, mouse splenocytes were collected to detect Gag peptide-specific T cell responses by IFN-γ ELISpot. The *gag*-C8.opt DNA vaccine induced significantly higher levels of IFN-γ T cell responses that were specific to the Gag peptides (38). Based on these analyses, the codon optimized *gag*-C8.opt DNA vaccine is included as a component in the PDPHV-201401 DNA vaccine.

After consultation with NIAID, a clinical adjuvant formulation GLA-SE (Infectious Disease Research Institute [IDRI], Seattle, Washington, USA) was selected as the adjuvant for the protein vaccine. GLA-SE is a stable oil-in-water emulsion (SE) containing glucopyranosyl lipid A (GLA), a synthetic analog of MPL. GLA is a potent TLR4 agonist and improves the immunogenicity of a wide variety of antigens by increasing the magnitude of the immune response and tailoring the response to the Th1-type cell-mediated paradigm.

IDRI and collaborators have tested GLA adjuvant formulations with antigens for several candidate infectious disease vaccines including leishmaniasis, schistosomiasis, tuberculosis, malaria, influenza, leprosy, and hookworm (39-44). It has been generally well tolerated in studies to date, without any significant safety concerns.

4.2 Env (A, B, C, A/E) / Gag (C) DNA plasmids

The polyvalent DNA vaccine contains equal amounts of 5 individual DNA plasmid components utilizing the same vector pSW3891 (45): 4 plasmids each containing a codon optimized gp120 gene sequence from 3 primary HIV-1 subtype A, B, C envelope proteins and a CRF01_AE consensus, and a fifth plasmid containing a codon optimized *gag* gene from subtype C (Table 4-1).

DNA vaccines

The 4 gp120-expressing (Env-A, B, C and A/E) and one Gag expressing (Gag-C) plasmids were constructed with the vector pSW3891 backbone. This backbone contains the following elements: 1) human cytomegalovirus (CMV) immediate-early promoter/Intron A enhancer for high-level gene expression in a wide range of mammalian cells; 2) the bovine growth hormone (BGH) polyadenylation signal for proper termination and processing of the recombinant transcript; 3) the tissue plasminogen activator (tPA) signal peptide sequence to lead the secretion of encoded proteins; 4) pUC origin for high copy replication and maintenance of the plasmid in E. coli; and 5) Kanamycin (Kan) resistance gene for selection in E. coli. The gp120 and *gag* cDNA that were cloned into the pSW3891 vector are described in Table 4-1. The codon optimized gp120 and *gag* cDNA genes were chemically synthesized at Geneart (Germany). Each codon optimized gp120 gene was individually cloned into NheI and BamHI sites in frame with tPA leader sequence of the pSW3891 vector. The *gag*-C gene was cloned into HindIII and BamHI sites of the pSW3891 vector.

Table 4-1 Five plasmids each encoding a codon optimized pg120 or gag gene sequence

DNA Vaccine Name	Vector Backbone	gp120 or *gag* Gene Insert	HIV-1 Isolate	GenBank Accession No. of original viral gene	Size (bp)
PDPHV-ENV-A	pSW3891	gp120-A2-opt	92UG037.1	U09127	6163
PDPHV-ENV-B	pSW3891	gp120-JR-FL-opt	JR-FL	U63632	6181
PDPHV-ENV-C	pSW3891	gp120-C2-opt	93MW965.26	U08455	6151
PDPHV-ENV-E	pSW3891	gp120-AE-Cons-opt	Subtype A/E consensus	N/A	6163
PDPHV-GAG-C	pSW3891	*gag*-C8-opt	96BW15C02	Af110974	6166

The DNA vaccines were manufactured at Waisman Biomanufacturing (Madison, WI) under GMP-compliant conditions.

No adjuvant will be used for the DNA vaccines in HVTN 124. Previous HIV-1 gp120 and *gag* DNA vaccines using the same plasmid vector pSW3891 were evaluated in the DP6-001 phase 1 clinical trial and effectively primed the immune system (5, 26, 46). The final Drug Product for the polyvalent DNA vaccine mixture in saline has the 5 plasmids vialed together by the manufacturer.

4.3 Recombinant gp120 (A,B,C,A/E) protein with GLA-SE adjuvant

4.3.1 Recombinant gp120 protein vaccine

The recombinant gp120 (A,B,C,A/E) protein vaccine contains equal amounts of 4 gp120 proteins matching the Env genes used in the DNA vaccine, produced in stable CHO cell lines by Waisman Biomanufacturing (Madison, WI) under GMP compliant conditions. The gp120 proteins are highly glycosylated and consist of 5 relatively conserved domains (C1-C5) interspersed with 5 hypervariable domains (V1-V5).

Each of the 4 gp120 proteins will be produced and vialed individually.

4.3.2 GLA-SE adjuvant

Glucopyranosyl lipid adjuvant-stable oil-in-water emulsion (GLA-SE) provided by Infectious Disease Research Institute (IDRI, Seattle, WA) will be used as the adjuvant for recombinant gp120 protein vaccines. GLA-SE contains glucopyranosyl lipid A, a synthetic and non-toxic form of bacterial lipopolysaccharide. The GLA-SE (6% oil) will be vialed separately at a volume of 0.4 mL at a concentration of 15 mcg/mL GLA in a 2.0 mL vial. The excipients in the GLA-SE formulation are: Glycerin (isotonicity adjustment), D,L-α-tocopherol (Vitamin E) (anti-oxidant), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) (emulsifier/stabilizer), Poloxamer 188 (emulsifier/stabilizer), Ammonium phosphate, monobasic ($NH_4H_2PO_4$) (buffer), and Ammonium phosphate, dibasic ($[NH_4]_2HPO_4$) (buffer). All clinical lots were manufactured by

IDRI under cGMP guidelines. Bulk GLA was manufactured for IDRI by Avanti Polar Lipids (Alabaster, Alabama, USA).

4.3.3 Recombinant gp120 protein/GLA-SE formulation

The final mixture of the four proteins plus GLA-SE adjuvant formulation will be prepared onsite at the local CRS pharmacy.

4.4 Trial design rationale

The main objective of the HIV vaccine formulation PDPHV-201401 is to safely induce high quality antibody responses able to protect against HIV infection. Based upon evidence from the RV144 trial that antibody responses are associated with protection from HIV acquisition (14-24), in this trial serum binding antibodies to gp120 and serum neutralizing antibodies will be the major immunogenicity outcomes. The secondary objectives are key immunogenicity parameters that will characterize and rank the vaccination regimens in terms of response rates, magnitudes and durability of HIV-1 Env antibody and CD4+ T cell responses, and determine whether the current HIV vaccine formulations will move to more advanced phases of clinical studies.

4.4.1 Safety

In order to carefully evaluate the safety of each component of the vaccine strategy, HVTN 124 will be conducted in two parts. Part A will evaluate the safety and immunogenicity of the protein vaccine with adjuvant, independent of the DNA prime. In Part B, the prime-boost regimen of 3 DNA primes (months 0, 1, 3) followed by 2 protein/adjuvant boosts (months 6, 8) will be compared to a study arm consisting of the DNA and protein/adjuvant vaccines co-administered at the same timepoints (months 0, 1, 3, 6, 8). Therefore Part B will evaluate the safety of the DNA prime individually or in combination regimens.

The protocol will enroll each part of the study in stages, with frequent safety reviews (see Section 11.3). The group sizes in Part B will allow for reasonable precision in the characterizations of the safety endpoints, as well as reasonable power for comparisons between the groups with respect to immunogenicity endpoints, particularly with assays that might have less frequent responses, such as Tier 2 neutralizing antibodies (see Sections 6.1.1 and 6.1.2).

4.4.2 Dose (amount and number)

Multiple studies in the HVTN have used plasmid DNA vaccines at doses between 0.3 mg and 8 mg. All doses have been well tolerated, and even the lowest dose demonstrated priming (47). DNA doses given in the DP6-001 study were 1.2 or 7.2 mg. In that study, there did appear to be a dose-dependent effect, with the higher dose producing higher response rates and magnitudes of immune responses after administration of the DNA vaccine. However, there were no differences in immunogenicity between the low and high dose DNA arms after the protein

boost. Therefore, the 2 mg IM dose (0.4 mg/each DNA plasmid) has been selected for HVTN 124.

The dose of protein vaccine in HVTN 124 will be 400 mcg (100 mcg of each gp120 protein), which is similar to the 375 mcg total dose given in the DP6-001 study. Protein doses in HVTN studies have recently ranged from 100 mcg to 600 mcg of gp120. Gp120 has been well tolerated with alum or MF59, and other adjuvants currently under investigation at these doses. The dose of GLA-SE adjuvant in HVTN 124 will be 5 mcg with 2% oil. The GLA-SE dose has been determined based on immunogenicity and tolerability results obtained from previous clinical studies including influenza vaccine and cancer vaccine clinical studies by IDRI.

4.4.3 Schedule

The prime-boost schedule of vaccinations at 0, 1, 3, 6 and 8 months has been chosen to give a minimum of a 2-month rest period between the protein boost vaccinations in Groups 1 and 2. Other HVTN trials evaluating DNA prime followed by a protein or vectored vaccine boost strategies follow a schedule of two or three DNA prime immunizations followed by two boosts (eg, HVTN 092, HVTN 105, HVTN 106, HVTN 108, HVTN 111, HVTN 112, and HVTN 115). A similar schedule of vaccinations, including DNA immunizations at months 0, 1, 3 and protein immunizations at months 6 and 8, was selected for this study. This will allow for some informal comparisons across studies.

4.4.4 DNA + protein vaccination regimen

In addition to the polyvalent DNA prime-protein boost arm, HVTN 124 includes an arm to test the DNA vaccine given simultaneously with the adjuvanted protein vaccine for comparison. Assessments starting after the third vaccination in this group will evaluate when recipients have achieved peak immune responses to the combination.

An important objective is to determine which of the two regimens will achieve the highest response rate, magnitude, and durability of antibody responses, and to characterize any qualitative differences in immune responses to the two regimens.

4.4.5 Choice of placebo control

Sodium Chloride for Injection, USP 0.9% is nonreactogenic and well tolerated. It will be used for placebo groups and as a control to blind between groups in Part B.

4.5 Plans for future product development and testing

Once HVTN 124 is initiated, the UMMS team will work on the optimization of the manufacturing process to allow vaccine production on a much larger scale, and being able to provide the protein vaccine components premixed in one vial, to

prepare for a possible phase 2 study. Clinical safety and immunogenicity data from HVTN 124 will provide key information to drive the further advancement of this unique polyvalent DNA-protein HIV vaccine regimen to more advanced studies.

4.6 Preclinical safety studies

A multiple dose toxicology study was conducted by Wuxi AppTec Inc. at its toxicology test facility located in St Paul, Minnesota (study protocol number: C13474-1).

Table 4-2 Summary of preclinical safety studies

Study number	Product	Type of study	Animal	N	Dose groups	Route	Schedule (days)
C13474-1	PBS	Toxicology	Rabbit (Group 1)	10m, 10f	N/A	IM	1, 21, 42, 63, 84, 105
	GLA-SE	Toxicology	Rabbit (Group 2)	10m, 10f	0.005mg	IM	Same
	DNA+ Protein+ GLA-SE	Toxicology	Rabbit (Group 3)	10m, 10f	2mg DNA 0.4mg Protein 0.005mg GLA-SE	IM	Same

4.6.1 General toxicology study of proposed product combination in New Zealand White rabbits (Study #C13474-1)

Administration of Test and Control Articles

Route: Intramuscular Injection
Frequency: Days 1, 21, 42, 63, 84 and 105

Vaccinations were administered as detailed in Table 4-2. The dose consisted of bilateral single intramuscular injections of constant volume regardless of body weight. For each dose, the adjuvant (Group 2), or adjuvant/protein (Group 3) injections were administered on the right thigh and the DNA injections (Group 3) were administered on the left thigh. Each injection was administered at a shaved/marked site.

Group 1

For each rabbit of Group 1, 0.8 mL of PBS was drawn into a syringe for intramuscular injection into the left thigh; then, 1.0 mL of PBS was drawn into a syringe for intramuscular injection into the right thigh.

Group 2

For each rabbit of Group 2, 0.8 mL of PBS by intramuscular injection was administered to the left thigh; then, 1.0 mL of PBS + GLA-SE adjuvant mixture was administered by intramuscular injection to the right thigh.

Group 3

For each rabbit of Group 3, 0.8 mL of DNA vaccine from one vial was administered by intramuscular injection to the left thigh; then 1.0 mL of the 4 protein vaccines + GLA-SE adjuvant mixture (0.2 mL of each gp120 protein (0.6 mg/mL) with 0.4 mL of GLA-SE (15 mcg/mL) was administered by intramuscular injection to the right thigh.

Observations

The study monitored body temperatures, daily mortality/moribundity checks, feed consumption, general health observations, clinical health observations, Draize scoring, and body weight. Blood was sampled for serological testing, C-reactive protein (CRP) and serum protein electrophoresis (SPEP) at Day 1 (pre-dose baseline) and at Days 2, 8, 21 (pre-dose 2), 43, 49, and 63 (pre-dose 4). Samples were also drawn for potential evaluation of antigen-specific IgG antibodies, hematology, coagulation studies, chemistry. Final observations included gross necropsy, organ weights, and histopathology.

Conclusion for General Toxicology Study

It was concluded that under the conditions of this study, C13474-1 Multiple Dose Toxicology Study for a Modified Polyvalent HIV Vaccines Delivered Intramuscularly, when taking all the toxicology, Draize scoring, body temperature, serum protein, and pathology data into account, there was no evidence of systemic toxicity or adverse findings specifically attributed to the test article. Please see the IB for additional information.

In addition, an immunogenicity analysis was conduct to measure gp120-specific IgG responses in rabbits included in the toxicology study. High titer antibody responses were clearly detected in Group 3 at the end of study while there were no detectable gp120-specific IgG responses in Groups 1 and 2.

4.7 Preclinical immunogenicity studies

Table 4-3 Summary of preclinical immunogenicity studies

Study number	Product	Animal	N	Dose groups	Route	Schedule (weeks)	Assay
LNAV-01	JR-FL protein	NZW rabbits	3f	P 50 mcg / A 300 µl	IM	0, 2, 4, 8 10	Breadth of nAb (Fig. 4-2)
	JR-FL DNA	Same	3f	D 36 mcg	GG	0, 2, 4, 8, 10	
	JR-FL DNA >> JR-FL protein/IFA	Same	3f	D 36 mcg >> P 60 mcg / A 300 µl	GG>> IM	D 0, 2, 4 >> P 8, 10	
	JF-FL DNA >> polyvalent proteins /IFA	Same	3f	D 36 mcg >> P 60 mcg / A 300 µl	GG>> IM	D 0, 2, 4 >> P 8, 10	
LNAV-02	DP6-001 DNAs >> DP6-001 proteins /QS-21	NZW rabbits	5f	D 400 mcg >> P 100 mcg / A 50 mcg	IM	D 0, 2, 4 >> P 10 14	Titers and breadth of nAb
	New polyvalent DNAs >> proteins/IFA	Same	5f	D 400 mcg >> P 100 mcg / A 300 µl	IM	D 0, 2, 4 >> P 10 14	(Figs. 4-4 and 4-5)
LNAV-03	4-valent gp120 (ABCE) proteins + GLA-SE	NZW rabbits	5f	P 100 mcg + A 25 mcg	IM	0, 4, 8	ELISA (Fig. 4-6)
	4-valent gp120 (ABCE) proteins + GLA-SE	Same	5f	P 400 mcg + A 25 mcg	IM	0, 4, 8	
	4-valent gp120 (ABCE)	Same	5f	P 400 mcg	IM	0, 4, 8	
LNAV-04	Empty Vector >> DP6-001 proteins	NZW rabbits	2f	D 36 mcg >> 100 mcg / A 300 µl	GG>> IM	D 0, 4, 12 >> P 20, 28	ELISA (Fig. 4-7)
	DP6-001 DNAs >> DP6-001 proteins/IFA	Same	4f	D 36 mcg >> 100 mcg / A 300 µl	GG>> IM	D 0, 4, 12 >> P 20, 28	

4.7.1 Immunogenicity of JR-FL gp120 protein alone, JR-FL DNA alone, DNA prime + JR-FL protein boost (monovalent), and JR-FL DNA prime + 5-gp120 proteins boost (polyvalent proteins), with IFA included as part of protein boosts (LNAV study #1)

In order to provide a full description of the quality of antibody elicited by the DNA prime-protein boost approach, different prime-boost vaccination regimens (ie, DNA only, protein only, DNA plus protein and Incomplete Freund's Adjuvant [IFA]) in rabbits were tested using the gp120 antigen from HIV-1 JR-FL as a model antigen. Results demonstrated that incorporating DNA immunization as a prime in a heterologous prime-boost regimen was able to elicit a more diverse and conformational epitope profile, higher antibody avidity, and improved neutralizing activity than immunization with protein alone. In addition, polyvalent Env protein boost was more effective than monovalent protein boost in rabbits receiving the same JR-FL gp120 DNA prime immunization to elicit broader neutralizing activities against more resistant Tier-2 viruses (32). See Figure 4-2.

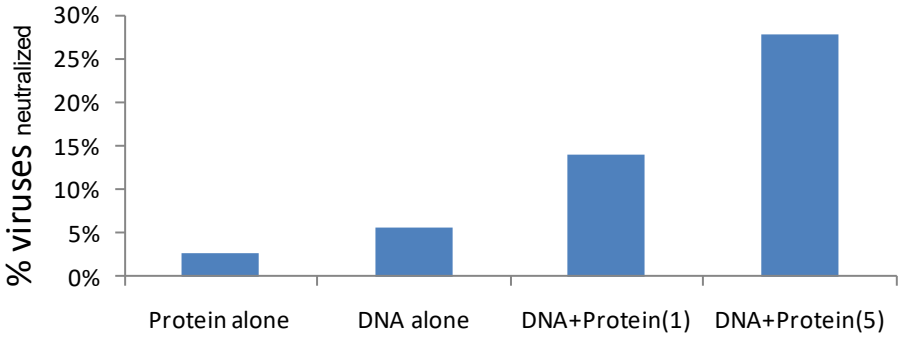

Figure 4-2. Breadth of neutralizing antibody responses induced by JR-FL gp120 protein alone (5 immunizations), DNA alone (5 immunizations), DNA prime (3 immunizations) + protein boost (2 immunizations with one gp120 protein and IFA), and DNA prime (3 immunizations) + protein boost (2 immunizations with five gp120 proteins and IFA). The neutralization was performed against the standard panel of 12 subtype B Tier 2 viruses in TZM-bl cells.

Additional studies using a mouse model have demonstrated that DNA prime-protein boost immunization was more effective than DNA or protein alone approaches in establishing gp120-specific B cell responses in both spleen and bone marrow when measured by B cell ELISpot analysis. This finding suggests that the DNA prime-protein boost strategy is effective in eliciting both circulating antibody secreting cell (ASC) and memory B cell responses (Shan Lu, unpublished data).

4.7.2 Immunogenicity of new polyvalent Env formulation (5-valent) vaccine versus DP6-001 (LNAV study #2)

The new gp120 immunogens were selected based on results of a comprehensive study in rabbits, which screened more than 60 primary Env immunogens. Only about 15% of primary gp120 immunogens were effective in eliciting broad

neutralizing antibody responses (Shan Lu, unpublished data). Five top candidate gp120 immunogens with the ability to prime broad nAb responses, each representing one of the five subtypes (A, B, C, D and A/E), were selected as candidates to form a new polyvalent formulation to compare with the immunogenicity of DP6-001 (which also contained five gp120 immunogens).

A limited antigenicity analysis was conducted to see if these gp120 immunogens could be recognized by several well-known broadly neutralizing mAbs, including recently discovered ones. As shown in Figure 4-3, different mAbs (VRC01, b12, 2G12, PGT128 and PG9) may recognize different subsets of the five gp120 immunogens included in the tested polyvalent formulation. (The final PDPHV-201401 formulation to be used as the clinical trial materials is quadrivalent, without gp120 from subtype D). Several gp120 immunogens can be recognized by more than one mAb. These would provide a good diversity of immunogen conformations and they may supplement each other in a polyvalent formulation. One interesting finding is that mAb PG9 showed very high and specific binding to the A/E consensus gp120 immunogen. The data suggested that the gp120 immunogens produced for this new vaccine have well preserved critical conformations, which are important for the induction of high quality antibody responses.

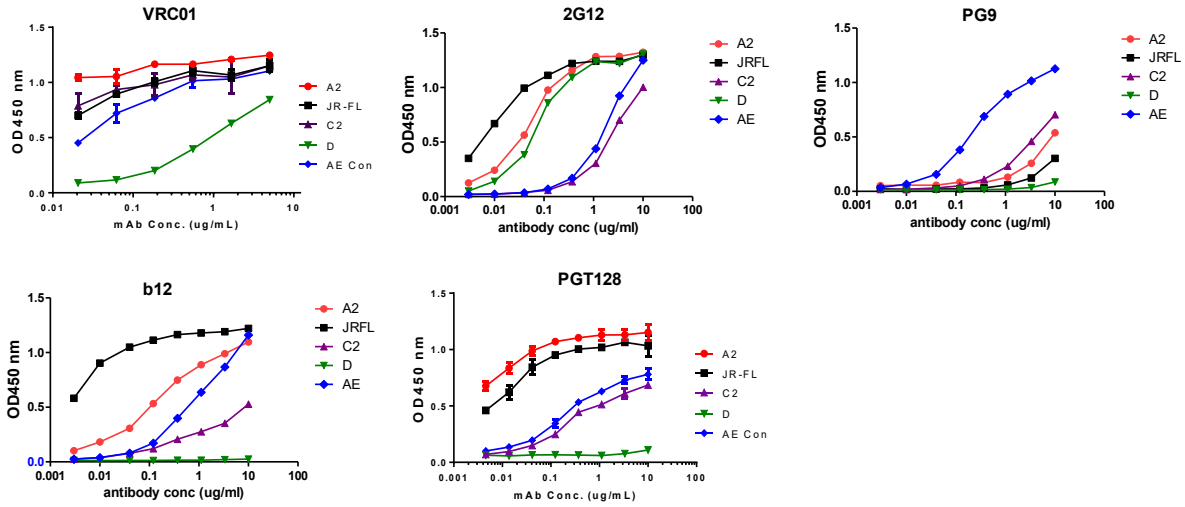

Figure 4-3. ELISA assay showing the recognition of five selected gp120 proteins (A2, JR-FL, C2, D and A/E) in by neutralizing mAb VRC01, b12, 2G12, PGT128 and PG9.

In a rabbit immunogenicity study, antibody responses with the new pentavalent DNA prime-protein boost regimen PDPHV-2011 were compared to sera elicited by the DP6-001 formulation and were found to produce improved nAb responses in rabbits compared to the first generation vaccine regimen DP6-001, as measured by both potency (Figure 4-4) and breadth (Figure 4-5).

The assay done at Monogram Biosciences used a standard panel, and a second assay used a panel of 26 pseudotyped viruses spanning Tier 1 to 3 viruses. Figure 4-4 shows that the nAb titers done at Dr. Montefiori's lab were similar against SF162 ($p = 0.062$) but significantly improved in pentavalent PDPHV-2011

compared to DP6-001 in testing against two Tier 1B viruses, SS1196.1 (p = 0.022) and 6535.3 (p = 0.041). The new regimen elicited higher nAb titers than the DP6-001 regimen. Figure 4-5 shows the neutralization assays conducted against an expanded panel of viruses, including many Tier 2 subtypes B and C viruses. Results from these analyses demonstrated that a higher percentage of viruses in the panel was neutralized by the PDPHV-2011 rabbit sera than the DP6-001 rabbit sera.

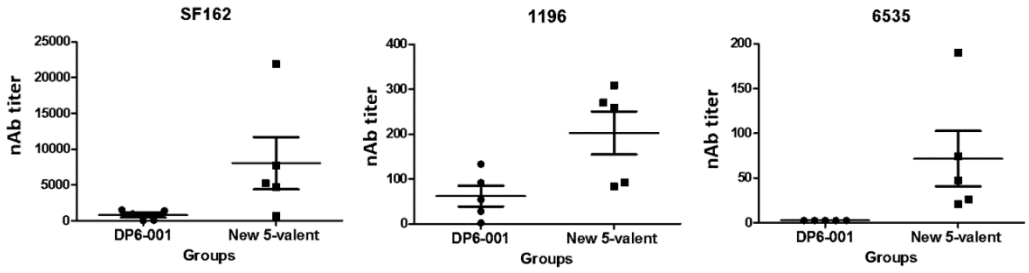

Figure 4-4. nAb titers against HIV-1 subtype B Tier 1 (SF162) and Tier 1b (1196 and 6535) pseudotyped viruses in rabbit sera immunized with DP6-001 or (5-valent) PDPHV-2011 gp120 DNA prime – protein boost.

A.

			IC50 NAb titers against HIV-1 pseudoviruses									
Vaccine (DNA prime - Protein boost)			DP6-001 gp120					new 5 valnet gp120				
Rabbit#			R729	R730	R731	R732	R733	R754	R755	R756	R757	R758
Tier	Clade	Virus ID										
1A	B	SF162	945	1537	27	78	1405	5266	7720	677	>21870	4718
1B	B	SS1196	28	133	54		92	83	259	92	309	270
	C	ZM197.PB7							16	14	38	21
	C	ZM109F.PB4			15					15	25	20
2	B	AC10.0.29							10			
		QH0692.42							23		20	
		SC422661.8				10					20	
		REJO4541.67									24	
		6535.3	22					47	21	26	190	74
		P6LN40				10						
		CAAN5342.A2			21						11	
		TRO.11									20	
		RHPA4259.7									26	12
		WITO4160.3			22						10	
	C	ZM249.PL1			24	22	>>90		13		41	19
		DU156.12#1	11	38	132	86	22	12	22	17	44	19
		DU172.17#3		14		34			19	11	28	17
		ZM135M.PL10a					53	12	19		31	19
		CAP45.200.E8							12		20	
		Du422.1									13	
		ZM53M.PB12										
		ZM214.pB7			29							
		ZM233M.PB6					15		21	19		22
		CAP210.2.00.E8			21		11				27	
3	B	PVO.4										
		TRJO4551.58							20			
Neg-control virus		MLV										

Figure 4-5. Frequency of pseudotyped viruses neutralized by rabbit sera immunized with either DP6-001 or the new 5-valent formulation (5 rabbits in each group). Rabbits were immunized with 3 DNA prime immunizations and 2 protein boost immunizations. Rabbit sera were collected at 2 weeks after the second protein boost. A: Rabbit sera that can neutralize any pseudotyped viruses in this assay (Tiers 1, 2 and 3) are highlighted in yellow

with neutralizing antibody titers (IC50) shown. The blank cells indicate that there was no detectable neutralizing activity (cut off at titer =/<10). B: Frequency of HIV-1 pseudotyped viruses neutralized by either DP6-001 (23%) or new 5-valent formulation (43.1%) based on data shown in panel.

4.7.3 Preclinical studies of GLA-SE adjuvant conducted at IDRI and UMMS (LNAV Study #3)

GLA-SE, an adjuvant developed by IDRI, has been tested in several toxicity studies in rabbits and rats conducted under GLP guidelines. Parameters evaluated included the following: morbidity/mortality; clinical signs; injection site reactogenicity; body weights; body temperatures; food consumption; ophthalmologic evaluations; clinical chemistry; hematology; coagulation; gross necropsy observations; organ weights; histopathology; and immunogenicity. In these studies, GLA-SE was found to be safe, well-tolerated, and immunostimulatory. No treatment-related adverse effects were observed in the parameters evaluated except for reversible injection site reactions (erythema, edema, inflammation) and a mild, transient acute inflammatory response (changes in hematologic values, increased fibrinogen levels) in some animals receiving GLA adjuvant formulations.

In addition, many preclinical and nonclinical studies in mice, guinea pigs, rats, rabbits, and non-human primates have demonstrated the immunostimulatory properties of GLA-SE with no significant safety signals. Thousands of mice have been immunized with candidate antigens for infectious disease vaccines (eg, leishmaniasis, tuberculosis, leprosy, malaria, influenza) formulated in GLA-SE. No safety issues have been observed. In addition, the potential for auto-antibody generation following GLA administration was evaluated through extensive immunohistochemistry assays for immune complexes and/or autoimmune pathology, and no signs of detrimental autoimmune effects were found. Studies have also assayed for anti-nuclear antibody and have not found abnormal levels in GLA-immunized mice.

More than 300 guinea pigs have been immunized with candidate tuberculosis vaccine antigens formulated with GLA. No safety issues have been observed. In addition, a safety study was conducted in guinea pigs in which various GLA adjuvant formulations (oil emulsions, aqueous suspensions, liposomes) were administered without antigen by three different injection routes (subcutaneous [SC], intramuscular [IM], and intradermal [ID]). Administration of GLA-SE by all three routes appeared to be safe and well-tolerated.

In several nonhuman primate studies using the adjuvant in male and female rhesus, male cynomolgus, and male aotus monkeys, the adjuvant was safe, well-tolerated, and increased the immune response to the co-administered antigen. No treatment-related adverse effects were observed in the safety parameters evaluated except for mild injection site reactions and a mild, transient acute phase inflammatory response characterized by increases in C-reactive protein (CRP), fibrinogen, and neutrophils.

Once GLA-SE was selected jointly by UMMS scientists and NIAID program officers in consultation with our Scientific Advisory Board members, one rabbit immunization study was conducted to confirm the ability of GLA-SE adjuvant in enhancing the gp120-specific antibody responses elicited by recombinant gp120 protein immunization.

The rabbits received the same quadrivalent gp120 protein immunization (A, B, C and A/E) as PDPHV-201401 at Weeks 0, 4 and 8 by intramuscular injection at two dosing levels (100 mcg or 400 mcg total protein at each immunization). The sera were collected to evaluate the gp120-antibody responses by ELISA (using plates coated with the 4-gp120 protein mix). The results demonstrated that GLA-SE could significantly enhance the gp120-specific antibody responses in rabbits (Figure 4-6).

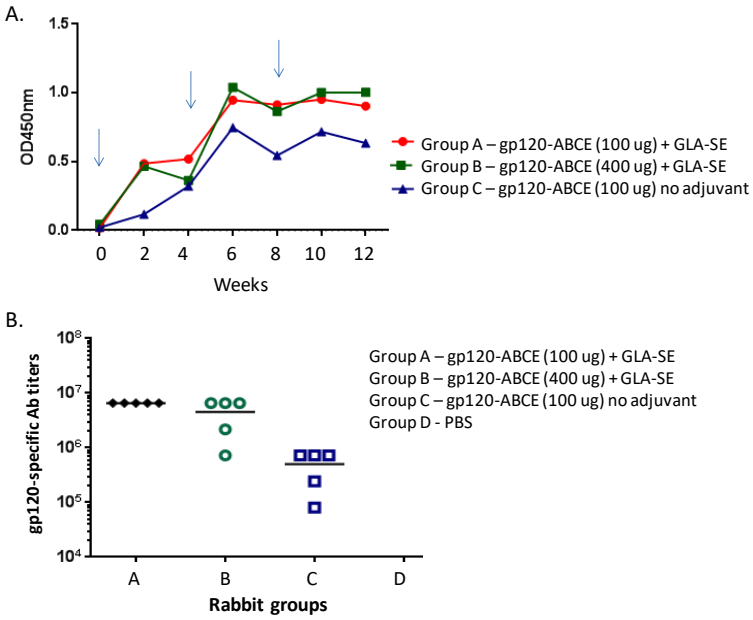

Figure 4-6. The gp120-specific serum antibody responses in rabbits immunized by the 4-valent gp120 proteins (gp120-ABCE) with or without GLA-SE adjuvant. A: the temporal antibody responses with sera at 1:3,000 dilutions. The arrows indicate the times of immunizations. B: The gp120-specific antibody titers at 2 weeks after the 3rd protein immunization.

4.7.4 Epitope mapping analysis (LNAV Study #4)

An epitope mapping study was performed that revealed that the DP6-001 DNA-primed, protein-boosted sera generated significant levels of conformation-dependent antibodies specific for the CD4 binding site (CD4bs) while animals immunized with protein alone were not efficient in generating such antibodies. Figure 4-7 shows that three mapped epitope areas (p30, p61-p64 and p113-117) overlapped with CD4bs as shown by their positions at the gp120 crystal structure. The importance of anti-CD4bs antibody in neutralizing HIV-1 has been described by studies with monoclonal antibody (mAb) b12, long term non-progressor HIV patients' sera reported from John Mascola's group, and the new mAb VRC01 (48, 49).

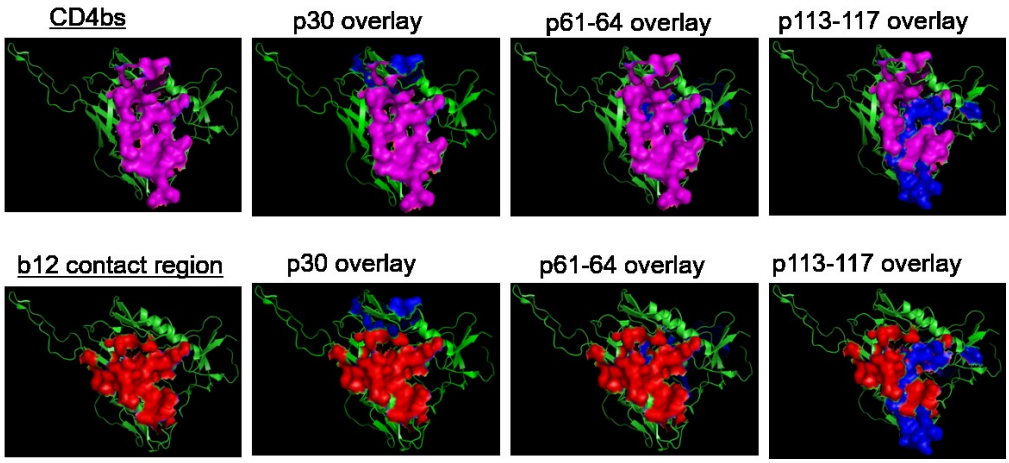

Figure 4-7. Identification of three clusters of targeted epitopes (in blue color, p30, p61-64 and p113-117) elicited by DP6-001 DNA prime-protein boost vaccine, overlapping either with known CD4bs (in purple color, upper row) or with the binding site for CD4bs mAb b12 (in red color, lower row).

4.8 Clinical studies

No previous clinical studies of the PDPHV-201401 vaccines have been performed. However, similar vaccines have been tested in an earlier study.

4.8.1 Phase 1 clinical study H11163 of the first generation regimen, DP6-001

Based on the results of the rabbit and non-human primate studies described above, a clinical formulation, DP6-001, was developed that included six DNA vaccine plasmids as the prime: one codon optimized *gag* gene from subtype C (96ZM651) plus 5 primary *env* genes representing 5 randomly selected Env antigens from four major subtypes, subtypes A (92UG037.8), B (92US715.6 [B] and Bal), C (96ZM651), and E (93TH976.17), followed by a boost using 5 matching recombinant gp120 proteins produced in CHO cells. This first generation regimen was tested in a phase 1 clinical trial (H11163) in healthy adults. The study was a randomized, 3-arm trial. The groups received 1.2 mg DNA ID (Group A), 1.2 mg DNA IM (Group B), or 7.2 mg DNA IM (Group C) without any adjuvant at weeks 0, 4, and 12, followed by one standard dose of protein (0.375mg) administered IM with the adjuvant QS-21 at weeks 20 and 28, except for the high dose DNA group who received the adjuvanted protein IM injection only at week 20, due to all study vaccinations being discontinued early.

In this study, a high level of local and systemic reactogenicity was observed, including injection site pain and erythema, fevers, myalgia, fatigue, headache, and arthralgias. Fevers were common within the first week after vaccination in Groups A and B, occurring in 2/3 of participants at one or more timepoints. All fevers were Grade 1 (38.0°C-39.0°C, 100.4-102.2°F) except for 1 Grade 2 fever (39.1°C-40°C, 102.3-104.0°F) in a Group B participant after the second DNA vaccination. Other adverse events included bilateral eye pain, sensitivity to light,

blurry vision, changes in sleep or appetite, increased liver function tests, decreased CD4+ T cell counts and 2 events of vasculitis (described below).

Three clinically distinct types of skin reactions were encountered. The most frequent skin reaction, in 39% who received at least two vaccine doses, was localized skin erythema. These reactions typically appeared 36–72 h after injection of either the DNA (8 reactions) or the protein vaccine (12 reactions), at the sites of prior ID or IM DNA inoculation ("recall reactions"). Several were biopsied and consistent with Delayed Type Hypersensitivity (DTH) reactions. DTH reactions were common in both the low dose DNA ID (66.7%) and high dose DNA IM groups (44.4%).

The second type of skin reaction was probably related to QS-21 based on prior reports of similar reactions to Env proteins formulated with QS-21. This pattern of localized skin erythema typically developed <24 h after vaccination with the protein vaccine, and was associated with greater induration, distinguishing it from the DTH reactions. However, a hypersensitivity reaction to the HIV antigens cannot be definitively excluded as a cause.

The third type of skin reaction occurred in two subjects, both developing vasculitis within days following inoculation of the protein vaccine. One low dose DNA IM participant received one dose of protein vaccine and experienced reactivation of Henoch-Schonlein Purpura (HSP), a past medical history that was not reported prior to enrollment. A participant in the high dose DNA IM group received one dose of protein vaccine and developed leukocytoclastic vasculitis (LCV). Following the LCV event, vaccinations were halted for the study (46).

LCV and HSP are categorized as immune-complex-mediated diseases. Several factors may have contributed to these vasculitis events: DNA priming immunization, HIV envelope antigens, and/or the use of QS-21 as an adjuvant and/or the protein/adjuvant combination. In any case, no significant skin or systemic reactions were observed in the DP6-001 rabbit toxicology study prior to the phase 1 clinical trial or in the additional toxicology study conducted in rabbits following the cases of vasculitis and DTH reactions.

The causes of the DTH reactions and the vasculitis events, and their relationship to each other, if any, are not clear. It is notable that several DTH events occurred prior to protein vaccination, and therefore cannot be attributed to boost effect or combination of protein vaccine and QS-21. In regard to the vasculitis events, the temporal relationship to vaccination (following protein boost after DNA prime) suggests various possible mechanisms, including: strong cytokine production, enhanced reactions to the multiplicity of Env antigens in the DNA and protein vaccine regimen, or due to the protein vaccine formulation with QS-21 adjuvant, and/or an amplified reaction to the protein-adjuvant combination after DNA priming. The potential relationship to HIV Env warrants consideration in the design and analysis of this and other vaccine trials (46). Future clinical trials will include study designs that would help clarify etiologies of potential adverse reactions.

Clinical immunogenicity data from Protocol H11163 evaluating DP6-001

High titer HIV-1 Env-specific antibody responses were generated with the DP6-001 DNA prime-protein boost formulation. In the groups receiving low doses of DNA, most volunteers did not have detectable levels of Env-specific antibody responses after three DNA immunizations. However, the antibody titers rose quickly after just one protein boost. The anti-gp120 IgG titers reached levels comparable to those observed in chronically infected HIV patients (ie, $1:10^5$ or higher) after only 1 or 2 protein boosts. By the end of the trial at week 52, 11 of 11 participants who had received low-dose DNA IM primes maintained significant levels of serum anti-gp120 IgG titers (median, 8000, range 200-104,000). In the high-dose DNA group, four out of six volunteers showed detectable Env-specific IgG responses even before the protein boost. Furthermore, Western blot analysis revealed that antibodies elicited by the polyvalent Env formulation DP6-001 were broadly reactive against a wide range of 11 heterologous primary HIV-1 gp120 antigens from subtypes A to G (5).

Three different laboratory studies were organized to assess the nAb activities in DP6-001 sera. The first study by David Montefiori found positive neutralizing antibody activities against a Tier 1 TCLA HIV-1 virus and different homologous pseudotyped viruses in immunized sera, whereas none of the 7 control samples had positive nAb against the above viruses (5).

The second neutralization study was conducted at Monogram Biosciences and used a high-throughput, pseudotyped virus assay system to test sera from volunteers who received low-dose DNA, collected two weeks after the second protein boost, against a panel of 11 heterologous primary viruses from subtypes A to E, as well as three sensitive viruses. High-titer nAb responses were identified in all human immune sera against the three sensitive viruses. The geometric mean nAb titers were 1:771 (range: 348~1184) against SF162, 1:696 (range: 418~2147) and 1:196 (range: 49~426) in the low dose DNA IM group. A majority of volunteers had positive nAb activities (nAb titer of 1:20 as cutoff) against at least half of the pseudotyped viruses expressing primary Env antigens included in the study (5). The levels of nAb were higher than what was reported in previous literature with different HIV vaccine designs that include a DNA component (47). The geometric mean nAb titers against HIV-1 primary pseudotyped viruses were 1:30 (20~90) in the low dose DNA IM vaccinees.

The third neutralization study was also conducted by David Montefiori and tested the ability of the DP6-001 vaccinee sera to neutralize standardized panels of Tier 2 viruses. Although positive neutralizing activity was higher than the negative control sera, most volunteer sera displayed viral inhibition of less than 50% at a serum dilution of 1:10.

Positive CMI responses were also observed with the DP6-001 formulation, supporting the effectiveness of DNA prime-protein boost approach to elicit a balanced antibody and CMI response. Overall, 83-100% of volunteers had positive Env-specific IFN-γ responses observed in this study, and these responses

were mediated predominantly by polyfunctional CD4+ T cells (5, 26). HIV-1 specific CD8+ T cell responses were also detected in the high dose group, but at a lower frequency (67% of subjects responded (26). CMI responses to Gag peptides increased in the high dose DNA group compared with the low dose DNA groups, in direct correlation with amounts of gag DNA in each arm.

Further comparisons were done on the specificities of antibody responses in sera from DP6-001 volunteers and volunteers enrolled in two other HIV vaccine trials that used different immunization regimens: HVTN 041, recombinant proteins with AS02A adjuvant; and HVTN 203, a canarypox viral vector prime-protein boost (alum adjuvanted). Analysis included testing sera from each regimen's volunteers for HIV-1 Env-specific binding antibody, nAb, antibody-dependent cell-mediated cytotoxicity (ADCC), and profiles of antibody specificity. While HVTN 041 had the highest binding antibody responses and nAb activities against the sensitive virus, DP6-001 sera showed the highest frequency of positive nAb activities against more resistant viral isolates with a significantly higher CD4 binding site antibody response compared to both HVTN 041 and HVTN 203. No differences were found in CD4-induced antibody responses, ADCC activity, or complement activation by Env-specific antibody among these sera (4). This study further supported the rabbit study finding that the DNA prime-protein boost approach is highly effective in eliciting antibodies targeting conformationally sensitive and functionally conserved epitopes (35).

4.8.2 Clinical studies of GLA-SE adjuvant

GLA-SE adjuvant has been used in a number of protein vaccines for phase 1 human clinical studies (Table 4-4).

Table 4-4 Summary of selected clinical studies of GLA-SE adjuvant

Study number [Reference]	Products	Type of study	Subjects	N	Dose groups	Route	Schedule
IDC 2008-001	Fluzone® + GLA-SE	Phase 1 randomized	Healthy adults (18 to 45) and healthy elderly (65+)	32 adults, 56 elderly	All groups received Fluzone + A: 0.5 mcg GLA-SE B: 1.0 mcg GLA-SE C: 2.5 mcg GLA-SE D: Fluzone alone E: SE	IM	Single dose
PSC-22; NCT01147068 (50)	PanBlok® pandemic influenza vaccine, rHA A/Indonesia/ 05/2005 [H5N1] + GLA-SE	Phase 1 randomized placebo controlled	Healthy adults	392	1: placebo 2: PanBlok (135 mcg) 3: PanBlok (45 mcg) 4: PanBlok (45 mcg) + 1.0 mcg GLA-SE 5: PanBlok (15 mcg) + 1.0 mcg GLA-SE 6: PanBlok (7.5 mcg)+ 1.0 mcg GLA-SE 7: PanBlok (3.8 mcg)+ 1.0 mcg GLA-SE	IM	Day 0, 21
IDRI-LVVPX-111	Recombinant fusion antigen LEISH-F3 + GLA-SE	Phase 1 randomized	Healthy adults	18	1: 20 mcg LEISH-F3+ 2 mcg GLA-SE 2: 20 mcg LEISH-F3+ 5mcg GLA-SE 3: 20 mcg LEISH-F3 alone	IM	Day 0, 28, 56
DMID 11-0031	LEISH-F3 + GLA-SE or MPL-SE or SE	Phase 1 randomized open label	Healthy adults	48	1: 20 mcg LEISH-F3+ 5 mcg GLA-SE 2: 20 mcg LEISH-F3+ 10mcg MPL-SE 3: 20 mcg LEISH-F3 + SE	IM	Day 0, 28, 168
NCT01154049 (43)	*Schistosoma mansoni* antigen Sm14 + GLA-SE	Phase 1 open label	Healthy men	20	50 mcg LEISH-F3+ 10mcg GLA-SE	IM	Day 0, 30, 60
NCT01540474	Recombinant malaria antigen FMP012 + GLA-SE	Phase 1 randomized	Healthy adults	30	1: 10 mcg FMP012 + 2 mcg GLA-SE 2: 10 mcg FMP012 + 5 mcg GLA-SE 3: 50 mcg FMP012 + 5 mcg GLA-SE	IM	1: Week 0, 4, 16 2: Week 2, 6, 16 3: Week 8, 12, 16
NCT02115815 (51)	Soluble RSV fusion protein (sF) + GLA-SE	Phase 1 randomized	Healthy adults > 60	144	1: 20 mcg RSV sF 2: 50 mcg RSV sF 3: 80 mcg RSV sF 4: 20 mcg RSV sF + 2.5 mcg GLA-SE 5: 50 mcg RSV sF + 2.5 mcg GLA-SE 6: 80 mcg RSV sF+ 2.5 mcg GLA-SE 7: placebo	IM	Single dose

The GLA/SE formulated with either recombinant influenza H5 hemagglutinin (HA) or the influenza split-virus vaccines containing A/Solomon Islands/3/2006 (HIN1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 were well

tolerated, safe, and immunogenic in healthy adults, and GLA-SE substantially improved the serum antibody responses (41, 50). In the study of GLA-SE with soluble RSV fusion protein (sF), immune responses were RSV sF dose-dependent and increased in the presence of adjuvant (51).

In IDC 2008-001, GLA-SE at 5 mcg was associated with fever and chills and subsequent doses were reduced. GLA-SE significantly increased influenza-specific antibody responses to Fluzone® and PanBlok® antigens, and had a dose-sparing effect. Please refer to the IB for additional information including numerous clinical studies. Overall, the study injections have been generally well tolerated with an acceptable safety profile, at the proposed dose of 5 mcg. There have been no serious adverse events related to study injection. Injection site reactions are common and may include pain, tenderness, erythema, and induration. Systemic reactions may include headache, fatigue, anorexia, fever, chills, myalgia, and arthralgia. Transient elevations in CRP levels were noted in one study. Hematologic changes may occur (including decreases in hemoglobin, WBC, and neutrophils). These reactions varied from study to study, were generally mild, resolved quickly, and are typical of vaccinations by the IM route.

4.9 Potential risks of study products and administration

Table 4-5 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 • Localized skin erythema • "Recall reactions" (Type IV Delayed-type hypersensitivity) at sites of previous vaccine administration • A vaccine-induced positive HIV antibody test result
Less common	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, nausea, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Decreased appetite (anorexia) • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, other transient lesions, itching, 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 • Muscle damage at the injection site
Unknown frequency	• Autoimmune disease, vasculitis
Theoretical risks	• 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 Env (A,B,C,A/E)/Gag (C)-expressing DNA plasmids and recombinant gp120 (A,B,C,A/E) protein/GLA-SE adjuvant, given individually, in combination, or as a prime-boost series in healthy HIV-1 uninfected adults

Primary endpoints 1:

- Frequency and severity of local injection site (including DTH) and systemic reactogenicity signs and symptoms, Adverse Events (AE) categorized by MedDRA system organ class, MedDRA preferred term, severity, and assessed relationship to study products; detailed description of all AEs meeting DAIDS criteria for expedited reporting by treatment arm throughout the trial

- The distribution of values of safety laboratory measures: white blood cells, neutrophils, lymphocytes, hemoglobin, alkaline phosphatase, platelets, ALT, AST, and creatinine at baseline and at follow-up visits post vaccination

- Number of participants with early discontinuation of vaccinations and reason for discontinuation

5.2 Secondary objectives and endpoints

Secondary objective 1:

To evaluate the serum IgG binding antibody responses to Env proteins elicited by each vaccination regimen

Secondary endpoint 1:

Magnitude and breadth of serum HIV-1 Env-specific IgG responses assessed by Binding Antibody Multiplex Assay two weeks after the last vaccination

Secondary objective 2:

To evaluate the serum neutralizing antibody responses elicited by each vaccination regimen in Part B

Secondary endpoint 2:

Serum neutralizing antibody responses against Tier 1A, Tier 1B, and selected Tier 2 viruses, assessed by TZM-bl assay, two weeks after the last vaccination in Part B

Secondary objective 3:

To evaluate each vaccination regimen with specific antibody assays to measure correlates of risk identified in the RV144 trial

Secondary endpoint 3:

Breadth of gp70-V1V2 IgG and gp120 IgA, assessed by Binding Antibody Multiplex Assay, and ADCC activities against HIV-1 subtypes A, B, C and A/E two weeks after the last vaccination in Part B

Secondary objective 4:

To evaluate HIV-specific T-cell responses elicited by each vaccination regimen

Secondary endpoint 4:

Frequency, magnitudes and quality of HIV-1 specific CD4+ and CD8+ T-cell responses as measured by ICS two weeks after the last vaccination

5.3 Exploratory objectives

Exploratory objective 1:

To evaluate serum IgG3 binding antibody responses to Env proteins 2 weeks after the third, fourth and fifth vaccinations and six months after the fifth vaccination in Part B

Exploratory objective 2:

To evaluate HIV-1 Env-specific B-cell responses elicited after the last vaccination in Part B

Exploratory objective 3:

To determine the frequency of circulating Tfh and plasmablasts in response to each vaccination regimen

Exploratory objective 4:

To assess the elicitation of mucosal immune responses in saliva, semen (men), cervicovaginal fluid (women), and rectal fluid

Exploratory objective 5:

To assess whether the diversity of gut microbiome correlates with vaccine responses using optionally provided stool specimens

Exploratory objective 6:

To assess the effects of BMI on the safety and immunogenicity of the vaccine regimens

Exploratory objective 7:

To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 60 healthy, HIV-uninfected adult participants.

The protocol is composed of two parts, Part A and Part B. Part A will assess the safety of recombinant gp120 (A, B, C, A/E) protein/GLA-SE adjuvant. Part B will evaluate the safety and immunogenicity of DNA prime and protein/adjuvant boost or DNA and protein/adjuvant co-administered. Part A will proceed prior to Part B. In Part A, 12 participants will be enrolled and randomized to receive either the protein or a placebo with a 5:1 ratio. In Part B, 48 participants will be enrolled into Group 2 and Group 3 with a 1:1 ratio. Within each group, the participants will be randomized to receive either the vaccine (DNA prime and protein/adjuvant boost in Group 2 and DNA+protein/adjuvant co-administered in Group 3) or a placebo with a vaccine:placebo ratio of 7:1. Therefore, in Group 2, 21 participants will receive the DNA prime and protein/adjuvant boost and 3 will receive the placebo; in Group 3, 21 participants will receive the DNA and protein/adjuvant co-administered and 3 participants will receive the placebo.

To ensure that both men and women will be adequately represented in the trial, the trial will attempt to enroll approximately 40% to 60% of each sex assigned at birth overall in each Part. Hence, at least 5 participants in Part A and 19 participants in Part B of each sex will be enrolled.

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 nine phase 1 and one phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of September 2014), indicate that 10% is a reasonable estimate for the rate of missing data at early timepoints (≤6 months) and 15% for later timepoints (>6 months). For this reason, the sample size calculations in Section 6.1.1 account for 15% enrolled participants having missing data for the primary immunogenicity endpoint.

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 serious adverse events (SAEs) (see Section 11) 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 the vaccine arm (n =10) in Part A, there is a 90% chance of observing at least 1 event if the true rate of such an event is 20.6% or more; and there is a 90% chance of observing no events if the true rate is 1% or less. For each vaccine arm (n =21) in

Part B, there is a 90% chance of observing at least 1 event if the true rate of such an event is 10.4% or more; and there is a 90% chance of observing no events if the true rate is 0.5% or less. For the vaccine arms combined from Part B (n=42), there is a 90% chance of observing at least 1 event if the true rate of such an event is 5.4% or more; and there is a 90% chance of observing no events if the true rate is 0.2% or less. As a reference, in HVTN vaccine trials from December 2000 through April 2014, about 4% of participants who received placebos experienced an SAE.

Binomial probabilities of observing 0, 1 or more, and 2 or more events for the arm of size 10 in Part A, an arm of size 21 in Part B, and for the combined vaccine arms in Part B of size 42 are presented in Table 6-1 for a range of possible true adverse event 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, 1 or more, and 2 or more events, for an arm (or combined arms) of size n=10, 21, and 42, for a range of possible adverse event rates

True event rate (%)	Pr(0/10)	Pr(1+/10)	Pr(2+/10)	Pr(0/21)	Pr(1+/21)	Pr(2+/21)	Pr(0/42)	Pr(1+/42)	Pr(2+/42)
1	90.4	9.6	0.4	81	19	1.9	65.6	34.4	6.6
4	66.5	33.5	5.8	42.4	57.6	20.4	18	82	50.5
10	34.9	65.1	26.4	10.9	89.1	63.5	1.2	98.8	93.2
20	10.7	89.3	62.4	0.9	99.1	94.2	<0.001	>99.9	99.9
30	2.8	97.2	85.1	0.1	99.9	99.4	<0.001	>99.9	>99.9
40	0.6	99.4	95.4	<0.001	>99.9	>99.9	<0.001	>99.9	>99.9

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 adverse event 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 (52). If none of the 42 participants receiving a vaccine regimen in Part B experience a safety event, 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 8.4%. For each individual vaccine arm in Part B (n=21), the 2-sided 95% upper confidence bound for this rate is 15.5%. For the protein only arm in Part A (n=10), the 2-sided upper confidence bound for this rate is 27.8%.

Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 10, 21, and 42

Observed event rate	Confidence interval (%)
0/10	[0, 27.8]
1/10	[1.8, 40.4]
2/10	[5.7, 51]
0/21	[0, 15.5]
1/21	[0.8, 22.7]

2/21	[2.7, 28.9]
0/42	[0, 8.4]
1/42	[0.4, 12.3]
2/42	[1.3, 15.8]

6.1.2 Sample size calculations for immunogenicity

The main goal of this trial regarding immunogenicity outcomes involves studying frequency, magnitude and breadth of IgG and IgA binding antibodies (measured by BAMA) and neutralizing antibodies (nAb) to various Env antigens, as well as studying frequency, magnitude, and quality of CD4 and CD8 T cell responses measured by intracellular cytokine staining. We consider statistical power and precision for studying the first aspect of this goal− immune response rate frequency to a given Env antigen or peptide pool in vaccinees in each Group in Part B. 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 (52). The n = 18 in each arm in Part B assumes a 15% of missing immunogenicity 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 in each vaccine arm (n =18)

No. of responses	Observed response rate (%)	95% Confidence interval
9/18	50	[29, 71]
11/18	61.1	[38.6, 79.7]
13/18	72.2	[49.1, 87.5]
14/18	77.8	[54.8, 91]
16/18	88.9	[67.2, 96.9]

As shown in Table 6-4, there is a limited power for a formal comparison of immunogenicity response rates between vaccine arms of size n = 18. For either 80% or 90% power, the sizes of differences that the trial is powered to detect are fairly large. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05.

Table 6-4 Power for comparison of response rates between 2 vaccine arms of size 18 in each arm (n_1=18, n_2=18)

True response rate in Arm 1 (%)	Minimum true response rate in Arm 2 (%) in order to detect the difference with	
	80% power	90% power
10	58	65
20	71	77
30	81	87
40	89	94
50	95	99

An alternative to formal superiority comparisons of arms is to rank the arms by their response rates. For arms of size 18 with assuming 15% missing immunogenicity data, we can assess the reliability of this study to select the best arm with respect to the magnitude of response rates. For a single assay, Table 6-5, shows for arms of size 18 various true response rates for which this study will correctly select the arm with the highest response rate with 0.8 or 0.9 probabilities. Each line in the table shows the results based on 40,000 simulated datasets of response rates for 2 arms of size 18 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 1 arm (53). If the difference in response 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) among the 2 vaccine arms of size (n_1=18, n_2=18)

Second best response probability	Best response probability	Difference
10%	21% (26%)	11% (16%)
20%	32% (39%)	12% (19%)
30%	44% (52%)	14% (22%)
40%	54% (62%)	14% (22%)
50%	64% (71%)	14% (21%)
60%	73% (80%)	13% (20%)
70%	83% (88%)	13% (18%)
80%	91% (94%)	11% (14%)
90%	98% (100%)	8% (10%)

Response magnitudes between arms will be compared among the positive responders when the response rates between the two arms are similar. Table 6-6 shows the minimum detectable difference in mean (or geometric mean for the $\log_{10}$-transformed) response magnitudes between the two arms of size 18 for 80% and 90% power based on an exact Wilcoxon test with a Type 1 error rate of 0.05, given each common true response rate. The calculations assume that the immunogenicity magnitudes (or after $\log_{10}$ transformation) are normally distributed with a common standard deviation in both groups.

Table 6-6 Minimum detectable difference in mean (or geometric mean for log_{10} -transformed) response magnitudes between two arms of size 18 (n_1=18, n_2=18), when response rates are similar

	Minimum detectable difference in mean (or geometric mean in log_{10}) response magnitude relative to standard deviation	
Common true response rate (%)	Power=80%	Power=90%
50	1.6	1.8
60	1.5	1.8
70	1.4	1.6
80	1.3	1.4
90	1.2	1.3
100	1.1	1.3

6.2 Randomization

A participant's randomization assignment will be computer generated and provided to the HVTN CRS pharmacist through a Web-based randomization system. The randomization will be done in blocks to ensure balance across arms in Part A and in each Group in Part B. The randomization will be stratified by gender. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments (except in emergency situations as specified in the HVTN MOP).

6.3 Blinding

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

Part A will be unblinded independently of Part B. Unblinding need not await completion of health contacts, which are not considered part of the "main study."

When a participant leaves the trial prior to study completion, the participant will be told he or she must wait until all participants in that part of the study (A or B) are unblinded to learn his or her treatment assignment.

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 124 PSRT should be consulted before emergency unblinding occurs.

6.4 Statistical analyses

This section describes the final study analyses, 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. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan (SAP) will address how to analyze the participant's safety data. Analyses are modified intent-to-treat 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 characteristics, 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 of systemic symptoms will be calculated. Wilcoxon rank sum 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. A separate listing will do the same for AEs of special interest (AESI). AESI for this protocol include but are not limited to autoimmune disorders; a sample list of AESI is provided in Appendix L.

6.4.3.3 Local laboratory values

Box plots 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 box plot will show the first quartile, the median, and the third quartile. Outliers (values outside the box plot) 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 Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (see section 9.11) will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also 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 post infection 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 post enrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be 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 (52). Because of the small numbers of control participants in each group, no adjustment will be made to the vaccine arm estimates for the false positive rates in the control arms. Barnard or Fisher's exact tests, as specified in the SAP, 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 general Barnard's is preferred since under most circumstances it is more powerful than Fisher's (54).

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 (55).

For quantitative assay data (eg, IgG binding Ab response from BAMA 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. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be used for graphical display of all of the study arms. Typically the results will be shown for each vaccine arm and for the set of placebo arms pooled into one group.

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.

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, ICS). If treatment arm differences for these assays are best summarized by a mixture model, then either Lachenbruch's test statistic (56) or an alternative two-part test (57) (as defined in the SAP) will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal response distributions. Lachenbruch's 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, linear mixed models or marginal mean models fit by 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 HVTN trials, missing 10-15% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data are missing for an endpoint (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 (58) will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE (59) methods, which are valid under MAR. All of the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

6.4.4.2 Multivariate display of immunogenicity endpoints

Data visualization techniques may be used to explore the relationship among immunogenicity readouts. The set of readouts may be based on one of the primary endpoints (eg, ICS), on the set of primary endpoints, or on immunogenicity endpoints that also include secondary or exploratory endpoints. To understand the relationship between pairs of readouts, scatter plots may be used when the number of readouts is small or for a larger number of readouts, a heat map showing the degree of correlation between any two pairs. Principal component analysis (PCA) and associated 'biplots' of the scores and loadings are particularly useful to understand associations between readouts, especially when readouts are correlated (60). PCA is a method to reduce the dimensionality of the number of readouts to a smaller set of values (principal components) that are normalized linear combinations of the readouts in such a way that the first principal component accounts for the most variability in the data and subsequent components, while maximizing variability, are uncorrelated with each other. A 'biplot' displays the first and second principal component scores and principal component loadings. The x-axis is the value from the first principal component and the y-axis is the second principal component, where each axis label includes the percentage of variation in the total set of readouts captured by the principal component. The top axis is the first principal component loadings and the right axis is the second principal component loadings. An arrow is drawn for each immunogenicity readout (eg, Env-specific CD4+ T cell polyfunctionality score, Env-specific CD8+ T cell total magnitude) from the origin to the point defined by its first two principal component loadings. The length of the arrow represents the amount of total variation of the set of readouts captured by the given readout. The direction of an arrow conveys the extent to which the variation of a readout is in the direction of the first or second principal component. The angle between two arrows conveys information about the correlation of the two readouts, with a zero degree angle denoting perfect correlation and a 90 degree angle denoting no correlation. Each arrow on the biplot is labeled by the immunogenicity readout it represents. A biplot is annotated with key meta-information such as the treatment arm (most common application) or a demographic category. Depending on the application, K-means clustering and hierarchical clustering may also be applied for multivariate graphical display of immunogenicity readouts.

6.4.4.3 Primary analyses of neutralization magnitude-breadth curves

Separate analyses of magnitude-breadth of neutralization will be conducted for the Tier 1B isolate panel and for the Tier 2 isolate panel, for all analyses whether primary, secondary, or exploratory.

For each panel, individual magnitude-breadth curves will be plotted by vaccine arm and pooled placebo, with average magnitude-breadth curves overlaid on the plots. For each panel, the area-under-the-magnitude-breadth curve (AUC-MB) (61) will be computed for each participant, as described in (62). Dunnett's procedure will be applied with 2-sided alpha = 0.05 to determine which of the two vaccine arms have a significantly higher mean AUC-MB than that of the pooled

placebo groups, as described in (63) (see their formula (1.1)). This procedure will be applied to construct 95% CIs about the 2 differences in mean AUC-MB for each vaccine regimen versus the pooled placebos (vaccine – placebo), which simultaneously have at least 95% coverage probability.

The vaccine regimens will be ranked by the estimated mean of the AUC-MB curves. The vaccine regimen with the highest estimated mean will be selected as the best regimen.

6.4.4.4 Secondary analyses of neutralization magnitude-breadth curves

Superiority comparisons of vaccine regimens

Simultaneous 95% CIs about the mean-differences in AUC-MBs for each vaccine regimen versus pooled placebo will be reported. These CIs are computed as the estimated mean-difference plus or minus $t_{N-2,025}$ multiplied by the square-root of S2 $(1/n_1 + 1/n_2)$, where $t_{N-2,025}$ is the 97.5^{th} percentile of a t-distribution with N – 2 degrees of freedom, where N is the total number of vaccine recipients evaluated (summing over the two vaccine regimens). In addition, S2 is an estimate of the common sample variance of the AUC-MB, whereas n_1 and n_2 are the sample sizes of evaluable participants for vaccine regimens 1 and 2 being compared. If the 95% CI comparing the two vaccine arms excludes zero then the two vaccine arms have a significant difference bounding the false positive rate at 5%. Nominal (unadjusted) 95% CIs comparing the vaccine arms will also be reported.

Omnibus comparison of magnitude-breadth distributions

The analyses of magnitude-breadth described above are based on the endpoint area-under-the-curve, which is interpreted as the average $\log_{10}$ IC50 to the set of isolates in the test panel. Use of this endpoint is maximally statistically powerful if 1 vaccine arm has greater magnitude and breadth than the comparator vaccine arm, but may miss an effect wherein 1 vaccine arm has greater magnitude and the comparator vaccine arm has greater breadth. Therefore, a secondary analysis may compare the distribution of magnitude-breadth curves among vaccine arms using the test statistic $\max|B_d^G|$ from Huang, et al (62) (see page 85), which is designed to detect general differences in magnitude-breadth curve distributions.

Selecting the best vaccine regimen

The best vaccine regimen will be deemed as that with the greatest value of the $\max|B_d^G|$ test statistic comparing its distribution of magnitude-breadth curves versus the pooled placebo group.

Superiority comparisons of vaccine regimens

Similarly, the $\max|B_d^G|$ test statistic will be used to compare the distribution of magnitude-breadth curves between the two vaccine regimens. Nominal (unadjusted) 95% CIs comparing the two vaccine arms will also be reported.

6.4.4.5 Analyses of IgG binding antibody magnitude and breadth

Secondary objectives 1 and 3 include the analysis of magnitude and breadth of IgG to panels of Env antigens. For each panel of Envs, the same statistical methods described above for assessing magnitude and breadth of neutralization responses will be used.

6.4.4.6 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. For each T-cell subset, the positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay. In general, the Mixture Models for Single-cell Assays (MIMOSA) statistical framework (64) and/or the Fisher's exact test-based positivity criteria will be used. Details of the positivity criteria will be discussed in the SAP. The magnitude of marginal response will be analyzed as described for quantitative 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. When 3 or more cytokines are being measured by the ICS assay, the polyfunctionality of ICS responses may also be analyzed as an exploratory endpoint. Besides descriptive plots of the magnitude of polyfunctional responses, the COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets) statistical framework (65) may also be used to perform joint modelling of multiple T-cell subsets of different cytokine combinations. For example, the functionality score (FS) and the polyfunctionality score (PFS) may be used to summarize the multi-parameter ICS responses.

6.4.4.7 Analysis of epitope mapping data

The minimal set of optimal length epitopes that can explain the observed T cells responses to a set of individually tested overlapping 15mer peptides may be obtained to assess the breadth and depth of the T cell responses based on the epitope mapping data. When HLA data are also available, computational HLA:peptide binding predictors (eg, NetMHCpan) may be used to more accurately identify each participant's T-cell epitopes.

Once estimates of breadth (number of epitopes) are determined for each participant by the above method or other methods, the distribution of participants with different numbers of total or antigen-specific reactive epitopes may be used to summarize the epitope mapping-based breadth data for each vaccine regimen. Poisson regression models with robust covariance ("sandwich") estimators or other count data analysis methods may be used to compare epitope breadth between vaccine regimens. Zero-inflated models may also be considered if there is interest in differentiating true zero breadth (ie, zero epitope recognized) and excess zeros (ie, no epitope mapping data due to negative T-cell response) in the data.

The set of epitopes may also be used to assess "coverage" of a representative set of circulating viruses by each participant's set of T-cell responses. The coverage provided by each epitope is defined by the fraction of viruses that match the sequence of the mapped epitope. The coverage provided by a participant's set of epitopes is defined by the fraction of viruses covered by at least one of the participant's epitopes.

6.4.4.8 Analysis of multiplexed immunoassay data

When a small panel of analytes (eg, ≤5) is being assessed in a multiplexed immunoassay, the analysis of response rates and response magnitudes will be evaluated and compared as described under the general approach. Details for calculating a positive response and response magnitude will be provided in the SAP. When a larger panel is being assessed, two approaches may be considered to evaluate the magnitude and breadth of these responses. First, Magnitude–Breadth (M-B) curves maybe employed to display individual- and group-level response breadth as a function of magnitude. Two choices are to compare the M-B curves among vaccine arms, as follows: a non-parametric Wilcoxon rank sum test on the subject-specific area-under-the M-B curve (AUC M-B) or a Kolmogorov-Smirnov type test on the 2 group-average M-B curves. Simulations can be used to obtain 2-sided p-values for the latter test. Second, a weighted-average score-like variable may be constructed to account for the correlations between analytes as an integrate magnitude of responses to multiple analytes. Similar group comparison methods described in the first approach may be adopted. Details of either approach will be described in the SAP.

6.4.5 Exploratory analyses of binding IgG antibodies including the Month 3.5 and Month 14 timepoints

To assess Exploratory objective 1, for each participant with IgG binding antibody data measured at the Month 3.5, 8.5, and 14 timepoints, the Scaled area-under-the (time) curve will be calculated based on linear segments between Month 0 and 3.5, 3.5 and 8.5, and 8.5 and 14, divided by 14 months. The individual participant IgG curves over time will be plotted stratified by vaccine arm and for the pooled placebo group, together with estimates of the mean at each of the three timepoints with a 95% confidence interval (based on sample averages and a t-statistic based confidence interval). Moreover, a two-sample t-test with unequal variances will be used to test whether the mean Scaled AUC differs between the two vaccine regimens, and correspondingly a t-statistic based 95% confidence interval about the mean vaccine arm difference in Scaled AUC will be reported.

6.4.6 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.

6.4.6.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study, as defined in Section 3, for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 124 PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.6.2 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, cell-mediated, innate or mucosal) or, 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.

In the phase 1 clinical trial that tested the polyvalent DNA prime-protein boost DP6-001 HIV vaccine regimen, a high level of reactogenicity was observed, including injection site pain and erythema, fevers, myalgia, fatigue, headache, and arthralgias. There were also AEs of delayed type hypersensitivity (DTH) reactions, and one person each developed Henoch-Schonlein purpura and leukocytoclastic vasculitis. The causes of the DTH reactions and the vasculitis events, and their relationship to each other, if any, are not clear. The next generation regimen has undergone significant changes to further enhance the breadth of nAb responses by carefully selecting optimal gene inserts. Additionally, the adjuvant to be administered with the protein vaccine differs from that in the phase 1 trial of DP6-001. However, as a precaution, the exclusion for autoimmune diseases also excludes people with connective tissue disease, or a history of vasculitis, and urinalysis will be performed as part of the safety evaluation after each vaccination.

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

7.1 Inclusion criteria

General and Demographic Criteria

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

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

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

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

5. **Willing to be contacted** 12 months after the last vaccination

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

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

HIV-Related Criteria:

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

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

10. 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. Low risk guidelines are found on the protocol web page under Study Materials on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn124)

Laboratory Inclusion Values

Hemogram/CBC

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

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

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

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

15. **Platelets** = 125,000/mm^3 to 450,000/mm^3

Chemistry

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

Virology

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

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

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

Urine

20. **Normal urine**:

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

Reproductive Status

21. **Volunteers who were born female**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

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

    - Agree to consistently use effective contraception (see Appendix A and Appendix B) for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment until after the last required protocol clinic visit. Effective contraception is defined as using 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 124 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.

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

7.2 Exclusion criteria

General

1. **Blood products** received within 120 days before first vaccination

2. **Investigational research agents** received within 30 days before first vaccination

3. **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, current smoker, known hyperlipidemia

4. **Intent to participate in another study** of an investigational research agent or any other study that requires non-HVTN HIV antibody testing during the planned duration of the HVTN 124 study

5. **Pregnant or breastfeeding**

6. **Active duty and reserve US military personnel**

Vaccines and other Injections

7. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 124 PSRT will determine eligibility on a case-by-case basis.

8. **Non-HIV experimental vaccine(s) received within the last 5 years** in a prior vaccine trial. Exceptions may be made by the HVTN 124 PSRT for vaccines that have subsequently undergone licensure by the FDA. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 124 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 124 PSRT on a case-by-case basis.

9. **Live attenuated vaccines** 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)

10. **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)

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

Immune System

12. **Immunosuppressive medications** received within 168 days before first vaccination (Not exclusionary: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatitis; or [4] a single course of oral/parenteral prednisone or equivalent at doses < 60 mg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment.)

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

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

15. **Autoimmune disease**, connective tissue disease, or history of vasculitis, , eg, leukocytoclastic vasculitis, Henoch-Schonlein purpura

16. **Immunodeficiency**

Clinically significant medical conditions

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

18. **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.

19. **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

20. **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.

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

22. **Asthma exclusion criteria:**

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

    Exclude a volunteer who:

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

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

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

25. **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.

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

27. **Malignancy** (Not excluded from participation: 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)

28. **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.

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

30. 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 11.4.

7.3.1 Delaying vaccinations for a participant

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

- Within 45 days prior to any study injection
  - Receipt of blood products or immunoglobulin
- Within 30 days prior to any study injection
  - Receipt of live attenuated vaccines
  - Receipt of allergy treatment with antigen injections
- Within 14 days prior to any study injection
  - Receipt of any vaccines that are not live attenuated vaccines (eg, pneumococcal)
- Pre-vaccination 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 124 Study Specific Procedures.

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

7.3.2 Participant departure from vaccination schedule

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

7.3.3 Discontinuing vaccination for a participant

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

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 124 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
  - Vasculitis, regardless of grade or relatedness
  - 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; upon review, the PSRT may allow continuation of vaccination if the event is grade 3 erythema and/or induration
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 124 PSRT is required

prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination

- DTH reaction at previous study agent injection sites

- Investigator determination in consultation with Protocol Team leadership (eg, for repeated nonadherence to study staff instructions).
- Participant misses more than 2 vaccinations(s) (see Section 7.3.2).

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

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

7.3.4 Participant termination from the study

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

- Participant refuses further participation,
- Participant relocates and remote follow-up or transfer to another HVTN CRS is not possible,
- HVTN CRS determines that the participant is lost to follow-up,
- Participant becomes HIV infected, or
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff).
- Any condition where termination from the study is required by applicable regulations.

8 Study product preparation and administration

CRS 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

Part A:

Group 1

Treatment 1 (T1):

400 mcg Recombinant gp120 (A,B,C,A/E) admixed with GLA-SE, to be administered as a 1 mL (IM) injection in the deltoid of the non-dominant arm (unless medically contraindicated) at months 0 and 2.

OR

Control 1 (C1):

Placebo for Recombinant gp120 (A,B,C,A/E) (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 1 mL (IM) injection in the deltoid of the non-dominant arm (unless medically contraindicated) at months 0 and 2.

Part B:

Group 2

Treatment 2 (T2):

2 mg Env (A,B,C,A/E) / Gag (C) DNA plasmids (labeled as PDPHV-201401 Plasmid) to be administered as a 0.8 mL (IM) injection in the deltoid of the LEFT arm (unless medically contraindicated) at months 0, 1 and 3

AND

Placebo for Recombinant gp120 (A,B,C,A/E) (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 1 mL (IM) injection in the deltoid of the RIGHT arm (unless medically contraindicated) at months 0, 1 and 3.

THEN

Placebo for Env (A,B,C,A/E) / Gag (C) DNA plasmids (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 0.8 mL (IM) injection

in the deltoid of the LEFT arm (unless medically contraindicated) at months 6 and 8

AND

400 mcg Recombinant gp120 (A,B,C,A/E) admixed with GLA-SE, to be administered as a 1 mL (IM) injection in the deltoid of the RIGHT arm (unless medically contraindicated) at months 6 and 8.

OR

Control 2 (C2):

Placebo for Env (A,B,C,A/E) / Gag (C) DNA plasmids (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 0.8 mL (IM) injection in the deltoid of the LEFT arm (unless medically contraindicated) at months 0, 1, 3, 6 and 8.

AND

Placebo for Recombinant gp120 (A,B,C,A/E) (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 1 mL (IM) injection in the deltoid of the RIGHT arm (unless medically contraindicated) at months 0, 1, 3, 6 and 8.

Group 3

Treatment 3 (T3):

2 mg Env (A,B,C,A/E) / Gag (C) DNA plasmids (labeled as PDPHV-201401 Plasmid) to be administered as a 0.8 mL (IM) injection in the deltoid of the LEFT arm (unless medically contraindicated) at months 0, 1, 3, 6 and 8.

AND

400 mcg Recombinant gp120 (A,B,C,A/E) admixed with GLA-SE, to be administered as a 1 mL (IM) injection in the deltoid of the RIGHT arm (unless medically contraindicated) at months 0, 1, 3, 6 and 8.

OR

Control 2 (C2):

Placebo for Env (A,B,C,A/E) / Gag (C) DNA plasmids (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 0.8 mL (IM) injection in the deltoid of the LEFT arm (unless medically contraindicated) at months 0, 1, 3, 6 and 8.

AND

Placebo for Recombinant gp120 (A,B,C,A/E) (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 1 mL (IM) injection in the deltoid of the RIGHT arm (unless medically contraindicated) at months 0, 1, 3, 6 and 8.

8.2 Study product formulation

8.2.1 GLA-SE (glucopyranosyl lipid adjuvant-stable emulsion, labeled as AP 10-205)

The GLA-SE (labeled as AP 10-205) adjuvant will be provided in 2 mL vials containing 6 mcg GLA in a 6% oil-in-water emulsion. Each sterile, single use vial contains 0.4 mL of product at a concentration of 15 mcg/mL. The product appears as a milky-white liquid. GLA-SE must be stored at 2-8°C and must not be frozen. The study product is described in further detail within the GLA-SE IB.

8.2.2 Env (A,B,C,A/E) / Gag (C) DNA plasmids

The Polyvalent DNA (PDPHV-201401 Plasmid) vaccine will be supplied in 2 mL vials containing 1 mL polyvalent DNA vaccine at a concentration of 2.5 mg/mL. The product must be stored at ≤ -60°C. (See study product IB for detailed vaccine components.)

8.2.3 Recombinant gp120 proteins (A, B, C & A/E)

Each gp120 protein (PDPHV-201401 Recombinant Proteins gp120A, gp120B, gp120C, gp120AE) will be supplied as individual vials each containing 0.3 mg gp120 protein in a total fill volume of 0.5 mL.

The final concentration per each vial of protein is 0.6 mg/mL. The product must be stored at ≤ -60°C. (See study product IB for detailed vaccine components.)

8.2.4 Placebo for Recombinant gp120 proteins (A, B, C & A/E) and Env (A,B,C,A/E) / Gag (C) DNA plasmids

Sodium Chloride for injection, 0.9% USP will be used as the placebo. It must be stored as recommended by the manufacturer.

8.3 Preparation of study products

8.3.1 Env (A,B,C,A/E) / Gag (C) DNA plasmids

One vial of polyvalent DNA (PDPHV-201401 Plasmid) vaccine 2.5 mg/mL will be required to prepare the study dose.

Prior to the preparation of the study dose, the pharmacist will remove one vial of polyvalent DNA vaccine from the freezer and allow to thaw completely at ambient room temperature. Preparation of the vaccine for injection can be

performed at the ambient temperature of the pharmacy, but the vaccine dose should be prepared and used within 2 hours of removing from storage at ≤ -60°C. Gently swirl and invert the vial for at least 10 inversions (do not shake vigorously).

Using aseptic technique, withdraw 0.8 mL of polyvalent DNA vaccine into a 1 mL syringe, remove the needle and cap syringe.

The final syringe for administration must be covered with an overlay and then labeled as "Polyvalent DNA vaccine or Placebo". The syringe must also be labeled for IM administration into the LEFT deltoid, with an expiration date and time of 2 hours.

Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in accordance with institutional or pharmacy policy for a biological safety level S1 product.

8.3.2 Recombinant gp120 proteins (A, B, C & A/E) admixed with GLA-SE

One vial of each gp120 protein (0.3 mg/0.5 mL) (4 vials total) and one vial of GLA-SE (6 mcg/0.4 mL) will be needed to prepare the study dose.

Prior to the preparation of the study dose, the pharmacist will remove one vial each of the recombinant gp120 proteins (gp120A, gp120B, gp120C, gp120AE) from the freezer and allow to thaw completely at ambient room temperature. Preparation of the vaccine for injection can be performed at the ambient temperature of the pharmacy, but the vaccine dose should be prepared and used within 2 hours of removing from storage at ≤ -60°C. Gently swirl and invert each vial for at least 10 inversions (do not shake vigorously). The pharmacist will also remove one vial of GLA-SE from the refrigerator.

Using aseptic technique, the pharmacist will withdraw 0.2 ml from each vial of gp120A, gp120B, gp120C, and gp120AE using a separate 1 mL syringe for each and inject them directly into the vial containing 0.4 mL GLA-SE. The pharmacist will then mix the vial containing the 1.2 mL mixture of gp120A, gp120B, gp120C, gp120AE, and GLA-SE. Gently swirl and invert the vial for at least 10 inversions (do not shake vigorously).

The pharmacist using aseptic technique will withdraw 1 mL of the polyvalent gp120 protein vaccine into a 3 mL syringe, remove the needle and cap the syringe.

The final syringe for administration must be covered with an overlay and then labeled as "Polyvalent protein vaccine or Placebo". For Group 1, the syringe must be labeled for IM administration into the deltoid of the non-dominant arm. For Groups 2 and 3, the syringe must be labeled for IM administration into the RIGHT deltoid. The syringe must also be labeled with an expiration date and time of 2 hours.

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

8.3.3 Placebo for Env (A,B,C,A/E) / Gag (C) DNA plasmids

The pharmacist using aseptic technique will withdraw 0.8 mL of Sodium Chloride for Injection, 0.9% USP into a 1 mL syringe, remove the needle and cap the syringe.

The final syringe for administration must be covered with an overlay and then labeled as "Polyvalent DNA vaccine or Placebo". The syringe must also be labeled for IM administration into the LEFT deltoid, with an expiration date and time of 2 hours following.

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

8.3.4 Placebo for Recombinant gp120 proteins (A, B, C & A/E)

The pharmacist using aseptic technique will withdraw 1 mL of Sodium Chloride for Injection, 0.9% USP into a 3 mL syringe, remove the needle and cap the syringe.

The final syringe for administration must be covered with an overlay and then labeled as "Polyvalent protein vaccine or Placebo". For Group 1, the syringe must be labeled for IM administration into the deltoid of the non-dominant arm. For Groups 2 and 3, the syringe must be labeled for IM administration into the RIGHT deltoid. The syringe must also be labeled with an expiration date and time of 2 hours following.

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

8.4 Administration

Administration of vaccine or placebo for Group 1 consists of 1 injection at each vaccination timepoint. Administration of vaccine or placebo for Groups 2 and 3 consists of 2 vaccinations at each timepoint.

All injections will be given by needle and syringe. Group 1 injections are given in the non-dominant deltoid, unless medically contraindicated. For Groups 2 and 3, Polyvalent DNA vaccine or placebo injection will be given in the LEFT deltoid, and Polyvalent protein vaccine or placebo will be given in the RIGHT deltoid, unless medically contraindicated.

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.

Any administrator of study product will be blinded to the individual participant's treatment assignment.

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.

8.5 Acquisition of study products

PDPHV-201401 Plasmid (the Env (A,B,C,A/E) / Gag (C) DNA plasmids) and PDPHV-201401 Recombinant Proteins gp120A, gp120B, gp120C, gp120AE (the Recombinant gp120 (A,B,C,A/E) vaccines) will be provided by the University of Massachusetts Medical School.

The GLA-SE (AP 10-205) adjuvant will be provided by Infectious Disease Research Institute (IDRI).

The placebo (Sodium Chloride for Injection, USP 0.9%) will not be provided through the protocol but must be purchased by the site.

The syringes and needles used for the preparation and delivery of the study products will not be provided through the protocol and must be purchased 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 J and Appendix K.

9.1 Informed consent

Informed consent is the process of working with participants so that they 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 their IRB/EC and any applicable 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 [informed consent forms] 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 RSC Protocol Registration Office.

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. The sample protocol-specific consent forms for the study are is located in Appendix A and Appendix B. A separate sample consent form for other uses of specimens is located in Appendix D.

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, Appendix B, and Appendix D. The consent form(s) must be developed in accordance with requirements of the following:

- CRS's IRB/EC and any applicable REs,
- CRS's institution, 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 Understanding (see Section 9.1.3)
- 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;
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - HBsAg,
  - Anti-HCV antibodies,
  - Syphilis test,
  - CBC with differential and platelets,

  - Chemistry panel (ALT, AST, alkaline phosphatase, and creatinine),
  - Urine dipstick (as described in Section 9.10), and
  - Urine or serum pregnancy test (participants who were born female)

- Pap smear (only for volunteers who were born female and are age 21 or older) Pap smear is not required:
  - if volunteer disagrees to provide cervicovaginal fluid samples,
  - if volunteer has reported having had a Pap smear within previous 5 years with most recent result normal or ASCUS [atypical squamous cells of undetermined significance] with no evidence of high-risk HPV), or
  - if high-risk HPV testing was not conducted, and volunteer has reported having had a Pap smear within the 3 years prior to enrollment, with the latest result normal or ASCUS.

- Administration of behavioral risk assessment questionnaire;

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

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

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

9.2.1 Use of screening results from another HVTN study

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

9.3 Enrollment and vaccination visits

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

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

- Abbreviated physical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of baseline reactogenicity parameters;
- Assessment of concomitant medications (as described in Section 9.2);
- Assessment of any new or unresolved AEs/intercurrent illnesses; and
- Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

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

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 memory tool and is instructed on how to complete it. The site will make arrangements to be in contact with the participant during the reactogenicity period (as described in Section 9.11).

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

- Risk reduction counseling (as described in Section 9.8);
- Pregnancy prevention assessment (as described in Section 9.2 and Section 9.9); and

- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation).

Additional procedures will be performed at scheduled visits as specified in Appendix H and Appendix K:

- HIV infection assessment including pretest counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of 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 control;
- Specimen collection (blood, mucosal, and/or stool) should be completed prior to vaccination);
- Clinical laboratory tests for study participants who agree to provide semen, rectal, or cervicovaginal fluid samples, including:
  - Syphilis;
  - Gonorrhea and chlamydia;
  - Trichomonas vaginalis (for participants providing cervicovaginal samples);
  - Bacterial vaginosis (for participants providing cervicovaginal samples); and
  - Yeast (for participants providing cervicovaginal samples, if clinically indicated).

9.4 Follow-up visits

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

- Risk reduction counseling (as described in Section 9.8);

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

Additional procedures will be performed at scheduled follow-up visits as specified in Appendix J, Appendix H and Appendix K:

- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;
- Administration of behavioral risk assessment questionnaire;
- HIV infection assessment including pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Specimen collection (blood, mucosal and/or stool);
- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.10); and

- Clinical laboratory tests for study participants who agree to provide (semen, rectal, or cervicovaginal) mucosal fluid samples, including:
  - Syphilis
  - Gonorrhea and chlamydia
  - Trichomonas vaginalis (for participants providing cervicovaginal samples),
  - Bacterial vaginosis (for participants providing cervicovaginal samples),
  - Yeast (for participants providing cervicovaginal samples, if clinically indicated)

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

9.5 Mucosal fluid sampling

Mucosal samples will be collected at the timepoints indicated in Appendix I from the study participants who agree to these procedures.

Participants who consent to provide cervicovaginal, rectal, or semen samples will be tested for the following infections at the mucosal sampling visits: gonorrhea, chlamydia, and syphilis. Participants who consent to provide cervicovaginal fluid samples will be tested for trichomoniasis and for bacterial vaginosis and (if clinically indicated) for hyphae/budding yeast. Test results will be provided to participants and all participants who test positive for 1 or more of these infections will receive counseling as well as treatment (or referral for treatment) as appropriate. Sample collection may not be performed or may be deferred to a later date within the visit window if a contraindication to sampling (eg, active GTI) is present (as indicated below).

Salivary fluid sampling (optional, both sexes): Participants willing to provide salivary samples should abstain from smoking, eating, or drinking anything other than water, brushing their teeth, using mouthwash, chewing gum or tobacco, or engaging in intimate oral activity for one hour prior to sample collection.

Rectal fluid sampling (optional, both sexes): For participants born female, a pregnancy test must be performed and be negative prior to any rectal mucosal sampling. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Rectal fluid sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, if still within the visit window.

Rectal sampling will not be performed (or may be deferred to a later date if still within the visit window) if there is a contraindication to rectal fluid sampling, such as an active infection or inflammation of the colorectal area [such as a herpes simplex virus (HSV)-2 outbreak or inflamed hemorrhoids or colitis/diarrhea] or if the participant has any active genital tract infection (GTI).

For 48 hours prior to sample collection, participants should abstain from:

- Receptive anal sex,
- Insertion of any foreign object or substance into the anus (including but not limited to cleaning products [creams, gels, lotions, pads, etc.], lubricant, enemas, and douching even with water), and
- Using perianal or intra-anal steroid or other anti-inflammatory cream in or around the anus.

Cervicovaginal fluid sampling (optional, only for participants who were born female): Participants who are 21 years of age and older must report having had a Pap smear within the 3-5 years prior to enrollment, with the latest result reported as normal or ASCUS (see Section 7.1). A pregnancy test must be performed on the day of cervicovaginal sampling. The pregnancy test can be performed after collection has taken place. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Cervicovaginal sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, if still within the visit window. In addition, cervicovaginal sampling will not be performed (or may be deferred to a later date if still within the visit window) if a participant is known to have an active ulcerative genital lesion or an active GTI at the scheduled timepoint. Participants providing cervicovaginal fluid samples should be advised as follows:

- Do not use anything with spermicide, lubricants, or topical/intravaginal medications (eg, topical yeast infection treatments) for 48 hours before the samples are collected;
- Do not douche for 48 hours before the samples are collected;
- Do not have vaginal sex and/or insert any foreign object or substance into the vagina for 48 hours before the samples are collected;

Semen sampling (optional, only for participants who were born male): For at least 48 hours prior to specimen collection, participants providing semen samples are asked:

- not to have sex, including insertive oral sex
- not to ejaculate

- not to use anything with lubricants
- not to put saliva on the penis

In addition, semen sampling will not be performed (or may be deferred to a later date if still within the visit window) if a participant is known or believes to have an active GTI at the scheduled timepoint.

9.6 Stool sampling

Two stool samples will be collected at the timepoints indicated in Appendix I from the study participants who agree to this procedure. These samples will be collected using swabs, either via rectal swabs or by taking swabs from stool. Information will be collected from these participants about dietary habits, antibiotic use, and gastrointestinal symptoms.

9.7 Health contact at 12 months after last vaccination visit

CRS staff will contact study participants at 12 months after the last vaccination to collect the information listed below. This contact may be accomplished by phone, text message, or email. Clinic visits will only be required if HIV confirmatory testing is necessary (see Section 9.8.1); however, a clinic visit may be arranged for other reasons.

- Confirmation of vital status; if deceased, attempt to learn cause and date of death;
- If participant is alive, report any of the following events:
  - New or updated AEs related to study product(s), including:
    - Life-threatening adverse experiences;
    - Persistent or significant disability/incapacity;
    - Hospitalizations and reasons;
    - Other important medical events that may jeopardize the participant or may require intervention to prevent 1 of the other outcomes listed above;
    - New chronic conditions requiring more than 30 days of medical intervention or medication;
  - AESI (Section 11.2.2). A sample list of AESI is provided in Appendix L. AESI are reported regardless of relationship to study product(s).
  - New diagnosis of HIV infection; and
  - Pregnancies and outcomes, including congenital anomalies/birth defects.

All such events will be recorded, and adverse events will be assessed for relationship to study product(s).

9.7.1 Interim contacts

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

9.8 HIV counseling and testing

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

Participants will be counseled routinely during the trial on the avoidance of HIV infection and on the potential negative social impacts of testing antibody positive due to the vaccine. They will also be counseled on the risks of HIV antibody testing outside of the HVTN CRSs and will be discouraged from doing so during study participation and/or during any period of vaccine-induced positive serology.

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

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

9.8.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

The study product may elicit an antibody 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 J and Appendix K. 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 J and Appendix K). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see *Study Specific Procedures* [SSP]), which is able to distinguish vaccine-induced antibody 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 antibody 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) unless or until HIV Ab testing is no longer the standard test in clinical settings.

9.8.2 VISP registry

Experimental HIV vaccines may induce antibody production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP). 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.

9.9 Contraception status

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

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

9.10 Urinalysis

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

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

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

9.11 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are followed until resolution and graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, except as noted in Section 11.2.2.

The reactogenicity assessment period is 7 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record symptoms using a postvaccination memory tool. Contacts between the participant and the site staff should take place daily between 1-3 days postvaccination for the

collection of reactogenicity data, and 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. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 to resolution.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, vaccine-related lesions, and lymph nodes. Events not listed on a CRF, or with an onset after the reactogenicity assessment period (day of vaccination and 7 full days after), or those meeting SAE/adverse events 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
1-7[b]	Between 12:00am and 11:59pm on the respective day	HVTN CRS staff or participant

[a] Day of vaccination

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

9.11.1 Assessment of systemic and local symptoms

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

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

9.11.2 Assessment of injection site

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

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

9.11.3 Assessment of lymph nodes

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

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

9.12 Evaluation of possible DTH or vasculitic reactions to vaccine

As described in Section 4.8.1, delayed-type hypersensitivity reactions were observed in recipients of DNA and protein vaccinations in the study of the DP6-001 vaccines. They were not seen in the preclinical safety studies of the DP6-001 products prior to the clinical trial, and there is no known predictive model for whether the PDPHV-201401 vaccines might also elicit DTH reactions. There were also two participants who developed vasculitis in the study of DP6-001. The safety of trial participants is the major priority of the HVTN and protocol team, and monitoring for DTH reactions or vasculitis that may be related to vaccination is an important part of this protocol.

The DTH reactions and vasculitis events in the study of the DP6-001 vaccines arose within a few days of vaccination. The DTH reactions typically occurred after the second or subsequent immunizations. If similar reactions were to occur in HVTN 124, they would likely appear within the 7-day reactogenicity period. DTH reactions may be difficult to distinguish from common vaccine reactogenicity such as injection site erythema, swelling, and pruritus. Vasculitis syndromes are systemic illnesses with variable presentations. In the case of DP6-001, both affected participants had a purpuric rash. The study includes urine dipstick assessments after each vaccination to look for protein or blood, which may be renal manifestations of vasculitis.

Any participant who develops a possible DTH reaction should be promptly recalled to clinic for assessment and evaluation of the injection sites and affected areas. Photography of skin lesions is optional for the participant, but encouraged. The participant may be asked to provide additional photographs of skin lesions if they continue during the days that follow the initial evaluation. Referral to a dermatologist to obtain a skin biopsy is encouraged.

For participants who have developed a possible vasculitis, the assessment should include a symptom-directed physical exam including inspection of injection sites and affected areas, CBC with differential, serum chemistries, urine dipstick (with urine microscopy, if indicated), ESR, CRP, and photography of injection sites and affected areas (optional for the participant, but strongly encouraged). HLA-typing may be requested from stored PBMC. The participant may be referred for

additional evaluation (eg, by a dermatologist and/or rheumatologist), and a skin biopsy may be obtained, with separate participant consent. Records of these contacts will be requested to complete the study record.

DTH reactions and vasculitis events that are related to vaccination must be promptly reported according to the AE notification and safety pause/AE review rules (Table 11-1) and, if applicable, the requirements for expedited reporting of AEs to DAIDS (see Section 11.2.3).

9.13 Visit windows and missed visits

Visit windows are defined in HVTN 124 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.

9.14 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, and chemistry panel), pregnancy testing, social impact assessment, and HIV test.

9.15 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. 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. Pregnancies and pregnancy outcomes will be reported.

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN 124 Site Lab Instructions and SSP provide further guidelines for operational issues concerning the clinical and processing laboratories. These documents include 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 H and Appendix I. For tests performed locally, the local lab may assign appropriate tube types.

10.2 Total blood volume

Required blood volumes per visit are shown in Appendix H and Appendix I. 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 timepoints in this study occur 2 weeks after the last vaccination for Parts A and B. Endpoint assays for humoral and cellular responses are performed on specimens collected from participants at the primary immunogenicity timepoints and may be performed on samples collected at baseline. Depending on the initial results, assays for humoral and cellular responses may be performed on samples collected from participants at other timepoints; the schedule is shown in Appendix H and Appendix I.

10.4 Endpoint assays: cellular

10.4.1 Flow cytometry (Parts A and B)

Flow cytometry will be used to examine vaccine-specific CD4+ and CD8+ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine. ICS parameters will include cytokines such as IFN-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-α, and may include other cytokines (such as cytokines relevant to Th2 and Th17 responses) to identify T cells of specific functionality . Markers of cytotoxic potential (eg, granzyme B and that identify circulating T follicular helper (Tfh) cells (e.g., CXCR5 and PD-1) 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 Binding antibody multiplex assay (Parts A and B)

HIV-1-specific total gp120 and gp70 V1V2 binding IgG antibodies will be assessed on serum samples from study participants taken at the primary immunogenicity timepoints and baseline. In addition, HIV-1-specific total binding IgA antibodies and binding to IgG subclasses (IgG1, IgG2, IgG3, and IgG4) may also be assessed. Specimens from other timepoints may also be assayed based on the results of the initial assay.

10.5.2 Neutralizing antibody assay (Part B)

HIV-1–specific nAb assays will be performed on serum samples from study participants taken at the primary immunogenicity timepoint(s). 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 timepoints. The TZM-bl assay will test neutralization of the vaccine strains and at least one highly neutralization-sensitive tier 1A virus. The global panel and/or clade-specific panels may be used to assess tier 2 virus neutralization (61, 66). Additional TZM-bl assays may be performed to test neutralization of Tier 1B strains.

10.5.3 Antibody-Dependent Cellular Cytotoxicity assay (ADCC) (Part B)

ADCC activity may be assessed using serum samples from study participants taken at the primary immunogenicity timepoints. 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 timepoints. For the Granzyme B flow-based cytotoxicity assay, participant sera are incubated with effector cells and gp120-coated target cells. ADCC is quantified as net percent granzyme B activity which is the percent of target cells positive for GranToxiLux (GTL), an indicator of granzyme B uptake, minus the percent of target cells positive for GTL when incubated with effector cells but without sera. For the Luciferase-based cytotoxicity assay, participant sera are incubated with infectious molecular clone (IMC)-infected cells and percent killing is measured through the use of Viviren luminescence.

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.

10.8.1 B-cell ELISpot (Part B)

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

10.8.2 B-cell Phenotyping (Part B)

Antigen-specific B cells induced by vaccination will be identified and characterized using fluorescently-labeled recombinant proteins in combination with a flow cytometry phenotyping panel. In particular, HIV Env-reactive B cells will be enumerated and may be further characterized for expression of memory, activation, inhibitory or other markers by protein and/or gene expression. Antigen-specific B cells may also be sorted for further analysis by B cell receptor sequencing or gene expression analysis. Lastly, functional properties may be confirmed by cloning of the B cell receptor (VH and VL) genes into an IgG expression vector in order to express the antibody for functional characterization using binding, neutralization or other assay.

10.8.3 Tfh phenotyping

Flow cytometry may be used to identify peripheral blood follicular helper T (pTfh) cells. Phenotyping of pTfh cells will be based on expression of CXCR5 and PD-1 on CD4+ T cells, and may include additional markers. For example, if flow cytometry panels are successfully developed to assess CD69, OX40, IL-21

and CD154 expression as a functional read-out, vaccine-specific Tfh cells may be assessed and isolated for further analysis.

10.8.4 B cell plasmablasts

Flow cytometry may be used to identify the early precursors for antibody-secreting B cells, called B cell plasmablasts, in the peripheral blood. Plasmablasts will be mainly characterized by CD19+ CD20- and high expression of CD27 and CD38. The flow cytometry panels might also include additional markers and assessment of antigen specificity.

10.8.5 Mucosal studies (Part B)

Saliva, cervicovaginal fluid, rectal fluid, and semen samples will be collected at timepoints indicated in Appendix I, then processed and assessed for HIV-specific IgA and IgG antibodies. Additional exploratory analyses may be performed on these samples.

10.8.6 Microbiome analysis

Swabs of stool will be shipped to a central laboratory. Specimens are processed to enable nucleic acid sequencing. 16s rRNA sequences will then be determined using sequencing approaches or other methods.

10.9 Specimen storage and other use of 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 covered by the protocol or the informed consent forms for the study (see Appendix A and Appendix B).

This research may relate to HIV, vaccines, the immune system, and other diseases. This could include 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 research on specimens ('other use') 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/REs if required.

As part of consenting for the study, participants document their initial decision to allow or not allow their specimens to be used in other research, and they may change their decision at any time. The participant's initial decision about other use of their specimens, and any later change to that decision, is recorded by their CRS in a Web-based tool that documents their current decisions for other use of their specimens. The HVTN will only allow other research to be done on specimens from participants who allow such use.

CRSs must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on specimen storage or 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 124 PSRT

The HVTN 124 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 124 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, clinical data manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 124 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. The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS. The SMB conducts additional special reviews at the request of the HVTN 124 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 124 PSRT and HVTN SMB (see Section 11.1.2);

11.1.4 HVTN Core roles and responsibilities in safety monitoring

- Daily monitoring of clinical data for events that meet the safety pause and HVTN 124 PSRT AE review criteria (see Section 11.4);

- Notifying HVTN CRSs and other groups when safety pauses or planned holds are instituted and lifted (see Section 11.4);

- Querying HVTN CRSs for additional information regarding reported clinical data; and

- Providing support to the HVTN 124 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 resubmitted 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 (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, available on the RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables, except:

- Unintentional weight loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health (see HVTN 124 Study Specific Procedures);

- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:

- Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area;
- Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area;
- Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage;
- Grade 4 is: Potentially life-threatening consequences (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue).

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 (see Section 11.2.3), and (2) if the AE meets the criteria for a safety pause/prompt AE review (see Section 11.4), and (3) if the AE is an AESI. A sample list of AESI is provided as Appendix L.

Sites are expected to notify HVTN clinical safety staff of any serious safety concern requiring their attention (see Table 11-1). Telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn124). 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 (local time) to confirm that the email has been received and reviewed. If email service is not available, the CRS should notify clinical safety staff 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/clinical-research-sites/safety-reporting/manual. The SAE Reporting Category will be used for this study.

The internet-based DAIDS Adverse Experience Reporting System (DAERS) must be used for expedited AE (EAE) reporting to DAIDS. In the event of system outages or technical difficulties, expedited AE reports may be submitted via the DAIDS EAE Form. This form is available on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids/paper-eae-reporting.

For questions about DAERS, please contact CRMSsupport@niaid.nih.gov or from within the DAERS application itself.

For questions about expedited AE reporting, please contact the DAIDS RSC Safety Office at (DAIDSRSCSafetyOffice@tech-res.com).

The study products for which expedited reporting are required are

- Env (A, B, C, A/E) / Gag (C) DNA plasmids or placebo
- Recombinant gp120 (A, B, C, A/E) proteins/GLA-SE adjuvant or placebo

In addition to the expedited Reporting Category identified above, any AE of vasculitis related to study product(s) must also be reported in an expedited manner.

While the participant is in the study reporting period (Section 3, Duration per participant), 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 routinely 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 is appropriate (eg, if 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 individual (eg, 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, see Section 11.4.

11.3 Safety reviews

11.3.1 Initial safety evaluation

Enrollment across all participating HVTN CRSs will be restricted to a maximum of 1 participant per day until 6 participants have been enrolled in Group 1. The HVTN 124 PSRT will review the cumulative safety data including at minimum local and systemic reactogenicity data reported for the first 72 hours postvaccination on each of these 6 participants, and will determine whether it is safe to proceed with full enrollment in Group 1. The PSRT may consult with the HVTN SMB on an ad hoc basis for this evaluation.

11.3.2 Safety evaluation for moving from Part A to Part B

In addition to monitoring participant safety throughout the study period, the HVTN 124 PSRT will review all cumulative safety data available from Group 1, when data is available for the first 6 participants, up to and including the 2-week visit after the second vaccination, and from the second 6 participants, up to and including the 2-week visit after the first vaccination. Based on the assessment of this safety data, the PSRT will make a decision regarding the appropriateness of moving to Part B. The PSRT may consult with the HVTN SMB on an ad hoc basis for this evaluation.

11.3.3 Interim safety evaluation for Part B

Part B will first open to enrollment to 10 participants, randomized between Group 2 and Group 3. The PSRT will review all cumulative safety data available from the first 10 participants in Part B, up to and including the 2-week visit after the second vaccination, to make a decision regarding the appropriateness of proceeding with full enrollment in Part B. Based on the assessment of this safety data, the PSRT will make a decision regarding the appropriateness of proceeding with full enrollment in Part B. The PSRT may consult with the HVTN SMB on an ad hoc basis for this evaluation.

11.4 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 124 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 124 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[a]	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and submit forms immediately	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately	Immediate HVTN 124 PSRT notification
SAE, related	Grade 3	Email and submit forms immediately	Immediate HVTN 124 PSRT notification and prompt HVTN 124 PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and submit forms immediately	Immediate HVTN 124 PSRT notification and prompt HVTN 124 PSRT AE review to consider pause
Vasculitis, related	Any grade	Phone immediately, email and submit forms immediately	Immediate pause
DTH responses, related	Any grade	Email and submit forms immediately	Prompt HVTN 124 PSRT notification and AE review to consider pause

[a] Phone numbers and email addresses are found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn124).

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

For all safety pauses, HVTN Core notifies the HVTN 124 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 notifies the SMB.

Once a trial is paused, the HVTN 124 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 124 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 124 PSRT notification or prompt HVTN 124 PSRT AE review is triggered, HVTN Core notifies the HVTN 124 PSRT as soon as possible during working hours (local time)—or, if the information was received during off hours, by the morning of the next workday. If a prompt HVTN 124 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

The HVTN requires that each CRS submit to its IRB/EC and any applicable RE 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 124 PSRT (see Section 11.5.2).

11.5 Review of cumulative safety data

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

Reviews proceed from a standardized set of protocol-specific safety data reports. These reports are produced by the SDMC and include queries to the HVTN CRSs. Events are tracked by internal reports until resolution.

11.5.1 Daily review

Blinded daily safety reviews are routinely conducted by HVTN Core for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 124 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the HVTN 124 PSRT reviews clinical safety reports on a weekly basis and conducts 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 124 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.

11.6 Study termination

This study may be terminated early by the determination of the HVTN 124 PSRT, a pertinent national regulatory authority, NIH, Office for Human Research Protections (OHRP), the Food and Drug Administration (FDA), or vaccine developer(s). 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;
- Exploratory and ancillary studies and sub-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 124 *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 or synthetic DNA, it must comply with regulations set forth in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016).* Information about the study must be submitted to site Institutional Biosafety Committees (IBCs) and Institutional Review Boards/Ethics Committees (IRBs/ECs). IBCs and IRBs/ECs must provide a written assessment of whether Recombinant DNA Advisory Committee (RAC) review is warranted. In exceptional cases that meet specific criteria outlined in the NIH Guidelines Appendix M-I-B, IBCs and IRBs/ECs assessment can include a request for RAC review. Regardless of the potential for RAC review, the HVTN and DAIDS will register the protocol with the NIH Office of Scientific Policy (OSP). The Protocol Team, jointly with the University of Massachusetts and IDRI, will prepare the NIH OSP registration documents.

Investigators at each site are responsible for obtaining IBC approval per NIH Guidelines *section IV-B7-a-(1).* IBC review and approval must be documented by the investigator and submitted as part of DAIDS's initial protocol registration for this trial before participants are enrolled at the site. If this protocol is amended, investigators should follow the requirements of their IBC.

The HVTN and DAIDS will ensure that reporting requirements to NIH OSP, as outlined in *Appendix M-I-C-1.Initiation of the Clinical Investigation*, *Appendix M-I-C-2.Additional Clinical Trial Sites. Appendix M-I-C-3.Annual Reports* and *Appendix M-I-C-4.Safety Reporting* are satisfied per the NIH Guidelines.

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 124 are described below.

Protocol history and modifications

Date: September 28, 2017

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 https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures
- Division of AIDS Protocol Registration Manual. Available at https://www.niaid.nih.gov/sites/default/files/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017. Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 124 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 124 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN 124 Site Lab Instructions. Accessible through the HVTN protocol-specific 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/publications/Pages/index.aspx
- Lab assay algorithm (available upon request)

- 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/products/guidelines/efficacy/article/efficacy-guidelines.html

- Participants' Bill of Rights and Responsibilities. Accessible through the HVTN website.

- NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Available at http://osp.od.nih.gov/sites/default/files/resources/NIH_Guidelines.pdf.

- 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://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf

- Title 21, Code of Federal Regulations, Part 50. Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50

- Title 45, Code of Federal Regulations, Part 46. Available at http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html

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

15 Acronyms and abbreviations

Ab	antibody
Ad	adenovirus
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANOVA	analysis of variance
ART	antiretroviral therapy
AST	aspartate 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
CIOMS	Council for International Organizations of Medical Sciences
CI	confidence intervals
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
CTL	cytotoxic T lymphocyte
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
DSMB	NIAID Data and Safety Monitoring Board
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
FDA	US Food and Drug Administration
FPR	false positive rate
GCP	Good Clinical Practice
GEE	generalized estimating equation
HAART	highly active antiretroviral therapy
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act

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
LTFU	loss to follow-up
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)
NICD	National Institute for Communicable Diseases (Johannesburg, South Africa)
NIH	US National Institutes of Health
OBA	NIH Office of Biotechnology Activities
OHRP	US Office for Human Research Protections
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PI	Principal Investigator
PSRT	Protocol Safety Review Team
PTE	potential T-cell epitope
RAB	DAIDS Regulatory Affairs Branch
RAC	NIH Recombinant DNA Advisory Committee
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
SFU	spot-forming unit

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

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Appendix A Sample informed consent form for Part A

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A,B,C,A/E) / *gag* (C) DNA and gp120 (A,B,C,A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1–uninfected adult participants

HVTN protocol number: HVTN 124

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 HIV vaccines. HIV is the virus that causes AIDS.

About 60 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.

This study tests 2 vaccines and another study product called an adjuvant, which we will explain in a later section. The study is divided into two parts, Part A and Part B. About 12 people will take part in Part A of this study. After we see the safety results from the first 6 people in Part A, we will decide whether or not to do Part B of the study.

You are being invited to join Part A of the study, which tests one HIV vaccine and a study adjuvant. We will call this HIV vaccine and study adjuvant the "study vaccine." They are described below.

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

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

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

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

3. **We do not know if the study vaccine in Part A will decrease, increase, or not change your risk of becoming infected with HIV if you are exposed to the virus.**

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org.

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 studies with a different vaccine, some people who got the vaccine had a *higher* risk of getting HIV than people 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. We 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. **The study vaccine in Part A is experimental.**

   Experimental means we do not know if the study vaccine will be safe to use in people, or if it will work to prevent HIV infection. These study vaccines are used only in research studies.

   Part A tests a protein vaccine. Proteins are natural substances that the body uses to build and maintain itself and also to protect itself against disease. The vaccine is made of man-made proteins that 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 against HIV. Antibodies are special proteins made by the body that can recognize and prevent infections.

   The protein vaccine was developed by scientists at the University of Massachusetts Medical School (Worcester, Massachusetts) with support from the Division of AIDS (DAIDS) at the National Institutes of Health (NIH).

   Sometimes vaccines work better when they are combined with another substance that helps to alert the immune system. These substances are called adjuvants. In

this study, the protein vaccine is mixed with an adjuvant called GLA-SE. The GLA-SE adjuvant was developed by Infectious Disease Research Institute located in Seattle, Washington.

The study vaccine has not been given to people before. It has been tested in mice and rabbits and appears safe. Even if something looks like it is safe or works in animals, it may not be true for people.

The GLA-SE adjuvant has been tested in mice, guinea pigs, rats, and rabbits. It has also been given to over 900 people in research studies of vaccines for other diseases. In those studies, there were no serious safety concerns with the GLA-SE adjuvant. This is the first time the GLA-SE adjuvant has been tested with this protein vaccine against HIV.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Vaccines can also cause pain, redness, swelling, or itching where you got the injection. Most people can still do their planned activities after getting a vaccine. Rarely, people have side effects that limit their normal activities or make them go to the doctor.

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

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 vaccine:

This section lists the side effects we know about. There may be others that we don’t yet know about, even serious ones. We will tell you if we learn about any new side effects.

Possible risks related to the protein vaccine include muscle damage at the site of the injection, the production of antibodies which might react with normal body tissues and cause an autoimmune disease, and temporary lab changes such as a low white blood cell count.

The GLA-SE adjuvant may also cause the same risks as shown above for vaccines in general. It may also cause lack or loss of appetite.

In an earlier study, people received two kinds of vaccines; one vaccine was a similar protein vaccine to this study but with a different adjuvant. Almost everyone had some injection site pain. A wide area of redness around the injection site was also common. After either type of vaccine, a couple of days later, some people developed redness and swelling at a previous injection site. After either

type of vaccine, some people also had injection site swelling, fevers, body aches, tiredness, headache, and joint aches. After receiving both types of vaccines, two people developed a kind of autoimmune disease called vasculitis that caused a rash in one person, and a rash along with kidney and liver injury in another person, who had had a similar illness before. These people recovered without any problem. We do not know if participants in this study will have similar effects, because the study vaccines and study adjuvant are different.

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 get a study product. Being in more than one study may not be safe.

   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 want 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.

We will review the screening results with you. The screening results may show you are not eligible to join the study, even if you want to.

Site: adapt the following section so it is applicable to the care available at your site

7. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here for free.**

   For the care that we cannot give, we will explain how we will help you get care elsewhere. For health problems that are unrelated to the study, we will not pay for care.

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

Site: If you want to include Appendix C, Approved birth control methods (for sample informed consent form), in this consent form, paste it below and delete paragraph below.

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 21 days before your first injection until after your last required protocol clinic visit. We will talk to you about effective birth control methods. They are listed on a handout that we will give to you.

Being in the study

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

9. **You will come to the clinic for scheduled visits about [#] times over [Insert period of time].**

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 main study ends (for example, to tell you about the study results).

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

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

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

Payments you receive for being in the study may be taxable. We 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.

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

Not everyone in Part A will get the study vaccine. Some people will get a placebo, a substance that does not contain vaccine or adjuvant. We will compare the results from people who got the placebo with results from people who got the study vaccine. In this study, the placebo is sterile salt water.

You have a 5-in-6 chance of getting the study vaccine. Whether you get the study vaccine or the placebo is completely random, like flipping a coin.

We have no say in whether you get the study vaccine or the placebo. We will not know which one you are getting, and neither will you. Only the pharmacist at this clinic will have this information while the study is going on.

You will have to wait until everyone in Part A completes their final study visits to find out whether you got the study vaccine or the placebo. This could take 1-2 years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

12. We will give you the study vaccine or the placebo on a schedule.

You will be in Part A of the study. You will get 1 injection with a needle and syringe into your upper arm, at each of 2 separate visits. The injections will be given 2 months apart.

Part A	**Month 0 (Day 0)**	**Month 2 (Day 56)**
10 people	Protein vaccine with GLA-SE adjuvant	Protein vaccine with GLA-SE adjuvant
2 people	Placebo	Placebo

You will have to wait in the clinic for about a half hour after each injection to see if there are any problems. Then for that night and for 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. Within 3 days of each injection, we will also ask you how you are doing. If you have a problem, we will continue to check on you until it goes away.

Because this is the first study using these products in people, it is important for you to call us if you have any symptoms, issues or concerns. We may ask you to return to the clinic. In the clinic, we may do some additional lab tests. If you have a reaction on your skin, we may take a picture of it. If we think it's needed, we may send you to a specialist outside this clinic. You may be asked for your

permission to take a skin biopsy. You will be given another consent form to explain this procedure. You would not have to pay for the specialist or for a skin biopsy. We will ask for these medical records to be shared with us so that we can use the information for this study.

13. In addition to giving you the study vaccine or placebo, we will:

- Do regular HIV testing, as well as counseling on your results and on how to avoid getting HIV;
- Do physical exams;
- Do pregnancy tests if you were born female;
- 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
- Take urine and blood samples;

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 20 mL and 170 mL (1 tablespoon to 3/4 cup). Your body will make new blood to replace the blood we take out.

Site: You may want to add a sentence to the end of the previous paragraph contextualizing the blood volumes described (eg, "To compare, people who donate blood in the US can give a total of about 500 mL in an 8-week period."). Modify the example for cultural relevance and alter blood volumes as necessary.

Site: Insert Appendix F Table of procedures (for informed consent form) in this section or distribute it as a separate sheet if it is helpful to your study participants. You are not required to do either.

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.

14. We will counsel you on avoiding HIV infection.

We will ask you personal questions about your HIV risk factors such as sexual behavior, alcohol, and drug use. We will talk with you about ways to keep your risk of getting HIV low.

15. The HVTN will test your samples to see how your immune system responds to the study products.

We will send your samples (without your name) to labs approved by the HVTN for this study, which are located in the United States. In rare cases, some of your samples may be sent to labs approved by the HVTN in other countries for research related to this study.

Researchers may also do genetic testing related to this study on your samples. 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. The genetic testing will only involve some of your genes, not all of your genes (your genome). The researchers will study only the genes related to the immune system and HIV and those that affect how people get HIV.

If you become HIV infected, the researchers may look at all of the genes of the virus found in your samples. The researchers will use this information to learn more about HIV and the study product(s).

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic, because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

When your samples are no longer needed for this study, the HVTN will continue to store them.

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

16. When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.

The HVTN calls these samples "extra samples". The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this 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. Your samples will be stored in the HVTN repository 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: Revise the previous sentence to 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 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 HVTN or other researchers. Once we share your samples and information, we may 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 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: If review by your institution's IRB/EC/RE is also required, insert a sentence stating this.]* IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

What information is shared with HVTN or other researchers? The samples and 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, sex, 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.

Researchers may also do genetic testing on your samples.

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

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 extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and 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 and information may be published. No publication will use your name or identify you personally.

17. 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.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs.

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],
- The University of Massachusetts Medical School and the people who work for them,
- The Infectious Disease Research Institute and the people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

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

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

- [Item 1]
- [Item 2]
- [Item 3]

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.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

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

This may happen if:

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

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

19. We will stop your injections if you become pregnant.

We will encourage you to stay in the study if you choose. We will discuss your study options with you.

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

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

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

Other Risks

21. 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, a sore, bleeding, and (rarely) muscle damage or infection where you got the injection. 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 an HIV study vaccine. The study vaccine may cause you to test positive on some types of HIV antibody tests, even if you are not infected with HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccine, a routine HIV test done outside this clinic may say you have HIV, even if you don't. For this reason, you should plan to get HIV tests only at this clinic during the study. Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccine.

If you have a positive test result caused by the study vaccine at any time, we can arrange 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 vaccine. 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, in some countries, 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 vaccine, 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. 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. If you or the baby continue to have VISP, we can arrange this testing for free for as long as it is needed.

Embarrassment/anxiety:

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

It is unlikely, but the genetic tests done on your samples 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 vaccine 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 vaccine might affect your HIV infection or how long it takes to develop AIDS.

We do not know if getting this study vaccine 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 vaccine. Currently, no HIV vaccine has been approved for use.

We do not know how the study vaccine will affect a pregnant participant or a developing baby.

Benefits

22. The study may not benefit you.

We do not know whether getting the study vaccine 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

23. If you join the study, you have rights and responsibilities.

You have many rights that we will respect. You also have responsibilities. We list these in the Participant's Bill of Rights and Responsibilities. We will give you a copy of it.

Leaving the study

24. Tell us if you decide to leave the study.

You are free to leave the study at any time and for any reason. Your care at this clinic and your legal rights will not be affected, but it is important for you to let us know.

We will ask you to come back to the clinic one last time for a physical exam, and we may ask to take some blood and urine samples. We will also ask about any personal problems or benefits you have experienced from being in the study. We believe these steps are important to protecting your health, but it is up to you whether to complete them.

Injuries

Site: Approval from HVTN Regulatory Affairs (at vtn.core.reg@hvtn.org) is needed for any change (other than those that the instructions specifically request or those previously approved by HVTN Regulatory Affairs) to the boxed text

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

Your health is important to us. *(Site: adjust the following 2 sentences if applicable to the care available at your site)* We will tell you about the care that we can give here. For the care that we cannot provide, we will explain how we will help you get care elsewhere.

If you become sick or injured in this study, there is a process to decide if it is related to the study products and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

The HVTN has limited funds to pay medical costs that it determines are reasonable. There may be other ways that study-related injuries can be funded. We can give you more information if you would like. *(Site: insert locale-appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

Some injuries are not physical. For example, you might be harmed emotionally by being in an HIV vaccine study. Or you might lose wages because you cannot go to work. However, there are no funds to pay for these kinds of injuries, even if they are study related.

You may disagree with the decision about whether your injury is study related. If you wish, the HVTN will ask independent experts to review the decision. You always have the right to use the court system if you are not satisfied.

Questions

26. 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 or title 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 or title and telephone number of the investigator or other study staff].

This study has been reviewed and approved by a committee called the
[name of local IRB/EC]. 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 or title and telephone number of person on IRB or other appropriate organization], at the committee.

If you want to leave this study, contact
[name or title 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

27. In Section 16 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used.

☐ I allow my extra samples and information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples and 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 genetic testing, growing more of my cells, or genome wide studies.

28. 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 Sample informed consent form for Part B

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A,B,C,A/E) / *gag* (C) DNA and gp120 (A,B,C,A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1–uninfected adult participants

HVTN protocol number: HVTN 124

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 2 HIV vaccines. HIV is the virus that causes AIDS.

About 60 people will take part in this study at multiple sites, 12 in Part A and 48 in Part B. 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.

The results from the first 6 people enrolled in Part A showed that one of the vaccines did not cause safety problems, so we are continuing with Part B. We will first have 10 people join. After we see results from them, we will decide if the remaining 38 people should join the study. The decision on whether to move forward is always based on the safety of the participants and how they respond to the study products.

You are being invited to join Part B of the study. Part B tests 2 vaccines and another study product called an adjuvant, which are described below. We will call the vaccines and study adjuvant the "study vaccines."

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

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

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

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

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

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org.

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 studies with a different vaccine, some people who got the vaccine had a *higher* risk of getting HIV than people 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. We 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. **The study vaccines are experimental.**

   Experimental means we do not know if the vaccines and adjuvant will be safe to use in people, or if they will work to prevent HIV infection. These study vaccines are used only in research studies.

   There are 2 vaccines in Part B of the study. One of them is a DNA vaccine. It contains DNA made in a laboratory. DNA is a natural substance in the body that tells the body how to make proteins. Proteins are natural substances that the body uses to build and maintain itself, and also to protect itself against disease. The DNA in the vaccine tells the body to make 5 proteins that are found in HIV. The body's immune system may respond to these proteins by making cells and antibodies that recognize and fight HIV. Antibodies are special proteins made by the body that can recognize and prevent infections.

   The second vaccine is a protein vaccine. The vaccine is made of 4 man-made proteins that 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 against HIV proteins.

The 2 vaccines were developed by scientists at the University of Massachusetts Medical School (Worcester, Massachusetts) with support from the Division of AIDS (DAIDS) at the National Institutes of Health (NIH).

Sometimes vaccines work better when they are combined with another substance that helps to alert the immune system. These substances are called adjuvants. In this study, the protein vaccine is mixed with an adjuvant called GLA- SE. The study adjuvant was developed by Infectious Disease Research Institute (Seattle, Washington).

We will call the protein vaccine mixed with the GLA-SE adjuvant the "protein vaccine."

The study vaccines have not been given to people before this study. They have been tested in mice and rabbits and appear safe. Even if something looks like it is safe or works in animals, it may not be true for people. Part A of this study tested the safety of the protein vaccine. This is the first study that will test the DNA and protein vaccines given together.

The GLA-SE adjuvant has been tested in mice, guinea pigs, rats, and rabbits. It has also been given to over 900 people in research studies of vaccines for other diseases. In those studies, there were no serious safety concerns with the GLA-SE adjuvant. This is the first time the GLA-SE adjuvant has been tested with this protein vaccine against HIV.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Vaccines can also cause pain, redness, swelling, or itching where you got the injection. Most people can still do their planned activities after getting a vaccine. Rarely, people have side effects that limit their normal activities or make them go to the doctor.

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

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:

This section lists the side effects we know about. There may be others that we don't yet know about, even serious ones. We will tell you if we learn about any new side effects.

The GLA-SE adjuvant may also cause the same risks as shown above for vaccines in general. It may also cause lack or loss of appetite.

Possible risks related to the study vaccines include muscle damage at the site of the injection, the production of antibodies which might react with normal body tissues and cause an autoimmune disease, temporary lab changes such as a low white blood cell count, and insertion of the study product's DNA into the body's DNA.

Since 1995, thousands of people have received experimental DNA vaccines for diseases such as hepatitis, human papilloma virus (HPV, also known as genital warts), and HIV. In these people, the DNA vaccines have not caused serious side effects. We expect the risks of the study vaccines in this study to be similar to those of other DNA vaccines.

In an earlier study, people received a similar DNA vaccine and similar protein vaccine, with a different adjuvant. Almost everyone had some injection site pain. A wide area of redness around the injection site was also common. After either vaccine, some people also had injection site swelling, fevers, body aches, tiredness, headache, and joint aches. After the DNA vaccine or the protein vaccine, a couple of days later, some people developed redness and swelling at a previous injection site. After receiving both vaccines, two people developed a kind of autoimmune disease called vasculitis that caused a rash in one person, and a rash along with kidney and liver injury in another person, who had had a similar illness before. These people recovered without any problem. We do not know if participants in this study will have similar effects, because the study vaccines and study adjuvant are different.

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 get a study product. Being in more than one study may not be safe. 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 want 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.

We will review the screening results with you. The screening results may show you are not eligible to join the study, even if you want to.

Site: adapt the following section so it is applicable to the care available at your site.

7. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here for free.**

   For the care that we cannot give, we will explain how we will help you get care elsewhere. For health problems that are unrelated to the study, we will not pay for care.

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

Site: If you want to include Appendix C, Approved birth control methods (for sample informed consent form), in this consent form, paste it below and delete paragraph below.

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 21 days before your first injection until after your last required protocol clinic visit. We will talk to you about effective birth control methods. They are listed on a handout that we will give to you.

Being in the study

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

9. **You will come to the clinic for scheduled visits about [#] times over [Insert period of time].**

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 main study ends (for example, to tell you about the study results).

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

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

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

Payments you receive for being in the study may be taxable. We 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.

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

Not everyone in this study will get the study vaccines. Some people will get a placebo, a substance that does not contain vaccine or adjuvant. We will compare the results from people who got the placebo with results from people who got the study vaccines. In this study, the placebo is sterile salt water.

You have a 7-in-8 chance of getting the study vaccines. Whether you get the study vaccines or the placebo is completely random, like flipping a coin.

We have no say in whether you get the study vaccines or the placebo. We will not know which one you are getting, and neither will you. Only the pharmacist at this clinic 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 whether you got the study vaccines or the placebo. This could be several years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

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

You will be in Part B of the study, in 1 of 2 groups. You will not know which group you will be assigned to. You will get 2 injections of the study products or placebo, one in each upper arm. This will happen 5 times during the study. The injections are given with a needle and syringe. People in group 2 will get the products one at a time, and people in group 3 will get them together.

Site: If a picture version of the injection schedule has been provided in a separate protocol appendix, you may insert it below in place of (or in addition to) the text version or give it as a separate document to volunteers if you believe it will be helpful to them. You are not required to do either.

Part B	Group*	Month 0 (Day 0)	Month 1 (Day 28)	Month 3 (Day 84)	Month 6 (Day 168)	Month 8 (Day 224)
21 people	2	DNA + Placebo	DNA + Placebo	DNA + Placebo	Protein with GLA-SE + Placebo	Protein with GLA-SE + Placebo
3 people		Placebo + Placebo	Placebo + Placebo	Placebo + Placebo	Placebo + Placebo	Placebo + Placebo
21 people	3	DNA+ Protein with GLA-SE	DNA+ Protein with GLA-SE	DNA+ Protein with GLA-SE	DNA+ Protein with GLA-SE	DNA+ Protein with GLA-SE
3 people		Placebo + Placebo	Placebo + Placebo	Placebo + Placebo	Placebo + Placebo	Placebo + Placebo
*(Group 1 was enrolled in Part A of the study.)						

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 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. Within 3 days of each injection, we will also ask know how you are doing. If you have a problem, we will continue to check on you until it goes away.

Because this is the first study using these products in people, it is important for you to call us if you have any symptoms, issues, or concerns. We may ask you to return to the clinic. In the clinic, we may do some additional lab tests. If you have a reaction on your skin, we may take a picture of it. If we think it's needed, we may send you to a specialist outside this clinic. You may be asked for your permission to take a skin biopsy. You will be given another consent form to explain this procedure. You will not have to pay for the specialist or for a skin biopsy. We will ask for these medical records to be shared with us so that we can use the information for this study.

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;

- Do pregnancy tests if you were born female;
- 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
- Take urine and blood samples;

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 240 mL (1 tablespoon to 1 cup). Your body will make new blood to replace the blood we take out.

Site: You may want to add a sentence to the end of the previous paragraph contextualizing the blood volumes described (eg, "To compare, people who donate blood in the US can give a total of about 500 mL in an 8-week period."). Modify the example for cultural relevance and alter blood volumes as necessary.

Site: Insert Appendix G, Table of procedures (for informed consent form) in this section or distribute it as a separate sheet if it is helpful to your study participants. You are not required to do either.

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.

13. If you agree, we will also collect saliva, rectal fluid, and cervical fluid or semen.

We want to see how the study vaccines affect the parts of the body where people may be exposed to HIV: their mouth, rectum, vagina, and penis. At the end of this form we will ask if you allow us to collect saliva, rectal fluid, and cervical fluid (if you were born female) or semen (if you were born male). You can decide not to give any of these samples and still be in the study.

We will ask you to avoid some activities before we collect these samples. This will help make sure your samples give accurate lab readings.

Saliva

We will collect saliva from you at 3 visits. We will ask you to spit into a container. We may give you chewing wax if you need it to help you produce more saliva. We want to collect 3 to 5 mL, which is less than a teaspoon.

For 1 hour before we collect your saliva, we will ask you to follow these instructions:

- Do not smoke, eat, or drink anything but water,

- Do not brush your teeth or use mouthwash,
- Do not chew gum or tobacco*, Site: localize as needed*
- Do not kiss or perform oral sex.

Rectal Fluid, Cervical Fluid, Semen

We would also like to collect rectal fluid and cervical fluid (if you were born female) or semen (if you were born male). We would do this at 3 visits. If you were born female, we will test you for syphilis, gonorrhea, chlamydia, trichomonas vaginalis, bacterial vaginosis, and yeast when we collect them. If you were born male, we will test you for syphilis, gonorrhea, and chlamydia when we collect them.

We will give you your test results. If you need care, we will tell you about the care we can give you here. We will also tell you about care we can help you get elsewhere.

Rectal fluid

Site: localize measurement units as needed

We will collect rectal fluid by placing up to 3 small absorbent sponges or swabs in the rectum using a plastic tube about 2 cm wide (a little less than an inch). The tube will go in about 6½ cm (about 2½ inches) and stay in place for 5 minutes.

For the 2 days before we collect your rectal fluid, we will ask you to follow these instructions:

- Do not have receptive anal intercourse (having someone else's penis in your anus).
- Do not put anything into your anus, including cleaning products (creams, gels, lotions, pads, etc.), lubricant, enemas or douches (even with water).
- Do not use any anti-inflammatory creams in or around your anus.
- We will not collect rectal fluid if you are pregnant, or if we think you may have an anal or rectal infection. You should tell us if your rectal area is sore.

We will not collect rectal fluid if you are menstruating or pregnant, or if we think you may have an infection of the rectum. If you are menstruating or have an infection, we may ask you to return to collect this sample at another time.

Cervical fluid (for persons born female)

If you are 21 or older, you must have had a Pap smear within the last 3 to 5 years with the most recent result being normal. If you have not had a Pap smear within the last 3 years and would like to get one, we will tell you where you can get one.

To collect cervical fluid, we will give you a menstrual cup (a small flexible plastic cup, shaped like a bell) to insert into your vagina. You will wear the cup for 6 hours. The study staff will explain how to insert and remove the cup, or they can do it for you here.

For the 2 days before we collect your cervical fluid, we will ask you these things:

- Do not use any spermicide, lubricants, douche (even with water), or medication in or around your vagina;
- Do not have vaginal intercourse or insert anything into your vagina.

We will not collect cervical fluid if you are menstruating or pregnant or if we think you may have a cervical or vaginal infection. If you are menstruating or have an infection, we may ask you to return to collect this sample at another time.

Semen (for persons born male)

You may provide the semen at home or here. We will give you a plastic cup and ask you to ejaculate into it. We must receive the semen sample within 2 hours or less after it is collected. For at least 2 days before providing the semen, we will ask you:

- not to have sex, including insertive oral sex (putting your penis in someone else's mouth);
- not to ejaculate;
- not to use anything with lubricants;
- not to put saliva on the penis.

You should tell us if you think you have an infection on your penis. If you have an infection, we may ask you to return to collect this sample at another time.

14. If you agree, we will also collect stool samples.

At the end of this form, we will ask if you allow us to collect stool samples. We would like to collect a small sample of your stool to look at the bacteria living in your stomach. We want to learn if your immune response to the study vaccines is influenced by these bacteria. We will ask you to do this two times during this study.

You may provide a stool sample at home or at the clinic. The clinic must receive the stool sample within 24 hours after it is collected. If you would like, we can collect a stool sample in the clinic by doing rectal swabs. If that is what you choose, we will briefly insert sterile swabs into your rectum.

You can decide not to give these samples and still be in the study.

15. We will counsel you on avoiding HIV infection.

We will ask you personal questions about your HIV risk factors such as sexual behavior, alcohol, and drug use. We will talk with you about ways to keep your risk of getting HIV low.

16. The HVTN will test your samples to see how your immune system responds to the study products.

We will send your samples (without your name) to labs approved by the HVTN for this study, which are located in the United States. In rare cases, some of your samples may be sent to labs approved by the HVTN in other countries for research related to this study.

Researchers may also do genetic testing related to this study on your samples. 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. The genetic testing will only involve some of your genes, not all of your genes (your genome). The researchers will study only the genes related to the immune system and HIV and those that affect how people get HIV.

If you become HIV infected, the researchers may look at all of the genes of the virus found in your samples. If you give stool, semen, saliva, cervical, or rectal fluid samples, the researchers may look at all of the genes of the bacteria found in your samples. In both cases, the researchers will use this information to learn more about HIV and the study products. The researchers may put this information about the virus and/or bacteria into a protected database so that other researchers can access it. They would not be able to link the information from your samples to you.

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic, because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

When your samples are no longer needed for this study, the HVTN will continue to store them.

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

17. When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.

The HVTN calls these samples "extra samples". The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this 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: choose one of the following two sentences. African sites should choose the sentence referencing the repository in South Africa. All other sites should choose the sentence referencing the repository in the United States].* Your samples will be stored in the HVTN repository in South Africa. Your samples will be stored in the HVTN repository 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: Revise the previous sentence to 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 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 HVTN or other researchers. Once we share your samples and information, we may 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 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: If review by your institution's IRB/EC/RE is also required, insert a sentence stating this.]* IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

What information is shared with HVTN or other researchers? The samples and 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, sex, 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.

Researchers may also do genetic testing on your samples.

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

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 extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and 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 and information may be published. No publication will use your name or identify you personally.

18. 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.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs.

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],
- The University of Massachusetts Medical School and people who work for them,
- The Infectious Disease Research Institute and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

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

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

- [Item 1]
- [Item 2]
- [Item 3]

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.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

19. 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 more injections.

This may happen if:

- you do not follow instructions,
- we think that staying in the study might harm you,
- you get HIV,
- you enroll in a different research study where you get 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.

20. We will stop your injections if you become pregnant.

We will encourage you to stay in the study if you choose. We will discuss your study options with you.

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.

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

Other Risks

22. 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, a sore, bleeding, and (rarely) muscle damage or infection where you got the injection. Taking blood can cause a low blood cell count (anemia), making you feel tired.

Risks of collecting semen, rectal and cervical fluids:

For semen collection, you may be asked not to ejaculate for a few days before providing the sample. You may find this inconvenient.

You may have some discomfort during sample collection, and minor bleeding during collection of rectal fluids. This does not usually last very long.

During any of these procedures, you may feel anxious or embarrassed. If you feel uncomfortable in any way, please tell us and we will try to help you.

Risks of rectal swab:

If you choose to provide a stool sample by rectal swabbing, you may feel pressure as the swab is inserted into your rectum, but it is usually not painful. Some people might have a little bit of bleeding.

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 may cause you to test positive on some types of HIV antibody 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 may say you have HIV, even if you don't. For this reason, you should plan to get HIV tests only at this clinic during the study. Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccines.

If you have a positive test result caused by the study vaccines at any time, we can arrange 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, in some countries, 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. 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. If you or the baby continue to have VISP, we can arrange this testing for free for as long as it is needed.

Embarrassment/anxiety:

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

It is unlikely, but the genetic tests done on your samples 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

23. 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

24. 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

25. 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

Site: Approval from HVTN Regulatory Affairs (at vtn.core.reg@hvtn.org) is needed for any change (other than those that the instructions specifically request or those previously approved by HVTN Regulatory Affairs) to the following section .

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

Your health is important to us. *(Site: adjust the following 2 sentences if applicable to the care available at your site)* We will tell you about the care that we can give here. For the care that we cannot provide, we will explain how we will help you get care elsewhere.

If you become sick or injured in this study, there is a process to decide if it is related to the study products and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

The HVTN has limited funds to pay medical costs that it determines are reasonable. There may be other ways that study-related injuries can be funded. We can give you more information if you would like. *(Site: insert locale-appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

Some injuries are not physical. For example, you might be harmed emotionally by being in an HIV vaccine study. Or you might lose wages because you cannot go to work. However, there are no funds to pay for these kinds of injuries, even if they are study related.

You may disagree with the decision about whether your injury is study related. If you wish, the HVTN will ask independent experts to review the decision. You always have the right to use the court system if you are not satisfied.

Questions

27. If you have questions or problems at any time during your participation in this study, use the following important contacts.

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

This study has been reviewed and approved by a committee called the
[name of local IRB/EC]. 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 or title and telephone number of person on IRB or other appropriate organization], at the committee.

If you want to leave this study, contact
[name or title 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

28. In Section 17 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used.

☐ I allow my extra samples and information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples and 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 genetic testing, growing more of my cells, or genome wide studies.

29. In sections 13 and 14 of this form, we told you about optional collection of stool samples, saliva, rectal fluid, and cervical fluid or semen. Please write your initials or make your mark in the boxes next to the options you choose.

☐ I agree to provide saliva.

☐ I do not agree to provide saliva.

☐ I agree to provide rectal fluid.

☐ I do not agree to provide rectal fluid.

☐ I agree to provide semen or cervical fluid.

☐ I do not agree to provide semen or cervical fluid

☐ I agree to provide stool samples.

☐ I do not agree to provide stool samples.

30. **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 C Approved birth control methods (for sample informed consent form)

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org.

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 21 days before your first injection until after your last required protocol clinic visit.

Effective birth control means using any of the following methods every time you have sex:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Male or female condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for one year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your "tubes tied") or confirmed successful placement of a product that blocks the fallopian tubes;
- You are having sex only with a female partner or partners;
- You only have oral sex; or,
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, you need to use male or female condoms to protect yourself from HIV infection.

If you join the study, we will test you for pregnancy at some visits, including before each study injection.

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

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A,B,C,A/E) / *gag* (C) DNA and gp120 (A,B,C,A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1–uninfected adult participants

HVTN protocol number: HVTN 124

Site: [Insert site name]

When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.

The HVTN calls these samples "extra samples". The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this 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. Your samples will be stored in the HVTN repository 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: Revise the previous sentence to 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 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 HVTN or other researchers. Once we share your samples and information, we may 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 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: If review by your institution's IRB/EC/RE is also required, insert a sentence stating this.]* IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

8. What information is shared with HVTN or other researchers?

The samples and 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.

Researchers may also do genetic testing on your samples.

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

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?

It is unlikely, but the genetic tests done on your samples 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.

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 extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and 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 and 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 or title 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 or title and telephone number of the investigator or other study staff].

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

13. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your choice about how your samples and information can be used.

☐ I allow my extra samples and information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples and 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 genetic testing, growing more of my cells, or genome wide studies.

Participant's name (print)	Participant's signature or mark	Date	Time

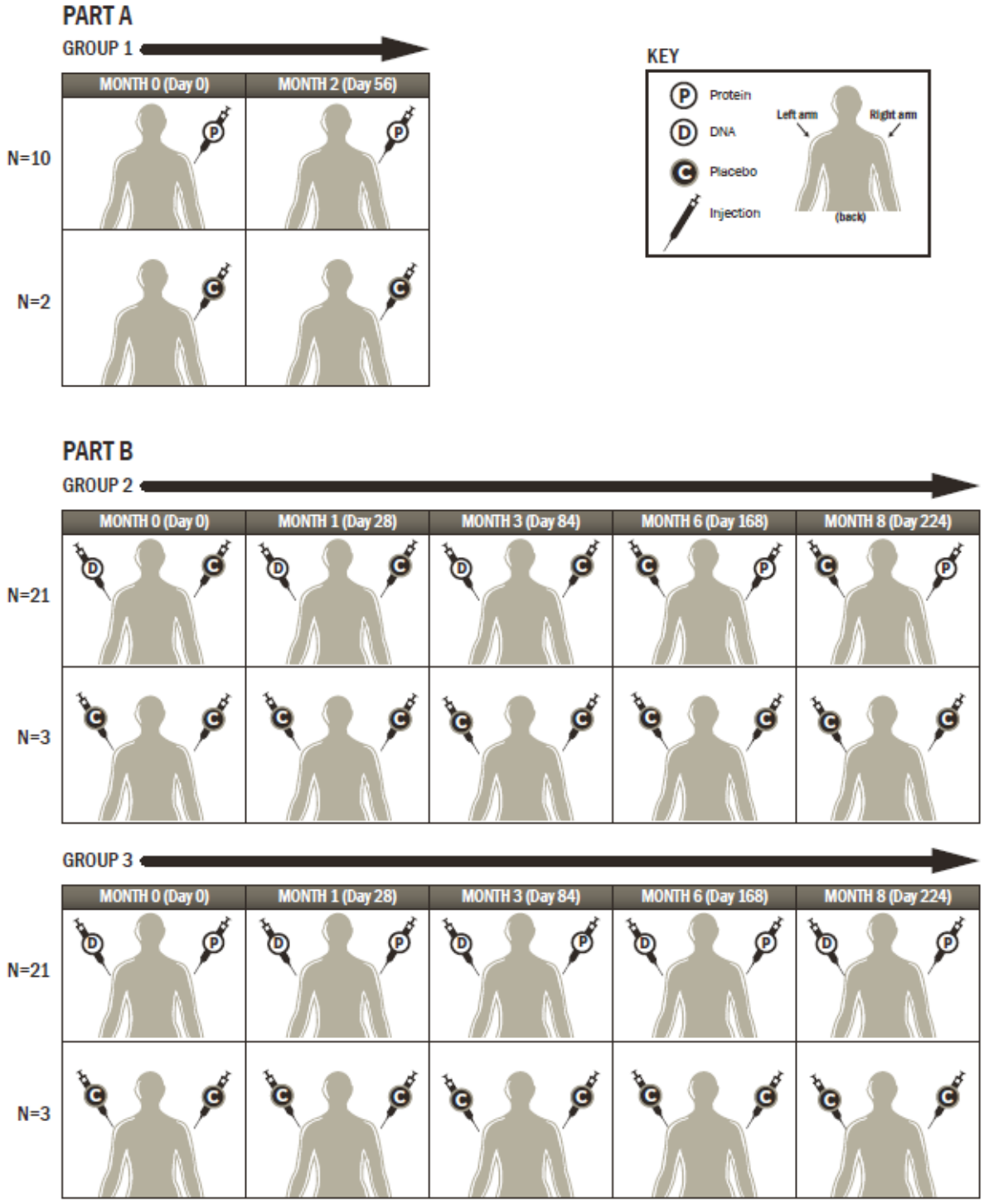
Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

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 E Injection schedule for Part A and B sample informed consent forms



Appendix F Table of procedures for Part A (for sample informed consent form)

			Time after first injection visit					
Procedure	Screening visit(s)	First injection visit	2 weeks	2 months	2.5 months	5 months	8 months	1 year + 2 months
Injections		√		√				
Medical history	√							
Complete physical	√						√	
Brief physical		√	√	√	√	√		
Urine test	√		√		√			
Blood drawn	√	√	√		√	√	√	
Pregnancy test (participants born female)*	√	√		√		√		
HIV testing & pretest counseling	√				√	√	√	
Risk reduction counseling	√	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√	
Health contact								√

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.

*Persons who had a total hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records), are not required to have a pregnancy test.

Appendix G Table of procedures for Part B (for sample informed consent form)

			Time after first injection visit												
Procedure	Part B Eligibility visit(s)	First injection visit	2 weeks	1 month	6 weeks	3 months	3½ months	6 months	6½ months	8 months	8 months + 1 week	8½ months	11 months	1 year + 2 months	1 year + 8 months
Injections		√		√		√		√		√					
Medical history	√														
Complete physical	√													√	
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√		
Urine test	√		√		√		√		√			√			
Blood drawn	√	√	√		√		√		√		√	√	√	√	
Pregnancy test (participants born female)[1]	√	√		√		√		√		√		√[2]	√	√[2]	
HIV testing & pretest counseling	√						√		√			√	√	√	
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Saliva, semen, cervical, and rectal fluid (optional)		√										√		√	
Genital Tract Infection testing (blood, urine and/or swabs) for people who agree to provide the optional samples		√										√		√	
Stool samples (optional)		√										√			
Health contact															√

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.

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

[2]Only for participants who agree to provide optional rectal and cervical fluid samples

Appendix H Laboratory procedures – Part A (1 of 2)

					Tube volume (mL)								
				Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**[10]	
				Day:	Screening visit[3]	D0	D14	D56	D70	D140	D224	D425	
				Week:		W0	W2	W8	W10	W20	W32	W61	
				Month:		M0	M0.5	M2	M2.5	M5	M8	M14	
						VAC1		**VAC2**					
Procedure	**Ship to**[1]	**Assay Location**[2]	**Tube**[4]	**Tube size (vol. capacity)**[4]									**Total**
BLOOD COLLECTION													
Screening or diagnostic assays													
Screening HIV test	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	5
HBsAg/anti-HCV	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	5
Syphilis	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	5
HIV diagnostics[9]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	10	10	20[9]	—	40
Safety labs													
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	5	—	5	5	—	—	20
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	5	—	5	5	—	—	20
Immunogenicity assays[6]													
Host genetics[7]	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	—	—	—	17
Cellular assays													
ICS	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	***42.5***	—	—	—	42.5
Humoral assays													
Binding Ab	CSR	HVTN Labs	SST	8.5mL	—	8.5	8.5	—	***8.5***	—	8.5	—	34
Specimen storage													
PBMC	CSR		ACD	8.5mL	—	119	102	—	76.5	—	102	—	399.5
Serum	CSR		SST	8.5mL	—	17	17	—	17	—	17	—	68
Visit total					**25**	**161.5**	**137.5**	**0**	**164.5**	**20**	**148**	**0**	**656**
56-Day total					**25**	**186.5**	**324**	**324**	**302**	**20**	**148**	**0**	
URINE COLLECTION													
Urine dipstick[11]	Local lab	Local lab			X	—	X	—	X	—	—	—	
Pregnancy test[8]	Local lab	Local lab			X	X	—	X	—	X	—	—	

Footnotes for Laboratory procedures – Part A (2 of 2)

[1]CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).
[2]HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, 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 9.4 (postenrollment).
[6]Immunogenicity assays will be performed at M0 (for binding Ab assay) and M2.5. 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]For participants born female. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or 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 9.14), blood should be drawn for HIV diagnostic testing, as shown for visit 7 above.
[10]For information concerning the Month 14 health contact, see Section 9.7. Clinic visits are not required except that any participant reporting a diagnosis of HIV infection from testing outside of the HVTN will be asked to come to the clinic to collect specimens for HIV testing with HVTN HIV diagnostic algorithms.
[11]And microscopy if needed.

Appendix I Laboratory procedures – Part B (1 of 2)

Procedure	Ship to[1]	Assay Location[2]	Tube[4]	Tube size (vol. capacity)[4]	Tube volume (mL)															Total
Visit:					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15[14]	
Day:					Screening visit[3]	D0	D14	D28	D42	D84	D98	D168	D182	D224	D231	D238	D334	D425	D607	
Week:						W0	W2	W4	W6	W12	W14	W24	W26	W32	W33	W34	W48	W61	W87	
Month:						M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M8	M8.25	M8.5	M11	M14	M20	
						VAC1		**VAC2**		**VAC3**		**VAC4**		**VAC5**						
BLOOD COLLECTION																				
Screening or diagnostic assays																				
Screening HIV test	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
HBsAg/anti-HCV	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
Syphilis	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	5[11]	—	5[11]	—	15
HIV diagnostics[9]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	—	—	10	—	10	—	—	10	10	20[9]	—	60
Safety labs																				
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	5	—	5	—	5	—	5	—	—	5	5	—	—	35
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	5	—	5	—	5	—	5	—	—	5	5	—	—	35
Immunogenicity assays[6]																				
Host genetics[7]	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	—	—	—	—	—	—	—	—	—	—	17
Cellular assays																				
ICS	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	—	—	42.5	—	42.5	—	—	***42.5***	—	68	—	195.5
pTfh and plasmablasts	CSR	HVTN Labs	ACD	8.5mL	—	42.5	—	—	—	—	—	—	—	—	42.5	—	—	—	—	85
B-cell ELISpot	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	—	17	—	17	—	—	17	—	17	—	85
B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	—	17	—	17	—	—	17	—	17	—	85
Humoral assays																				
Binding Ab	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	8.5	—	8.5	—	—	***8.5***	—	8.5	—	42.5
Neutralizing Ab	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	8.5	—	8.5	—	—	***8.5***	—	8.5	—	42.5
ADCC	CSR	HVTN Labs	SST	8.5mL	—	y	—	—	—	—	y	—	y	—	—	***y***	—	y	—	0
Specimen storage																				
PBMC	CSR		ACD	8.5mL	—	76.5	—	—	76.5	—	76.5	—	68	—	25.5	76.5	—	76.5	—	476
Serum	CSR		SST	8.5mL	—	17	—	—	17	—	17	—	8.5	—	8.5	17	—	17	—	102
Visit total					**25**	**204**	**10**	**0**	**103.5**	**0**	**207**	**0**	**190**	**0**	**76.5**	**212**	**20**	**237.5**	**0**	**1285.5**
56-Day total					**25**	**229**	**239**	**239**	**342.5**	**103.5**	**310.5**	**0**	**190**	**190**	**266.5**	**478.5**	**20**	**237.5**	**0**	
URINE COLLECTION																				
Urine dipstick[15]	Local lab	Local lab			X	—	X	—	X	—	X	—	X	—	—	X	—	—	—	
Pregnancy test[8]	Local lab	Local lab			X	X	—	X	—	X	—	X	—	X	—	X[10]	X	X[10]	—	
Chlamydia/Gonorrhea[11]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	X	—	X	—	
CERVICAL/VAGINAL SWAB COLLECTION[12]																				
Trichomonas vaginalis	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	X	—	X	—	
Bacterial vaginosis	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	X	—	X	—	
Yeast	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	X	—	X	—	
MUCOSAL COLLECTION (OPTIONAL)																				
Saliva	CSR	HVTN Labs			—	X[13]	—	—	—	—	—	—	—	—	—	X	—	X	—	
Semen	CSR	HVTN Labs			—	X[13]	—	—	—	—	—	—	—	—	—	X	—	X	—	
Cervical Secretions	CSR	HVTN Labs			—	X[13]	—	—	—	—	—	—	—	—	—	X	—	X	—	
Rectal Secretions	CSR	HVTN Labs			—	X[13]	—	—	—	—	—	—	—	—	—	X	—	X	—	
STOOL COLLECTION (OPTIONAL)																				
Stool	CSR	HVTN Labs			—	X[16]	—	—	—	—	—	—	—	—	—	X	—	—	—	

Footnotes for Laboratory procedures – Part B (2 of 2)

[1]CSR Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).
[2]HVTN Laboratories include Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, 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 9.4 (postenrollment).
[6]Immunogenicity assays will be performed at M0 (for binding Ab assay) and M8.5. 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]For participants born female. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or 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 9.14), blood should be drawn for HIV diagnostic testing, as shown for visit 14 above.
[10]Pregnancy testing at the indicated visit is only required of participants who are born female and are providing a cervical and/or rectal fluid sample.
[11]Syphilis testing by serology and Chlamydia and Gonorrhea testing by urine will only be performed if the participant agrees to provide a semen, cervical, or rectal mucosal sample.
[12]Cervical/vaginal swabs will only be collected from participants who agree to provide a cervical fluid sample and for yeast if clinically indicated.
[13]Optional mucosal specimens may be collected as part of screening and prior to the enrollment visit once the participant has been found to have met mucosal specimen collection criteria as specified in Section 9.5.
[14]For information concerning the Month 20 health contact, see Section 9.7. Clinic visits are not required except that any participant reporting a diagnosis of HIV infection from testing outside of the HVTN will be asked to come to the clinic to collect specimens for HIV testing with HVTN HIV diagnostic algorithms.
[15]And microscopy if needed.
[16]Optional stool specimen must be collected prior to first vaccination.
y = SST collected for binding Ab and neutralizing Ab assays will also cover specimen needs for ADCC; no separate blood draw is needed.

Appendix J Procedures at HVTN CRS - Part A (1 of 2)

Visit:	**01**[1,2]	**02**[2]	**03**	**04**	**05**	**06**	**7**	**8**[3]	**Post**
Day:		D0	D14	D56	D70	D140	D224	D425	
Month:		M0	M0.5	M2	M2.5	M5	M8	M14	
Procedure	Scr.	VAC1		VAC2					
Study procedures									
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		—	—
Risk reduction counseling	X	X	X	X	X	X	X	—	—
Pregnancy assessment[4]	X	X	—	X	—	X	—	—	—
Pregnancy prevention assessment[5]	X	X	X	X	X	X	X	—	—
Behavioral risk assessment	X	—	—	—	—	X	X	—	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	—	—
Social impact assessment questionnaire	—	—	—	X	—	—	X	—	—
Outside testing and belief questionnaire	—	—	—	—	—	X	X	—	—
Concomitant medications	X	X	X	X	X	X	X	—	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	—	—
HIV infection assessment[6]	X	—	—	—	X	X	X	—	—
Confirm HIV test results provided to participant	—	X	—	—	—	X	X	X	—
Health contact	—	—	—	—	—	—	—	X	—
Specimen collection[7]	X	X	X	X	X	X	X	—	—
Vaccination procedures									
Vaccination[8]	—	X	—	X	—	—	—	—	—
Reactogenicity assessments[9]	—	X	—	X	—	—	—	—	—
Poststudy									
Unblind participant	—	—	—	—	—	—	—	—	X

Footnotes for Procedures at HVTN CRS – Part A (2 of 2)

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

[2]Specimens indicated for day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination

[3]See Section 9.7. Clinic visits are not required except that any participant reporting a diagnosis of HIV infection from testing outside of the HVTN will be asked to come to the clinic to collect specimens for HIV testing with HVTN HIV diagnostic algorithms.

[4]For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination. Pregnancy test to determine eligibility may be performed at screening, but must also be done on Day 0 prior to vaccination. Persons who have undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[5]Pregnancy prevention assessment is required only for participants who were born female and are capable of becoming pregnant.

[6]Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.

[7]See Clinical procedures sections 9.2, 9.3, 9.4, 9.5, 9.6, Laboratory procedures section 10, and Appendix H.

[8]Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated.

[9]Reactogenicity assessments performed daily for at least 7 days post vaccination (see Section 9.11).

Appendix K Procedures at HVTN CRS - Part B (1 of 2)

Visit:	**01[1,2]**	**02[2]**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15[3]**	**Post**
Day:		D0	D14	D28	D42	D84	D98	D168	D182	D224	D231	D238	D334	D425	D607	
Month:		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M8	M8.25	M8.5	M11	M14	M20	
Procedure	Scr.	VAC1		VAC2		VAC3		VAC4		VAC5						
Study procedures																
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	X	X	X	—	—	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Pregnancy assessment[4]	X	X	—	X	—	X	—	X	—	X	—	X[5]	X	X[5]	—	—
Pregnancy prevention assessment[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Behavioral risk assessment	X	—	—	—	—	X	—	X	—	—	—	—	X	X	—	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Social impact assessment questionnaire	—	—	—	—	—	—	—	X	—	—	—	—	X	X	—	—
Outside testing and belief questionnaire	—	—	—	—	—	—	—	X	—	—	—	—	X	X	—	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
HIV infection assessment[7]	X	—	—	—	—	—	X	—	X	—	—	X	X	X	—	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	X	—	X	—	—	X	X	X	—
Stool sample (optional)	—	X	—	—	—	—	—	—	—	—	—	X	—	—	—	—
Dietary and GI symptom questionnaire[8]	—	X	—	—	—	—	—	—	—	—	—	X	—	—	—	—
Health contact	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—

Procedures at HVTN CRS – Part B (2 of 2)

Visit:	01[1,2]	02[2]	03	04	05	06	07	08	09	10	11	12	13	14	15[3]	Post
Day:		D0	D14	D28	D42	D84	D98	D168	D182	D224	D231	D238	D334	D425	D607	
Month:		M0	M0,5	M1	M1.5	M3	M3.5	M6	M6.5	M8	M8.25	M8.5	M11	M14	M20	
Procedure	Scr.	VAC1		VAC2		VAC3		VAC4		VAC5						
Specimen collection[9]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Vaccination procedures																
Vaccination[10]	—	X	—	X	—	X	—	X	—	X	—	—	—	—	—	—
Reactogenicity assessment[11]	—	X	—	X	—	X	—	X	—	X	—	—	—	—	—	—
Poststudy																
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

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

[2]Specimens indicated for day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination.

[3]See Section 9.7. Clinic visits are not required except that any participant reporting a diagnosis of HIV infection from testing outside of the HVTN will be asked to come to the clinic to collect specimens for HIV testing with HVTN HIV diagnostic algorithms.

[4]For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination. Pregnancy test to determine eligibility may be performed at screening, but must also be done on Day 0 prior to vaccination. Persons who have undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing

[5]Pregnancy testing at the indicated visits is only required of participants who are born female and are providing a cervical and/or rectal fluid sample.

[6]Pregnancy prevention assessment is required only for participants who were born female and are capable of becoming pregnant.

[7]Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.

[8]For participants providing optional stool sample.

[9]See Clinical procedures sections 9.2, 9.3, 9.4, 9.5, 9.6, Laboratory procedures section 10, and Appendix I.

[10]Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated.

[11]Reactogenicity assessments performed daily for at least 7 days post vaccination (see Section 9.11).

Appendix L Adverse events of special interest

AEs of special interest (AESI) for this protocol include but are not limited to potential immune-mediated diseases; representative examples of AESI are listed below. Updates to AESI will be provided as an appendix to the HVTN 124 *Study Specific Procedures*.

Gastrointestinal disorders	Liver disorders	Metabolic diseases
• Celiac disease • Crohn's disease • Ulcerative colitis • Ulcerative proctitis	• Autoimmune cholangitis • Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis	• Addison's disease • Autoimmune thyroiditis (including Hashimoto thyroiditis) • Diabetes mellitus type I • Grave's or Basedow's disease
Neuroinflammatory disorders	**Musculoskeletal disorders**	**Skin disorders**
• Acute disseminated encephalomyelitis, including site specific variants (eg, non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis) • Cranial nerve disorders, included paralyses/paresis (eg, Bell's palsy) • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy • Multiple sclerosis • Myasthenia gravis, including Eaton-Lambert syndrome • Narcolepsy • Optic neuritis • Transverse Myelitis	• Antisynthetase syndrome • Dermatomyositis • Juvenile chronic arthritis (including Still's disease) • Mixed connective tissue disorder • Polymyalgia rheumatic • Polymyositis • Psoriatic arthropathy • Relapsing polychondritis • Rheumatoid arthritis • Scleroderma, including diffuse systemic form and CREST syndrome • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Systemic lupus erythematosus • Systemic sclerosis	• Alopecia areata • Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis • Cutaneous lupus erythematosus • Erythema nodosum • Morphoea • Lichen planus • Psoriasis • Sweet's syndrome • Vitiligo
Vasculitides	**Others**	
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis	• Antiphospholipid syndrome • Autoimmune hemolytic anemia • Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Autoimmune myocarditis/cardiomyopathy • Autoimmune thrombocytopenia • Goodpasture syndrome • Idiopathic pulmonary fibrosis • Pernicious anemia • Raynaud's phenomenon • Sarcoidosis • Sjögren's syndrome • Stevens-Johnson syndrome • Uveitis	

Appendix M Protocol Signature Page

A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A,B,C,A/E) / *gag* (C) DNA and gp120 (A,B,C,A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1–uninfected adult participants

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies

Investigator of Record Name (print)	Investigator of Record Signature	Date

DAIDS Protocol Number: HVTN 124

DAIDS Protocol Version: Version 1.0

Protocol Date: September 28, 2017