HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 111

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV clade C DNA and of MF59-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

DAIDS DOCUMENT ID 12017

CLINICAL TRIAL SPONSORED BY

Division of AIDS (DAIDS)
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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, in accordance with Good Participatory Practices (GPP) and all local and national guidelines.

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

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

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

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

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

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

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

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

2 IRB/EC review considerations

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

This trial is being conducted in Southern Africa, with partial funding from the US NIH. Due to this, the trial is subject to both US and local regulations and guidelines on the protection of human research subjects and ethical research conduct. Where there is a conflict in regulations or guidelines, the regulation or guideline providing the maximum protection of human research subjects will be followed.

In compliance with international and local (as appropriate) Good Clinical Practice guidelines, each research location has a locally based Principal Investigator (PI) who is qualified to conduct (and supervise the conduct of) the research; and the research addresses an important local health need for an HIV vaccine. In addition, the investigators take responsibility for the conduct of the study and the control of the study products, including obtaining all appropriate regulatory and ethical reviews of the research. Each participating site has a standard operating procedure for ensuring that participants have the necessary information to make a decision whether or not to consent to the research.

The sections below address each of the review concerns by IRBs/ECs regarding how the research will be conducted.

2.1 Minimized risks to participants

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

This protocol minimizes risks to participants by (a) correctly and promptly informing participants about risks so that they can join in partnership with the researcher in recognizing and reporting harms; (b) respecting local/national blood draw limits; (c) performing direct observation of participants postvaccination and collecting information regarding side effects for several days postvaccination; (d) having staff properly trained in administering study procedures that may cause physical harm or psychological distress, such as blood draws, vaccinations, HIV testing and counseling and HIV risk reduction counseling; (e) providing HIV risk reduction counseling and checking on contraception use (for women); and (f) providing safety monitoring.

2.2 Reasonable risk/benefit balance

45 CFR 46.111 (a) 2 and 21 CFR 56 (a) 2: Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result.

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

2.3 Equitable subject selection

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

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

2.4 Appropriate informed consent

45 CFR 46.111 (a) 4 & 5 and 21 CFR 56.111 (a) 4 & 5: Informed consent is sought from each prospective subject or the subject's legally authorized representative as required by 45 CFR 46.116 and 21 CFR Part 50; informed consent is appropriately documented as required by 45 CFR 46.117 and 21 CFR 50.27

The protocol specifies that informed consent must be obtained before any study procedures are initiated and assessed throughout the trial (see Section 9.1). Each site is provided training in informed consent by the HVTN as part of its entering the HVTN. The HVTN requires a signed consent document for documentation, in addition to chart notes or a consent checklist.

2.5 Adequate safety monitoring

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

This protocol has extensive safety monitoring in place (see Section 11). Safety is monitored daily by HVTN Core and routinely by the HVTN 111 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). The privacy of participants is protected by assigning unique identifiers in place of the participant's name on study data and specimens. In addition, each staff member at each study site in this protocol signs a Confidentiality Agreement with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV clade C DNA and of MF59-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

Primary objectives

Primary objective 1

To evaluate the safety and tolerability of clade C DNA and bivalent gp120 protein and MF59 adjuvant in each vaccine regimen.

Primary objective 2

To evaluate the immune responses at the Month 6.5 timepoint (2 weeks after the 4th vaccination) of clade C DNA and bivalent gp120 protein and MF59 adjuvant in each vaccine regimen.

Study products and routes of administration

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

- **Protein + MF59**: Bivalent Subtype C gp120/MF59: clade C TV1.C gp120 Env and clade C 1086.C gp120 Env, each at a dose of 100 mcg, mixed with MF59 adjuvant, administered IM via needle and syringe

- **Placebo**: Sodium Chloride for Injection, 0.9%, administered IM via needle and syringe or Biojector

Table 3-1 Schema

Group	N	Deltoid	Month 0 (Day 0)	Month 1 (Day 28)	Month 3 (Day 84)	Month 6 (Day 168)
Groups 1-3: All injections administered via needle and syringe.						
1	30	Left	DNA	DNA	DNA	DNA
		Right	Placebo	Placebo	Protein + MF59	Protein + MF59
2	30	Left	DNA	DNA	Placebo	DNA
		Right	Protein + MF59	Protein + MF59	Placebo	Protein + MF59
3	6	Left	Placebo	Placebo	Placebo	Placebo
		Right	Placebo	Placebo	Placebo	Placebo
Groups 4-6: DNA (and DNA placebo) injections administered via Biojector. Protein + MF59 (and protein + MF59 placebo) injections administered via needle and syringe.						
4	30	Left	DNA	DNA	DNA	DNA
		Right	Placebo	Placebo	Protein + MF59	Protein + MF59
5	30	Left	DNA	DNA	Placebo	DNA
		Right	Protein + MF59	Protein + MF59	Placebo	Protein + MF59
6	6	Left	Placebo	Placebo	Placebo	Placebo
		Right	Placebo	Placebo	Placebo	Placebo

Participants

132 healthy, HIV-uninfected volunteers aged 18 to 40 years; 120 vaccinees, 12 placebo recipients

Design

Multicenter, randomized, controlled, double-blind trial

Duration per participant

12 months of scheduled clinic visits

Estimated total study duration

16 months (includes enrollment and follow-up)

Study sponsor

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

Study product providers

- DNA-HIV-PT123: IPPOX Foundation (Lausanne, Switzerland)
- Bivalent Subtype C gp120/MF59: Novartis Vaccines (Cambridge, MA, USA)

Core operations

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

Statistical and data management center (SDMC)

HVTN SDMC, FHCRC (Seattle, Washington, USA)

HIV diagnostic laboratory

HIV Sero-Molecular Laboratory–National Institute for Communicable Diseases (HSML-NICD) (Johannesburg, South Africa)

Endpoint assay laboratories

- Cape Town HVTN Immunology Laboratory (CHIL) (Cape Town, South Africa)
- Duke Human Vaccine Institute (DHVI), Duke University Medical Center (Durham, North Carolina, USA)
- Neutralizing Antibody (nAb) Assay Laboratory, Duke University Medical Center (Durham, North Carolina, USA)
- Antibody-Dependent Cellular Cytotoxicity (ADCC) Laboratory, Duke University Medical Center (Durham, North Carolina, USA)
- FHCRC/University of Washington (Seattle, Washington, USA)
- South Africa Immunology Laboratory and National Institute for Communicable Diseases (SAIL-NICD) (Johannesburg, South Africa)

Study sites

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

Safety monitoring

HVTN 111 PSRT; HVTN SMB

3.1 Protocol Team

Protocol leadership

Chair	Mina Hosseinipour Malawi CRS 265-175-8938 mina_hosseinipour@med.unc.edu	*Statisticians*	Barbara Metch SCHARP, FHCRC 206-667-4656 bmetch@scharp.org
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Other contributors to the original protocol

Core medical monitor	Philipp Mann HVTN Core, FHCRC	*Statistical research associate*	Niya Gu SCHARP, FHCRC
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	Song Ding IPPOX Foundation	*Clinical trials manager*	Marianne Hansen HVTN Core, FHCRC
	Susan Barnett Novartis Vaccines	*Project manager*	Lisa Sunner SCHARP, FHCRC
	Brian Burke Novartis Vaccines	*HVTN program manager*	Gina Escamilla SCHARP, FHCRC
Laboratory Program representative	Eva Chung HVTN Laboratory Program, FHCRC	*DAIDS Project Officer*	Michael Pensiero DAIDS, NIAID
Regulatory affairs	Liz Briesemeister HVTN Core, FHCRC	*Protocol development coordinator*	Ryan Jensen HVTN Core, FHCRC
Clinic coordinator	Gift Kamanga Malawi CRS	*Community engagement unit representative*	Genevieve Meyer HVTN Core, FHCRC
Community Advisory Board (CAB) member	Lindiwe Mvubu Isipingo CRS	*Community educator/recruiter*	Nelecy Chome Malawi CRS
DAIDS protocol pharmacist	Scharla Estep DAIDS, NIAID 240-627-3046	*Technical editor*	Erik Schwab HVTN Core, FHCRC

4 Background

4.1 Building on RV144

Following the RV144 study in Thailand that demonstrated modest (31%) preventive efficacy for an HIV vaccine regimen comprising ALVAC-HIV® (vCP1521) and clade B/E gp120 Env protein (AIDSVAX® B/E) [4], a large number of consultations with independent groups of scientists collected through the NIH, MHRP, and the Global Vaccine Enterprise were held providing input into the next steps for the field. Several consensus concepts emerged: (1) the need to evaluate the RV144 pox-protein prime-boost approach in a higher incidence population in a region of the world most affected by the HIV epidemic (ie, Southern Africa); (2) the consequent need to manufacture vaccines that more closely match those clades/subtypes that circulate in the proposed trial population (clade C); (3) at least one of the vaccine concepts to be tested should be analogous to the ALVAC/gp120 regimen used in RV144; and (4) the program to be developed should build upon the "correlates" analysis done after RV144 [5] to confirm the correlates identified in RV144 and to continue to define additional correlates of risk (CoR) for HIV vaccines. These recommendations led to the formation of the Pox Protein Public Private Partnership (P5), a group of vaccine developers, funders, and implementers, which was created to build on the RV144 results with the goal of improving the pox-protein regimen and enhancing the level and/or duration of protection seen in the RV144 study, with the hope of producing an effective prophylactic HIV vaccine with the potential to have a major public health impact.

Two distinct clinical trial programs have been developed by P5 collaborators to satisfy these aims for the Southern Africa region. Firstly, the "*Phase 3 Program*", will evaluate a clade C-adapted ALVAC-Bivalent gp120/MF59 vaccine regimen in South Africa with a goal of eventual vaccine licensure. The second, the "*Correlates Program*", focuses on the broader goal of advancing the field by ultimately discovering immune response biomarkers that predict HIV vaccine protective efficacy, called correlates of protection (CoPs) [6]. This program will address this goal by evaluating multiple vaccine regimens containing combinations of next-generation vaccine products as well as different adjuvant systems to identify those vaccine regimens exhibiting potent yet diverse immunological profiles, thus providing the greatest potential to confirm previous and identify new CoR in an efficacy trial. Up to thirteen different vaccine regimens will be evaluated in several phase 1/2a trials, including HVTN 111; and a maximum of 4 of these candidate vaccine regimens will be selected based on safety and immunogenicity data collected through the primary immunogenicity timepoint of Month 6.5 by the Correlates Program Oversight Committee to advance to a phase 2b proof-of-concept efficacy trial.

The design of the efficacy trial with up to four active vaccine arms and a placebo arm will allow for an efficient and simultaneous assessment of the safety and efficacy of the vaccine regimens to prevent HIV-1 infection, including between-regimen comparisons. In addition, it may provide further validation of the immune correlates of risk identified in the RV144 Immune Correlates Study, allow for identification of additional correlates of risk, and allow for assessment of identified correlates of risk as correlates of protection.

4.1.1 The RV144 trial

The RV144 trial was conducted by the US Military HIV Research Program and the Thailand Ministry of Health in a community-based sample of more than 16 000 HIV-1–uninfected participants in Thailand and results were published in 2009 [4]. This community-based study enrolled individuals aged 18 to 30 years with varying degrees of HIV risk. The clinical trial evaluated the heterologous prime-boost combination of canarypox prime ALVAC-HIV (vCP1521), expressing clade E env and clade B gag and pol, followed by the AIDSVAX® clades B/E gp120 protein boost. These products were based on viruses commonly circulating in Thailand at the time. This vaccine regimen demonstrated 31.2% efficacy when compared with placebo (n = 51 vs. n = 74, respectively; p = 0.04) at 3.5 years [4]. Although evaluation of vaccine efficacy at 12 months post vaccination was not included in the pre specified analysis, substantially greater reduction in acquisition was observed one year post vaccination (estimated 60.5%, p = 0.02) with the vaccine effect waning over time to 31% cumulative through 3.5 years [7].

4.1.2 Correlates of risk (CoR) in RV144

To better understand how the RV144 vaccine regimen reduced the risk of HIV infection, a large consortium of independent laboratories worked together systematically to ensure maximal information could be derived from samples obtained from participants who were vaccinated but became infected compared with those vaccinated but uninfected at the end of the trial. A case control study was performed on 41 infected vaccine recipients, 205 uninfected vaccine recipients (5:1) and 40 placebo recipients (20 infected and 20 uninfected) within the RV 144 clinical trial to identify CoR [5]. Among the 6 primary immunological variables selected for the correlates analysis (5 different antibody [Ab] responses and CD4+ T cell cytokine production) that were measured at the 2 weeks after the final vaccination visit (ie, at or near peak immunogenicity), 2 immune CoR of HIV acquisition were identified among vaccine recipients in the RV144 case control study. The first was the presence of immunoglobulin G (IgG) Ab that bound to a scaffolded gp70 V1V2 recombinant protein; this variable correlated inversely with infection rate (ie, higher V1V2 Ab→lower infection rate). The second was plasma Env-specific binding IgA, which correlated directly with infection rate (ie, lower immunoglobulin A [IgA] Ab to Env→lower infection rate). The other 4 primary variables correlated inversely with infection rate only when the level of IgA binding was low. Notably, neither low levels of V1V2 Ab nor high levels of Env-specific IgA were associated with higher rates of infection than those found in the placebo group [5].

Recently, several studies have further enhanced our understanding of the efficacy seen in RV144. Rolland and colleagues demonstrated a sieve effect in the vaccine recipients, specifically that the vaccine induced better protection against viruses that matched the vaccine sequence at position 169 in the V2 loop of Env [8]. These data further substantiate the importance of antibodies directed against this region in protecting against infection [5]. Yates and colleagues noted that Env V1V2-specific IgG3 was the immunoglobulin subclass showing the strongest correlation with prevention of HIV acquisition in RV144 [9]. Chung and colleagues demonstrated that the IgG3 subclass was much better at engaging Fc-mediated effector responses when compared to the other subclasses, thereby providing a possible mechanism explaining the association of Env V1V2 IgG3 with a lower rate of HIV acquisition [10]. In sum, these CoR studies point to the importance of functional Ab responses, directed against a specific region of Env, in mediating the differing rates of HIV acquisition observed in RV144. They lay the groundwork for directing immune analyses planned for future HIV vaccine clinical trials.

4.2 The Global Burden of HIV

The most recent UNAIDS report indicates that an estimated 2.3 million new HIV infections occurred worldwide in 2012, a 33% decline from 2001 [11]. With emphasis on early diagnosis and treatment, efforts to improve linkages to care, as well as with access to a number of behavioral prevention strategies, the overall number of new infections and deaths attributable to AIDS has declined. However, as people are living longer after infection, the estimated number of individuals living with HIV continues to increase [11]. Although these statistics are encouraging and represent a decrease from previous years, these trends are not evident everywhere and the burden of the epidemic varies considerably between countries and sub-populations.

4.2.1 HIV in sub-Saharan Africa

While universal access to antiretroviral HIV treatment is a global ideal, in many regions of Sub-Saharan Africa limited access undermines the prevention potential of widespread antiretroviral therapy. Moreover, the costs and health care burden of delivering ever-increasing amounts of treatment in resource constrained settings pose significant challenges. In addition, while studies conducted over the past few years have confirmed the promise of antiretroviral chemoprophylaxis, it is well recognized that one way to eradicate a global viral epidemic is to design, mass produce, and then systematically immunize the target population with an effective prophylactic vaccine. Although the results of the RV144 trial are modest, these provide the first indications that a prophylactic vaccine can reduce HIV acquisition risk.

With more than 6 million people living with HIV as of 2012, and more than 350,000 new infections each year, South Africa's epidemic remains the largest in the world and the Sub-Saharan region bears the preponderant burden of the HIV epidemic with almost 70% of all infections worldwide [11,12]. The vast majority of newly acquired infections in this region occur during unprotected heterosexual intercourse and subsequent transmission to newborns and breastfed babies. Clearly, effective methods for preventing the acquisition and transmission of HIV-1 are urgently and desperately needed for this region.

4.3 Rationale for HVTN 111

DNA, HIV gp120 proteins, and the combination of the two, as well as the combination of each of these immunogens with other constructs have been widely evaluated. However, these investigations included a variety of different vaccine candidates in different combinations, doses and incongruent injection schedules. They were implemented in diverse populations and the immunological assessments were performed in a variety of different laboratories using different assays. The HVTN has planned a series of phase 1/2a studies to compare the immunogenicity of several complementary vaccine regimens using consistent assays performed in central laboratories. Data from each of these studies will be analyzed individually and as a component of this suite of studies. This will add value to individual studies as well as, collectively, the overall vaccine agenda.

HVTN 111, as one of the suite of studies, is designed to evaluate the immune response profiles elicited by two combination vaccine regimens containing DNA and adjuvanted protein without a viral vector.

4.3.1 Rationale for DNA prime, protein boost

A variety of preclinical studies using the DNA prime followed by protein boost approach with HIV immunogens have been published since the 1990s. Quantitative and qualitative differences were found between the antibodies induced by Env-based DNA/protein vs. Env protein alone or DNA alone in rabbits [13,14]; and small NHP SHIV challenge studies showed protection [15,16].

DNA-protein combination vaccine regimens have recently been investigated in clinical trials. Two studies conducted by the NIAID Vaccine Research Center (VRC) in 2008-2010 assessed influenza H5 DNA priming followed by boosting with H5/N1 monovalent inactivated vaccine (MIV) and showed that the combination approach enhanced the magnitude of hemagglutination inhibition antibodies to protective levels in 81% of vaccine recipients with geometric mean titers 4-fold higher than MIV given twice [17]. In addition, this effect was most evident when the booster vaccination was given at least 12-24 weeks after the prime as compared to 4-8 week intervals [18].

Three HIV vaccine clinical trials have been conducted using the DNA prime/Env protein boost platform. The first study was conducted by Lu and colleagues at the University of Massachusetts Medical School. In this study, participants who received 2 dose levels of a multi-clade HIV DNA prime followed by a multi-clade HIV gp120 protein with QS-21 adjuvant boost demonstrated Env-specific interferon gamma (IFN-γ) ELISpot responses in over 90% of participants 2 weeks after the final vaccination, which persisted at 1 year (5 months after the final vaccination) in over 80% of participants. Also, Env-specific binding antibodies were induced at a higher magnitude than those seen from recombinant envelope vaccine regimens alone and these responses persisted in most participants at 1 year with only a modest decrease in titer [19]. Furthermore, the DNA plus protein platform elicited a different Ab profile than recombinant HIV envelope protein alone or a pox-vector prime plus protein boost [20].

HVTN 049 evaluated DNA priming at months 0, 1 and 2 followed by trimeric gp140 clade B protein in MF59 boosting at months 6 and 9 versus protein only at months 0, 3 and 9 and found that following the protein boost, the DNA plus gp140 protein prime-boost regimen induced: 1) substantial levels of binding antibodies against Env in 100% of vaccinees; 2) significantly higher titers of homologous neutralizing antibodies (nAbs); 3) CD4+ T-cell responses to Env antigens of significantly greater magnitude (among the group that was primed with 1mg of *env* DNA), in comparison with the group that was immunized with gp140 protein alone, and 4) a polyfunctional CD4+ T cell response pattern that differed qualitatively from the CD4+ responses in the protein alone group, shifting towards a Th_1 response by DNA priming [21]. In HVTN 049, antibodies were induced that were avid, reacted with envelopes of multiple clades (A, B, C, A/E, G) and mediated infectious virion capture [Georgia Tomaras, personal communication]. Also, in a comparison between multiple different vaccination strategies from several HVTN clinical trials with differing combinations of DNA, MVA, Ad5 vectors, and Env protein vaccinations, as well as RV144, the DNA/protein strategy in HVTN 049 led to the highest magnitude memory B cell responses [22].

HVTN 088 evaluated an HIV gp140 clade C protein in MF59 adjuvant administered to participants who had received HIV DNA boosted by HIV gp140 clade B protein in MF59 as a part of HVTN 049 or other HVTN trials conducted 5-7 years earlier. Even before administration of the clade C gp140 boost, a significant portion of the HVTN 049 participants still had detectable T-cell and binding Ab responses, suggesting remarkably

durable memory responses induced by the DNA + protein combination. This impression was further confirmed by the finding of strong and rapid boosting responses after the administration of the clade C gp140 protein [23] and see Section 4.8.2.2.

The findings from these clinical trials provide intriguing clues as to an approach that may facilitate the induction of nAbs and long-lasting memory B and T cell responses and provides justification to examine the DNA prime/ protein boost combination regimen in this study. Co-administration of the DNA with the protein as a boost increases the number of DNA vaccinations beyond two priming doses, as 3 DNA primes have been shown to be beneficial in some clinical trials of other HIV DNA vaccine candidates [24]. While boosting with DNA that is co-administered with protein has not yet been investigated in humans, boosting with co-administration of vector and protein has been shown to enhance immunogenicity as compared to vector or protein boost alone [25]. By extrapolation, then, it is hypothesized that the combined DNA/protein boost will elicit strong humoral and cellular responses. Therefore, HVTN 111 will evaluate DNA prime with DNA+protein+MF59 boost.

4.3.2 Rationale for simultaneous administration of DNA and protein vaccines

Co-administration of a protein from the initial vaccination timepoint has the potential benefits of more rapidly eliciting both Ab and T-cell responses from the first vaccination. The Pavlakis group demonstrated the potential immunologic advantages of co-administration of protein with DNA in NHP. In this 4-arm study rhesus macaques received either DNA alone, DNA co-administered with protein, 2 DNA priming injections followed by 2 protein boosts, or sham DNA vaccination. The DNA vaccine plasmids expressed SIV Env, Gag, Pol, Nef, Tat, and Vif, were given with rhesus IL-12 DNA adjuvant administered IM with electroporation. The protein vaccine consisted of inactivated SIVmac239 particles. Vaccinations were given at month 0, 2, 4 and 9, and followed by repeated low-dose mucosal challenge with heterologous SIVsmE660. The 2 groups that received DNA vaccine (DNA alone, or DNA co-administered with protein) at all 4 timepoints had higher levels of vaccine-induced SIV-specific IFN-γ^+ T cells. However, the group that received co-administered DNA and protein had the highest levels of Env binding Abs and higher avidity that persisted at a higher rate than the other groups. In addition, Env binding Abs and avidity correlated with slower SIV acquisition and cytotoxic CD4+ effector memory inversely correlated with peak viral load [26,27].

In another study, they compared DNA alone, EM-005 adjuvanted gp120 protein alone, and co-administration of both. This study also demonstrated significantly higher Env humoral responses upon DNA with protein co-immunization. The study further showed a positive combined effect of EM-005 adjuvanted protein concurrently administered with EP delivered DNA, indicating the importance of inclusion of an appropriate adjuvant to maximize the humoral immune responses in non-human primates [26,27]. Some differences between these pre-clinical studies and the proposed clinical trial are of note: 1) the use of DNA adjuvanted with IL-12, 2) the difference in the adjuvant used with the protein, 3) the DNA delivery method (EP in the pre-clinical studies vs Biojector or needle/syringe in the proposed clinical trial) and 4) the fact that DNA and protein were delivered to the same injection site in the pre-clinical studies, whereas the clinical study proposes contralateral injections for the individual products. The injections at separate sites have also been evaluated in the AUP512 study (see section 4.7.2) and this approach permits the distinct benefit to consign local reactogenicity symptoms to a specific product. As the protein vaccine is adjuvanted, adding another adjuvant with the DNA vaccine (namely IL-12) adds a level of complexity not yet warranted for evaluation when

supportive "dual-adjuvant" synergistic immunogenicity clinical data does not yet exist and is, therefore, not considered in the proposed trial. For the rationale for Biojector and the adjuvant, see sections 4.3.3 and 4.3.4. In addition, the Haigwood group recently demonstrated in rabbits that DNA plus protein co-administration was superior to protein administration alone in terms of antibody kinetics, magnitude, avidity, and neutralization potency [28].

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

Protein was concurrently administered (at different anatomical sites) with DNA or NYVAC in the NHP AUP512 study (see Section 4.7.2) and demonstrated earlier binding antibody immune responses.

Concurrent protein administration with NYVAC, as well as concurrent administration of protein and DNA-HIV-PT123 is currently being evaluated in HVTN 096/EV04, and this is the only clinical trial of which we are aware that has concurrently administered DNA and protein (DNA-HIV-PT123 and AIDSVAX® B/E have been given simultaneously at months 0 and 1, followed by simultaneous boosting with a NYVAC-HIV and AIDSVAX® B/E). This study is still blinded and final results are pending (see Section 4.8.1 for interim results).

4.3.3 Rationale for MF59

Adjuvants are known to enhance the potency, quality, and longevity of antigen-specific immune responses [29], and the availability of novel commercial adjuvants that are potent and safe has been heralded as an important contribution that may advance HIV vaccines [30,31]. The MF59 adjuvant, an oil-in-water emulsion, is licensed for several flu vaccines in multiple countries, and in pre-clinical models [32] has demonstrated recruitment of antigen presenting cells and upregulation of cytokines, chemokines, and receptors. The adjuvant has improved antibody affinity maturation [33], improving both epitope breadth and binding affinity, and elicits a balanced Th1 and Th2 response. It also increases T-cell proliferation by enhanced surface expression of MHC class II and co-stimulatory molecules [34].

4.3.4 Rationale for Biojector

The optimal delivery system for HIV vaccines is not clear and the immune responses to the vaccine may differ according to administration method. Additionally, tolerability of the technique may differ. The various delivery systems have different costs which can influence the overall cost of an effective vaccine program.

Needle and syringe are typically used for vaccinations. A needleless delivery system may pose advantages with respect to prevention of needle-stick injuries at the clinic. The Biojector 2000 Needle-Free Injection Management System™, manufactured by Bioject (Tualatin, OR) has now been used in many HIV Vaccine trials (eg RV 172 [35], HVTN 505, VRC008, HVTN 076, HVTN 077, HVTN 083) and has demonstrated good safety and tolerability.

Biojector has been reported to induce a small skin lesion. These papules or scabs are infrequently recorded on diary cards completed by study participants and resolve without treatment. The Biojector® is also associated with some pain, redness, swelling and/or bruising at the injection site [36].

However, more importantly, the delivery system may enhance immune responses. In a single study of limited sample size (20 per arm), DNA delivery with this device primed for greater IFN-γ ELISpot, CD8+ T-cell, and antibody responses after rAd5 boosting, as compared to delivery with needle and syringe [37,38].

As the direct comparison of DNA delivery via Biojector versus needle and syringe has not been widely evaluated in clinical trials - especially not in combination with a protein boost - the proposed study can help assess this valuable scientific question.

4.4 Study product descriptions

4.4.1 DNA: DNA-HIV-PT123

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

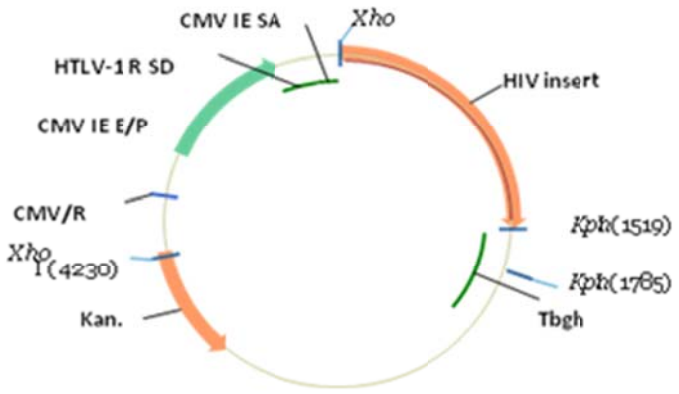

Figure 4-1 Example DNA-HIV-PT123 plasmid map

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

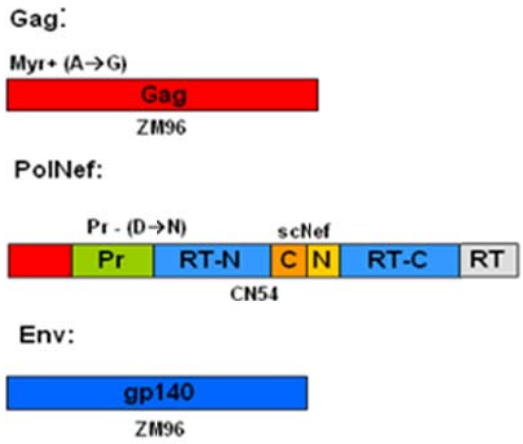

Figure 4-2 DNA-HIV-PT123 plasmid inserts schematics

Additional information on the construction of these plasmids is provided in the DNA-HIV-PT123 Investigator's Brochure (IB).

4.4.2 Bivalent Subtype C gp120

4.4.2.1 Constructs

Bivalent Subtype C gp120, manufactured by Novartis Vaccines at Rentschler Biotechnologie (Laupheim, Germany), consists of two subtype C recombinant monomeric proteins, TV1.C gp120 and 1086.C gp120. These recombinant gp120s represent the HIV Env surface glycoprotein containing the receptor binding domain. Each gp120 is modified from its wild type full-length form (gp160) by replacement of the native signal sequence and deletion of the entire gp41 C-terminal portion of the glycoprotein containing the TM and cytoplasmic domains. The combination of the 2 subtype C gp120 proteins and the MF59 is referred to as Bivalent Subtype C gp120/MF59.

4.4.2.2 Manufacturing and formulation

Each protein is expressed in Chinese hamster ovary (CHO) cells under conditions favorable for secretion of monomeric protein. Following fermentation, each protein is extensively purified from culture supernatants, including further enrichment for monomer.

Following clone selection, a fed batch cell culture at 500L or 1000L scale is employed for cell propagation. Once the cells reach optimum cell density, the culture is harvested and purified using standard methods. The conditioned media is concentrated by ultrafiltration. The manufacturing process utilizes a weak cation exchange chromatography step, CM-Fractogel, which provides purification as well as viral reduction. Concentration (by ultrafiltration) is then used, followed by exchange into the formulation buffer.

After these process steps, both subtype C gp120 protein processes include viral reduction filtration (nanofiltration) followed by 0.22 µm filtration and bulk fill. Both TV1.C and 1086.C bulk drug substances are stored frozen at not more than -60°C. The formulations are similar for both drug substances, containing Env antigen, sodium citrate, and sodium chloride, pH 6.0-7.0.

The qualitative composition per dose of each subtype C gp120 vaccine protein is provided in Table 4-1.

Table 4-1 Qualitative composition of Subtype C gp120 drug substances vials

Ingredient	Function
gp120 protein	active
Sodium Citrate, Dihydrate	buffer
Citric Acid, Monohydrate	buffer
Sodium Chloride	tonicity modifying agent
Water for injections	diluting agent

Additional information is provided in the Bivalent Subtype C gp120/MF59 IB.

4.4.3 MF59 adjuvant

The Novartis MF59 adjuvant is an oil-in-water emulsion with a squalene internal oil phase and a citrate buffer external aqueous phase. Two non-ionic surfactants, sorbitan trioleate and polysorbate 80, serve to stabilize the emulsion. The qualitative composition is shown in the table below.

Table 4-2 Qualitative composition of MF59

Name of Ingredients	Function
Squalene	oil phase
Polysorbate 80	surfactant
Sorbitan Trioleate	surfactant
Sodium Citrate, dihydrate	buffer
Citric Acid, monohydrate	buffer
Water for Injection	aqueous phase
Nitrogen	inert gas

The full dose of MF59 utilized in the marketed Fluad® vaccine (containing 9.75 mg of squalene) will be utilized for formulation with subtype C recombinant envelope gp120 proteins (described above).

The MF59 (full name: MF59C.1) manufacturing process consists of five manufacturing steps: raw materials dispensing and blending, premixing, emulsification, sizing filtration, and filling.

The MF59 bulk resulting at the end of the process is filled into the vials with an overlay of nitrogen and stored protected from light at 2-8° C.

Additional information is provided in the Bivalent Subtype C gp120/MF59 IB.

4.4.4 Bivalent Subtype C gp120/MF59 for injection

A final dose of 100mcg of each recombinant Env protein will be mixed with MF59 adjuvant. The composition of 1 dose of the resulting vaccine is shown in Table 4-3.

Table 4-3 Composition of 0.5 mL dose of Bivalent Subtype C gp120/MF59 for injection

Ingredient	Amount in1 dose	Function
Drug Substances		
TV1.C gp120 protein 1086.C gp120 protein	100 mcg 100 mcg	active active
Adjuvant (MF59)		
Squalene	9.75 mg	oil phase
Polysorbate 80	1.175 mg	surfactant
Sorbitan Trioleate	1.175 mg	surfactant
Excipients		
Sodium Citrate, Dihydrate Citric Acid, Monohydrate Sodium Chloride Water for injections	0.72 mg 0.009 mg 4.38 mg qs to 0.5 mL	buffer buffer tonicity modifying agent solvent

4.5 Trial design rationale

HVTN 111 has 4 active groups and 2 placebo groups and will compare DNA priming administered at Months 0 and 1 followed by DNA + Protein + MF59 boosting at Months 3 and 6 versus DNA + Protein + MF59 coadministered at Months 0, 1, and 6.

In groups 1-3 all injections will be administered via needle and syringe. In groups 4-6 the DNA injections (and DNA placebo injections) will be administered via Biojector. Protein + MF59 injections (and protein + MF59 placebo injections) will be administered via needle and syringe.

Each of the vaccine regimens included in HVTN 111 will address specific questions in the field and help identify regimens with diverse immune responses warranting evaluation in a future efficacy trial aimed at discovery CoR.

4.5.1 Dose rationale

4.5.1.1 DNA-HIV-PT123 dose

The choice of the 4mg dose for the DNA-HIV-PT123 vaccine is based on: 1) the dose response study conducted by the Vaccine Research Center (VRC), NIAID [39] with a DNA vaccine using a similar backbone as DNA-HIV-PT123, that demonstrated that there is a trend toward a greater magnitude and frequency of T cell responses in recipients of the 4-or 8-mg dose than in the recipients of the 2-mg dose; 2) the previous clinical studies with the DNA-HIV-PT123 and NYVAC-HIV combination [24,40,41]; 3) the preclinical immunogenicity studies in non-human primates (NHP) with DNA-HIV-PT123 (see Section 4.7); and 4) the repeated-dose toxicity study of DNA-HIV-PT123 and NYVAC-HIV (see Section 4.6.1) that used the same clinical material at the same dose as this study and demonstrated that the selected dose was safe and well tolerated in animals.

4.5.1.2 Bivalent Subtype C gp120/MF59 dose

For the Bivalent Subtype C gp120/MF59 vaccine component, 100 mcg each of the two gp120 subtype C proteins (TV1 gp120 and 1086 gp120) will be admixed with the oil-in-

water emulsion MF59 (9.75 mg squalene) by the Pharmacist at each CRS prior to IM administration.

Bivalent Subtype C gp120/MF59 vaccine has not yet been administered to humans. The 200 mcg total dose was selected based on previous clinical experience with similar proteins. Limited dose range studies performed with Novartis (formerly Chiron) subtype B SF2 gp120 and subtype E gp120 protein candidates indicated that 50 mcg and 100 mcg, totaling 150 mcg, doses with MF59 adjuvant were immunogenic and well tolerated [42].

4.5.2 Schedule

The vaccination schedule of the DNA prime followed by DNA+protein boost regimens is based upon the schedule used in RV144. The schedule for the DNA and protein co-administration regimen is similar to the prime/boost regimen, yet administers placebo instead of active product at month 3. In previous trials with adjuvanted protein, peak humoral immunogenicity was obtained after 3 doses of protein, while peak cellular responses are observed after 2 doses. A longer rest between the 2nd and 3rd vaccinations is preferred for antibody maturation.

4.5.3 Choice of control

Sodium Chloride for Injection, 0.9% will serve as the placebo for the DNA vaccine and the Bivalent Subtype C gp120/MF59 vaccine.

4.6 Summary of preclinical safety studies

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

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

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

Group	Test/Control Article Days 0, 14, 28 and 42	Dose (mg)	Volume (mL)	Test/Control Article Days 56 and 70[1]	Dose (PFU)	Volume (mL)1	Main Study Necropsy (Day 72)	Recovery Study Necropsy (Day 84)	Total # Animals
1	Saline	N/A	1	Saline	N/A	2	5M, 5F	5M, 5F	10M/10 F
2	DNA-HIV-PT123	4	1	NYVAC-HIV-PT1 and NYVAC-HIV-PT4[1]	$\geq 5 \times 10^6$ PFU per NYVAC	2	5M, 5F	5M, 5F	10M/10 F

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

New Zealand White rabbits (20/sex, total 40 rabbits) were randomly assigned to receive IM injections of either DNA-HIV-PT123 on Days 0, 14, 28 and 42 followed by NYVAC-HIV-PT-1 and NYVAC-HIV-PT-4 on Days 56 and 70 or to saline control on the same

days. NYVAC-HIV-PT1 and NYVAC-HIV-PT4 were administered separately (right and left hind limbs) in 2 separate injections of 1 ml each on Days 56 and 70. The control group received saline control delivered as a single injection on Days 0, 14, 28 and 42, and delivered as 2 separate injections of 1 mL each on Days 56 and 70.

Clinical symptoms, body weight, temperature, ophthalmology, injection site reactions and safety laboratory parameters were assessed. Five animals/sex/group were sacrificed 2 days or 2 weeks following the final vaccination (Day 72 or 84). Each animal underwent a complete necropsy with organ weights. All tissues collected from animals assigned to the main study (Day 72 necropsy) were examined microscopically. For those animals assigned to the recovery study (Day 84 necropsy) only the injection site with surrounding muscle and gross lesions were microscopically examined.

In accordance with the toxicity report, all animals survived to scheduled sacrifice. No abnormal findings or statistically significant changes in physical and cageside examinations, ophthalmological examination, body weights, administration site, urinalysis and gross necropsy considered to be related to treatment were observed. Body temperatures were briefly elevated following the NYVAC administration. Some changes were observed in clinical pathology and microscopic observations, most of which could be correlated to an acute inflammatory and immune response that is expected with administration of a test material intended for use as a vaccine. Most changes were temporary and reversible and therefore not considered to constitute a safety concern. Overall, it was concluded that the study results demonstrated that administration of DNA-HIV-PT123 in combination with NYVAC-HIV-PT1 and NYVAC-HIV-PT4 was safe and well-tolerated in New Zealand White Rabbits. The vaccine was immunogenic in all treated animals following the complete immunization schedule.

4.6.2 Toxicity studies of HIV Env vaccines

Nonclinical *in vivo* Good Laboratory Practice (GLP) toxicology studies were conducted with early candidate subtype B and E gp120 Env protein vaccine candidates that were subsequently advanced to phase 1-2 clinical trials. More recently, similar subtype B gp140 and subtype C gp140 vaccine candidates with MF59 have been tested in nonclinical safety studies. The subtype C gp140 previously tested was from the same strain (HIV-1 TV1) as one of the components (TV1 gp120) in the proposed Bivalent Subtype C gp120/MF59 vaccine, and hence is very similar in sequence. Overall, toxicology studies revealed that both the subtype B gp140 and subtype C gp140 vaccines with MF59 were well tolerated and testing revealed no adverse local or systemic effects.

Data from the following nonclinical studies are included in the IB:

- Subchronic IM toxicity study of Biocine® HIV Thai E gp120/SF 2 gp120 vaccine in rabbits
- Repeat dose toxicity of IM HIV DNA/PLG prime followed by IM subtype B gp140/MF59 in rabbits
- Repeat dose toxicity of intranasal (IN) subtype B gp140 with an LTK63 adjuvant followed by IM subtype B gp140 with MF59 in rabbits
- Repeat dose toxicity of IM SAAVI DNA-C2 followed by IM SAAVI MVA-C with subtype C gp140/ MF59 in rabbits

4.6.3 Toxicity studies of MF59

MF59 is not associated with any potential for systemic toxicity and it has a low order of local reactogenicity. In repeat-dose rabbit studies, clinical pathology findings of increased fibrinogen and minor inflammatory and degenerative changes at the injection site are consistent with the effects of IM injections of an immunological adjuvant. These findings are readily reversible within days to 1 - 2 weeks. In repeat-dose toxicology studies in dogs, there were no effects on cardiovascular or central nervous system (safety pharmacology) parameters. MF59 is not genotoxic (Ames test) or clastogenic (mouse micronucleus), is not a dermal sensitizer (Guinea pig), and was not teratogenic (rat and rabbit) or a developmental toxicant (rat).

Pivotal toxicology studies performed with MF59 include:

- single- and repeat-dose toxicity (including local tolerability),
- genotoxicity,
- sensitization, and
- embryofetal and developmental toxicity.

4.7 Preclinical immunogenicity studies

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

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

The immunogenicity and safety of DNA-HIV-PT123 in combination with NYVAC or NYVAC-KC and protein was evaluated in this non-human primate study. The results discussed below focus on the safety and immunogenicity results from groups that have DNA prime. Groups 1-3 are therefore also referred to as DNA prime groups and groups 4-5 are also referred to as No-DNA prime groups.

The study design is provide in Table 4-5 shown below. The study was initially designed with 6 vaccinations for groups 1, 2 and 3 (last vaccination at week 32) and 4 vaccinations for groups 4 and 5 (last vaccination at week 24). This is also referred to as “Primary Vaccination” in this study. Based on the initial immunogenicity data, a late boost at week 49 was added.

Table 4-5 AUP444 study design

Gp	Size	Wk 0	Wk 4	Wk 8	Wk 12	Wk20	Wk 24	Wk28	Wk32	Wk 49
1	8	DNA[1] (IM)	DNA (IM)	DNA (IM)		NYVAC –KC[2] (Scarification)		Protein[4] (IM)	protein (IM)	protein (IM)
2	8	DNA (IM)	DNA (IM)	DNA (IM)		NYVAC -KC (IM)		protein (IM)	protein (IM)	protein (IM)
3	8	DNA (IM)	DNA (IM)	DNA (IM)		NYVAC[3] (IM)		protein (IM)	protein (IM)	protein (IM)
4	8	NYVAC -KC (IM)	NYVAC -KC (IM)		NYVAC –KC + protein (IM)		NYVAC –KC + protein (IM)			NYVAC –KC + protein (IM)
5	8	NYVAC (IM)	NYVAC (IM)		NYVAC + protein (IM)		NYVAC + protein (IM)			NYVAC + protein (IM)

[1] DNA: DNA-HIV-PT123 with a total dose of 4mg
[2] NYVAC-KC: NYVAC-KC-HIV-PT1 and NYVAC –KC-HIV-PT4 with a final dose of $2x10^8$pfu/mL
[3] NYVAC: NYVAC-HIV-PT1 and NYVAC –HIV-PT4 with a final dose of $2x10^8$pfu/mL
[4] Protein: TV1gp120 with a final dose of 100µg plus MF59 adjuvant

4.7.1.1 Summary of AUP444 cellular responses

- DNA-HIV-PT123 was greatly immunogenic with an average of 0.5% cytokine secreting cells (Figure 4-3) after DNA immunization ;
- The DNA-HIV-PT123 prime groups induced balanced CD4 and CD8 T-cell responses as shown by flow cytometry after the 3 DNA immunizations (see Figure 4-3);
- DNA-HIV-PT123 prime induced broad T-cell responses as indicated by the large number of targeted peptide pools encompassing Env, Gag, Pol and Nef HIV proteins (see Figure 4-4);
- The DNA-HIV-PT123 prime groups induced more balanced Env and Gag/Pol responses; while the No-DNA prime groups (Group 4-5) induced predominantly Env T-cell responses (see Figure 4-4);
- The DNA-HIV-PT123 prime groups induced more potent CD4 and CD8 T-cell responses both in terms of magnitude and polyfunctionality (see Figure 4-5 and Figure 4-6 and Figure 4-7).

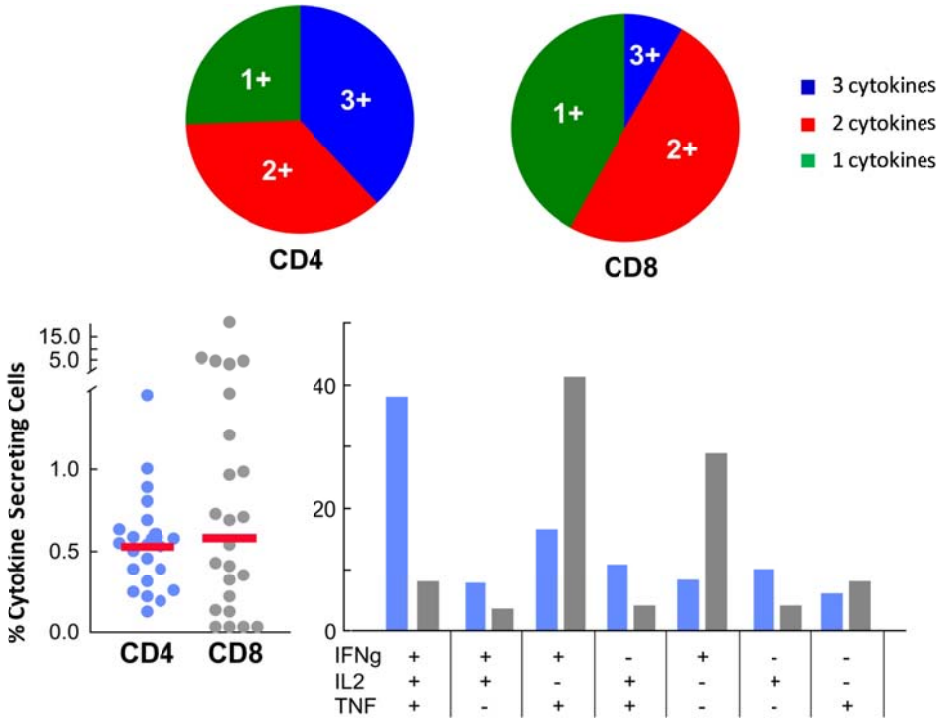

Figure 4-3: AUP444 - DNA prime induces polyfunctional and balanced CD4 and CD8 T-cell responses (Week 10, 2 weeks post 3xDNA prime)

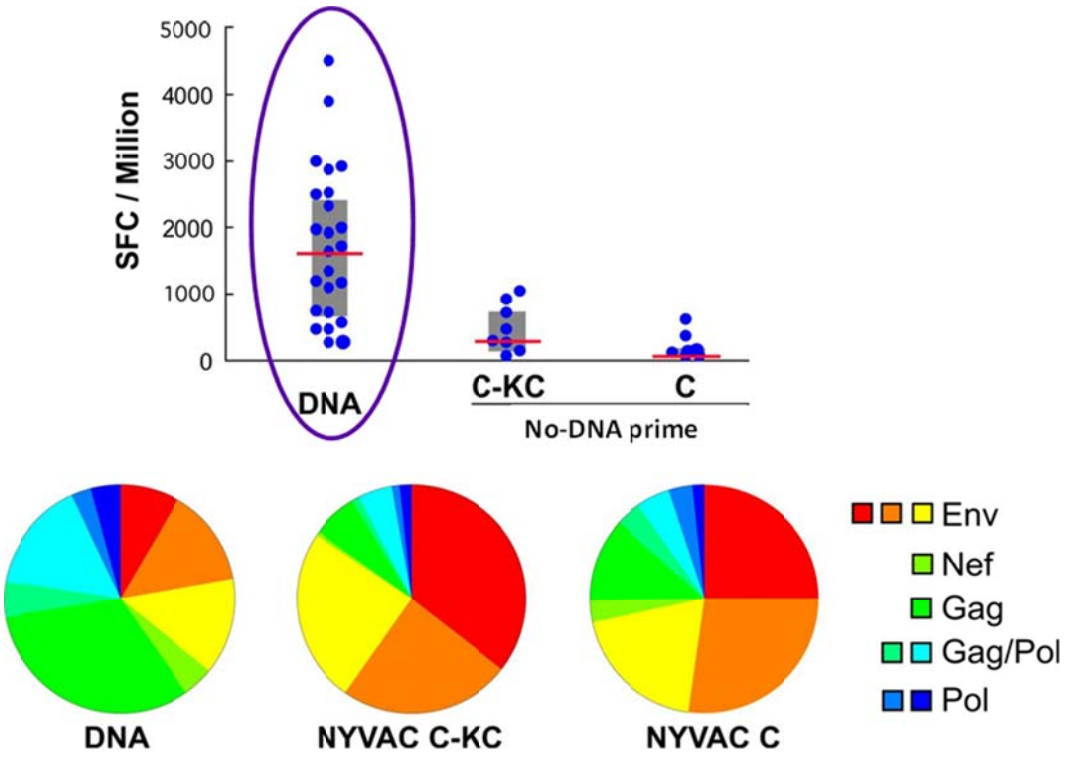

Figure 4-4 AUP444 - Breadth of responses comparing DNA prime groups (group 1-3) at week 10 (2 weeks post 3 DNA injections) vs No-DNA prime groups (group 4-5) at week 8 (4 weeks post 2 NYVAC injections)

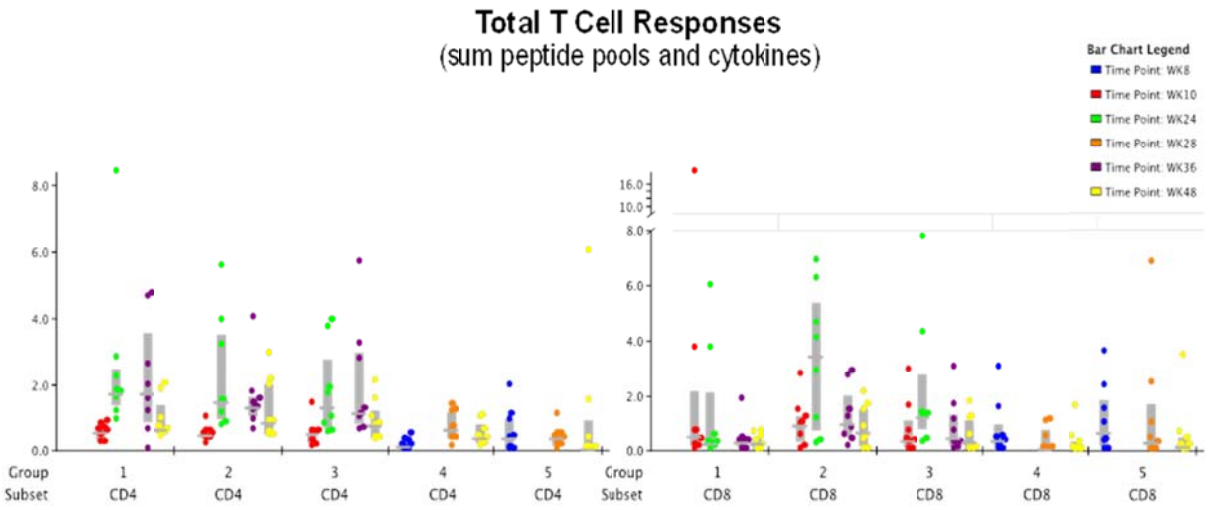

Figure 4-5 AUP444 - Total T Cell Responses

CD4 T Cell Function

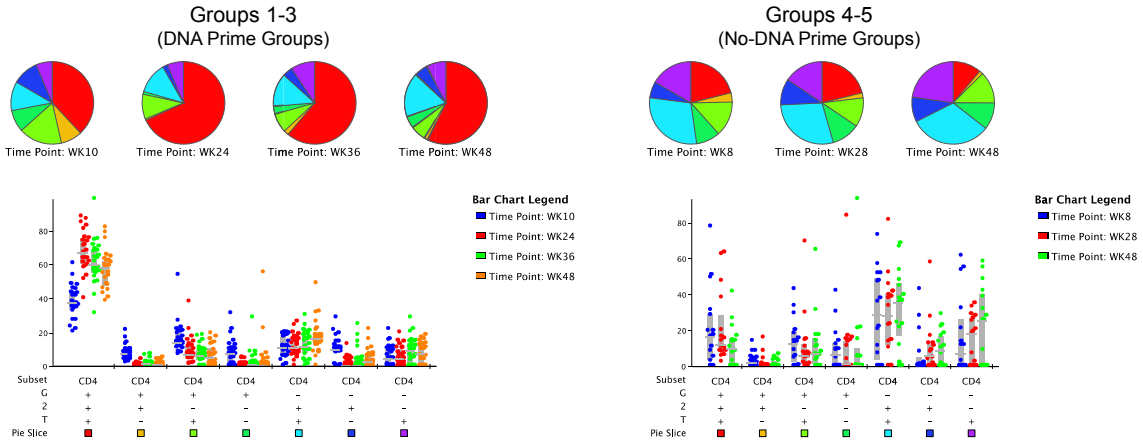

Figure 4-6 AUP444 - CD4 T Cell Responses

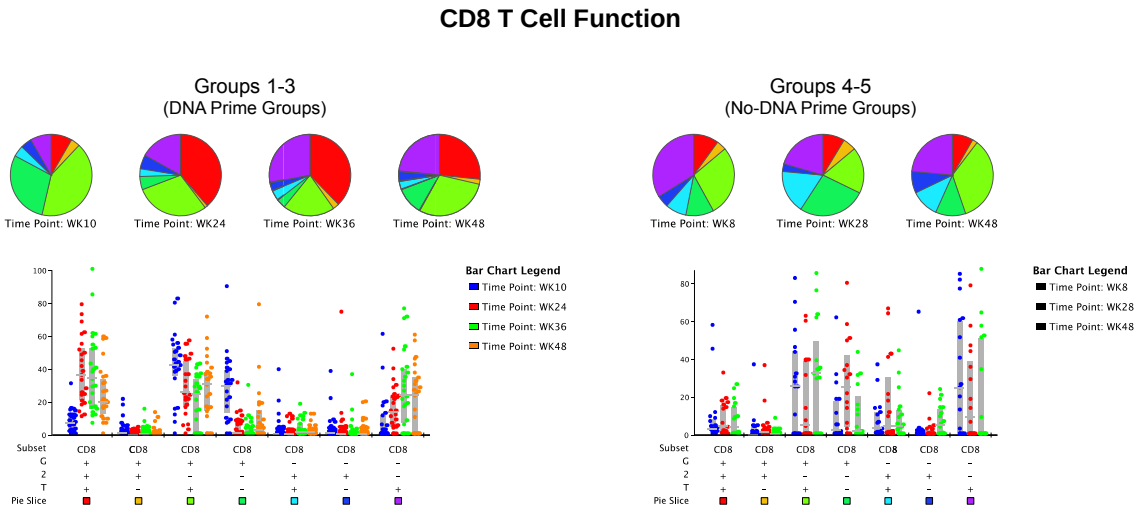

Figure 4-7 AUP444 - CD8 T Cell Responses

4.7.1.2 Summary of AUP444 humoral responses

Prior to the late protein only boost at week 49, No-DNA prime groups induced significantly greater Nab activity against Tier 1 viruses and SHIVs (TZM.bl assay, Figure 4-8), and significantly greater titers of ADCC activity (Figure 4-9) as well as a trend to greater cross clade binding IgG Ab titers, as compared to DNA prime groups. Post the week 49 late boost, DNA prime groups showed a substantial increase in Ab responses, comparable to the No-DNA prime groups (Figure 4-8 and Figure 4-9). In contrast, no significant increase in T-cell responses (data not shown) was observed with the DNA prime groups after the week 49 boost.

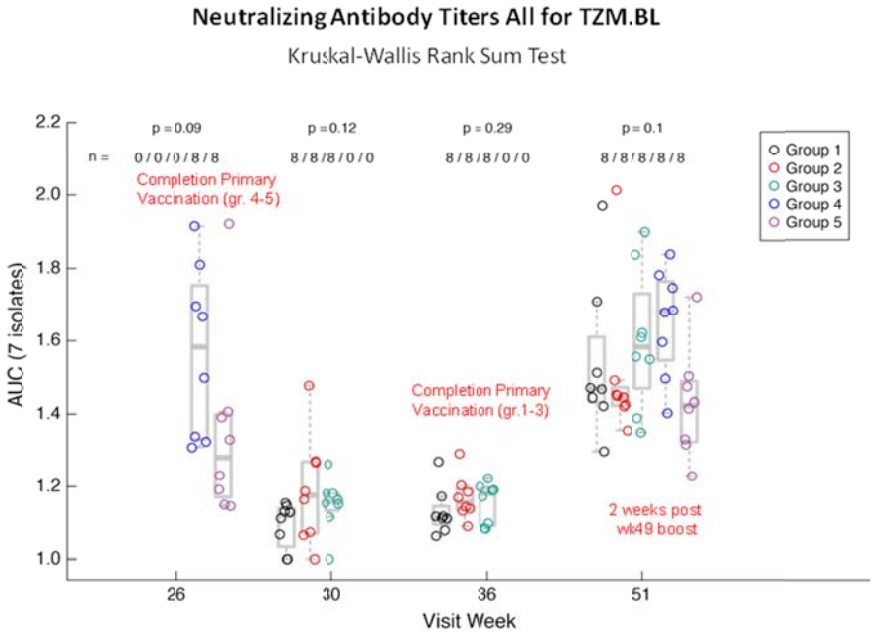

Figure 4-8 Neutralizing antibody responses after completion of primary vaccination regimens and post week 49 late boost

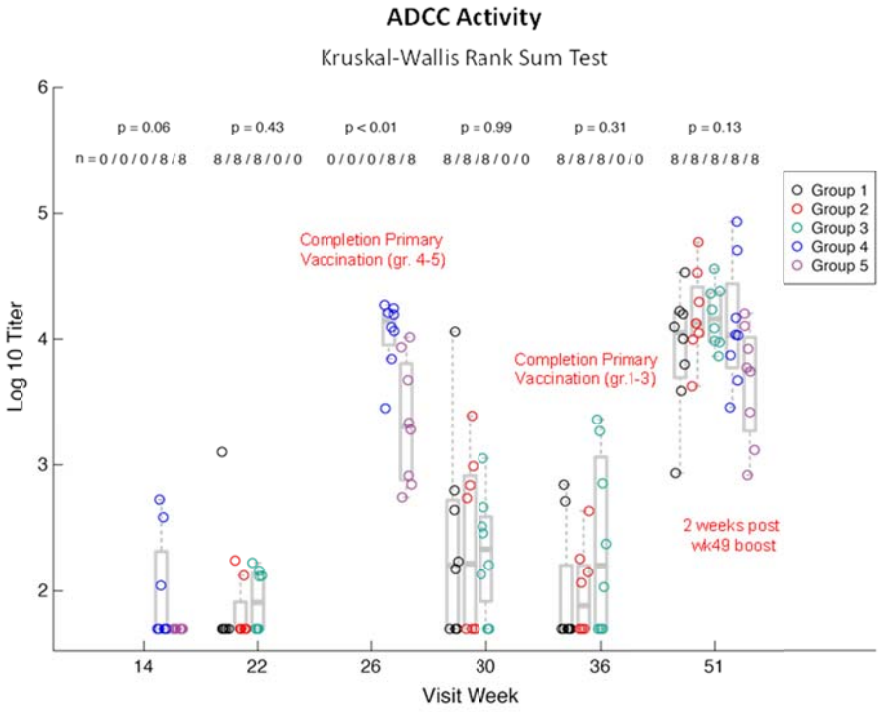

Figure 4-9 AUP444 - ADCC Activity after completion of primary vaccination regimens and post week 49 late boost

In summary, no apparent toxicity was observed during the course of the AUP444 study and the vaccines were well tolerated. Dose site observations were generally mild in nature and quickly resolved. The immunogenicity data obtained in this study demonstrated that DNA-HIV-PT123 combined with NYVAC or NYVAC-KC were highly immunogenic in terms and CD4 and CD8 T-cell responses as assessed by qualitative and quantitative endpoints. The immunogenicity profile of the DNA, NYVAC and protein regimen is distinctively different from the regimen with NYVAC and protein combination.

4.7.2 AUP512: protein schedule vaccination study

The objective of the AUP512 NHP study is to compare the safety and immunogenicity among: 1) DNA or NYVAC prime / NYVAC + protein boost versus DNA or NYVAC in combination with gp120 protein prime followed by NYVAC/gp120 protein boost; 2) DNA+gp120 protein at all timepoints versus Groups 1-4. Groups 1-4 are being studied in HVTN 096 (see Section 4.8.1).

In order to better understand the kinetics of the antibody response, the study was amended by adding the week 36 and 48 boost. The study design is provided in Table 4-6.

Table 4-6 Study design of AUP512

Gp	Size	Wk 0	Wk 4	Wk12	Wk 24	Wk 36	Wk 48
1	12	NYVAC	NYVAC	NYVAC + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein
2	12	NYVAC + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein
3	12	DNA + protein	DNA + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein
4	8	DNA	DNA	NYVAC + protein	NYVAC + protein	NYVAC + protein	NYVAC + protein
5	8	DNA + protein	DNA + protein	DNA +protein	DNA + protein	DNA + protein	DNA + protein
Total	52						

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

Preliminary immunogenicity data demonstrated that:

- IgG binding antibody responses against Env (Figure 4-10 and Figure 4-11) are detectable in Groups #2, #3 and #5 at week 6 (after two immunizations and co-administration with gp120 proteins). These responses are further boosted at week 14 after the 3rd immunization. IgG binding antibody responses against Env are detectable in Groups #1 and #4 only at week 14 (after the first protein co-administration);

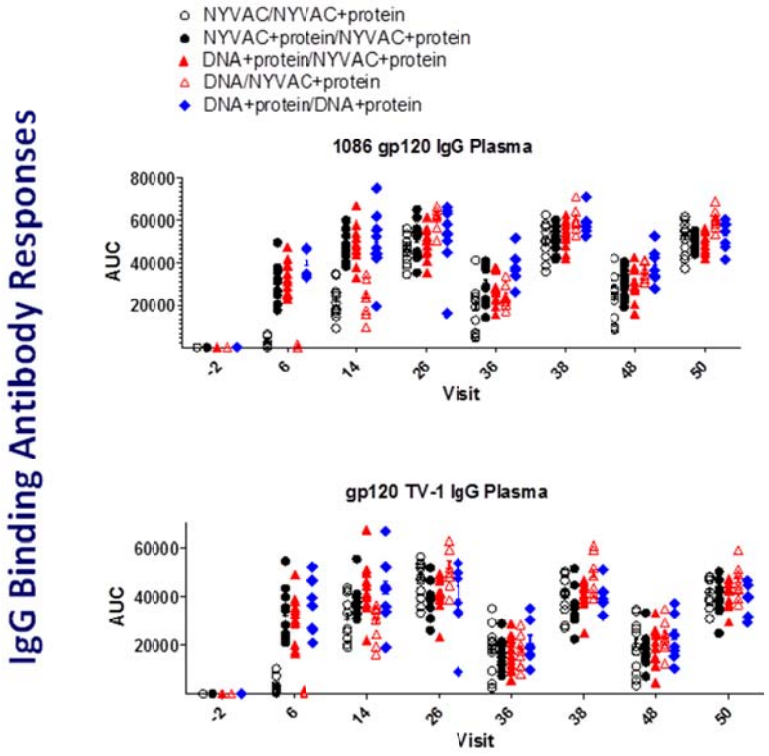

Figure 4-10 AUP512 - IgG binding Ab responses

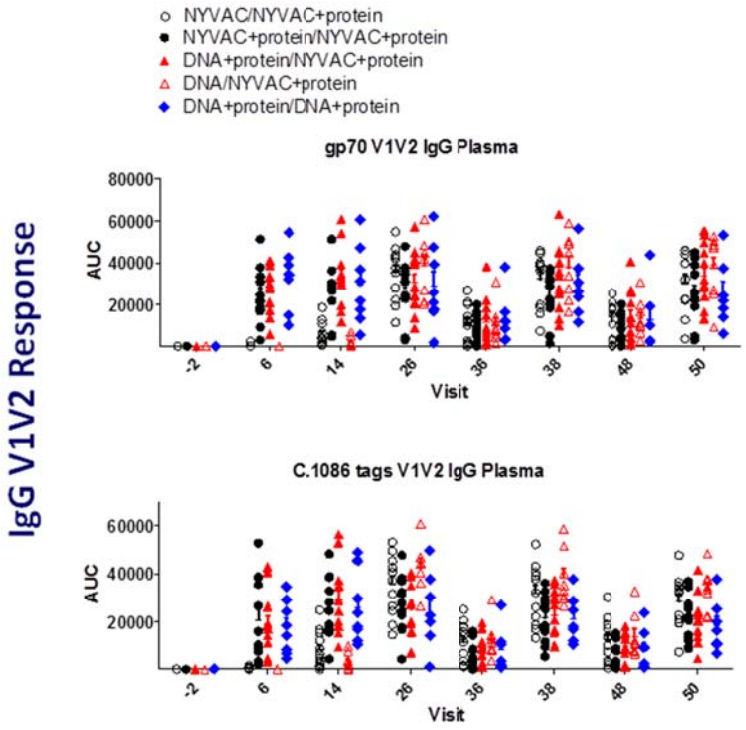

Figure 4-11 AUP512 - IgG V1V2 Ab responses

- Peptide microarray for mapping of linear epitope has been completed on a subset of 15 AUP512 animals (3 from each of the 5 groups) that are among the top binders for Env and/or gp70-V1V2 binding in the Luminex assay. For each animal wk0 and wk26 samples were tested. The results obtained indicate:
  - Animals in AUP 512 developed binding Abs targeting multiple epitopes:
  - C1, C1-V1, V2, C3, V3, C5 of gp120.
  - No binding was seen in gp41.
  - V3-response dominates in most animals, followed by C5, C3, and C1.
  - No significant difference was seen among the groups for binding patterns.
- Appearance of neutralizing antibodies followed the same kinetics of the IgG binding antibodies (Figure 4-12);

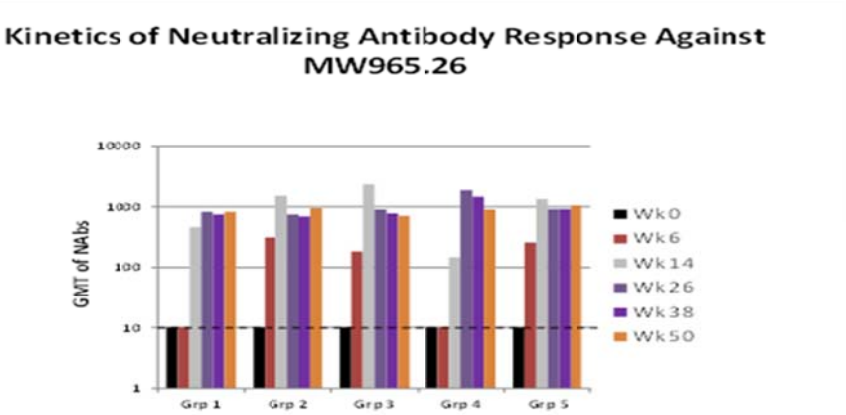

Figure 4-12 AUP512 - Kinetics of Neutralizing Ab responses against MW965.26

- T-cell responses were greater in the DNA prime/NYVAC boost group (Figure 4-13).

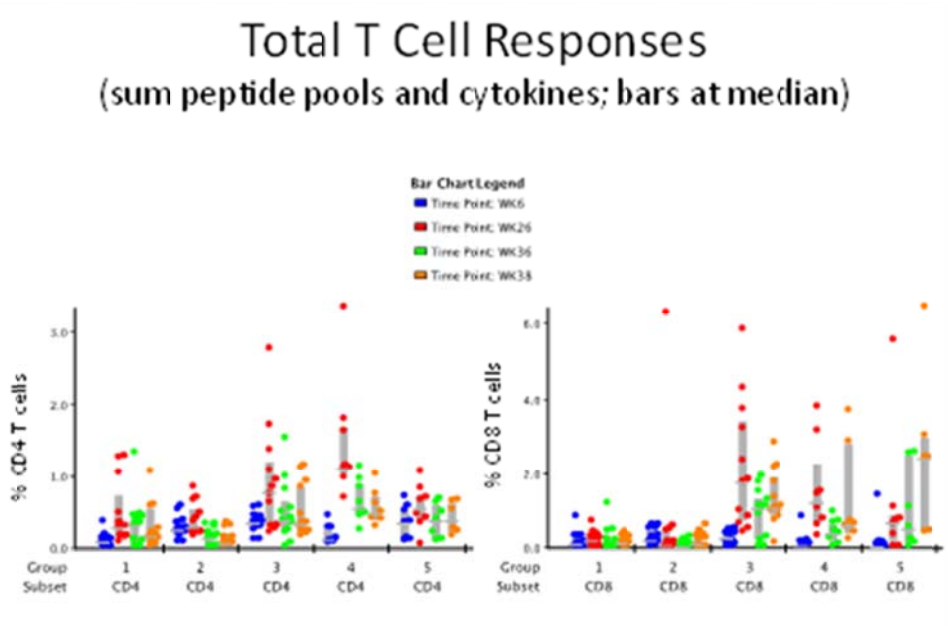

Figure 4-13 AUP512 - Total T-cell responses

AUP512 demonstrated that earlier immunization with protein (week 0 vs week 14) induces earlier IgG binding and neutralizing antibody responses, and heterologous DNA prime and NYVAC boost regimen induces greater T-cell responses compared to homologous NYVAC or DNA prime boost regimen.

With regard to safety, no apparent toxicity was observed during the course of this study and the vaccines were well-tolerated. Minimal dose site reactions were observed and any changes in clinical pathology were generally mild in nature, often preexisting conditions or incidental findings. Overall, the study animals were devoid of any signs of clinical abnormality. The only scorable reporting was inappetence and occasional Grade 1 local reactions. The days with the most inappetence correlate with anticipated recovery times from either fasting or sedation.

4.8 Clinical studies

4.8.1 Clinical studies with DNA-HIV-PT123

HVTN 096/EV04: DNA-HIV-PT123, NYVAC (NYVAC-HIV-PT1 and NYVAC-HIV-PT4), and AIDSVAX® B/E are currently being tested in an early phase clinical trial. HVTN 096/EV04 is being conducted exclusively in Lausanne, Switzerland under Swissmedic regulatory authority and entitled "*A phase 1 double blind placebo-controlled clinical trial to evaluate the safety and to compare the priming ability of NYVAC alone versus NYVAC + AIDSVAX® B/E, and DNA alone versus DNA + AIDSVAX® B/E when followed by NYVAC + AIDSVAX® B/E boosts in healthy, HIV-1–uninfected adult participants*." The HVTN 096/EV04 study design is shown in Table 4-7.

Table 4-7 Study design of HVTN 096/EV04

Group	N	Month 0	Month 1	Month 3	Month 6
1	20	NYVAC[1]	NYVAC	NYVAC+ AIDSVAX® B/E[3]	NYVAC+ AIDSVAX® B/E
	4	Placebo[4]	Placebo	Placebo	Placebo
2	20	NYVAC+ AIDSVAX® B/E	NYVAC+ AIDSVAX® B/E	NYVAC+ AIDSVAX® B/E	NYVAC+ AIDSVAX® B/E
	4	Placebo	Placebo	Placebo[4]	Placebo
3	20	DNA[2]	DNA	NYVAC+ AIDSVAX® B/E	NYVAC+ AIDSVAX® B/E
	4	Placebo	Placebo	Placebo[4]	Placebo
4	20	DNA+ AIDSVAX® B/E	DNA+ AIDSVAX® B/E	NYVAC+ AIDSVAX® B/E	NYVAC+ AIDSVAX® B/E
	4	Placebo	Placebo	Placebo[4]	Placebo
Total	80/16				

[1]NYVAC: NYVAC-HIV-PT1 and NYVAC-HIV-PT4 containing clade C ZM96 gp140 and ZM96 gag- CN54 pol-nef respectively, each delivered at a dose of ≥ 5x106 plaque-forming units (PFU), administered IM.
[2]DNA: DNA-HIV-PT123 containing clade C ZM96 gag and gp140, CN54 pol-nef, delivered at a dose of 4 mg, administered IM.
[3]AIDSVAX® B/E: 300mcg of subtype B (MN) HIV gp120 glycoprotein and 300mcg of subtype E (A244) HIV gp120 glycoprotein absorbed onto 600mcg of aluminum hydroxide gel adjuvant, administered IM.
[4]Placebo: Sodium Chloride for injection, 0.9% administered IM.

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

Interim immunogenicity data analysis has shown that the DNA prime/NYVAC-AIDSVAX boost regimen induces higher number of responders and greater magnitude of both CD4 and CD8 T-cell responses. In particular, CD4+ T cell response rates and

magnitudes for any PTE are greater in the DNA primed compared to NYVAC primed groups, with or without protein co-administration during the prime.

Co-administration of protein with DNA or NYVAC at time 0 leads to early (after 2nd immunization) induction of both binding and neutralizing antibody responses in group 2 and 4. Of note there is a trend to more durable antibody responses in the groups with protein co-administration at month 0 (groups 2 and 4) as compared to those with protein co-administration at month 3 (groups 1 and 3).

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

4.8.2 Clinical studies with Novartis HIV-1 subunit protein vaccines

Although Novartis Bivalent Subtype C gp120/MF59 vaccine has not yet been administered to humans, other closely related recombinant monomeric (gp120) subunit vaccine formulations from Novartis Vaccines (formerly Chiron) have been tested in clinical trials. In addition, recombinant oligomeric (o-gp140) Env proteins for subtypes B and C from Novartis have been or are currently in clinical trials. Overall, in these studies, recombinant HIV-Env proteins manufactured by Novartis were well tolerated and immunogenic. In most cases, recombinant HIV-Env proteins (either gp120 or gp140) were CHO-based and administered with MF59, Novartis' proprietary oil-in-water emulsion adjuvant [43]. MF59 safety has been established in clinical studies as well as in commercial products. A seasonal influenza vaccine adjuvanted with MF59 (Fluad®) is licensed in the EU and other countries for use in the elderly. MF59 is also used in a prepandemic H5N1 influenza vaccine (Aflunov®) licensed in the EU for use in adults, and in two pandemic H1N1 influenza vaccines (Focetria® and Celtura®), licensed in the EU and other countries for use in adults and children. More than 100 million doses of MF59-adjuvanted influenza vaccines have been distributed in licensed products.

Recombinant monomeric (gp120) vaccine candidates studied include Chiron's early gp120-based candidates from subtypes B and E, most of which were CHO-based and administered with MF59. More than 1200 subjects participated in the evaluation of the Chiron HIV SF2 gp120/MF59 vaccine and the Chiron HIV CM235 Thai E gp120/MF59 vaccine [42,44-48]. Two clinical trials were conducted using Novartis CHO-based subtype B gp140 recombinant Env protein with MF59. There are 3 ongoing phase 1 studies with Novartis CHO-based subtype C gp140/MF59 being conducted by the NIH-sponsored HVTN in the US and the RSA. Table 4-8 summarizes clinical trial experience with Novartis gp120 and gp140 recombinant vaccine candidates.

Table 4-8 Novartis recombinant gp120 and gp140 vaccines in human clinical trials [46]

Candidate vaccine	# receiving Novartis protein	Protocol	Status
Yeast derived recombinant subtype B SF2 Env 2-3 protein with MF59 and MTP-PE	60	AVEG 005 A/B/C	Completed
SF-2 gp120 (CHO) with MF59 and MTP-PE	50	AVEG 007 A/B/C	Completed
SF2 gp120 (CHO)/MF59 and ALVAC	40	AVEG 012A 012B	Completed
SF2 gp120 (CHO) with MF59, SAF/2, SAF2 + MDP	107	AVEG 015	Completed
SF2 gp120 (CHO)/MF59 and ALVAC	47	AVEG 022A	Completed
SF2 gp120 (CHO) with MF59	24	AVEG 024	Completed
SF2 gp120 (CHO)/MF59 and ALVAC	85	AVEG 026	Completed
SF2 gp120 (CHO)/MF59 and ALVAC	22	AVEG 029	Completed
SF2 gp120 (CHO) +/- yeast derived p24/MF59 and ALVAC	56	AVEG 032	Completed
SF2 gp120 (CHO) with MF59	126	AVEG201	Completed
SF2 gp120 (CHO)/MF59 and ALVAC	140	AVEG 202/HIVNET 014	Completed
SF2 gp120 & CM235 gp120 (CHO)/MF59 and ALVAC	45	RV132	Completed
Subtype B (SF162) gp140 (CHO) /MF59 and Subtype B DNA/PLG	90	HVTN 049	Completed
Subtype B (SF162) gp140 (CHO) /MF59 IN with LTK63	20	C86P1	Completed
Subtype C (TV1) gp140 (CHO)/MF59 and SAAVI DNA-C2 and SAAVI MVA-C	24	HVTN073E	Completed
Subtype C (TV1) gp140 (CHO) & ISS TAT	30	ISS P-002	Ongoing
Subtype C (TV1) gp140 (CHO)/MF59	20	HVTN 088	Ongoing
Subtype C (TV1) gp140 (CHO)/MF59 and SAAVI DNA-C2 and SAAVI MVA-C	114	HVTN 086	Ongoing
Bivalent Subtype C gp120/MF59 and ALVAC-HIV (vCP2438)	210	HVTN 100	Planned

In general, these recombinant protein vaccines were immunogenic and well tolerated with no unusual or serious vaccine-associated AEs reported. Most of the reactions were mild to moderate in nature, and of short duration [4,42,44-50].

4.8.2.1 Summary of safety, reactogenicity, and tolerability from recent human experience

There were 3 clinical trials conducted recently using Novartis CHO-based subtype B gp140 with MF59. In addition there are 3 ongoing clinical trials using Novartis CHO-based subtype C gp140.

A phase 1 single-center trial (C86P1) was conducted using Novartis CHO-based subtype B gp140 recombinant Env protein in Great Britain by the Mucosal Vaccines for Poverty Related Diseases (MUVAPRED) Consortium to assess safety, tolerability, and immunogenicity of IN administration of subtype B gp140 with and without the mucosal adjuvant LTK63 (detoxified mutant heat labile protein) followed by IM boosting with subtype B gp140/MF59. This study enrolled 30 healthy volunteers aged 18-45, with 20 to receive gp140. The protocol was amended to halt further IN administration of LTK63 following a report of an AE (ie, facial nerve paralysis) with a possible association with the LTK63 adjuvant in another study [51]. During the study, there was 1 SAE reported of Bell's Palsy (facial nerve paralysis) considered possibly related to the study vaccine LTK63 in a subject who never received any subtype B gp140 protein or any protein with MF59 adjuvant. IN vaccination was reactogenic resulting in upper respiratory tract

symptoms including nasal congestion, nasal discomfort, pharyngolaryngeal pain and rhinorrhea. The subtype B gp140 MF59 was well tolerated following IM boost.

Another completed study with Novartis subtype B gp140 MF59 was a multicenter, placebo-controlled trial (HVTN 049) conducted by the HVTN in the United States [21]. Subjects received1 of three doses of a DNA/PLG vaccine (subtype B *gag* DNA/PLG and subtype B *env* DNA/PLG microparticles, at doses of 250/250, 500/500, or 1000/1000 mcg) or placebo (5 to 1 ratio) as a single IM injection at 0, 1 and 2 months, followed by a boost of subtype B gp140 with MF59 (or placebo) at 6 and 9 months. An additional group of subjects received subtype B gp140 with MF59 without DNA prime, administered at 0, 3, and 9 months. Overall 96 healthy, HIV-1–uninfected adult subjects were enrolled and 86 subjects completed all planned vaccinations. There were no SAEs reported as related to study vaccine. There were four events reported as SAEs that were not considered related to the study vaccine. A death attributed to cocaine overdose occurred in1 subject, 10 days after receipt of the second dose of the placebo. One subject had a Grade 3 increase in creatine phosphokinase (CPK) to 2311 U/L, 14 days after the first DNA prime vaccination, which resolved within a week. Another subject had a Grade 4 increase in CPK to 4806 U/L 15 days after the first DNA prime vaccination, which resolved within two weeks. Both subjects reported to have initiated new exercise programs. One subject experienced severe fatigue 20 days after the fourth immunization (including1 dose of subtype B gp140/MF59), attributed to working two jobs and long hours. Overall, the regimens were generally well tolerated.

A third study, HVTN 073E, was conducted in the US and the Republic of South Africa (RSA) as an extension to the previous HVTN 073/SAAVI03 study. This extension study examined the safety and immunogenicity of two boosting doses of Novartis subtype C gp140/MF59 or placebo in subjects who previously received 3 vaccinations of SAAVI DNA-C2 and two vaccinations of SAAVI MVA-C. This study enrolled 27 subjects. There was 1 report of endometrial intra-epithelial neoplasia resulting in hospitalization for hysterectomy, which was assessed as unrelated to study agents.

There are 2 ongoing phase 1 studies with Novartis subtype C gp140/MF59 being conducted by the HVTN in the US and RSA as well as a phase 1 trial being conducted by the Istituto Superiore di Sanità (ISS) in Italy. One of these trials, HVTN 088, is being conducted in the United States in order to evaluate the safety and immunogenicity of a long-interval, cross-clade subtype C gp140/MF59 boost in subjects previously administered subtype B gp120/MF59 or subtype B gp140/MF59 in previous trials. This includes subjects from the HVTN049 DNA/PLG prime, gp140/MF59 boost study described above. The study is fully enrolled with 16 previously vaccinated subjects and 20 naive controls. Individuals were identified who had received a clade B Env protein with MF59 4-17 years earlier, most in combination with a DNA or ALVAC prime. These individuals were enrolled in HVTN 088 to receive a clade C protein boost in an open label phase 1 trial. There have been 3 SAEs reported in this trial, 1 involving traumatic injury, 1 instance of gastroenteritis, and 1 of appendicitis. All of these were assessed as unrelated to study agents.

The second ongoing HVTN study, HVTN 086, is being conducted in the RSA. It is evaluating the safety and immunogenicity of various combinations of SAAVI DNA-C2, SAAVI MVA-C, and Novartis subtype C gp140/MF59. This study enrolled 184 subjects. To date, 6 SAEs have been reported in this study, 1 case of acute tonsillitis that required hospitalization, 1 of schizophrenia requiring hospitalization (later determined to be a pre-existing condition), 1 of pelvic inflammatory disease, 1 soft-tissue injury, 1 instance of

anemia, and1 instance of alcohol-related cardiomyopathy. All were assessed as not related to the study products.

The ISS study (ISS P-002) being conducted in Italy is examining the safety and immunogenicity of subtype C gp140 co-administered with ISS TAT compared to subtype C gp140 alone or TAT alone. The study includes intradermal and IM injections (100 mcg for subtype C gp140 and 7.5 mcg for ISS TAT). This study does not include MF59. There have been no SAEs reported in this study.

4.8.2.2 Summary of immunogenicity from recent human experience

The immunogenicity of Novartis recombinant proteins has been demonstrated consistently in all clinical trials and in both of the recently completed studies using Novartis CHO-based subtype B gp140 MF59. In the HVTN 049 DNA/PLG prime protein boost study, the primary cellular immunogenicity endpoints included interferon gamma (IFN-γ) enzyme-linked immunospot (ELISpot) and ICS responses. Immunogenicity was assessed 14 days after each vaccination. Env-specific IFN-γ ELISpot response rates did not increase substantially compared to baseline after the three DNA/PLG prime vaccinations, but did rise after the first protein/MF59 boost. nAb titers against the homologous SF162 isolate were detectable in two subjects after the third DNA/PLG priming vaccination and in 13 subjects after the first protein boost. Neutralization was boosted to high titer in all but 1 subject following the second protein boost. Similarly in the group of subjects who received subtype B gp140/MF59 without a DNA/PLG prime, a nearly complete response to the SF162 isolate was observed at the second vaccination (all but1 subject) which lasted through the third vaccination. Binding Ab titers against Env, measured by enzyme-linked immunosorbent assay (ELISA), were detected following the first subtype B gp140/MF59 boost and were very high following the second boost administration.

The C86P2 MUVAPRED IN study, demonstrated immunogenicity with considerable IgG and IgA Ab responses to subtype B gp140 in serum, cervical, and vaginal secretions of subjects following IN administration of subtype B gp140 with the adjuvant LTK63 and an IM boost with subtype B gp140 and M59 adjuvant. nAb responses against the homologous SF162 were also detected in all groups following IM boost with subtype B gp140 and MF59 adjuvant.

In addition, although the subtype C gp140/MF59 HVTN 088 long interval boost study is currently ongoing in the long term safety follow up stage, all vaccine administrations have been completed and preliminary results are available. Sixteen previously primed volunteers and 20 naïve volunteers each received 2 doses of the subtype C gp140/MF59 given 6 months apart. HIV-1 specific CD4+ and CD8+ T-cell responses were measured by an ICS assay. Ab responses were measured with a Luminex binding Ab assay and a nAb assay in TZM-bl Cells. Despite the long interval (4-17 years from prior protein/MF59 administration), 31% of primed participants demonstrated CD4+ T-cell responses to Env at baseline, which increased to 75% after a single protein boost. IgG and IgA responses to subtype C gp140/MF59 were present in 64% (IgG) and 7% (IgA) of primed participants at baseline, and rose to 93% and 85%, respectively, after1 dose of protein. 71% of primed participants demonstrated nAb against Tier 1 clade B isolate MN at baseline. After a single booster dose of protein, 100% of the primed participants neutralized MN and 93% showed neutralizing activity against a clade C isolate, MW965.26. Unprimed participants did not demonstrate CD4+ responses or Ab responses to Env until after the second dose, which elicited IgG and IgA responses to TV1 trimeric

Env in 88% and 50%, respectively. nAb developed to MN in 38% and to MW965.26 in 88% of the unprimed participants.

4.9 Potential risks of study products and administration

Table 4-9 summarizes the potential risks associated with administration of the study products.

Table 4-9 Summary of potential risks of study products and administration

Common	• Mild to moderate injection site pain/itching, tenderness, erythema, or swelling/induration/edema • Malaise/fatigue, myalgia, or headache in the first few days following injection • A vaccine-induced positive HIV Ab test result • Papule or scab formation at or near the site of Biojector® injection
Less common	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, diarrhea, arthralgia, rash, nausea, vomiting, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Transient changes in clinical laboratory values • Lymphadenopathy • Injection site hematoma, bruising/ecchymosis, laceration, other transient lesions, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection • Injection site warmth • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis • Abdominal pain
Theoretical risks	• Muscle damage at the injection site • Autoimmune disease • 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 clade C DNA and bivalent gp120 protein and MF59 adjuvant in each vaccine regimen.

Primary endpoints 1

- Severe local and systemic reactogenicity signs and symptoms (pain, tenderness, erythema, induration, fever, malaise/fatigue, myalgia, headache, nausea, vomiting, chills, arthralgia) to 3 days after each vaccine/placebo dose

- AEs by body system, Medical Dictionary for Regulatory Activities (MedDRA) preferred term, severity, and assessed relationship to study products to 30 days after each vaccine/placebo dose

- SAEs, AESIs, and new chronic conditions requiring medical intervention for ≥ 30 days throughout the study

- Laboratory measures: white blood cells (WBC), neutrophils, lymphocytes, hemoglobin, platelets, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), and creatinine at baseline and following vaccinations

- AEs leading to early participant withdrawal or early discontinuation of study products and reason for discontinuation throughout the study.

Primary objective 2

- To evaluate the systemic immune responses at the Month 6.5 timepoint (2 weeks after the 4th vaccination) of clade C DNA and bivalent gp120 protein and MF59 adjuvant in each vaccine regimen.

Primary endpoints 2

- HIV-specific total IgG binding antibody response breadth and magnitude as assessed by multiplex assay.

- Anti –V1/V2 scaffold IgG binding antibody responses as assessed by multiplex assay.

- Neutralizing antibody responses against HIV-1 isolates.

- HIV-specific CD4+ and CD8+ T-cell responses as assessed by flow cytometry.

5.2 Secondary objectives and endpoints

Secondary objective 1

- To compare the immune responses at the Month 6.5 timepoint (2 weeks after the 4th vaccination) among participants receiving DNA via Biojector® versus those receiving DNA via needle and syringe

Secondary endpoint 1

- HIV-specific total IgG binding antibody response breadth and magnitude as assessed by multiplex assay.
- Anti –V1/V2 scaffold IgG binding antibody responses as assessed by multiplex assay.
- Neutralizing antibody responses against HIV-1 isolates.
- HIV-specific CD4+ and CD8+ T-cell responses as assessed by flow cytometry.

Secondary objective 2

- To further evaluate the immune responses of each vaccine regimen at the Month 6.5 timepoint (2 weeks after the 4th vaccination).

Secondary endpoint 2

- Contingent upon results from the primary immunogenicity objective described above (primary objective 2), additional immunogenicity assays may be performed on blood and mucosal samples based on the HVTN Laboratory Assay Algorithm.

5.3 Exploratory objectives

Exploratory objective 1

- To evaluate the durability of immunogenicity of each vaccine regimen measured at Month 12 (6 months after the 4th vaccination).

Exploratory objective 2

- To evaluate immune responses at the Month 1.5 timepoint (2 weeks after the 2nd vaccination) for participants receiving DNA via Biojector® as compared to those receiving it via needle and syringe

Exploratory objective 3

- To evaluate immune responses in mucosal specimens.

Exploratory objective 4

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

Exploratory objective 5

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

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 132 healthy, HIV-uninfected adult participants ages 18-40 years from Southern Africa. Participants will be randomized to 6 treatment groups which consist of 4 vaccine groups of size 30 each and 2 placebo groups of size 6 each. Group 1 will evaluate DNA priming at months 0 and 1 followed by a boost of DNA and Bivalent Subtype C gp120/MF59® at months 3 and 6, with the DNA administered by needle and syringe. Group 2 will evaluate priming with DNA and Bivalent Subtype C gp120/MF59® at months 0 and 1 followed by a boost of the same products at month 6, with the DNA administered by needle and syringe. Groups 4 and 5 will evaluate the same vaccine regimens as Groups 1 and 2, with the DNA administered via Biojector. For Groups 2 and 5, placebo injections will be given at month 3 to preserve blinding of participants and staff to the vaccine regimen. Randomization will be in a ratio of 5:5:1:5:5:1 (vaccine:vaccine:placebo using needle and syringe for DNA administration:vaccine:vaccine:placebo using Biojector for DNA administration). To ensure that women will be adequately represented in the trial and yet maintain relative balance between sexes, enrollment will be limited to no more than approximately 50% participants born male and no more than approximately 60% participants born female. Hence, when approximately 66 men are enrolled, recruitment of men will stop, or when approximately 79 women are enrolled, recruitment of women will stop.

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, low cell viability of processed peripheral blood mononuclear cells (PBMCs) or high background. Immunogenicity data from nine phase 1 and one phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of June 2011), indicate that 15% is a reasonable estimate for the rate of missing data at month 6.5. For this reason, the sample size calculations in Section 6.1.2 account for 5 enrolled participants on each of the vaccine groups 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 each vaccine group of the study (n =30), there is a 90% chance of observing at least 1 event if the true rate of such an event is 7.4% or more; and there is a 90% chance of observing no events if the true rate is 0.4% or less. Safety evaluation may be performed for the vaccine groups pooled across DNA administration method or across regimens. With a sample size of 60, there is a 90% chance of observing at least 1 event if the true rate of such an event is 3.8% 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 6.5% of participants from South Africa who received placebos experienced an SAE.

Probabilities of observing 0, 1 or more, and 2 or more events among groups of size 30 and 60 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 regimens.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among groups of size 30 and 60, for different true event rates

True event rate (%)	Pr(0/30)	Pr(1+/30)	Pr(2+/30)	Pr(0/60)	Pr(1+/60)	Pr(2+/60)
1	0.74	0.26	0.04	0.55	0.45	0.12
4	0.29	0.71	0.34	0.09	0.91	0.70
6.5	0.13	0.87	0.59	0.02	0.98	0.91
10	0.04	0.96	0.82	0.002	>0.99	0.99
20	0.001	>0.99	0.99	<0.0001	>0.99	>0.99
30	<0.0001	>0.99	>0.99	<0.0001	1	1

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 30 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 11.4%. If none among 60 participants experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events is 6.0%.

Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for groups of size 30 or 60

Observed event rate	Confidence interval (%)
0/30 = 0.0%	(0.0, 11.4)
1/30 = 3.3%	(0.6, 16.7)
2/30 = 6.7%	(1.8, 21.3)
3/30 = 10.0%	(3.5, 25.6)
5/30 = 16.7%	(7.3, 33.6)
10/30 = 33.3%	(19.2, 51.2)
20/30 = 66.7%	(48.8, 80.8)
0/60 = 0.0%	(0.0, 6.0)
1/60 = 1.7%	(0.3, 8.9)
2/60 = 3.3%	(0.9, 11.4)
3/60 = 5.0%	(1.7, 13.7)
5/60 = 8.3%	(3.6, 18.1)
10/60 = 16.7%	(9.3, 28.0)
20/60 = 33.3%	(22.7, 45.9)

6.1.2 Sample size calculations for immunogenicity

The main goals of this trial regarding immunogenicity outcomes involve a preliminary estimation of systemic response rates for each vaccine group with regard to HIV-specific total IgG binding antibody and anti-V1/V2 scaffold IgG binding antibody as assessed by

multiplex assay, neutralizing antibody against HIV-1 isolates, HIV-specific CD4+ and CD8+ T-cell response as assessed by flow cytometry, and possible other assay outcomes to be determined after assessment of the primary endpoints. No adjustment for multiple comparisons will be made for the multiple endpoints. 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 a vaccine group is shown in Table 6-3. Calculations are done using the score test method [52]. The n = 25 assumes a 15% loss of data.

Table 6-3 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the vaccinees (n = 25)

No. of responses	Observed response rate (%)	Confidence interval
3	12.0	(4.2, 30.0)
5	20.0	(8.9, 39.1)
8	32.0	(17.2, 51.6)
10	40.0	(23.4, 59.3)
13	52.0	(33.5, 70.0)
15	60.0	(40.7, 76.6)
18	72.0	(52.4, 85.7)
20	80.0	(60.9, 91.1)

As shown in Table 6-4, there is limited power for a formal comparison of immunogenicity response rates between any two vaccine groups of size n = 25 and, hence, formal comparisons are not listed in the primary study objectives. For power of either 80% or 90%, 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 groups of size n=25

	Minimum true response rate in other group (%) in order to detect a difference	
True response rate one group (%)	80% power	90% power
20	61	67
30	71	77
40	81	86
50	89	93
60	95	98
70	1	N/A

A secondary objective is a comparison of the response levels between administration methods (Biojector and needle/syringe) for each vaccine regimen at month 6.5 after the DNA and Bivalent Subtype C gp120/MF59® boost. Since we will be comparing response levels across multiple assays it is useful to standardize the response to the standard deviation scale for purposes of describing the power to detect a difference. In Table 6-5, the mean difference between groups can be expressed in terms of the number of standard deviations difference in the means between groups. The study has good power to detect differences when the difference in means between groups is > 0.8 of the standard deviation. These calculations use an exact 2-sided Wilcoxon test with a Type I error rate of 0.05 and assume equal standard deviations for the two groups.

Table 6-5 Power for comparison of response levels between 2 groups of size n=25.

Difference in means between 2 groups (SD scale)	Power
0	4.7%
0.1	6.5%
0.2	10.3%
0.3	17.2%
0.4	27.4%
0.5	39.6%
0.6	51.8%
0.7	66.6%
0.8	76.2%
0.9	86.6%
1.0	91.9%

6.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through the SDMC's Web-based randomization system. The randomization will be done in blocks to ensure balance across groups. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments.

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participants' treatment group assignments (eg, vaccine or placebo) but not to the administration method (Biojector or needle/syringe). Study product assignments are accessible to those HVTN CRS pharmacists, DAIDS protocol pharmacists and contract monitors, and SDMC staff who are required to know this information in order to ensure proper trial conduct. Any discussion of study product assignment between pharmacy staff and any other HVTN CRS staff is prohibited. The HVTN SMB members also are unblinded to treatment assignment in order to conduct review of trial safety.

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

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 111 PSRT should be consulted before emergency unblinding occurs.

6.4 Statistical analysis

This section describes the final study analysis, unblinded as to treatment group assignment. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. The analysis is a modified intent-to-treat analysis in that individuals who are randomized but

not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

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

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

Immunogenicity data from this study may be combined with other phase 1/2a studies within the Correlates Program. Comparable eligibility criteria and validated assays for primary immunogenicity endpoints will be used to mitigate the potential bias introduced by combining data across studies conducted over an extended period of time.

The active treatment groups of this trial form a 2 by 2 factorial design crossing the two prime-boost strategies with the two administration methods. This allows us to evaluate the two prime-boost strategies by combining information across the two administration methods. Similarly, we can evaluate each of the administration methods by combining information across the two prime-boost strategies. When evaluating continuous immune response outcomes we will consider linear models with main effects for each of the two factors along with an interaction term. For binary outcomes we will consider logistic regression models. Interpretation of the main effects depends on the presence or lack of an interaction, which the study will have low power to detect [53], therefore we will treat these analyses as exploratory and descriptive.

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 groups 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 group and the percentages displayed graphically by group. 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. Kruskal-Wallis tests will be used to test for differences in severity between groups.

6.4.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment group 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 groups 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 potential immune-mediated disorders; a sample list of AESI is provided in Appendix G.

6.4.3.3 Local laboratory values

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

For each local laboratory measure, summary statistics will be presented by treatment group and timepoint, as well as changes from baseline for postenrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the DAIDS AE Grading Table (see Section 11.2) will be tabulated by treatment group 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 group.

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 after 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 after 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 group 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] No adjustment will be made to the vaccine group estimates for the false positive rates in the placebo groups.

All of the primary assays, and likely some of the secondary assays, will have quantitative assay data, including IgG binding antibody magnitude from the multiplex assay, neutralizing antibody titers, area under the magnitude-breadth curve [AUC-MB] for the neutralizing antibody assay, and percentage of positive cells from the ICS assay. Quantitative data will be displayed graphically and tabular summaries of the distributions by antigen, treatment group, and timepoint will be made.

For comparisons in which the response rate for one of the groups is low (eg, $\leq 20\%$ for the class), statistical testing will use Fisher's exact test comparing the two response rates as most of the continuous data readouts would be left censored at the lower limit of detection. For comparisons in which the response rates for both groups are high (eg, $\geq 75\%$), the difference between groups will be tested using the continuous readouts 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, CD4+ T cell response). If treatment group differences for these assays are best summarized by a mixture model, then Lachenbruch's test statistic [54] will be used to evaluate the composite null hypothesis of equal response rates in the 2 groups and equal response distributions among responders in the 2 such groups. This test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between groups will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between groups will be estimated using the methods described above.

Based upon previous AIDS Vaccine Evaluation Group (AVEG) and HVTN trials, missing 10-15% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with nonparametric tests and generalized linear models fit by generalized estimating equation (GEE) methods, missing data need to be missing completely at random (MCAR). MCAR assumes that the probability of an observation being missing does not depend upon the observed responses or upon any unobserved covariates but may depend upon covariates included in the model (eg, missing more among whites than nonwhites). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then nonparametric tests and GEE methods will be used, because violations of the MCAR

assumption will have little impact on the estimates and hypothesis tests. These models will include as covariates all available baseline predictors of the missing outcomes.

If a substantial amount of immunogenicity data are missing (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right- censored, then the linear mixed effects models of Hughes [55] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [56] methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

6.4.5 Analyses prior to end of scheduled follow-up visits

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

6.4.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study, as defined in Section11, for review by the HVTN SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 111 PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity

An unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key HVTN members and investigators. Distribution of reports will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the scheduled follow-up visits.

7 Selection and withdrawal of participants

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

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

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

7.1 Inclusion criteria

General and Demographic Criteria

1. **Age** of 18 to 40 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; provides answers to a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly

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

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

HIV-Related Criteria:

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

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

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

Laboratory Inclusion Values

Hemogram/Complete blood count (CBC)

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

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

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

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

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

Chemistry

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

Virology

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

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

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

Urine

19. **Normal urine**:

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

Reproductive Status

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

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

- Agree to consistently use effective contraception (Appendix B) for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment through the last required protocol clinic visit.

  - Effective contraception is defined as using 1 of the following methods:

    - Condoms (male or female), or
    - Diaphragm or cervical cap,

    PLUS 1 of the following methods:

    - Intrauterine device (IUD),
    - Hormonal contraception (in accordance with applicable national contraception guidelines),
    - 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 after vasectomy); or
    - Any other contraceptive method approved by the HVTN 111 PSRT

  - Or not be of reproductive potential, such as having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation;

  - Or be sexually abstinent.

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

Other

23. **Volunteers 21 years of age and older who were born female consenting to provide cervical samples:** pap smear within the 3 years prior to enrollment, with the latest result reported as normal or ASCUS (atypical squamous cells of undetermined significance); for those 21 years and older that have not had a pap smear within the last 3 years prior to enrollment, must be willing to undergo a pap smear with the result reported as normal or ASCUS prior to sample collection.

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: 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 during the planned duration of the HVTN 111 study

5. **Pregnant or breastfeeding**

Vaccines and other Injections

6. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 111 PSRT will determine eligibility on a case-by-case basis.

7. **Non-HIV experimental vaccine(s) received within the last 5 years** in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure by the FDA. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 111 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 111 PSRT on a case-by-case basis.

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

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

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

Immune System

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

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

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

14. **Autoimmune disease**

15. **Immunodeficiency**

Clinically significant medical conditions

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

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

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

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

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

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

21. **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.

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

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

24. **Hypertension**:

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

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

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

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

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

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

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

7.3 Participant departure from vaccination schedule or withdrawal

This section concerns an individual participant's departure from the vaccination schedule. Pause rules for the trial as a whole are described in Section 11.3.

7.3.1 Delaying vaccinations for a participant

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

- Within 45 days prior to any study injection
  - Receipt of blood products or immunoglobulin

- Within 30 days prior to any study injection
  - Receipt of live attenuated vaccines other than influenza vaccine
  - Receipt of allergy treatment with antigen injections

- Within 14 days prior to any study injection
  - Receipt of influenza vaccine or any vaccines that are not live attenuated vaccines (eg, pneumococcal)

- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.

- Pregnancy: for participants who become pregnant, no study vaccinations will be given; except for participants who may have been pregnant during the study but are no longer pregnant as shown by two negative urine pregnancy tests taken from two different urine samples that may be collected on the same day; in this circumstance, the HVTN 111 PSRT should be consulted to determine if the participant may resume vaccinations.

Vaccinations should not be administered outside the visit window period specified in the HVTN 111 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 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 111 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 (Vaccinations will be stopped while a participant is pregnant. If the participant is no longer pregnant and can be vaccinated within an appropriate visit window, vaccinations may resume, see Section 7.3.1);

  - Any grade 4 local or systemic reactogenicity symptom, or AE that is subsequently considered to be related to vaccination;

  - Any grade 3 clinical AE (exception: fever or vomiting and subjective local and systemic symptoms) that is subsequently considered to be related to vaccination;

  - Any grade 3 or 4 lab abnormality confirmed by a repeated value that is subsequently considered to be related to vaccination. Upon PSRT review the participant may be allowed to continue study vaccinations.
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 111 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination; or

- Investigator determination in consultation with Protocol Team leadership (eg, for repeated nonadherence to study staff instructions).

- A study participant who misses a study injection is permitted to continue with subsequent study injections that can still be scheduled within the time interval specified in the *HVTN 111 Study Specified Procedures* (SSP) unless there is a protocol-mandated reason for discontinuation.

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 9.6.1).

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,

- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff), or

- Any condition where termination from the study is required by applicable regulations.

8 Study product preparation and administration

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

8.1 Vaccine regimen

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

Group 1

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%) to be administered as 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 1;

THEN

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 3 and 6;

AND

Bivalent Subtype C gp120/MF59 (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and MF59C.1) to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Group 2

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0, 1 and 6

AND

Bivalent Subtype C gp120/MF59 (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and MF59C.1) to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 0, 1 and 6

AND

Placebo for DNA (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Month 3

AND

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%) to be administered as 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Month 3.

Group 3

Placebo for DNA (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM in the LEFT deltoid (unless medically contraindicated) at Months 0, 1, 3 and 6

AND

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%) to be administered as 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0, 1, 3 and 6.

Group 4

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL IM via Biojector® in the LEFT deltoid (unless medically contraindicated) at Months 0 and 1;

AND

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%) to be administered as 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0 and 1;

THEN

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL IM via Biojector® in the LEFT deltoid (unless medically contraindicated) at Months 3 and 6;

AND

Bivalent Subtype C gp120/MF59 (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and MF59C.1) to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Group 5

DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL IM via Biojector® in the LEFT deltoid (unless medically contraindicated) at Months 0, 1 and 6

AND

Bivalent Subtype C gp120/MF59 (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and MF59C.1) to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 0, 1 and 6

AND

Placebo for DNA (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM via Biojector® in the LEFT deltoid (unless medically contraindicated) at Month 3

AND

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%) to be administered as 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Month 3.

Group 6

Placebo for DNA (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM via Biojector® in the LEFT deltoid (unless medically contraindicated) at Months 0, 1, 3 and 6

AND

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%) to be administered as 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0, 1, 3 and 6.

8.2 Study product formulation

DNA-HIV-PT123 (labeled as DNA-HIV-PT123 [4 mg/mL]) is supplied as a 4 mg/mL DNA solution in a 2 mL sterile glass vial containing a volume to deliver 1 mL of a clear, colorless solution. The product must be stored at -20^{0}C ± 5^{o}C. The study product is described in further detail in the Investigator's Brochure (IB).

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

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

Bivalent Subtype C gp120 composed of two different proteins:

TV1.C gp120 protein [labeled as TV1.C gp120]: The TV1.C gp120 protein will be provided in a 2 mL glass vial containing approximately 0.55 mL (440 mcg protein) of a clear colorless to slightly yellow liquid. The product must be stored frozen at -61°C or colder.

1086.C gp120 protein [labeled as 1086.C gp120]: The 1086.C gp120 protein will be provided in a 2 mL glass vial containing approximately 0.55 mL (440 mcg protein) of a clear colorless to slightly yellow liquid. The product must be stored frozen at -61°C or colder.

The study product is described in further detail in the Bivalent Subtype C gp120/MF59 IB.

MF59 [labeled as MF59C.1] is supplied as an oil-in-water emulsion. The MF59 adjuvant has a milky white opaque appearance and is provided in a glass vial containing a

total volume of 0.7 mL. The product must be stored refrigerated at 2 - 8° C. Do not freeze.

The study product is described in further detail in the Bivalent Subtype C gp120/MF59 IB.

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%)

Sodium Chloride for Injection, 0.9%, will be used as the placebo for Bivalent Subtype C gp120/MF59. Product must be stored as directed by the manufacturer.

8.3 Preparation of study products

8.3.1 DNA-HIV-PT123 (Groups 1 and 2)

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

Once thawed, the pharmacist, using aseptic technique, will gently swirl the vial and then withdraw 1 mL into a syringe. The syringe should be no smaller than 1.5 mL syringe and no larger than a 5 mL syringe.

The syringe should be labeled as “DNA 4 mg or Placebo 1 mL” and have an overlay to maintain blinding. The syringe must also be labeled for administration in the LEFT deltoid. The study product should be administered as soon as possible after preparation.

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

8.3.2 Placebo for DNA (Group 2 and 3)

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a syringe. The syringe should be no smaller than 1.5 mL syringe and no larger than a 5 mL syringe.

The syringe should be labeled as “DNA 4 mg or Placebo 1 mL” and have an overlay to maintain blinding. The syringe must also be labeled for administration in LEFT deltoid. The study product should be administered as soon as possible after preparation.

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

8.3.3 DNA-HIV-PT123 (Groups 4 and 5 - Biojector®)

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

Once thawed, the pharmacist, using aseptic technique, will gently swirl the vial and then withdraw 1mL of the DNA vaccine from the vial into the Biojector® syringe and cap the syringe.

The Biojector® syringe should be labeled as "DNA 4 mg or Placebo 1 mL". The syringe must also be labeled for administration in the LEFT deltoid. The study product should be administered as soon as possible after preparation.

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

8.3.4 Placebo for DNA (Group 5 and 6 – Biojector®)

Using aseptic technique, the pharmacist will withdraw 1mL of Sodium Chloride for Injection, 0.9% into the Biojector® syringe and cap the syringe.

The Biojector® syringe should be labeled as "DNA 4 mg or Placebo 1 mL". The syringe must also be labeled for administration in LEFT deltoid. The study product should be administered as soon as possible after preparation.

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

8.3.5 Bivalent Subtype C gp120/MF59

One vial of TV1.C gp120 protein, one vial of 1086.C gp120 protein, and one vial of MF59C.1 will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the TV1.C gp120 and 1086.C gp120 from the freezer and allow to thaw at room temperature. The pharmacist will also remove the MF59C.1 vial from the refrigerator and mix by repeated gentle swirling and inversion (do not shake vigorously).

Using aseptic technique, the pharmacist will gently swirl the contents of the vial containing TV1.C gp120 and then withdraw 0.35 mL of TV1.C gp120 from the correct vial and inject it into the vial containing MF59C.1. The pharmacist will then gently swirl the vial containing 1086.C gp120 after which, using aseptic technique, the pharmacist will withdraw 0.35 mL of 1086.C gp120 from the correct vial and inject it into the MF59C.1 vial (which contains TV1.C gp120 and MF59C.1). After gentle swirling and inversion (do not shake vigorously) the pharmacist, using aseptic technique, will withdraw 0.5 mL of the mixed preparation for dosing into a 1 or 2 mL syringe.

The syringe should be labeled as "Bivalent Subtype C gp120/MF59 or Placebo 0.5 mL" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid and "Gently roll the syringe prior to administration". This study product should be administered as soon as possible after preparation.

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

8.3.6 Placebo for Bivalent Subtype C gp120/MF59

Sodium Chloride for Injection 0.9% will be needed to prepare the dose.

The pharmacist, using aseptic technique, will withdraw 0.5 mL of the sodium chloride for injection into a 1 or 2 mL syringe.

The syringe should be labeled as "Bivalent Subtype C gp120/MF59 or Placebo 0.5 mL" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid and "Gently roll the syringe prior to administration". This study product should be administered as soon as possible after preparation.

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

8.4 Administration

8.4.1 DNA-HIV-PT123 (DNA) vaccine or Placebo (Sodium Chloride for Injection, 0.9%) Groups 1, 2 and 3

All injections are to be given using standard IM injection technique after preparation of the site with alcohol. Syringes labeled as "DNA 4 mg or Placebo 1 mL" should be administered in the LEFT deltoid unless medically contraindicated.

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.

8.4.2 DNA-HIV-PT123 (DNA) vaccine or Placebo (Sodium Chloride for Injection, 0.9%) Groups 4, 5, and 6.

A 1 mL injection of DNA vaccine or DNA placebo will be administered IM using the Biojector® at Months 0, 1, 3 and 6. Biojector Syringes labeled as "DNA 4 mg or Placebo 1 mL" should be administered in the LEFT deltoid unless medically contraindicated.

The Biojector® 2000 Needle-Free Injection Management System™ will be used as directed by the Biojector® company. Neither the material being injected nor injection site skin preparation require deviation from standard procedures. The injection site is disinfected and the area allowed to dry completely. The skin around the injection site is held firmly while the syringe is placed against the injection site at a 90° angle. The actuator is pressed and the material is released into the muscle and held firmly for 3 seconds. After the injection, the site is covered with a sterile covering and pressure applied with 3 fingers for 1 minute. Biojector® utilizes sterile, single-use syringes for variable dose, up to 1mL, medication administration. The study product is delivered under pressure by a compressed CO_2 gas cartridge that is stored inside the Biojector®. When the Biojector's actuator is depressed, CO_2 is released, causing the plunger to push the study product out of the sterile syringe through the skin and into the underlying tissue. The study product is expelled through a micro-orifice at high velocity in a fraction of a second to pierce the skin. The CO_2 does not come in contact with the injectate and the syringe design prevents any back splatter or contamination of the device by tissue from the subject.

8.4.3 Bivalent Subtype C gp120/MF59 or Placebo (Sodium Chloride for Injection, 0.9%) Groups 1 - 6

All injections are to be given using standard IM injection technique after preparation of the site with alcohol.

For all syringes labeled as "Bivalent Subtype C gp120/MF59 or Placebo" the person administering the injection should gently roll the syringe prior to administration of the study product. The product should then be administered in the RIGHT deltoid unless medically contraindicated.

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.

8.4.4 All Injections (Groups 1 – 6)

If an injection needs to be administered in an alternate body site (e.g. thigh) due to a medical contraindication, the injection should not be administered in the same deltoid as the other injection. The appropriate study staff should document this clearly. Under this circumstance, this is NOT a protocol violation.

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

8.5 Acquisition of study products

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

The Biojector and Biojector syringes (including CO_2 cartridges) will be supplied by HVTN Core.

Bivalent Subtype C gp120 (TV1.C gp120 and 1086.C gp120) and the MF59 adjuvant will be provided by Novartis Vaccines.

Placebo for all study products (Sodium Chloride for Injection, 0.9%) will not be provided through the protocol and must be obtained by the site.

Once an HVTN CRS is protocol registered and all importation requirements have been met, the pharmacist can obtain study products (not including the Biojector, CO_2 cartridges or syringes) 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 schedules of clinical procedures are shown in Appendix F.

9.1 Informed consent

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

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

An HVTN CRS may employ recruitment efforts prior to the participant consenting. For example, some HVTN CRSs use a telephone script to prescreen people before they come to the clinic for a full screening visit. Participants must sign a screening or protocol-specific consent before any procedures are performed to determine eligibility. HVTN CRSs must submit recruitment and prescreening materials to IRB/EC and any applicable Regulatory Entity (RE) for human subjects protection review and approval.

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

9.1.1 Screening consent form

Without a general screening consent, screening for a specific study cannot take place until the site receives protocol registration from the DAIDS 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. A sample protocol-specific consent form for the main study is located in Appendix A. A separate sample consent form for other uses of specimens is located in Appendix 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 and Appendix D. The consent form(s) must be developed in accordance with requirements of the following:

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

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

The sample informed consent form includes instructions throughout the document for developing specific content.

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

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

9.1.3 Assessment of Understanding

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

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

IRB/EC and any applicable RE may require that a participant has signed either a screening or protocol-specific consent document prior to administering the Assessment of Understanding. The consent process (including the use of the Assessment of

Understanding) should be explained thoroughly to the IRB/EC and any applicable RE, whose recommendations should be followed.

9.2 Pre-enrollment procedures

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

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

- Medical history, documented in the case history record;
- Assessment of 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 (record the complete generic name for all medications);
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - CBC with differential and platelet count,
  - Chemistry panel (ALT, AST, ALP, and creatinine),
  - Urine dipstick (urinalysis if indicated, see Section 9.8),
  - HBsAg,
  - Anti-HCV Ab,
  - Syphilis test,
  - Urine or serum pregnancy test (volunteers who were born female);
  - Pap smear (Only for volunteers who were born female and who agree to provide cervical samples; not required if volunteer has had Pap smear within previous 3 years with most recent result normal or ASCUS or less than 21 years old;
- 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 9.6; and

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

9.2.1 Use of screening results from another HVTN study

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

9.3 Enrollment and vaccination visits

Enrollment is simultaneous with first vaccination. The time interval between randomization and enrollment should not exceed 4 working days. The HVTN CRS randomizes the participant via the Web-based randomization system as described in the HVTN 111 Study Specific Procedures. 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 may be given the postvaccination symptom log and instructed on how to complete it. The site will make arrangements to obtain daily reports of reactogenicity events from the participant during the reactogenicity period (as described in Section 9.9).

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 9.6);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.7); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation).

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

- 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; and
- 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;
- Specimen collection (blood and/or mucosal) should be completed prior to vaccination;
- For participants who agree to mucosal sampling collection (see Appendix F):
    - Urine test for gonorrhea and chlamydia;
    - Vaginal swab for Trichomonas and bacterial vaginosis (for participants providing cervical samples);
    - Vaginal swab (if indicated) for hyphae/budding yeast (for participants providing cervical samples);

- Syphilis serology; and
- Mucosal specimen collection.

9.4 Follow-up visits

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

- Risk reduction counseling (as described in Section 9.6);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.7); 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 F:

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

- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.8); 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.
- For participants who agree to mucosal sampling collection (see Section 9.5):
  - Urine test for gonorrhea and chlamydia;
  - Vaginal swab for Trichomonas and bacterial vaginosis (for participants providing cervical samples);
  - Vaginal swab (if indicated) for hyphae/budding yeast (for participants providing cervical samples);
  - Syphilis serology;
  - Pregnancy test (see Section 9.5); and
  - Mucosal specimen collection.

9.5 Mucosal sampling

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

Participants who consent to provide cervical, rectal, or semen samples will be tested for the following infections at the mucosal sampling visits: gonorrhea, chlamydia, and syphilis. Participants who were born female who consent to provide cervical 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.

Rectal fluid sampling (both sexes): For participants born female, a pregnancy test must be performed and be negative prior to any rectal mucosal sampling. Rectal secretion sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, within the visit window. In addition, rectal sampling will not be performed (or may be deferred to a later date within the visit window) if there is a contraindication to rectal secretion sampling, such as an active infection or inflammation of the colorectal area (such as an herpes simplex virus (HSV)-2 outbreak or inflamed hemorrhoids or colitis/diarrhea) or if the participant has any active genital tract infection (GTI).

Participants should abstain for 48 hours prior to sample collection 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.

Cervical sampling (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 years prior to enrollment, with the latest result reported as normal or ASCUS. A pregnancy test must be performed and must be negative prior to any cervical sampling. Cervical sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, within the visit window. In addition, cervical sampling will not be performed (or may be deferred to a later date within the visit window) if a participant has an active ulcerative genital lesion or is known to have an active GTI at the scheduled timepoint. Participants providing cervical secretion 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 (only for participants who were born male): Participants providing semen samples are asked to refrain from ejaculation for at least 48 hours prior to specimen collection. In addition, mucosal sampling will not be performed (or may be deferred to a later date within the visit window) if a participant is known to have an active GTI at the scheduled timepoint.

9.6 HIV counseling and testing

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

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

Study staff will take particular care to inform study participants of the likelihood of routine HIV testing being offered or performed outside the study CRS at emergency rooms, clinics, and medical offices. Such testing has become more likely due to the

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

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

9.6.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

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

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

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

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

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

9.6.2 VISP registry

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

seropositivity" (VISP) (see Section 9.6.1). In order to provide poststudy HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of poststudy testing and counseling services to HIV vaccine recipients. The registry contains the names of all study participants, unless they request that their names be removed.

9.7 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.

9.8 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.

9.9 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are followed until resolution and graded according to the Division of AIDS Table for

Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004 (Clarification August 2009).

The reactogenicity assessment period is 3 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record symptoms using a postvaccination symptom log and to be in contact with the site daily during the assessment period. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 3 to resolution. Participants are instructed to contact the clinic for events that arise during the period between vaccination and the next scheduled visit. In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, vaccine-related lesions, and lymph nodes. Events not listed on a CRF, or with an onset after the reactogenicity assessment period (day of vaccination and 3 full days after), or those meeting criteria for SAE/adverse events requiring expedited reporting to regulatory authorities, are recorded on an adverse experience log form.

Table 9-1 Schedule of reactogenicity assessments

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

[a] Day of vaccination

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

9.9.1 Assessment of systemic and local symptoms

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

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

9.9.2 Assessment of injection site

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

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

9.9.3 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).

9.10 Visit windows and missed visits

Visit windows are defined in HVTN 111 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.

9.11 Early termination visit

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

9.12 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given during the pregnancy, but remaining visits and study procedures should be completed unless medically contraindicated. For participants who are no longer pregnant, see Section 7.3.1. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome.

10 Laboratory

10.1 HVTN CRS laboratory procedures

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

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

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

10.2 Total blood volume

Required blood volumes per visit are shown in Appendix E. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated, or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 500 mL in any 56-day (8-week) period.

10.3 Primary immunogenicity timepoint

The primary immunogenicity timepoint in this study is month 6.5 (ie, 2 weeks after the 4^{th} vaccination visit). Endpoint assays for humoral and cellular responses are performed on participants at the primary immunogenicity timepoint and may be performed at baseline. Depending on the number of responders observed, assays for humoral and cellular responses may be performed on participants at other timepoints; the schedule is shown in Appendix E.

10.4 Endpoint assays: humoral

10.4.1 HIV-1 multiplex Ab assay

Total binding IgG antibodies to HIV-1 Env proteins (including V2 regions of interest) will be assessed on plasma/serum and mucosal secretion samples from study participants taken at the primary immunogenicity timepoints and baseline. Specimens from other timepoints as well as other HIV antigens and Ab isotypes may also be assayed based on the results of the initial assay.

10.4.2 Neutralizing Ab (nAb) assay

HIV-1–specific nAb assays may be performed on serum samples from study participants taken at the primary immunogenicity timepoint. Specimens from the baseline and other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoint. Tier 1 assays will test neutralization of HIV-1 strains represented in the highly neutralization-sensitive tier 1 viruses. The tier 2 assays will test neutralization of a panel of heterologous primary isolates [57].

10.5 Endpoint assays: cellular

10.5.1 Flow cytometry

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

10.6 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-1 disease progression may also be assessed.

10.7 Lab assay algorithm

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

10.8 Exploratory studies

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

10.9 Other use of stored specimens

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

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

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

The protocol sample informed consent form is written so that the participant either explicitly allows or does not allow their samples to be used in other research when they sign the form. Participants who initially agree to other use of their samples may rescind their approval once they enter the study; such participants will remain in this study and their samples will only be used for the studies described in this protocol. If a participant decides against allowing other research using his or her samples, or at any time rescinds prior approval for such other use, the study site investigator or designee must notify HVTN Regulatory Affairs in writing. In either case, HVTN Regulatory Affairs directs the HVTN Lab Program not to use samples from these participants for such other uses.

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

10.10 Biohazard containment

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

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

11 Safety monitoring and safety review

11.1 Safety monitoring and oversight

11.1.1 HVTN 111 PSRT

The HVTN 111 PSRT is composed of the following members:

- DAIDS medical officer representative,
- Protocol chair and cochair,
- Protocol Team leader,
- Core medical monitor,
- Clinical safety specialist (CSS), and
- A medical officer from an organization in Southern Africa designated by the study sponsor will also participate in the PSRT.

The clinician members of the HVTN 111 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, project manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 111 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 evaluations of cumulative reactogenicity events, AEs, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS and pertinent national regulatory authorities. To increase the sensitivity for detecting potential safety problems, the SMB will review safety data aggregated across multiple protocols that use the same or similar vaccine candidates. The SMB conducts additional special reviews at the request of the HVTN 111 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 111 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 111 PSRT AE review criteria (see Section 11.3);
- Notifying HVTN CRSs and other groups when safety pauses or planned holds are instituted and lifted (see Section 11.3);
- Querying HVTN CRSs for additional information regarding reported clinical data; and
- Providing support to the HVTN 111 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

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

11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004 (Clarification dated August 2009), available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/, except that unintentional weight loss of less than 10% loss in body weight from baseline is not required to be reported as an AE.

Unsolicited AEs will be collected over 30 days after each vaccination visit. All collected 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 (Section 11.2.3), (2) if the AE meets the criteria for a safety pause/prompt AE review (Section 11.3), and (3) if the AE is a potential immune-mediated disease that may be listed as an AE of special interest (AESI). A sample list of AESI is provided in Appendix G.

Certain AEs will be collected and reported throughout the entire study:

- SAEs/EAEs,
- AESIs; a sample list of AESIs is provided in Appendix G,
- New chronic conditions requiring medical intervention for ≥ 30 days,
- Newly diagnosed or treated STIs,
- AEs leading to early participant withdrawal or early discontinuation of study product(s) administration.

CRSs are expected to notify the CSS 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/hvtn111). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

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

In addition, site Investigators of Record (IoRs) or their designees are required to submit AE information in accordance with IRB/EC and any applicable RE requirements.

11.2.3 Expedited reporting of AEs to DAIDS

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

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

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

Under ICH E2A (*Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*), an SAE is defined as any untoward medical occurrence that at any dose:

- results in death,

- is life-threatening (Note: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death, if it were more severe),
- requires patient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect, or
- is a medically important event or reaction

Medical and scientific judgment should be exercised when deciding if other situations are serious. Such instances could include medical events that may not be immediately life-threatening or result in death or hospitalization, but which may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions not resulting in hospitalization, or development of drug dependency or drug abuse.

The expedited reporting period for this study comprises the entire study period for each individual participant (from study enrolment until study completion or discontinuation from the study).

The study products that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS and pertinent national regulatory authorities are:

- DNA-HIV-PT123/Placebo
- Bivalent Subtype C gp120/MF59/Placebo

11.2.4 Expedited reporting of AEs to pertinent national regulatory authorities

The study sponsor or designee(s) prepares and files expedited reports to appropriate regulatory authorities within the timelines required by pertinent national regulatory authorities.

Site IoRs/designees will submit AE information and any other relevant safety information to their ECs/IRBs in accordance with EC/IRB requirements.

11.3 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccination with the product related to the event that triggered the pause will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 111 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 111 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

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

Event and relationship to study products	Severity	HVTN CRS action	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and fax forms immediately[a]	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and fax forms immediately[a]	Immediate HVTN 111 PSRT notification
SAE, related	Grade 3	Email and fax forms immediately	Prompt HVTN 111 PSRT AE review to consider pause
AE[b], related	Grade 4 or Grade 3	Email and fax forms immediately	Prompt HVTN 111 PSRT 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/hvtn111).

[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 111 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 111 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 111 PSRT assessment, the trial sponsor or designee(s) notifies pertinent national regulatory authorities as needed.

If an immediate HVTN 111 PSRT notification or prompt HVTN 111 PSRT AE review is triggered, HVTN Core notifies the HVTN 111 PSRT as soon as possible during working hours (US Pacific Time)—or, if the information was received during off hours, by the morning of the next work day. If a prompt HVTN 111 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 protocol-related safety information (such as 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 111 PSRT (see Section 11.4.2).

11.4 Review of cumulative safety data

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

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

11.4.1 Daily review

Blinded daily safety reviews are routinely conducted by HVTN Core for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 111 PSRT AE review criteria.

11.4.2 Weekly review

During the injection phase of the trial, the HVTN 111 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 111 PSRT. HVTN Core reviews reports of clinical and laboratory AEs. Events identified during the review that are considered questionable, inconsistent, or unexplained are referred to the HVTN CRS clinic coordinator for verification.

11.5 Study termination

This study may be terminated early by the determination of the HVTN 111 PSRT, HVTN SMB, a pertinent national regulatory authority, NIH, Office for Human Research Protections (OHRP), 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;
- Ancillary studies, and
- Destruction of specimens.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 111 *Study Specific Procedures.*

12.1 Social impacts

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

Social harms are tabulated by the SDMC and are subjected to descriptive analysis. The goal is to reduce their incidence and enhance the ability of study staff to mitigate them when possible.

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

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

Because this study is evaluating products containing recombinant DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant DNA Molecules.* Information about the study must be submitted to site Institutional Biosafety Committees (IBC) and must be approved before participants are enrolled at the site. Investigators at each site are responsible for obtaining IBC approval and periodic review of the research per NIH guidelines *section IV-B07-b-(6)* and *section IV-B-2-b.* IBC review and approval must be documented by the investigator and submitted as part of initial protocol registration for this trial. If this protocol is amended, investigators should follow the requirements of their IBC.

12.3 Specific regulatory considerations for Republic of South Africa and other Southern African countries

The Republic of South Africa has laws regarding the use, manufacture, importation, and experimentation of products which are genetically modified. These are contained in the Genetically Modified Organism (GMO) Act 15 of 1997, administered by the South African National Department of Agriculture, Pretoria. The Registrar of GMO shall be consulted on all formal developments relating to this protocol and clinical trial, and as required, a formal application will be made to the Registrar of GMO to review the HVTN 111 clinical trial, to obtain approval for the proposed clinical trial and for the importation of the study products.

Any regulations specific to other countries containing CRSs at which HVTN 111 will be implemented will also be observed.

12.4 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 111 are described below.

Protocol history and modifications

Date: October 6, 2014

Protocol version: 1.0
Protocol modification: Original protocol

14 Document references (other than literature citations)

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

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines. Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf.
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/
- Division of AIDS Protocol Registration Manual. Available at http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Version 1.0, December 2004. (Clarification dated August 2009) Available at http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/safetyandpharmacovigilance/manualforexpeditedreporting.aspx
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 111 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 111 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN Site Lab Reference Manual. Accessible through the HVTN website.
- HVTN Manual of Operations. Accessible through the HVTN website.
- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at http://www.iata.org/ps/publications/dgr/Pages/index.aspx.
- Lab assay algorithm. Accessible through the HVTN website.
- HVTN algorithm for diagnosis of HIV infections. Part of the HVTN Site Lab Reference Manual (see above).

- International Conference on Harmonisation (ICH) E6 (R1), Guideline for Good Clinical Practice. Available at http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/good-clinical-practice.html

- International Conference on Harmonisation (ICH) E2A, *Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*. Available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E2A/Step4/E2A_Guideline.pdf

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

- NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research. Available at http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html.

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

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

- Title 21, Code of Federal Regulations, Part 50. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=21:1.0.1.1.19&idno=21

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

15 Acronyms and abbreviations

Ab	antibody
Ad26	adenovirus serotype 26
AE	adverse event
AESI	adverse events of special interest
ALP	alkaline phosphate
ALT	alanine aminotransferase
ART	antiretroviral therapy
ASCUS	atypical squamous cells of undetermined significance
AST	aspartate aminotransferase
AVEG	AIDS Vaccine Evaluation Group
BAMA	binding antibody multiplex assay
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
CAB	Community Advisory Board
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CBC	complete blood count
CCID	cell cultural infectious dose
CDC	US Centers for Disease Control and Prevention
CEF	chick embryo fibroblast
CFR	Code of Federal Regulations
CHIL	Cape Town HVTN Immunology Laboratory
CHO	Chinese hamster ovary
CI	confidence interval
CoR	correlate of risk
CRF	case report form
CPK	creatine phosphokinase
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
CSS	Clinical Safety Specialist
CTL	cytotoxic T lymphocyte
DAERS	DAIDS Adverse Event Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
DHVI	Duke Human Vaccine Institute
DSMB	NIAID Data and Safety Monitoring Board
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
ELISA	enzyme-linked immunosorbent assay

ELISpot	enzyme-linked immunospot
EMA	European Medicines Agency
FDA	US Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
GLP	Good Laboratory Practice
GM	geometric mean
GMO	genetically modified organism
GMP	Good Manufacturing Practice
GPP	Good Participatory Practice
GSID	Global Solutions for Infectious Diseases
GTI	genital tract infection
HBsAG	hepatitis B surface antigen
HCRISA	Hutchinson Centre Research Institute - South Africa
HCV	hepatitis C virus
HLA	human leukocyte antigen
HPTN	HIV Prevention Trials Network
HSML	HIV Sero-Molecular Laboratory
HSV	herpes simplex virus
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICF	informed consent form
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IDRI	Infectious Disease Research Institute
IFN-γ	interferon gamma
IgA	immunoglobulin (isotype) A
IgG	immunoglobulin (isotype) G
IL	interleukin
IM	intramuscular
IN	intranasal
IP	IFN-γ–induced protein
IRB	Institutional Review Board
ISS	Istituto Superiore di Sanità
IUD	intrauterine device
MAR	missing at random
MCAR	missing completely at random
MCC	(South Africa) Medicines Control Council
MCP	monocyte chemotactic protein
MedDRA	Medical Dictionary for Regulatory Activities

MMR	measles, mumps, and rubella
MUVAPRED	Mucosal Vaccines for Poverty Related Diseases
nAb	neutralizing antibody
NAEPP	National Asthma Education and Prevention Program
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
NK	natural killer (cells)
OHRP	US Office for Human Research Protections
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PHRU	Perinatal HIV Research Unit
PI	Principal Investigator
PSRT	Protocol Safety Review Team
RAB	DAIDS Regulatory Affairs Branch
RE	regulatory entity
RNA	ribonucleic acid
RSA	Republic of South Africa
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SAIL	South African Immunology Laboratory
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPF	specific pathogen free
SPT	DAIDS Safety and Pharmacovigilance Team
SSP	Study Specific Procedures
TB	tuberculosis
Tfh	T follicular helper (CD4+)
TM	transmembrane
TNF	tumor necrosis factor
VISP	Vaccine induced seropositivity
WBC	white blood cell

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

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Appendix A Sample informed consent form

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV clade C DNA and of MF59-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 111

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 132 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 United States National Institutes of Health (NIH) and the Bill & Melinda Gates Foundation are paying for the study.

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

   - Are the study vaccines safe to give to people?
   - Are people able to take the study vaccines without becoming too uncomfortable?
   - How do people's immune systems respond to the study vaccines? (Your immune system protects you from disease.)
   - Will giving the study vaccine with the Biojector 2000 change people's immune responses? The Biojector 2000 is a device that gives the injections without a needle. We will give some participants one of the study vaccines with the Biojector 2000. All participants will still get injections by needle and syringe.

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

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

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

   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 other studies, 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. The clinic staff can tell you about the differences.

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

4. These study vaccines are experimental.

The study vaccines are called DNA-HIV-PT123 and Bivalent Subtype C gp120. Gp120 is a protein. From here on, we will call them the DNA vaccine and the Protein vaccine, or the study vaccines. They are experimental HIV vaccines. That means we do not know whether the study vaccines will be safe to use in people, or whether they will prevent HIV infection. These study vaccines are used only in research studies.

The DNA vaccine:

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

The DNA vaccine is being tested in people in 2 studies in the US and in Switzerland. These studies are giving the DNA vaccine with other vaccines. As of June 2014, over 180 people received the DNA vaccine. So far, it has not made them too uncomfortable or caused them serious health problems. However, studies with a small number of people do not tell us everything about the safety of the study vaccines.

The Protein vaccine:

The Protein vaccine was developed by Novartis Vaccines (USA).

The Protein vaccine will be mixed with an adjuvant. An adjuvant is a substance added to the vaccine to help the immune system respond better. In this study the adjuvant is called MF59. MF59 is commonly used in licensed flu vaccines in many countries. It has also been in other vaccines that have been given to over 50,000 people in research studies without causing any serious health problems.

The Protein vaccine has not been given to people before. It has been tested in mice, rabbits, and monkeys and it did not cause any health concerns. Animal testing may not always tell us what will happen with humans. Similar Protein vaccines have been given to more than 10,000 people in research studies. In these studies, the protein vaccines did not cause serious health problems or make people too uncomfortable.

This Protein vaccine will be given to 210 participants in another study in South Africa that starts before this one. If we learn anything from that study that might affect your willingness to be in this study, we will tell you.

General risks of vaccines:

All vaccines can cause fever, chills, rash, itching, aches and pains, and redness and swelling where the injection was given. Vaccines can also cause nausea, headache,

dizziness, and feeling tired. Most people can still do their planned activities after getting a vaccine. Rarely, people experience side effects that limit their normal activities or make them go to the doctor.

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

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

Risks of the study vaccines:

The DNA vaccine:

In studies with this and similar DNA vaccines, the most common complaints were mild to moderate pain or itching in the area where they got the injection, headache, and feeling tired.

The Protein vaccine:

This study vaccine has not been tested in people before. In studies with similar products, some people had redness, swelling, pain, tenderness, or itching in the area where they got the injection. Some people had headache. A small number of people had, joint pain, flu-like symptoms, nausea, rash, vomiting, diarrhea, or swollen lymph nodes after getting an injection. A very small number of people had abdominal pain. People who have these symptoms may only have a few of them.

There may be other risks of the study vaccines that we don't yet know about. We will tell you if we learn anything new that may affect your willingness to stay in the study.

Joining the study

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

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

If you join this study, you may not be allowed to join other HIV vaccine or HIV prevention studies now or in the future. You cannot be in this study while you are in another study where you receive a study product. Also during the study, you should not donate blood or tissue.

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

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

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

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

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

   We will also do blood and urine tests. These tests tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for: Hepatitis B, Hepatitis C, and syphilis. We will ask you about medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were born female, we will test you for pregnancy. We will also ask if you have ever been allergic to eggs, egg products, or the antibiotic Neomycin.

   We will review the screening results with you, and offer you counseling and referral if you need medical care. We will not pay for this medical care. The screening results may show you are not eligible to join the study, even if you want to.

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

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

   You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 3 weeks before your first injection until 6 months after your last study injection. We will talk to you about effective birth control methods. They are listed on a handout that we will give to you. *Site: Delete the preceding sentence if you include the birth control sheet in this consent form.* If you join the study, we will test you for pregnancy at some visits, including before each study injection.

Being in the study

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

8. **You will come to the clinic for scheduled visits about [#] times over 12 months.**

   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).

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

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

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

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

10. Not everyone in this study will get the study vaccines.

Out of every 11 people who join the study, one of them will get *only* placebos. Everyone else will get a combination of study vaccines *and* placebos. Placebos are substances that do not contain vaccine. In this study, the placebo is sterile salt water. We will compare the results from people who got only the placebos with results from people who got the study vaccines and placebos. Everyone in this study will get some placebos.

There are 6 groups in this study. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.* You do not choose the group you are in. You will be assigned to one at random, just like flipping a coin.

- In 2 of the groups, people will get *only* placebos.
- In the other 4 groups, people will get the study vaccines *and* placebos.

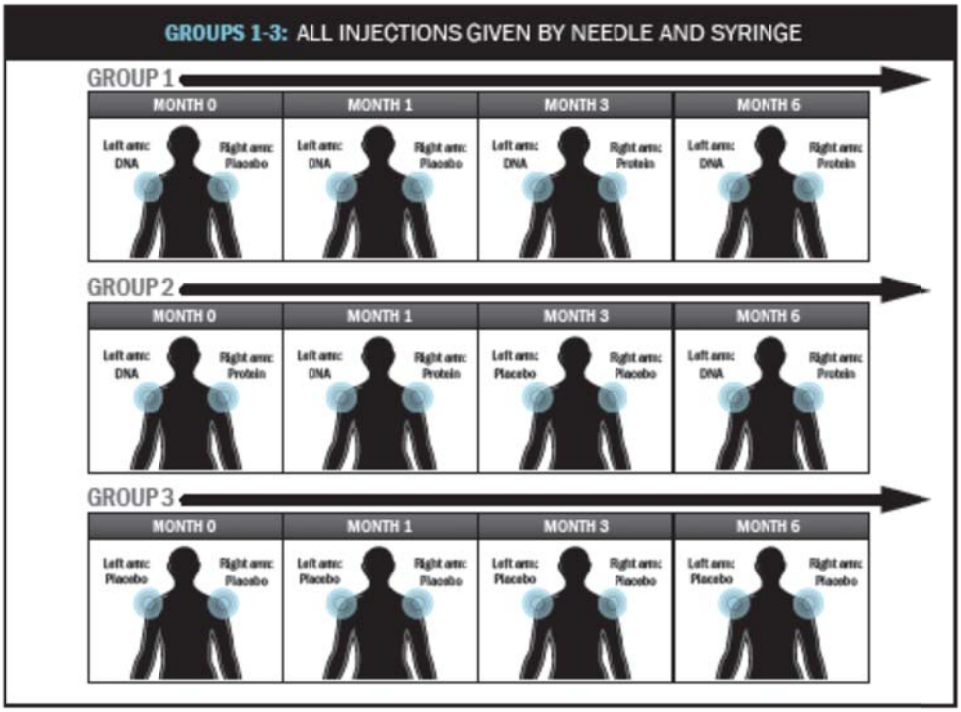
The clinic staff have no say in whether you get the study vaccines and/or the placebos. They will not know which study products you are getting, and neither will you. Only the pharmacist at your site will have this information while the study is going on.

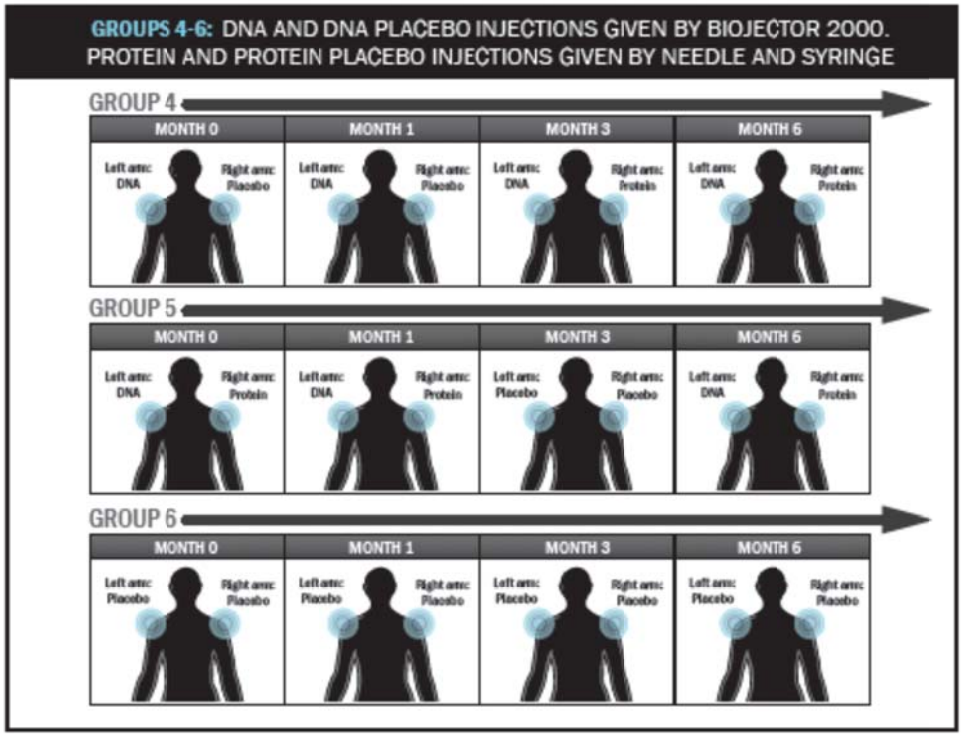
You will have to wait until everyone completes their final study visits to find out what study products you got. This could be 2-5 years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

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

People in groups 1-3 will get all injections with a needle and syringe. People in groups 4-6 will get the Protein vaccine or its placebo with a needle and syringe. They will get the DNA vaccine or its placebo with a device called Biojector 2000. It is a device that pushes the study product through the skin without using a needle. The US Food and Drug Administration has approved this system for delivering injections into muscles.

Everyone in this study will get 2 injections (1 in each arm) at each injection visit. The DNA vaccine or its placebo will go into your left arm. The Protein vaccine or its placebo will go into your right arm. You will get a total of 8 injections during 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. That night and for three more days, you will need to report to the clinic staff how you feel. To help you do this, we can give you tools and show you how to use them. We will ask you the ways we can contact you. If you do not contact us each of the three days after your injections, we will contact you by the ways you wish to be contacted.

Also, please contact clinic staff if you have any issues or concerns after receiving an injection. If you have a problem, we will continue to check on you until it goes away.

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

- Do regular HIV testing, as well as counseling on your results and on how to avoid getting HIV;
- Perform 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 blood and urine 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 200 mL (2 teaspoons to a little less than 1 cup/ 12 tablespoons). Your body will make new blood to replace the blood we take out.

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

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

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

13. If you agree, we will also collect 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 rectum, vagina, and penis. At the end of this form we will ask if you allow us to collect 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 would like to collect these samples at 3 visits. When we collect the samples, we will test you for gonorrhea, chlamydia and syphilis. If you were born female, we will also test you for pregnancy, trichomoniasis, bacterial vaginosis and if needed, for a yeast infection. We will explain what these tests are for and we will give you the results. If you need care, we will tell you about the care we can give you at this clinic. We will also tell you about care we can help you get elsewhere.

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

Rectal Fluid

We will collect rectal fluid by placing a small absorbent sponge or swab in the rectum using a plastic tube about 2 cm wide (a little less than an inch). The tube will go in about 6.35 cm (2 ½ inches). This will take about 5 minutes.

For the 2 days before we collect your rectal fluid, we will ask you to not do these things:

- have receptive anal intercourse
- put anything into your anus, including cleaning products (creams, gels, lotions, pads, etc.), lubricant, or enemas
- douche (even with water),
- use any anti-inflammatory creams in or around your anus.

We will not collect rectal fluid if you are menstruating, pregnant, or if we think you may have an anal or rectal infection. You should tell us if your rectal area is sore.

Cervical Fluid

You must have had a Pap smear within the last 3 years with the most recent result being normal. (If you haven't had a Pap smear within the last 3 years and would like to get one, we will tell you where you can get one.)

We will collect cervical fluids by using either a soft sponge inserted into the opening of your cervix, or by using something call a Softcup inserted into your vagina. If we use a soft sponge to collect cervical fluids, we will insert a speculum (a device that holds your vagina open) into your vagina and place the sponge in the opening of the cervix. This is similar to getting a pap smear. If we use the Softcup, we will explain how to use it.

For the 2 days before we collect your cervical fluid, we will ask you to not do these things:

- use any spermicide, lubricants, douche (even with water), or medication in or around your vagina;
- 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.

Semen

You may provide the semen at home or at the clinic. We will ask you to ejaculate into a plastic cup, which we will give to you. The clinic must receive the semen sample within 2 hours or less after it is collected. We will ask you not to ejaculate for 2 days before

providing the semen. You should tell us if you think you have an infection on your penis. If you have an infection we may not use your sample.

14. We will test your samples for this study.

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

Also, the researchers may:

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

- Do limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. Limited genetic testing involves only some of your genes, especially genes related to the immune system and diseases, not all of your genes (your genome).

These tests are for research purposes only. The lab will not give the results to you or this clinic, and the results will not become part of your study record.

Most of these tests will be done in labs in South Africa. Some samples may be shipped to US labs for testing.

15. We will counsel you on avoiding HIV infection.

We will ask you personal questions about your HIV risk factors such as sexual behavior and drug use. We will talk with you about ways to keep your risk of getting HIV low. Some topics we may discuss include:

- What you think may cause risky behavior for you.

- Methods to avoid getting HIV.

These may include not having sex, using condoms, or behavior changes, such as cutting down on alcohol. We will talk with you about which methods of HIV prevention may be right for you.

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

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

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

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

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

While most of your samples will be stored in South Africa, some may be shipped to the US for some tests, including genetic tests.

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

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

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

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

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

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

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

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.

- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

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

People who may see your information are:

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

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

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

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

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, its study monitors, and its chosen representatives,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- IPPOX Foundation and Novartis Vaccines 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]

The results of this study may be published. No publication will use your name or identify you personally.

We may share information from the study with other researchers. We will not share your name or information that can identify you.

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

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

This may happen if:

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

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

19. We will stop your injections if you become pregnant during the study.

We will encourage you to stay in the study if you choose. The clinic staff 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, and about other studies you may want to join. *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

Other Risks

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 with a needle and syringe and/or with a Biojector 2000 device. These procedures can cause a cut or sore, bleeding, bruising, pain, fainting, soreness, redness, swelling, itching, muscle damage, and (rarely) infection where the needle was inserted. Taking blood can cause a low blood cell count (anemia), making you feel tired.

Injections given with the Biojector® may cause pain, redness, swelling and bruising. People may be startled by the sound the Biojector makes when it injects the study product. Some people may get a small red bump and then a scab on the arm where the Biojector injection was given. This usually goes away within a few days.

Personal problems/discrimination/testing HIV antibody positive:

About 10 to 20% of people who join HVTN studies report personal problems or discrimination because of joining an HIV vaccine study. Family or friends may worry, get upset or angry, or assume that you are infected with HIV or at high risk and treat you unfairly as a result. Rarely, a person has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

The body makes antibodies to fight or prevent infection. Most vaccines cause the body to make antibodies as a way of preventing infection. Your body may make antibodies to HIV because you received HIV study vaccines. The study vaccines are likely to cause you to test positive on some types of HIV tests, even if you are not infected with HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccines, a routine HIV test done outside this clinic is likely to say you have HIV, even if you don't. For this reason, you should plan to get HIV tests only at this clinic during the study. Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccines.

If you receive a positive test result caused by the study vaccines at any time, we can provide you with free HIV testing for as long as you need it. If this happens, we do not know how long you will test positive due to the study vaccines. If you receive a positive HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

It is unlikely, but you could test negative at the end of the study and positive some time later, even though you don't have HIV. This could happen if different HIV tests come into use. We will give you a phone number to call for more information.

Site: Modify the following paragraph if applicable. If someone believes you are infected with HIV even if you are not, you could face discrimination and other problems. For example, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military in some countries. If you do have a positive HIV antibody test caused by the study vaccines, you will not be able to donate blood or organs. Your family and friends may treat you differently. We will give you a brochure that tells you more about testing HIV positive because of an HIV vaccine, and how you can avoid some of these problems.

If you become pregnant during or after the study and have VISP, we don't know if the antibodies could be passed to your baby. We know that this happens with other vaccines, like tetanus vaccine. These antibodies from the mother are not a danger to the baby, and they go away over time. If the baby continues to have VISP, we can do this testing for free for as long as it is needed. For most babies antibodies from the mother last for about six months.

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

Embarrassment/anxiety:

You may feel embarrassed when we ask about your HIV risks, such as having sex and using drugs. Also, waiting for your HIV test results or other health test results could make you feel anxious. You could feel worried if your test results show that you are infected with HIV. If you feel embarrassed or anxious, please tell us and we will try to help you.

Risks of disclosure of your personal information:

We will take several steps to protect your personal information. Although the risk is very low, it is possible that your personal information could be given to someone who should not have it. If that happened, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information if you would like it.

Risks of genetic testing:

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

Unknown risks:

We do not know if the study vaccines will increase, decrease, or not change your risk of becoming infected with HIV if exposed. If you get infected with HIV, we do not know how the study vaccines might affect your HIV infection or how long it takes to develop AIDS.

We do not know if getting these study vaccines will affect how you respond to any future approved HIV vaccine. It could be that a future HIV vaccine may not work as well for you because you got the study vaccines. Currently, no HIV vaccine has been approved for use.

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

Benefits

22. The study may not benefit you.

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 study participants.

Your rights and responsibilities

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

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

Leaving the study

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

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

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

Injuries

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

Your health is important to us. We will help you get the medical care you need. We will tell you about the care that we can give you at this clinic. We will also tell you about the care that we can help you get elsewhere.

We call an injury or illness study-related if it occurs as a direct result of the administration of the study products or study-related procedures. The clinic staff will treat you for study-related problems or tell you where you can get the treatment you need. If a study-related injury occurs you have not waived any of the legal rights which you otherwise would have as a participant in this study by signing this form.

Only South African sites keep the paragraph below:

If you get sick or injured because of the study vaccines, insurance has been purchased to cover your medical treatment. This policy will follow the guidelines for payment of study-related illness or injury approved by the Association of the British Pharmaceutical Industry ("ABPI Guidelines"). You can get a copy of these ABPI Guidelines from us if you wish.

In addition, the HVTN also has limited funds from the U.S. government to pay for your treatment for study related injuries.

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

You, your usual health care provider and/or your health insurance carrier will continue to be responsible for the cost of your usual medical care outside this study, and for medical expenses that are determined not directly related to study procedures or products.

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

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

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

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

Your permissions and signature

27. In section 13 of this form, we told you about collecting 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 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.

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

28. In Section 16 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please write your initials or make your mark in the box next to the option you choose.

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

OR

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

OR

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

29. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

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

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

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

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix B Approved birth control methods (for sample informed consent form)

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby.

If you were born female and are sexually active in a way that could lead you to get pregnant, you must agree to use effective birth control, from 3 weeks before your first study injection until 6 months after your last study injection.

Effective birth control means using 1 of the following methods every time you have sex:

- Male or female condoms; or,
- Diaphragm or cervical cap;

PLUS 1 of the following methods:

- Birth control drugs that prevent pregnancy—given by pills, patches, vaginal rings, or inserts under the skin;
- Intrauterine device (IUD); or
- You are only having sex with a partner who has had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.).

You do not have to use birth control if:

- 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 sexually abstinent (no sex at all)

Sites may delete the bullets below, if desired.

- You are having sex only with a female partner or partners;
- You only have oral sex;

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

Appendix C Table of procedures (for sample informed consent form)

Procedure	Screening visit(s)	Time after 1st injection visit (in months)										
		First injection visit	¼	1	1½	3	3½	6	6¼	6½	9	12
Injection		√		√		√		√				
Medical history	√											
Complete physical	√											√
Brief physical		√	√	√	√	√	√	√	√	√	√	
Urine test	√		√							√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√
Pregnancy test (participants born female)	√	√		√		√		√		√*		√*
HIV testing & pretest counseling	√					√		√			√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√
Pap smear*	√											
Rectal fluids/cervical fluids/semen samples		√								√		√
Genital Tract Infection testing (urine, blood and swab for females)**		√								√		√

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.

* For participants who were born female and who agree to provide cervical and/or rectal fluid samples.

**For participants who agree to provide rectal fluids/cervical fluids/semen samples.

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 HIV clade C DNA and of MF59-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 111

Site: [Insert site name]

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

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

1. Do I have to agree?

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

2. Where are the samples stored?

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

While most of your samples will be stored in South Africa, some may be shipped to the US for some tests, including genetic tests.

3. How long will the samples be stored?

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

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

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

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

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

6. Will the HVTN sell my samples and information?

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

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

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

8. What information is shared with other researchers?

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

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

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

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.

- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

10. What are the risks of genetic testing?

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

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

People who may see your information are:

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

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

Questions

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

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

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

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

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

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

OR

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

OR

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

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

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

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

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

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Appendix E Laboratory procedures

					Visit:	1	2	3	4	5	6	7	8	9	10	11	12	
					Day:	D0	D7	D28	D42	D84	D98	D168	D175	D182	D273	D364	
					Weeks	Screening visit[3]	W0	W1	W4	W6	W12	W14	W24	W25	W26	W36	W48	
					Month		M0	M0.25	M1	M1.5	M3	M3.5	M6	M6.25	M6.5	M9	M12	
							VAC1		**VAC2**		**VAC3**		**VAC4**					
							DNA + Placebo OR DNA + Prot + MF59 OR Placebo		**DNA + Placebo OR DNA + Prot + MF59 OR Placebo**		**DNA + Prot + MF59 OR Placebo**		**DNA + Prot + MF59 OR Placebo**					
Procedure	**Ship to[1, 2]**	**Assay location[2]**	**Tube Type[4]**	**Tube size (vol. capacity)[4]**														**Total**
BLOOD COLLECTION																		
Screening/Diagnostic																		
Screening HIV test	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	**5**
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	**5**
HIV in-study diagnostic test[9]	HSML-NICD	HSML-NICD	EDTA	10mL		—	—	—	—	—	10	—	10	—	—	10	20	**50**
Safety labs																		
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL		5	—	5	—	5	—	5	—	—	5	—	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—	5	—	5	—	5	—	—	5	—	—	**25**
STI Serology																		
Syphilis[11]	Local lab	Local lab	SST	5mL		5	5[11]	—	—	—	—	—	—	—	5[11]	—	5[11]	**20**
Immunogenicity & Virologic assays[6]																		
HLA host genetics[7]	BARC	FHCRC	ACD	8.5mL		—	17	—	—	—	—	—	—	—	—	—	—	**17**
Cellular assays																		
ICS	BARC	FHCRC / CHIL	ACD	8.5mL		—	68	—	—	34	—	—	—	—	***68***	—	68	**238**
pTfh and Plasmablast	BARC	FHCRC / CHIL	ACD	8.5mL		—	17	17	—	—	—	—	—	17	—	—	—	**51**
Humoral assays																		
Binding Ab Assay	BARC	DHVI-Duke	SST	8.5mL		—	8.5	—	—	8.5	—	—	—	—	***8.5***	—	8.5	**34.0**
Neutralizing Ab Assay	BARC	Duke-NAB / SAIL-NICD	SST	8.5mL		—	8.5	—	—	—	—	—	—	—	***8.5***	—	8.5	**25.5**
Ab Avidity	BARC	DHVI-Duke	SST	8.5mL		—	y	—	—	—	—	—	—	—	***y***	—	y	**0**
ADCC	BARC	Duke-ADCC	SST	8.5mL		—	y	—	—	—	—	—	—	—	***y***	—	y	**0**
STORAGE																		
Serum storage	BARC	—	SST	8.5mL		—	17	—	—	—	—	—	—	—	8.5	17	8.5	**51**
PBMC storage	BARC	—	ACD	8.5mL		—	34	—	—	—	—	—	—	—	17	—	17	**68**
Visit total						**25**	**175**	**27**	**0**	**53**	**10**	**10**	**10**	**17**	**126**	**27**	**136**	**615**
56-Day total						**25**	**200**	**227**	**227**	**255**	**63**	**73**	**10**	**27**	**153**	**27**	**136**	
URINE COLLECTION																		
Urinalysis	Local lab	Local lab				X	—	X	—	—	—	—	—	—	X	—	—	
Pregnancy Test[8]	Local lab	Local lab				X	X	—	X	—	X	—	X	—	X[10]	—	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)[13]																		
Semen	BARC	DHVI-Duke				—	X	—	—	—	—	—	—	—	X	—	X	
Cervical Secretions	BARC	DHVI-Duke				—	X	—	—	—	—	—	—	—	X	—	X	
Rectal Secretions	BARC	DHVI-Duke				—	X	—	—	—	—	—	—	—	X	—	X	

y = 8.5mL of SST blood collected for the Binding Ab and Neutralizing Ab assays will also cover specimen needs for the Ab avidity and ADCC assays; no separate blood draw is needed.

[1] BARC = Bio Analytical Research Corporation South Africa (Pty) Ltd (Johannesburg, South Africa)

[2] HVTN Laboratory Program includes laboratories at HSML-NICD, FHCRC, CHIL, DHVI-Duke, Duke-NAB, Duke-ADCC and SAIL-NICD. HSML-NICD = HIV Sero-Molecular Laboratory-National Institute for Communicable Diseases (Johannesburg, South Africa); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); CHIL = Cape Town HVTN Immunology Laboratory (Cape Town, South Africa); DHVI-Duke = Duke Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA); Duke-NAB = Neutralizing Antibody Assay Laboratory, Duke University Medical Center (Durham, North Carolina, USA); Duke-ADCC = Antibody-Dependent Cellular Cytotoxicity Laboratory, Duke University Medical Center (Durham, North Carolina, USA); SAIL-NICD = South African Immunology, Laboratory–National Institute for Communicable Diseases (Johannesburg, South Africa)

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

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

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

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

[7] Genotyping may be performed on enrolled participants using cryopreserved PBMC collected at baseline, initially in participants who demonstrate vaccine-induced T-cell responses at postvaccination timepoints.

[8] Pregnancy 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 9.11), blood should be drawn for HIV diagnostic testing, as shown for visit 12 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 secretion sample.

[11] Syphilis testing by serology and Chlamydia and gonorrhea testing by urine will only be performed if the participant agrees to provide a mucosal sample.

[12] Cervical/vaginal swabs will only be collected from participants who agree to provide a cervical secretion 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 specified in the SSP.

Appendix F Procedures at HVTN CRS

Visit:	01[a]	02	03	04	05	06	07	08	09	10	11	12	Post
Day:		D0	D7	D28	D42	D84	D98	D168	D175	D182	D273	D364	
Month:		M0	M0.25	M1	M1.5	M3	M3.5	M6	M6.25	M6.5	M9	M12	
Procedure	Scr.	VAC1		VAC2		VAC3		VAC4					
Study procedures[b]													
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—	—
Signed protocol consent	X	—	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	X	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	—	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	X	X	X	—
Pregnancy prevention assessment[c]	X	X	X	X	X	X	X	X	X	X	X	X	—
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	—
Social impact assessment questionnaire	—	—	—	—	—	X	—	X	—	—	—	X	—
Outside testing and belief questionnaire	—	—	—	—	—	—	—	X	—	—	—	X	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	—
Intercurrent illness/adverse experience[d]	—	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[e]	X	—	—	—	—	X	—	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	X	—	X	—	—	X	X
Local lab assessment													
Urine dipstick	X	—	X	—	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	X	—	X	—	X	—	X[n]	—	X[n]	—
CBC, differential, platelet	X	—	X	—	X	—	X	—	—	X	—	—	—
Chemistry panel (see Section 9.2)	X	—	X	—	X	—	X	—	—	X	—	—	—
Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—
Syphilis	X	X[m]	—	—	—	—	—	—	—	X[m]	—	X[m]	—
Pap smear[g]	X	—	—	—	—	—	—	—	—	—	—	—	—
Chlamydia/gonorrhea (urine)[h]	—	X	—	—	—	—	—	—	—	X	—	X	—
Trichomonas vaginalis[i]	—	X	—	—	—	—	—	—	—	X	—	X	—
Bacterial vaginosis[i]	—	X	—	—	—	—	—	—	—	X	—	X	—
Yeast[j]	—	X	—	—	—	—	—	—	—	X	—	X	—
Mucosal sample collection (optional)													
Rectal secretions, cervical secretions, semen	—	X	—	—	—	—	—	—	—	X	—	X	—
Vaccination procedures													
Vaccination[k]	—	X	—	X	—	X	—	X	—	—	—	—	—
Reactogenicity assessments[l]	—	X	—	X	—	X	—	X	—	—	—	—	—
Poststudy													
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	X

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

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

[c] Pregnancy prevention compliance occurs only with participants who were born female and are capable of becoming pregnant.

[d] AEs to be collected and reported through 30 days after each vaccination (see Section 11.2.2).

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

[f] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination. Pregnancy test to determine eligibility may be performed at screening or on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[g] Only for volunteers who were born female and who agree to provide cervical samples. Per Section 9.2; Pap smear not required if volunteer has had Pap smear within previous 3 years with most recent result normal or ASCUS or less than 21 years old.

[h] Urine testing for Chlamydia and gonorrhea will be done only if the participant consents to provide mucosal samples. Specimen collection for this testing will take place at the time of mucosal sampling, prior to vaccination (if scheduled).

[i] This testing will be done for participants providing cervical mucosal samples. Specimen collection for this testing will take place at the time of mucosal sampling, prior to vaccination (if scheduled).

[j] This testing will be done for participants providing cervical mucosal samples only if clinically indicated. Specimen collection for this testing will take place at the time of mucosal sampling, prior to vaccination (if scheduled).

[k-] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration. Lab tests may be drawn within the 3 days prior to vaccination.

[l] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.9).

[m] Syphilis testing will only be performed at the indicated visit if the participant agrees to provide mucosal samples.

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

Appendix G 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 111 Study Specific Procedures*

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (eg Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: eg non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy). • Narcolepsy	• Systemic lupus erythematosus and associated conditions • Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome • Idiopathic inflammatory myopathies, including dermatomyositis • Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still's disease • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis • Mixed connective tissue disorder	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localized Scleroderma (Morphea)
Vasculitides	**Blood disorders**	**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.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia • Autoimmune aplastic anemia • Autoimmune neutropenia • Autoimmune pancytopenia	• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy) • Autoimmune myocarditis/cardiomyopathy Sarcoidosis • Stevens-Johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon