HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 108

A phase 1/2a clinical trial to evaluate the safety and immunogenicity of HIV clade C DNA, and of MF59®- or AS01$_B$-adjuvanted clade C Env protein in various combinations, in healthy, HIV-uninfected adult participants

DAIDS DOCUMENT ID 12007

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CLINICAL TRIAL SPONSORED BY

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

STUDY PRODUCTS PROVIDED BY

IPPOX Foundation
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Novartis Vaccines
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Rixensart, Belgium

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HVTN 108, Version 2.0

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

In all public health research, the risk-benefit ratio may be difficult to assess because the benefits to a healthy participant are not as apparent as they would be in treatment

protocols, where a study participant may be ill and may have exhausted all conventional treatment options. However, this protocol is designed to minimize the risks to participants while maximizing the potential value of the knowledge it is designed to generate.

2.3 Equitable 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 108 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 the United States, research participants in HVTN protocols are protected by a Certificate of Confidentiality from the US NIH, which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form. In addition, each staff member at each study site in this protocol signs a Confidentiality Agreement with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A phase 1/2a clinical trial to evaluate the safety and immunogenicity of HIV clade C DNA, and of MF59®- or AS01B-adjuvanted clade C Env protein in various combinations, in healthy, HIV-uninfected adult participants

Primary objectives

Primary objective 1

- To evaluate the safety and tolerability of DNA and/or different doses of clade C bivalent gp120 protein with either MF59® or $AS01_B$ adjuvant in various regimens in HIV uninfected healthy adults.

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, bivalent gp120 protein/MF59, and/or bivalent gp120 protein/$AS01_B$ 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 96ZM651 *gag*, 2) clade C 96ZM651 *gp140*, and 3) clade C CN54 *pol-nef*, delivered at a total dose of 4 mg, administered IM as a single 1 mL dose.

- **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 as a single 0.5 mL dose.

- **Protein/$AS01_B$**: Bivalent Subtype C gp120/$AS01_B$: clade C TV1.C gp120 Env and clade C 1086.C gp120 Env, each at a dose of 20 mcg or 100 mcg, mixed with $AS01_B$ adjuvant, administered IM as a single 0.75 mL dose.

- **Placebo**: Sodium Chloride for Injection, 0.9%, administered IM at volumes to match the active products.

Table 3-1 Schema

Group	N	Dose of each protein	Deltoid	Month 0 (Day 0)	Month 1 (Day 28)	Month 3 (Day 84)	Month 6 (Day 168)
1	30	100 mcg	Left	DNA	DNA	DNA	DNA
			Right	Placebo + Placebo*	Placebo + Placebo*	Protein/MF59 + Placebo*	Protein/MF59 + Placebo*
2	50	100 mcg	Left	DNA	DNA	DNA	DNA
			Right	Placebo + Placebo*	Placebo + Placebo*	Protein/AS01$_B$ + Placebo*	Protein/AS01$_B$ + Placebo*
3	50	20 mcg	Left	DNA	DNA	DNA	DNA
			Right	Placebo + Placebo*	Placebo + Placebo*	Protein/AS01$_B$ + Placebo*	Protein/AS01$_B$ + Placebo*
4	30	100 mcg	Left	DNA	DNA	Placebo	DNA
			Right	Protein/MF59 + Placebo*	Protein/MF59 + Placebo*	Placebo + Placebo*	Protein/MF59 + Placebo*
5	50	100 mcg	Left	DNA	DNA	Placebo	DNA
			Right	Protein/AS01$_B$ + Placebo*	Protein/AS01$_B$ + Placebo*	Placebo + Placebo*	Protein/AS01$_B$ + Placebo*
6	50	20 mcg	Left	DNA	DNA	Placebo	DNA
			Right	Protein/AS01$_B$ + Placebo*	Protein/AS01$_B$ + Placebo*	Placebo + Placebo*	Protein/AS01$_B$ + Placebo*
7	50	20 mcg	Left	Placebo	Placebo	Placebo	Placebo
			Right	Protein/AS01$_B$ + Placebo*	Protein/AS01$_B$ + Placebo*	Placebo + Placebo*	Protein/AS01$_B$ + Placebo*
8	24	N/A	Left	Placebo	Placebo	Placebo	Placebo
			Right	Placebo + Placebo*	Placebo + Placebo*	Placebo + Placebo*	Placebo + Placebo*

* Two distinct placebo volumes for protein/adjuvant will be needed to maintain the blind since Protein/AS01$_B$ and Protein/MF59 consist of different injection volumes.

Participants

334 healthy, HIV-uninfected volunteers aged 18 to 40 years; 310 vaccinees, 24 placebo recipients

Design

Multicenter, randomized, controlled, double-blind trial

Duration per participant

12 months of scheduled clinic visits (main study) followed by 1 phone, text message, or e-mail contact 6 months after the last scheduled clinic visit to monitor AESIs

Estimated total study duration

24 months (includes enrollment, follow-up, and AESI contact)

Investigational New Drug (IND) sponsor

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

Study product providers

- DNA-HIV-PT123: IPPOX Foundation (Lausanne, Switzerland)
- Bivalent Subtype C gp120: Novartis Vaccines (Cambridge, MA, USA)
- MF59: Novartis Vaccines (Cambridge, MA, USA)
- $AS01_B$: GlaxoSmithKline Biologicals (Rixensart, Belgium)

Core operations

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

Statistical and data management center (SDMC)

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

DF/Net Research (Seattle, Washington, USA)

HIV diagnostic laboratory

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

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 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 the US and Southern Africa to be specified in the Site Announcement Memo

Safety monitoring

HVTN 108 PSRT; HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

Protocol leadership

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4 Background

4.1 Rationale for trial concept

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 1 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 post-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.

The P5 partnership is committed to evaluating multiple vaccine regimens and will address this goal by assessing regimens containing combinations of next-generation vaccine products as well as different adjuvant systems to identify those exhibiting potent yet diverse immunological profiles, thus providing the greatest potential to confirm previous and identify new CoR in an efficacy trial. More than a dozen different vaccine regimens will be evaluated in several phase 1/2a trials; and a number of these candidate vaccine regimens will be selected based on safety and immunogenicity data collected through the primary immunogenicity timepoint of Month 6.5 to advance to phase 2b proof-of-concept efficacy testing.

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 1 year postvaccination (estimated 60.5%, 95% CI 22-80) with the vaccine effect waning over time to 31% cumulative through 3.5 years [6].

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 and 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, higher immunoglobulin A [IgA] Ab to Env→higher 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 [7]. 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 [8]. 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 [9]. In sum, these CoR studies point to the importance of 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

According to the most recent Gap Report of the Joint United Nations Program on HIV/AIDS, there were an estimated 35 million people living with HIV globally in 2013. There were 2.1 million new HIV infections globally and 1.5 million AIDS deaths in 2013. Therefore, despite the well-recognized benefits from scaling-up ART and certain HIV prevention strategies, the global burden of HIV remains enormous [10].

4.2.1 HIV in sub-Saharan Africa

While universal access to ART is a global ideal, in many regions of sub-Saharan Africa limited access undermines the prevention potential of widespread ART. 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 approximately 6.3 million people living with HIV as of 2013, South Africa's epidemic remains the largest in the world and the Sub-Saharan region bears the preponderant burden of the HIV epidemic with approximately 70% of all infections worldwide. Ten countries (Ethiopia, Kenya, Malawi, Mozambique, Nigeria, South Africa, Uganda, United Republic of Tanzania, Zambia and Zimbabwe) account for 81% of all people living with HIV in the region and half of those are in only 2 countries—Nigeria and South Africa. The vast majority of newly acquired infections in this region occur during unprotected heterosexual intercourse and subsequent transmission to newborns and breastfed babies. Approximately 340,000 new HIV infections and 200,000 HIV-related deaths occurred in South Africa in 2013 [10]. These statistics give strong support to recent statements from top scientists and opinion leaders who have voiced the persistent unmet need for a preventive HIV vaccine [11].

4.2.2 HIV in the United States

Approximately 1 million people are living with HIV in the United States. Access to potent and safe ART has transformed a life-threatening disease into a manageable chronic condition for many of these individuals. Life expectancy has risen steadily, to the point where individuals, diagnosed early in infection and at a high CD4 count, may have a similar life-expectancy to the general population. Better tolerated treatment is now often provided as early as possible to reduce the contribution of HIV on non-AIDS related morbidity, and to prevent onward transmission.

However, despite these advances in HIV care, HIV morbidity remains high and prevention efforts for the highest risk groups have had only partial success. The estimated incidence rates remain high at 15.8/100,000 overall, and 4 times as high among African Americans. Concernedly, the rates for young Americans, aged 20-29 years, increased between 2008 and 2011. In addition, 65% of new infections were estimated to be among men who have sex with men, and rates were increasing. It is unlikely that early treatment strategies and the availability of pre-exposure prophylaxis alone will be able to stop this epidemic, and that therefore an effective vaccine will be essential [12].

The P5 partnership will investigate new vaccine products and vaccination strategies in populations from the US and Sub-Saharan Africa. This will ensure the study of correlates of risk and protection is representative of the global HIV epidemic, resulting in the greatest impact on populations at risk in the US and globally.

4.3 Rationale for phase 1/2a vaccine regimen selections

The vaccine regimens included in the multiple phase 1/2a trials were selected based on the rationale that they will have robust yet distinct immune profiles for selection of future phase 2b efficacy testing and to address specific questions in the field that are pertinent for identifying CoR. These questions include the following:

- What are the immune responses elicited by vaccine regimens containing DNA and adjuvanted protein without a pox vector?
    - When DNA is administered alone as a prime followed by DNA plus protein/adjuvant boost?

  - When DNA and protein/adjuvant are co-administered at each vaccination?

- What immune responses are elicited by the Bivalent Clade C gp120 + $AS01_B$ alone regimen?

- How do the adjuvants MF59 versus $AS01_B$ affect the immune responses elicited by each vaccine regimen?

- Does a higher dose (100 mcg) of each Env protein in the Bivalent Clade C gp120 vaccine product adjuvanted with $AS01_B$ dampen immune responses as compared to a lower dose (20mcg) within each vaccine regimen?

- How does priming with DNA versus priming with ALVAC versus priming with NYVAC affect HIV-specific immune responses when followed by ALVAC or NYVAC + protein/adjuvant boosting?

- What are the immune responses elicited by a vaccine regimen comprising ALVAC and Bivalent Subtype C gp120 when the protein component is administered with MF59 and with alum adjuvants, or without adjuvant?

Including all vaccine regimens in a single phase 1/2a trial would be prohibitively complex. Therefore a suite of studies has been developed, allowing the opportunity to address the pertinent questions in a programmatically feasible manner. The MF59 versus $AS01_B$ question will be addressed in several of these studies – HVTN 108 among them – as it applies to several vaccine product regimens. Similarly, since the $AS01_B$/protein dose question may provide different results depending upon the full vaccine regimen evaluated, this question will be addressed in several studies as well. However, only 1 $AS01_B$ adjuvanted Env protein dose for each vaccine regimen will be considered for advancement to phase 2b testing.

HVTN 108 is one of the phase 1/2a studies planned within this program. Evaluation of DNA/protein and protein only regimens will provide an opportunity to compare the immunogenicity of these simpler, non-vector vaccine regimens. This study has 7 active arms and 1 placebo arm and will compare DNA priming administered at Months 0 and 1, followed by DNA + Protein + MF59 or $AS01_B$ boosting at Months 3 and 6, versus DNA + Protein + MF59 or $AS01_B$ co-administered at Months 0, 1, and 6. In addition, this study will evaluate Protein + $AS01_B$ administered at Months 0, 1, and 6. Furthermore, to assess whether too high a protein dose in the presence of $AS01_B$ adjuvant may overstimulate the immune system and thereby suppress responses, 2 different doses of each of the bivalent Env proteins will be evaluated: 20 mcg versus 100 mcg for each of the DNA containing regimens (see section 4.5.4).

How to identify the regimens which will be further assessed in a future efficacy trial will depend on the ‘adequate take/potency’ (ATP) as defined in Section 6.1.2. Meeting the ATP criteria is a necessity to move forward to efficacy testing, however in itself does not suffice for a regimen to qualify for further evaluation. Because many or all of the regimens of this study could hypothetically meet the ATP, further down-selection will be performed using secondary and exploratory objectives as described in Sections 5.2 and 5.3. In this way, those individual regimens which are able to elicit an immune response with a unique profile, and therefore warrant further evaluation, can be moved forward into a future efficacy trial. Such an efficacy trial could potentially include 1 or more of the vaccine regimens evaluated in this study, together with regimens from other phase 1/2a studies.

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.

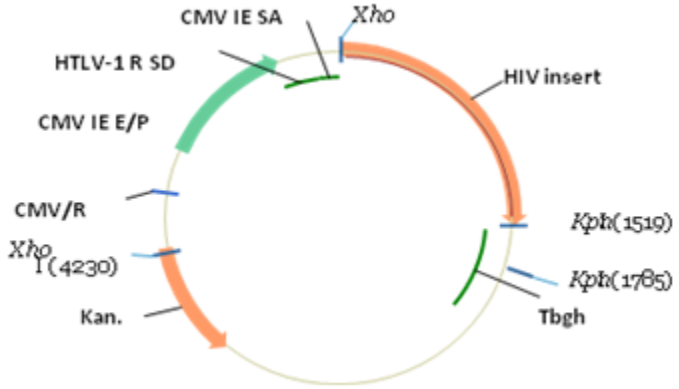

Figure 4-1 Example DNA-HIV-PT123 plasmid map

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.

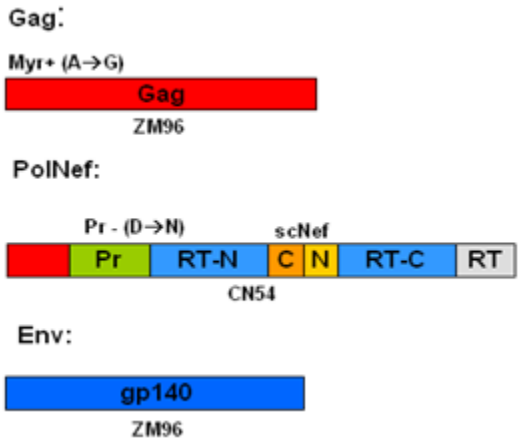

Figure 4-2 DNA-HIV-PT123 plasmid inserts schematics

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 proteins

4.4.2.1 Constructs

Bivalent Subtype C gp120, manufactured by Rentschler Biotechnologie (Laupheim, Germany), consists of 2 separate subtype C recombinant monomeric proteins, TV1.C gp120 and 1086.C gp120. These recombinant gp120s represent the receptor binding domain of the HIV envelope glycoprotein. 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.

4.4.2.2 Manufacturing and formulation

Each protein is expressed in CHO cells under conditions favorable for secretion of monomeric protein. Following fermentation, each protein is extensively purified as described in the following paragraph.

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. Harvest clarification was performed using a series of depth filters followed by bioburden reduction using a sterilizing-grade filter. The harvest was collected in single use disposable bags and purified including further enrichment for monomer. Additional processing utilizes multiple filtration steps and a series of chromatography steps that remove process related impurities.

Both TV1.C and 1086.C bulk drug substances are stored frozen at not more than -61°C. The formulations are similar for both drug substances, containing Env antigen, sodium citrate, and sodium chloride, pH 6.5-7.0. Each of the HIV gp120 proteins as final products are tested for pH, appearance, identity, strength (concentration), purity, potency, as well as safety and content uniformity following US Pharmacopoeia methods where applicable.

The 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	solvent

Additional information is provided in the 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 bulk formula is shown in the Table 4-2.

Table 4-2 Composition of MF59 per liter

Name of Ingredients	Quantity per Litre*	Function	Reference to Standards
Squalene	39.0 g	oil phase	In-house specification
Polysorbate	4.7 g	surfactant	USP/NF
Sorbitan Trioleate	4.7 g	surfactant	USP/NF
Sodium Citrate, dihydrate	2.65 g	buffer	USP/NF
Citric Acid, monohydrate	0.17 g	buffer	USP/NF
Water for Injection	q.s. 1 L	aqueous phase	Ph.Eur. and USP/NF
Nitrogen	overlay	inert gas	USP/NF

*An overage of up to 10% is included to compensate for manufacturing losses.

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).

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

4.4.4 $AS01_B$ adjuvant

The $AS01_B$ Adjuvant System component, which consists of a liposomal formulation containing two immunoenhancers, 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and QS-21, is a proprietary current Good Manufacturing Practice (cGMP)-grade adjuvant developed by GlaxoSmithKline (GSK) Biologicals.

Additional information is provided in Section 4.5.3 and in the Bivalent Subtype C gp120/$AS01_B$ IB.

4.4.5 Bivalent Subtype C gp120/MF59 for injection

The combination of the 2 subtype C gp120 proteins and the MF59 is referred to as Bivalent Subtype C gp120/MF59. 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 in 1 dose	Function
Drug Substances		
TV1.C gp120 protein	100 mcg	active
1086.C gp120 protein	100 mcg	active
Adjuvant (MF59)		
Squalene	9.75 mg	oil phase
Polysorbate	1.175 mg	surfactant
Sorbitan Trioleate	1.175 mg	surfactant
Excipients		
Sodium Citrate, Dihydrate	1.39 mg	buffer
Citric Acid, Monohydrate	0.051 mg	buffer
Sodium Chloride	4.38 mg	tonicity modifying agent
Water for injection	qs to 0.5 mL	solvent

4.4.6 Bivalent Subtype C gp120/AS01$_B$ for injection

The combination of the 2 subtype C gp120 proteins and the AS01$_B$ adjuvant is referred to as Bivalent Subtype C gp120/AS01$_B$. A final dose of 20mcg of each recombinant Env protein will be mixed with AS01$_B$ adjuvant and a final dose of 100mcg of each recombinant Env protein will be mixed with AS01$_B$ adjuvant. The composition of 1 dose of each of the resulting vaccines are shown in Table 4-4 and Table 4-5.

Table 4-4 Composition of 0.75 mL dose of Bivalent Subtype C gp120/AS01$_B$ for injection with 20 mcg of each gp120 protein

Ingredient	Amount in 1 dose	Function
Drug Substances		
TV1.C gp120 protein	20 mcg	active
1086.C gp120 protein	20 mcg	active
Adjuvant (AS01$_B$)		
Liposome		Vesicles for MPL and QS-21
MPL	50 mcg	Immunoenhancer
QS21	50 mcg	Immunoenhancer
Excipients		
Sodium citrate, dihydrate	0.07 mg	buffer
Citric acid, monohydrate	<0.01 mg	buffer
Sodium chloride	7.06 mg	tonicity modifying agent
Sodium phosphate dibasic (Na_2HPO_4)	0.15 mg	buffer
Potassium phosphate monobasic (KH_2PO_4)	0.54 mg	buffer
Water for injection	q.s. ad 0.75 mL	solvent

Table 4-5 Composition of 0.75 mL dose of Bivalent Subtype C gp120/AS01$_B$ for injection with 100 mcg of each gp120 protein

Ingredient	Amount in 1 dose	Function
Drug Substances		
TV1.C gp120 protein	100 mcg	active
1086.C gp120 protein	100 mcg	active
Adjuvant (AS01$_B$)		
Liposome		Vesicles for MPL and QS-21
MPL	50 mcg	Immunoenhancer
QS21	50 mcg	Immunoenhancer
Excipients		
Sodium citrate, dihydrate	0.34 mg	buffer
Citric acid, monohydrate	0.02 mg	buffer
Sodium chloride	8.77 mg	tonicity modifying agent
Sodium phosphate dibasic (Na_2HPO_4)	0.15 mg	buffer
Potassium phosphate monobasic (KH_2PO_4)	0.54 mg	buffer
Water for injection	q.s. ad 0.75 mL	solvent

4.5 Trial design rationale

DNA, Protein, 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 with incongruent injection schedules. They were implemented in different populations and the immunological assessments were performed in a variety of different laboratories using different assays. HVTN 108, in the context of the suite of P5 trials, is in the unique position to compare the suggested regimens directly. Subsequently, the immunogenicity and safety profiles can also be compared unconditionally. In order to assess the impact of the different adjuvants in the respective doses on prime/boost versus simultaneous DNA/Protein injections, each individual combination is evaluated as its own regimen. This setup allows forming a rational decision on which regimen(s) warrant further evaluation in an efficacy trial. Furthermore, the overall number of participants will provide sufficient safety data to comfortably move into a phase 2b efficacy set up (see also Section 6.1).

4.5.1 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]. In addition, nonhuman primate (NHP) SHIV challenge studies showed protective antibody responses [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) enzyme-linked immunospot (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 prime plus protein boost approach elicited a unique Ab profile compared to recombinant HIV envelope protein alone or a pox-vector prime plus protein boost, characterized by higher neutralizing antibodies against more resistant viral isolates and higher CD4 binding site antibodies [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]. 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/ DNA+ protein boost regimen in this study. Co-administration of the DNA with the protein as a boost increases the number of DNA vaccinations beyond 2 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 108 will evaluate DNA prime with DNA+ protein boost.

4.5.2 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 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 co-administered with DNA or NYVAC in the NHP AUP512 study (see Section 4.7.3) and this approach induced earlier binding antibody immune responses.

Protein co-administered with NYVAC, as well as protein co-administered with 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 co-administered DNA and protein (DNA-HIV-PT123 and AIDSVAX B/E have been given simultaneously at months 0 and 1, followed by simultaneous boosting with a NYVAC-HIV and AIDSVAX B/E). This study is still blinded and final results are pending (see Section 4.8.1 for interim results).

4.5.3 Rationale for MF59 and $AS01_B$

An equally unique and scientifically compelling aspect of these trial designs is the use of 2 different adjuvants with the HIV envelope (env) glycoproteins in the boosts. 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 up-regulation 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]. Remarkably durable memory responses (T-cell and binding and neutralizing Ab responses) were induced by a DNA-prime/gp120 and MF59 boost regimen in HVTN 049 and HVTN 088 (see results in Section 4.5.1).

The Adjuvant System AS01 is a liposome-based adjuvant which contains 2 immunostimulants, 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) and QS-21 [35]. MPL is a non-toxic derivative of the lipopolysaccharide from Salmonella *minnesota* and is a TLR4 agonist [36]. It can stimulate NF-κB transcriptional activity and subsequent cytokine production [36]. MPL directly activates APCs such as dendritic cells (DCs) to produce cytokines and elevated levels of costimulatory molecules [37-39]. QS-21 is a natural saponin molecule extracted from the bark of the South American tree *Quillaja saponaria Molina* [40] (reviewed in [35,41]). The early evaluations of QS-21 as an adjuvant demonstrated that it could promote high Ag-specific Ab responses and $CD8^+$ T-cell responses in mice [42,43] and high Ag-specific Ab responses in humans [44]. However, specific receptors and signaling pathways induced by saponin-based adjuvants have yet to be clearly defined, most likely through the activation of the inflammasome as observed with QuilA [45]. AS01 has been selected in a number of human vaccine candidates because of its association with enhanced and durable immune responses, both humoral and cellular [35,46-54] and recently with protection [46,47]. The ability of AS01 to improve adaptive immune responses was shown in mice to be linked to a transient stimulation of the innate immune system characterized by the induction of a specific pattern of cytokines and innate immune cell recruitment that occurred rapidly and transiently at the muscle injection site and draining lymph node postinjection, consistent with the rapid drainage of the vaccine components to the draining lymph node, and also linked to the generation of a high number and heterogeneous dendritic cell population in the draining lymph node that was efficient at priming T cells [55].

GSK PRO HIV-002 evaluated the gp120 20 mcg / NefTat 20 mcg (clade B) candidate HIV vaccine formulated with 1 of 3 different Adjuvant Systems ($AS02_A$, $AS02_V$ and $AS01_B$) each in 60 healthy HIV-seronegative adults. The vaccine candidates were administered at Month 0, Month 1, Month 3 and Month 6. All vaccine formulations induced strong HIV-specific CD4+ T-cell responses characterized by high lymphoproliferative capacity and IL-2 production that were still detectable 18 months after the last immunization, with significantly stronger responses seen in the $AS01_B$ group (Figure 4-3 A and B). Broad coverage of CD4+ T-cell responses was demonstrated against gp120, and to a lesser extent Nef, derived from the most common circulating clades (B, C and circulating recombinant form [CRF]-01) (Figure 4-3 C).

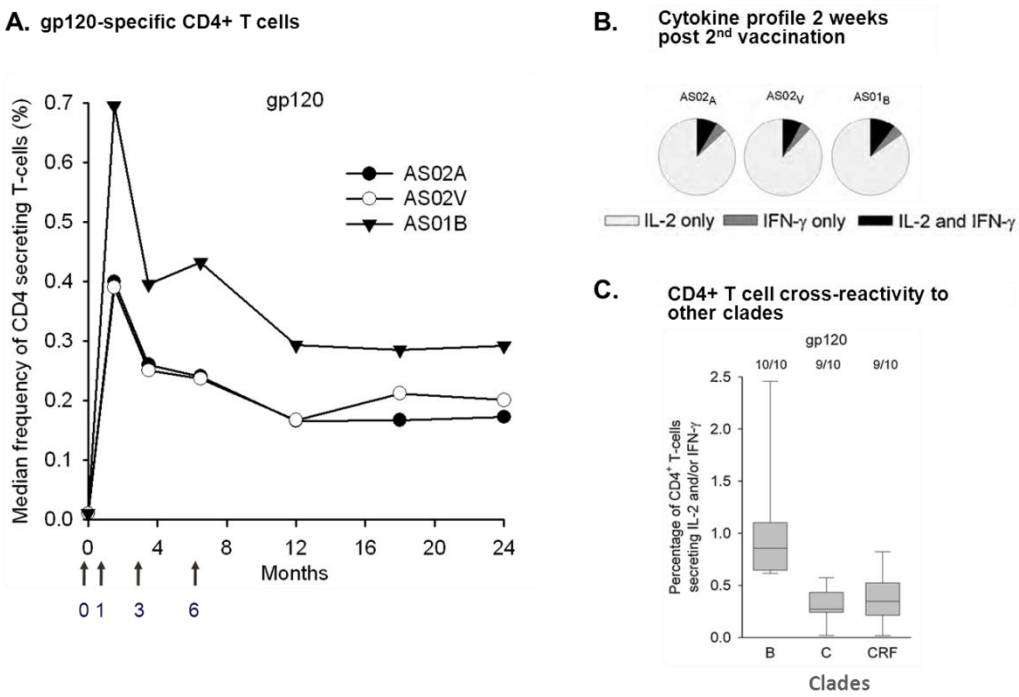

Figure 4-3 CD4+ T cell responses following vaccination with the gp120/NefTat vaccine candidate formulated with 1 of 3 different Adjuvant Systems ($AS02_A$, $AS02_V$ and $AS01_B$) in study PRO HIV-002

Strong antibody responses against gp120, Nef and Tat were observed in all 3 groups. Responses were detected already after the second vaccine dose and peaked 2 weeks after the third or fourth vaccine dose.

The demonstration of superior CD4+ T-cell induction by $AS01_B$ has guided selection of this Adjuvant System for further HIV vaccine evaluation [54].

Retrospective testing of the GSK PRO HIV-002 clinical study evaluating gp120W6.1D/NefTat in different Adjuvant Systems has been performed by Dr. Georgia Tomaras' lab, which had conducted the correlate analysis of the RV144 trial. Samples collected at various timepoints from 30 subjects vaccinated with gp120W6.1D (20 mcg) /NefTat/$AS01_B$ were tested. The analysis of the isotype-specific responses showed that after the vaccination that all subjects had detectable IgG1 responses, however most of these subjects also had detectable IgA against gp120 vaccine protein [56].

In all cases, IgG1 was found to have the highest serum concentration among the IgG subclasses (mean concentration of serum antibody to gp120 was 5 µg/ml). IgG2 vaccine-induced antibodies were detected in a minority (8/30, 27%) of subjects. When IgG3 and IgG4 vaccine-induced antibodies were detected, they were found in concentrations less than IgG2 vaccine-induced antibodies.

The V1V2 specificity was also assessed and the results showed that after vaccine dose 3, 100% of the subjects had total anti-V1V2 concentrations comparable to the high levels seen in RV144 and that this response was persistent, with total IgG anti-V1V2 detected in 87% of the subjects 18 months after the administration of the last vaccine dose (4-dose schedule) (see Figure 4-4) [57].

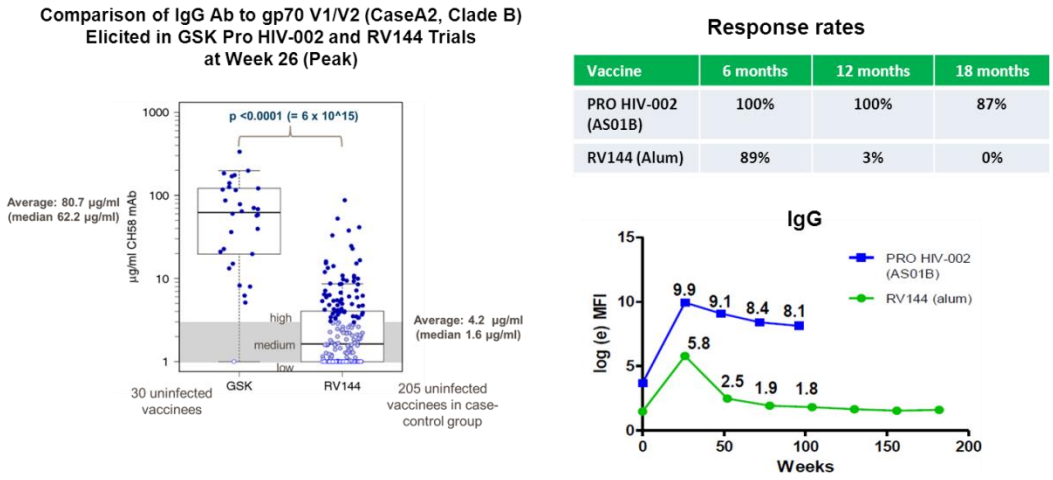

Figure 4-4 Total IgG Ab to gp70 V1V2 (Case A2, Clade B) elicited in GSK Pro HIV-002 and RV144 trials

Vaccination with gp120W61D ($AS01_B$) induces increased total IgG but not high IgG3 response magnitude and half-life. IgG3 V1V2 response rates and magnitude significantly declined 12-18 months post last boost (see Figure 4-5) [57].

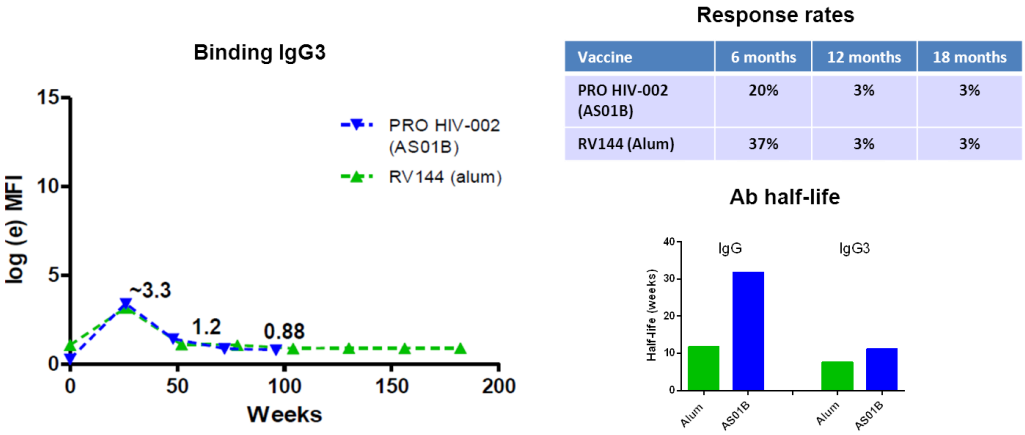

Figure 4-5 IgG3 Ab to gp70 V1V2 (Case A2, Clade B) elicited in GSK Pro HIV-002 and RV144 trials

A subset of GSK PRO HIV-005 (see Section 4.8.3) volunteers primed 3 years before with 2 doses F4 10 mcg / $AS01_B$ received a booster dose of F4 10 mcg / $AS01_B$ (GSK ECR-004). Before the administration of the booster dose, all participants were still seropositive for anti-F4 antibodies as well as high levels of CD4+ T-cell responses were still detected. The F4/ $AS01_B$ booster induced strong F4-specific CD4+ T-cell responses, with similar frequencies and polyfunctional phenotypes as following primary vaccination, and high anti-F4 antibody concentrations, reaching higher levels than those following primary vaccination (see Figure 4-6) [49].

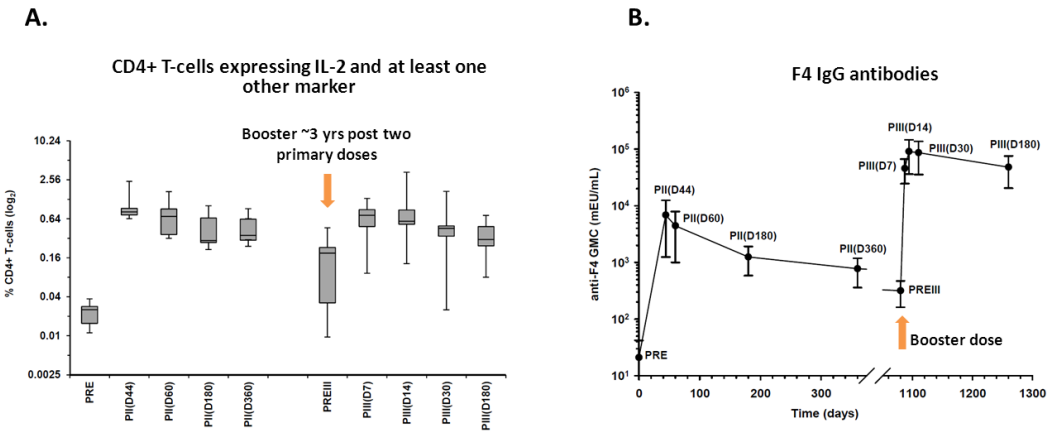

Figure 4-6 CD4+ T cell and antibody responses following primary and booster doses of the F4 / AS01$_B$ vaccine candidate in studies PRO HIV-005 and ECR-004.

4.5.4 Rationale for evaluation of protein plus AS01$_B$ alone arm and 2 protein doses with AS01$_B$

Clinical trials assessing gp120 with a GlaxoSmithKline Biologicals (GSK) Adjuvant System provide evidence that an Env protein with AS01$_B$ can elicit strong humoral and cellular responses. Currently, it is unclear whether too high a protein dose in the presence of the AS01$_B$ adjuvant leads to suboptimal vaccine responses.

HVTN 041 evaluated a combination vaccine (NefTat and gp120W61D) formulated with AS02$_A$ (oil-in-water emulsion of a combination of MPL and QS-21) administered at 0, 1 and 3 months with varying doses (5, 20 and 100 mcg in 20 subjects each) of the gp120 vaccine component (the NefTat antigen dose was constant at 20mcg). The vaccine was safe and well tolerated at all dose amounts and Nef-, Tat-, and gp120-specific binding antibodies were induced in all individuals that received the respective antigen, lasting up to 9 months after the final immunization (Figure 4-7).

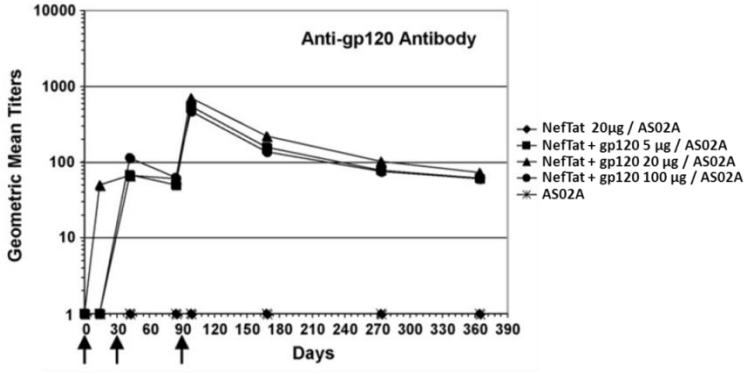

Figure 4-7 Kinetic of binding IgG antibodies induced by the NefTat/gp120/AS02$_A$ vaccine candidate in study HVTN 041

Antibodies able to neutralize the T-cell laboratory-adapted strain of HIV-1W6.1D were detected in the majority of vaccinees, but did not neutralize primary isolates. Envelope specific ADCC was detected in most of the individuals receiving gp120. Robust and persistent HIV-specific lymphoproliferative responses were detected against all subunits proteins in the majority of immunized participants. Most immune responses were similar

across all doses, except for a significant dampening effect on the CD4+ T-cell responses occurring at the highest gp120 dose (100mcg) measured by lymphoproliferative and ICS assays [52] (see Figure 4-8).

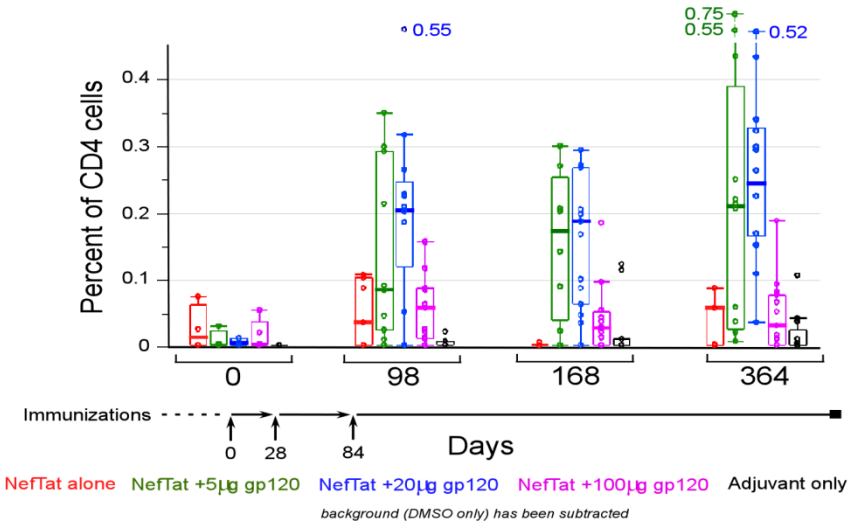

Figure 4-8 gp120-specific CD4+ T cell responses (IFN-γ and/or IL-2) induced by the NefTat/gp120/AS02$_A$ vaccine candidate in study HVTN 041

GSK PRO HIV-005 evaluated an HIV vaccine candidate consisting of a recombinant fusion protein (F4) containing 4 HIV-1 clade B antigens (Gag p24, Pol-RT, Nef, and Gag p17) adjuvanted with AS01$_B$ in 3 different doses: 10, 30, and 90 mcg. Each dose of the AS01$_B$-adjuvanted vaccine candidate was administered twice in a group of 50 subjects, following a Month 0 and Month 1 schedule. Three control groups of 10 subjects received a dose of 10, 30 or 90 mcg without AS01$_B$. The 10 mcg AS01$_B$-dose elicited a statistically significantly higher CD4+ T-cell response as compared to the other doses ($p < 0.0001$ at 2 weeks post second vaccination), while anti-F4 IgG responses were elicited in 100% of vaccinees with no significant difference in GMC titers amongst the groups [58].

The protein dose question with AS01$_B$ warrants evaluation within the context of the specific immunogens and vaccine regimens being tested using updated methodologies. The dampening effect of high protein doses on the immune response has not been observed with Env proteins adjuvanted with MF59 however, so this specific question is limited to the AS01$_B$ adjuvanted arms. These observations raise important hypotheses to test in this and other planned phase 1/2 trials. The protein plus AS01$_B$ alone question warrants testing as well in the context of immune responses as they relate to the correlates in RV144 (see Section 4.1.2).

As mentioned in section 4.5.3 protein alone adjuvanted by AS01$_B$ induced anti-V1V2 antibody concentrations comparable to RV144 and detectable levels of IgG1 in 100% of the subjects.

4.5.5 Dose rationale

4.5.5.1 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 [59] 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 [24,60,61]; 3) the preclinical immunogenicity studies NHP with DNA-HIV-PT123 (see Section 4.7.1); 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.

4.5.5.2 Bivalent Subtype C gp120 dose

For the Bivalent Subtype C gp120/MF59 vaccine component, 100 mcg each of the 2 gp120 subtype C proteins (TV1 gp120 and 1086) 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. 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 [62].

As discussed in 4.5.3, the Bivalent Subtype C gp120 dose for evaluation with $AS01_B$ is based on previous dose escalation studies. Both a 20mcg and 100mcg per Env component will be tested to determine if the higher dose results in suppression of any immune parameters when administered with this adjuvant system.

The dose of the $AS01_B$ / Bivalent Subtype C gp120 administered alone will be 20mcg of each Env component for consistency with the PRO HIV-002 trial described in 4.5.4.

4.5.6 Schedule

The vaccination schedule of the DNA prime followed by DNA+protein boost regimens is based upon the schedule used in RV144, but replaces the canarypox (ALVAC) vector immunogen with a DNA plasmid construct. The schedule for the DNA and protein co-administration and protein alone regimens is similar to the prime/boost regimens, yet administers placebo instead of active product at month 3. In previous trials with adjuvanted protein, peak immunogenicity is obtained after 3 doses of protein, while a longer rest between the 2^{nd} and 3^{rd} vaccinations is preferred for antibody maturation.

4.5.7 Choice of control

Sodium Chloride for Injection, 0.9% delivered IM will serve as the placebo for the DNA vaccine, the Bivalent Subtype C gp120/MF59 vaccine, and the Bivalent Subtype C gp120 + $AS01_B$ 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 4-6). The study was conducted at Spring Valley Laboratories, Inc. in Sykesville, MD, US.

Table 4-6 Study design of the toxicity study with DNA-HIV-PT123 and NYVAC-HIV-PT1 and NYVAC-HIV-PT4

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

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

New Zealand White rabbits (20/sex, total 40 rabbits) were randomly assigned to receive IM injections of either DNA-HIV-PT123 on Days 0, 14, 28 and 42 followed by NYVAC-HIV-PT-1 and NYVAC-HIV-PT-4 on Days 56 and 70 or to saline control on the same days. NYVAC-HIV-PT1 and NYVAC-HIV-PT4 were administered separately (right and left hind limbs) in 2 separate injections of 1 ml each on Days 56 and 70. The control group received saline control delivered as a single injection on Days 0, 14, 28 and 42, and delivered as 2 separate injections of 1 mL each on Days 56 and 70.

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

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

4.6.2 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 to 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) [63].

Pivotal toxicology studies performed with MF59 include [63]:

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

4.6.4 Toxicity studies of $AS01_B$

Toxicology studies performed with $AS01_B$ included:

- Repeated dose studies (Rabbit, Rat)
- Local tolerance studies (Rabbit)
- Safety/pharmacology studies (Rat, Dog)
- Genotoxicity (micronucleus) studies (Rat)

The pre-clinical toxicity studies indicated that no treatment-related systemic toxicity was associated with single or repeated IM injection of $AS01_B$, alone or in combination with

antigens. In addition, no adverse treatment-related effects on male mating performance, fertility, early embryonic development, pre- and post-natal survival, growth or development of the offspring up to Day 25 of age were associated with repeated IM injection of $AS01_B$, alone or in combination with antigens. $AS01_B$ was considered safe with regard to cardio-respiratory side effects in two different animal species. Moreover, single or repeated IM injection of $AS01_B$ did not adversely affect RBC production in the bone marrow. The only treatment-related effects of vaccine administration were mild local injection site reactions. Hematology parameters such as increased WBC and neutrophil counts, both related to the local inflammation reaction, were transiently affected.

Details of these studies and more information are included in the Bivalent Subtype C gp120/$AS01_B$ IB.

4.7 Preclinical immunogenicity studies

4.7.1 Preclinical immunogenicity studies with DNA-HIV-PT123

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.

4.7.2 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 received 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 provided in Table 4-7. 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 4-7 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

4.7.2.1 Summary of AUP444 cellular responses

- DNA-HIV-PT123 was greatly immunogenic with an average of 0.5% cytokine secreting cells 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;
- 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;
- 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;
- The DNA-HIV-PT123 prime groups induced more potent CD4 and CD8 T-cell responses both in terms of magnitude and polyfunctionality.

4.7.2.2 Summary of AUP444 humoral responses

Prior to the late protein only boost at week 49, the no-DNA prime groups induced significantly greater Nab activity against Tier 1 viruses and SHIVs (TZM.bl assay), and significantly greater titers of ADCC activity 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. In contrast, no significant increase in T-cell responses was observed with the DNA.

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.

4.7.3 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 4-8.

Table 4-8 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 are detectable in Groups #2, #3 and #5 at week 6 (after 2 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);

- 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 week 0 and week 26 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 dominated 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;

- T-cell responses were greater in the DNA prime/NYVAC boost group.

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 regimens induces greater T-cell responses compared to homologous NYVAC or DNA prime boost regimens.

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.

4.7.4 Dose-range Immunogenicity study of Bivalent Clade C gp120 (TV1.C and 1086) / $AS01_B$ in mice

The objective of this experiment was to assess the dose response relationship of bivalent Clade C gp120 (TV1.C and 1086) antigens in CB6F1 mice when formulated in combination with AS01B, in terms of antigen-specific cellular and humoral responses. Animals were immunized intramuscularly on Day 0, 14, and 28 with 10 mcg, 2 mcg, 0.4 mcg, or 0.08 mcg of bivalent (TV1.C and 1086) gp120 antigens formulated with 50 mcL of $AS01_B$. The induced T cell and antibody responses were characterized at 7 and 14 days post third dose, respectively.

The bivalent 1086-TV1.C / $AS01_B$ vaccine formulation elicited 1086C and TV1.C-specific CD4+ T cell responses for all tested doses. No statistically significant differences were observed between doses, however, a trend for higher 1086C-specific CD4+ T cell responses was observed with lower doses of 1086-TV1.C / $AS01_B$ (Figure 4-9-Panel A). This was not observed when measuring the TV1.C-specific CD4+ T cell responses (Figure 4-9-Panel B). The vaccine-induced CD8+ T cell responses at 7 days after third immunization were low to undetectable for both, TV1 and 1086 responses. The bivalent (1086C-TV1.C) gp120 antigens adjuvanted in $AS01_B$ induced dose-dependent high levels of 1086C and TV1.C-specific antibody responses at 14 days post second and third immunization (Figure 4-9-Panel C). Moreover, for all antigen doses tested, similar levels of 1086C and TV1.C specific total immunoglobulin (Ig) responses were observed, suggesting that there is no negative impact on the humoral responses when combining 1086C and TV1.C antigens in $AS01_B$. Anti-V1V2 total Ig responses were also detected in all the groups (Figure 4-9-Panel D).

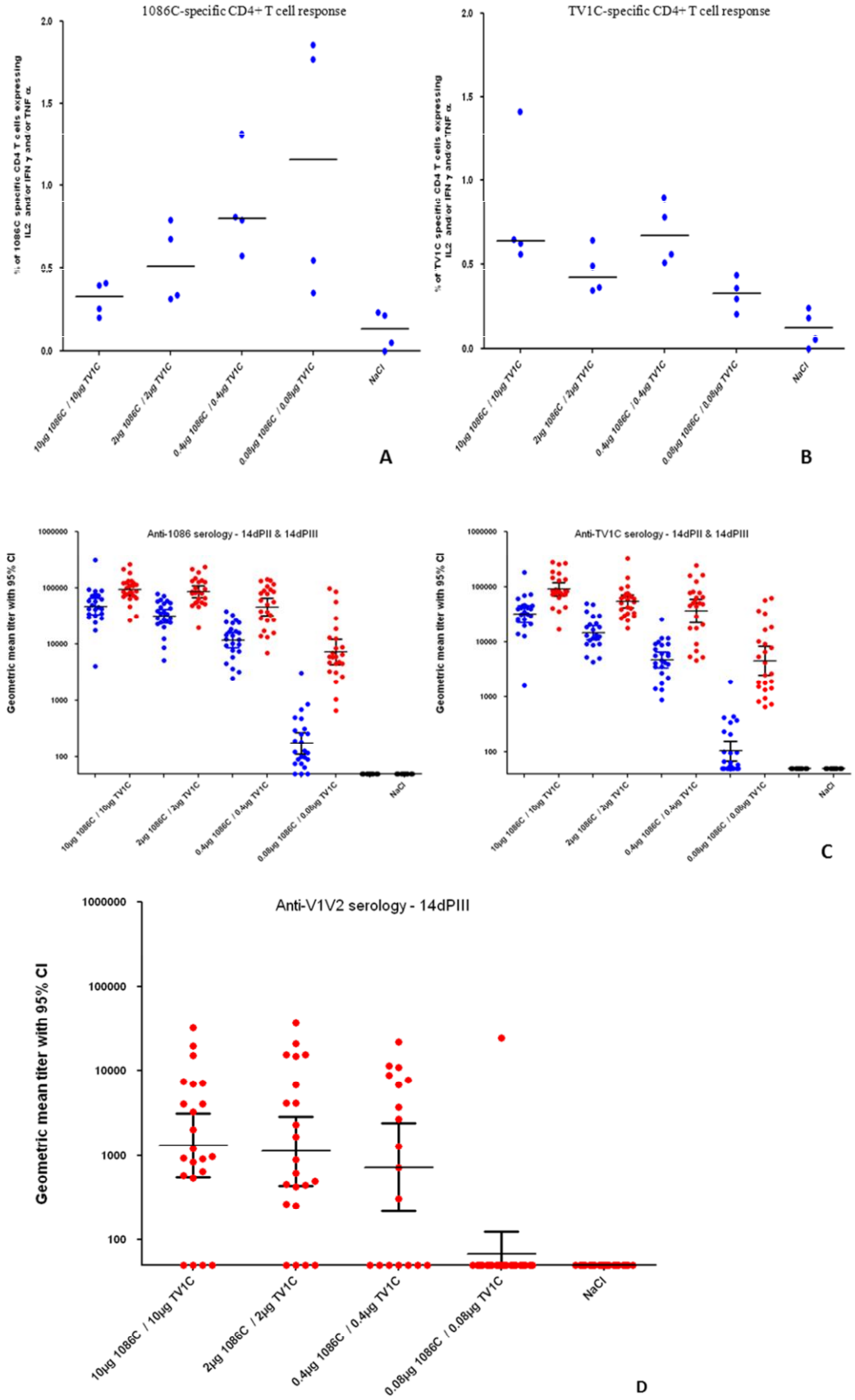

Figure 4-9: Animals were intramuscularly immunized with 10 µg, 2 µg, 0.4 µg or 0.08 µg of bivalent (TV1 and 1086) antigen formulated in 50 µL of AS01B at Days 0, 14 and 28. Percentage of CD4+ T cells secreting IFN-γ and/or IL-2 and/or TNFα was measured at 7 days

post-third immunization (Panels A and B). Intracellular staining performed on peripheral blood lymphocytes after a 6-hour re-stimulation with TV1 and 1086 clade C gp120 antigens. 4 pools of 6 mice with median are represented. Anti-TV1 and anti-1086 (Panel C) and anti-gp70-V1V2 (Clade B/Case A2) (Panel D) binding antibody titers measured by ELISA 14 days post-third immunization (Blue dots=14 days post-dose 2; Red dots=14 days post-dose 3).

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. The HVTN 096/EV04 study design is shown in Table 4-9.

Table 4-9 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. As of October 31st, 2014 there have been no SAEs reported that were deemed related to the study products. There was 1 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 most common complaints have been mild or moderate malaise/fatigue, myalgia, headache and injection site pain. The most common AE deemed by the site investigator to be related to study product was lymphadenopathy (n=9, 9.4%).

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 potential T-cell epitopes 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 from the first injection 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 at the month 12 timepoint in group 4 (DNA / protein prime with NYVAC / protein boost). Additionally, co-administration of protein with DNA or NYVAC from priming substantially reduces the generation of IgA binding Ab responses to gp140 and gp120.

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 possibly 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.

HVTN 105 (BB-IND 15997): assesses several vaccine regimens of DNA-HIV-PT123 in combination with AIDSVAX B/E. The study opened in July, 2014 and is being conducted at multiple sites in the US. The study is fully enrolled and as of April 6, 2015, no SAEs have been reported. No AEs graded severe or higher have been reported, and all AEs deemed related to the study product have been assessed as mild. The DNA-HIV-PT123 vaccine has been well-tolerated. The most common complaints have been mild or moderate injection site pain/tenderness, headache, and malaise/fatigue.

4.8.2 Clinical studies with Novartis Vaccines HIV-1 subunit protein vaccines with MF59

Although Novartis Bivalent Subtype C gp120 proteins have not yet been administered to humans, other closely related recombinant monomeric (gp120) subunit vaccine formulations from Novartis Vaccines and Diagnostics (formerly Chiron) have been tested in many 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 [64]. 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 pre-pandemic H5N1 influenza vaccine (Aflunov®) licensed in the EU for use in adults, and in 2 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 [62,65-69]. 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, 2 of them are being conducted by the NIH-sponsored HVTN in the US and the RSA. Additionally, several HVTN phase

1/2a studies testing the protein/MF59 combination from HVTN 108 are scheduled to start as early as Q1 2015. Table 4-10 summarizes clinical trial experience with Novartis gp120 and gp140 recombinant vaccine candidates.

Table 4-10 Novartis recombinant gp120 and gp140 vaccines in human clinical trials* [67]

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	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	11	ISS P-002	Terminated*
Subtype C (TV1) gp140 (CHO)/MF59	36	HVTN 088	Completed
Subtype C (TV1) gp140 (CHO)/MF59 and SAAVI DNA-C2 and SAAVI MVA-C	110	HVTN 086	Completed
Bivalent Subtype C gp120/MF59 and ALVAC-HIV (vCP2438)	210	HVTN 100	Planned

* Due to slow enrollment and expiration of the study product stability program.

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 [4,62,65-71].

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

There were 2 clinical trials conducted recently using Novartis CHO-based subtype B gp140 with MF59. In addition there have been 4 recent 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 [72]. 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, pharyngo-laryngeal 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 received 1 of 3 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 1000/1000mcg) 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 4 events reported as SAEs that were not considered related to the study vaccine. A death attributed to cocaine overdose occurred in 1 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 2 weeks. Both subjects reported to have initiated new exercise programs (one weight lifting and the other aerobics and weight lifting). One subject experienced severe fatigue 20 days after the fourth immunization (including 1 dose of subtype B gp140/MF59), attributed to working 2 jobs and long hours. Overall, the regimens were well tolerated.

A third study, HVTN 073E, was conducted in the US and RSA as an extension to the previous HVTN 073/SAAVI 102 study. This extension study examined the safety and immunogenicity of 2 boosting doses of Novartis subtype C gp140/MF59 or placebo in subjects who previously received 3 vaccinations of SAAVI DNA-C2 and 2 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 reported as an SAE and assessed as unrelated to study agents.

Two other recent phase 1 studies with Novartis subtype C gp140/MF59 have been conducted by the HVTN in the US and the RSA. In addition, 1 phase 1 trial was conducted by the Istituto Superiore di Sanità (ISS) in Italy. One of these trials, HVTN 088, was 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 included subjects from the HVTN049 DNA/PLG prime, gp140/MF59 boost study described above. The study enrolled 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 HVTN088 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 HVTN study, HVTN 086, was conducted in the RSA. It evaluated the safety and immunogenicity of various combinations of SAAVI DNA-C2, SAAVI MVA-C, and Novartis subtype C gp140/MF59. All scheduled clinic visits have been completed, though study participants remain subject to annual contacts to assess their health status. 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, and 1 instance of alcohol-related cardiomyopathy. All were assessed as not related to the study products.

The ISS study (ISS P-002) conducted in Italy examined 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 did not include MF59. This study was stopped early due to slow enrollment and subsequent expiration for the study product stability program. No SAEs were 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 IFN-γ ELISpot and ICS responses. Immunogenicity was assessed 14 days after each vaccination. Env-specific IFN-γ ELISpot response rates did not increase substantially after the 3 1000-mcg DNA/PLG prime vaccinations (6/27) compared to baseline, but did rise after the first protein/MF59 boost (14/25). nAb titers against the homologous SF162 isolate were undetectable after the third 1000 mcg DNA/PLG priming vaccination. Neutralization was boosted to high titers in all subjects 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 but 1 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 [21].

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.

Available data from HVTN 073E show that boosting with protein improves binding and neutralizing antibody responses for DNA/MVA primed subjects. All participants who received the protein boost had binding antibody responses to TV1.21 gp140 2 weeks after the last protein boost and these responses persisted in all participants 6 months later. After the last protein boost, neutralizing antibody responses to MW965.26 were seen in all participants and persisted in 75% of participants for at least 6 months. Notably, neutralizing antibody responses, which were not detected in the main HVTN 073/SAAVI 102 dataset, were detected after a single protein boost in the study extension. In the absence of priming, several protein administrations are typically required to elicit neutralizing antibody responses. That these responses were detected after a single dose

indicates efficient priming by the DNA/MVA regimen and points to protein boosts as an effective way to boost such responses.

In addition, in the subtype C gp140/MF59 HVTN 088 long-interval boost study 16 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), 5 of 16 (31%) of primed participants demonstrated CD4+ T-cell responses to Env at baseline, which increased to 12 of 16 (75%) after a single protein boost. IgG and IgA responses to Con S gp140were present in 64% (IgG) and 7% (IgA) of primed participants at baseline, and rose to 93% and 85%, respectively, after 1 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 vaccine-matched oligomeric TV1 Env in 88% and 50%, respectively. nAb developed to MN in 38% and to MW965.26 in 88% of the unprimed participants.

Three of the 4 vaccine regimens in HVTN 086 contained Novartis subtype C gp140/MF59, either as a boost following pox-vector (MVA) primes, administered concurrently with a pox-vector (MVA) vaccine, or in a concurrent pox-vector/protein boost and second protein boost following DNA plasmid primes. Ab responses were measured with a Luminex binding Ab assay and a nAb assay in TZM-bl cells. All three vaccine regimens containing subtype C gp140/MF59 elicited high rates (82–100%) and magnitudes of IgG binding Ab against a range of gp120 and gp140 antigens. All vaccine regimens had evidence of nAb to (Tier 1) HIV strains MN.3, MW965.26, and SF162.LS, with the MVA- and DNA-primed regimens showing the highest titers to MW965.26, a clade C strain. HIV-1 specific CD4+ and CD8+ T-cell responses to global potential T-cell epitopes (PTEg) were measured by an ICS assay. The MVA- and DNA-primed vaccine regimens elicited the highest rates of CD4+ T cells producing IFN-γ and/or IL-2 (77% and 45%, respectively), while the regimen with concurrent MVA/protein administration elicited the lowest rate (13%).

4.8.3 Clinical studies with GSK HIV-1 subunit protein vaccines with $AS01_B$

Immunogenicity data for studies HVTN 041, PRO HIV-005, ECR-004 and PRO HIV-002 are presented in Section 4.5.3 and 4.5.4.

The safety, reactogenicity and tolerability profiles of the vaccine regimens investigated in these studies were considered acceptable and are presented below.

HVTN 041 evaluated a combination vaccine (NefTat and gp120W61D) formulated with AS02A (which is an oil-in-water emulsion of a combination of MPL and QS-21, while AS01 is a liposomal formulation of the same components) administered at 0, 1 and 3 months with varying doses (5, 20 and 100 mcg) of the gp120 vaccine component (the NefTat antigen dose was constant at 20mcg).

The vaccinations were well tolerated and none were discontinued because of vaccine-associated reactogenicity. The most common local symptoms were pain and tenderness. Systemic symptoms were generally mild with the exception of moderate myalgia and headache occurring in 20 and 25%, respectively. Both symptoms were evenly distributed in the 3 NefTat and gp120W61D groups (20% in each group for myalgia and from 10

[2/20 subjects] to 40% [8/20 subjects] per group for headache). Severe pain was observed in 2/20 subjects of 2 NefTat and gp120W61D groups (5 mcg and 100 mcg of gp120). Severe tenderness was observed in 2/20 subjects of 1 NefTat and gp120W61D group (5 mcg of gp120). Severe malaise was observed in 2/20 subjects of 1 NefTat and gp120W61D group (20 mcg of gp120). All vaccine-related reactogenicities were transient and had either improved or resolved within 48 h of onset. Grade 4 laboratory abnormalities were observed in 1 single participant who was administered the AS02A-adjuvanted NefTat 20 mcg / gp120W61D 5 mcg vaccine. This participant had an elevated CPK value which may have been due to creatine supplements taken prior to exercise and likely not vaccine related. There were 6 serious adverse events during the trial that occurred within 24 hours postvaccination and were felt to be probably or definitely due to the immunizations. These included 2 severe local site reactions in group NefTat 20 mcg and gp120W61D 5 mcg (associated to limitation of limb movement in 1 case), 2 occurrences of severe malaise in 2 subjects of group NefTat 20 mcg / gp120W61D 20 mcg, a febrile illness (103.2 ◦F) in 1 subject of group NefTat 20 mcg / gp120W61D 20 mcg, and an episode of generalized urticaria in 1 subject from group NefTat 20 mcg / gp120W61D 100 mcg. All of these serious adverse events significantly improved within 24 h of onset. It is important to note that none of these SAEs would be regarded as such in accordance with the current ICH E2A definitions. Grade 3 and Grade 4 related Adverse Events were considered SAEs per the "SAE reporting manual" active at the time of the study.

PRO HIV-002 evaluated the gp120W61D 20 mcg / NefTat 20 mcg candidate HIV vaccine formulated with 1 of 3 different Adjuvant Systems (AS02A, AS02V and AS01B), each in 60 healthy HIV-seronegative adults. The vaccine candidates were administered at Month 0, Month 1, Month 3 and Month 6.

During the 7-day follow-up period after vaccination, almost all doses (90.4% to 98.8%) were followed by at least 1 solicited or unsolicited symptom considered to be related to vaccination with no difference between groups. The reactogenicity profiles obtained in the present study were expected and confirmed the previous results obtained with these adjuvants. During the course of the study, a total of 14 SAEs were reported in 12 subjects, none of them was causally related to vaccination.

Local and general solicited symptoms could be divided into 4 groups given their frequency per dose given into brackets (by decreasing order):

- Pain (between 86.3% and 96.3%) with few grade 3 symptoms (≤ 3.4%).

- Fatigue (between 33.8% and 46.6%), feverishness (between 22.9% and 53.4%), headache (between 35.0% and 48.3%) and myalgia (between 25.8% and 43.3%). Few cases of grade 3 myalgia and fatigue were reported (≤ 4.2%). For feverishness the frequency of grade 3 symptoms was 10.1% and for headache was 5.9% in the gp120/ NefTat/ $AS01_B$ group.

- Redness, swelling, nausea and fever: the frequency of those symptoms did not exceed 30.3%. Few grade 3 nausea and fever were reported (≤ 2.1%). The frequency of grade 3 redness and swelling was 20.2% and 13.9% respectively in the gp120/ NefTat/ $AS01_B$ group.

- Diarrhea and vomiting: the frequency of these symptoms was less than or equal to 7.5%. No grade 3 diarrhea was observed, and only 0.4% of the doses were followed by grade 3 vomiting.

Almost all general symptoms (fatigue, feverishness, headache, myalgia, nausea and fever, vomiting) were considered as related to the vaccination, except for diarrhea.

The duration of general symptoms was less than or equal to 2 days in most cases whereas the duration of local symptoms was often more than 2 days. In the gp120/ NefTat/AS01B group the overall frequency of the general symptoms tended to be higher after the 2nd, 3rd, or 4th dose of vaccine compared to the first dose (but not for all symptoms and all groups).

The incidence of unsolicited AEs ranged from 78.3% to 83.3%. The most frequently reported symptoms were upper respiratory tract infections and headache, followed by injection site induration, injection site pruritus and pharyngeal pain. Less than half unsolicited symptoms were causally related to vaccination (incidence between 31.7% and 36.7%). The most frequent symptoms causally related to vaccination were administration site reactions (injection site induration, pruritus or warmth) and asthenia. Few grade 3 or grade 3 related symptoms occurred: 18 subjects (incidence between 6.7% and 13.3%. according to group) experienced grade 3 unsolicited symptoms, among which a majority were non-related infectious conditions (cystitis, gastroenteritis, otitis, pyelonephritis, tonsillitis, tooth abscess, upper respiratory tract infection and vaginal candidiasis). 6 of them had symptoms considered to be causally related to vaccination (lymphadenopathy, palpitations, application site hyperesthesia, asthenia, muscle rigidity, headache, and tremor).

No significant changes in laboratory parameters (hematology, biochemistry, urinalysis) and vital signs were observed throughout the study.

PRO HIV-005 evaluated an HIV vaccine candidate consisting of a recombinant fusion protein (F4) containing 4 HIV-1 clade B antigens (Gag p24, Pol-RT, Nef, and Gag p17) adjuvanted with AS01B in 3 different doses: 10, 30, and 90mcg. Each dose of the AS01B-adjuvanted vaccine candidate was administered twice in a group of 50 subjects, following a Month 0 and Month 1 schedule. Three control groups of 10 subjects received a dose of 10, 30 or 90 mcg F4 in water for injection (WFI).

Reactogenicity was higher during the 7-day period after each vaccine dose in the F4/AS01 groups than in the F4 in WFI groups. The incidence of local and general symptoms tended to be higher in the F4/AS01 groups after the second vaccine dose. Pain was the most common solicited local symptom, reported after 96.0%–98.0% of doses in the F4/AS01 groups and after 10.0%–30.0% of doses in the F4 in WFI groups (grade 3 severity after 5.0%-10.1% of doses in the F4/AS01 groups). Fatigue was the most common solicited general symptom, reported after 66.0%-77.8% and 30.0%-45.0% of doses in the F4/AS01 and F4 in WFI groups, respectively (grade 3 severity after 7.0%-12.1% of doses in the F4/AS01 groups). Fever of more than 39°C was reported in 3.0%, 2.0% and 1.0% of the doses in the respective 10, 30 and 100 mcg F4/AS01 groups). No solicited local or general grade 3 symptoms were reported in the F4 in WFI groups. No differences in reactogenicity were observed between the antigen dose levels in the F4/AS01 groups. During the 30-day postvaccination period, 60.0%–84.0% of subjects in the F4/AS01 groups reported unsolicited symptoms, compared with 50.0%–70.0% in the F4 in WFI groups. In the F4/AS01 groups, unsolicited symptoms (mainly chills and injection site reactions) were considered causally related to vaccination in 30.0%–44.0% of subjects and were of grade 3 severity in <10.0%. All related symptoms were transient and resolved without sequelae, generally within 2–3 days. Six serious adverse events were reported in the F4/AS01 groups, all considered unrelated to vaccination No subjects died during the study period, and no subject withdrew because of adverse events.

ECR 004, a subset of volunteers primed 3 years before with 2 doses F4 10 mcg / AS01B (PRO HIV-005) received a (third) single booster dose of F4 10 mcg / AS01B.

93.3% (ie, 14 out of 15 subjects) of the subjects reported at least 1 AE and 40% (ie, 6 out of 15 subjects) of the subjects in the group F4 reported at least 1 grade 3 symptom considered by the investigator to be related to vaccination. Pain was the most frequent solicited local AE. Grade 3 pain (13.3% or in 2 out of 15 subjects), redness (in 6.7% or in 1 out of 15 subjects) or swelling (in 13.3% or in 2 out of 13 subjects) were reported for a maximum duration of 2 days. Fatigue and headache were the 2 most frequently reported solicited general AEs. Fever was recorded in 6 out of 15 subjects (40%) within the 2 days after vaccination. Grade 3 fatigue (in 20% or in 3 out of 15 subjects) or fever (in 1 out of 15 subjects) was reported with a maximum duration of 1 day. Grade 3 unsolicited AEs were reported by 13.3% (ie, 2 out of 15 subjects). Out of these cases, 2 cases were considered by the investigator to be causally related to vaccination: 1 case of chills and 1 case of insomnia. All of the grade 3 unsolicited AEs were resolved without sequelae. No subjects died, experienced an SAE, or withdrew due to an AE.

4.9 Potential risks of study products and administration

Table 4-11 summarizes the potential risks associated with administration of the study products.

Table 4-11 Summary of potential risks of study products and administration

Common	• Mild to moderate injection site pain, tenderness, erythema, or swelling/induration/edema • Fever, malaise/fatigue/weakness, chills, myalgia, nausea, or headache in the first few days following injection • A vaccine-induced positive HIV Ab test result
Less common	• Severe injection site pain or tenderness • Flu-like syndrome, arthralgia, rash, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Diarrhea, vomiting • Lymphadenopathy • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, other transient lesions, warmth, itching, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection • Abdominal pain • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis • Muscle damage at the injection site
Theoretical risks	• Autoimmune disease • Neuromuscular system disorders: Neuralgia, paresthesias, neuritis, convulsions, encephalomyelitis, muscle weakness, and Guillain-Barré syndrome • Thrombocytopenia • 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 DNA and/or different doses of clade C bivalent gp120 protein with either MF59 or $AS01_B$ adjuvant in various regimens in HIV uninfected healthy adults.

Primary endpoints 1

- Severe local and systemic reactogenicity signs and symptoms (pain, tenderness, erythema, induration, fever, malaise/fatigue, myalgia, headache, nausea, vomiting, chills, arthralgia) up to 7 days after each vaccine dose

- AEs by body system, Medical Dictionary for Regulatory Activities (MedDRA) preferred term, severity, and assessed relationship to study products up to 30 days after each vaccine 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 administration 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, bivalent gp120 protein/MF59, and/or bivalent gp120 protein/$AS01_B$ 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 further evaluate the systemic immune responses of each vaccine regimen at the Month 6.5 timepoint (2 weeks after the 4th vaccination)

Secondary endpoint 1

- Contingent upon results from the primary immunogenicity objective described above (primary objective 2), additional immunogenicity assays may be performed on blood 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 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 3

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

Exploratory objective 4

- 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 334 healthy, HIV-uninfected adult participants ages 18-40 in Southern Africa and the United States. Participants will be randomized to 8 treatment groups (7 vaccine groups and 1 placebo group). Vaccine groups 1-3 share the same DNA prime and test 3 different boosts consisting of DNA coadministered with a protein/adjuvant combination (gp120 proteins at 100 mcg with MF59 adjuvant, gp120 proteins at 100 mcg with $AS01_B$, or gp120 proteins at 20 mcg with $AS01_B$) at 2 timepoints. In groups 4-6 the DNA is coadministered with 1 of the protein/adjuvant combinations at 3 timepoints. An additional vaccine group (group 7) will test the proteins alone at the 20 mcg dose adjuvanted with $AS01_B$ administered at 3 timepoints. Groups 2, 3 and 5-7 will enroll 50 participants per group. Groups 1 and 4 evaluating the proteins adjuvanted with MF59 will enroll 30 participants per group as these two vaccine regimens are also being evaluated in HVTN 111 at sites in Southern Africa (HVTN 111 , n=30 per group). Evaluation of these 2 regimens will pool data from HVTN 108 and HVTN 111 for a total of 60 participants per group. The primary purpose of the placebo group (Group 8 n=24) is to ensure blinding. To ensure that both men and women will be adequately represented in the trial, the trial will enroll at least approximately 40% of each sex overall. Hence, when approximately 200 participants of 1 sex are enrolled, recruitment of persons born of that sex 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 17 phase 1 and 2 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of September 2014), 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 for n=50 account for 7 enrolled participants on each group 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. Various statistical considerations for safety assessment are addressed below based on groups with n=50. The statistical properties for the combined safety data from the MF59 regimens in 108 and 111 (n=60 for each) will be slightly better than the properties for n=50 discussed below.

The ability of the study to detect serious adverse events (SAEs) (See Section 11.2) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each group of the study (n =50), there is a 90% chance of observing at least 1 event if the true rate of such an event is 4.5% or more; and there is a 90% chance of observing no events if the true rate is 0.2% or less. As a reference, in HVTN vaccine trials from December 2000 through April 2014, about 4% of participants who received placebos experienced an SAE.

Probabilities of observing 0, 1 or more, and 2 or more events among groups of size 50 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 a vaccine regimen.

Table 6-1 Probability of observing no events, 1 or more events, and 2 or more events, among groups of size 50, for different true event rates

True event rate (%)	Pr(0/50)	Pr(1+/50)	Pr(2+/50)
1	0.61	0.39	0.09
4	0.13	0.87	0.60
10	0.005	0.99	0.97
20	<0.001	>0.99	>0.99
30	<0.001	>0.99	>0.99

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 [73]. If none of the 50 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate for such an event is 7.1%.

Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for groups of size 50

Observed event rate	Confidence interval (%)
0/50 = 0.0%	(0.0, 7.1)
1/50 = 2.0%	(0.4, 10.5)
2/50= 4.0%	(1.1, 13.5)
5/50 = 10.0%	(4.3, 21.4)
10/50 = 20.0%	(11.2, 33.0)
20/50 = 40.0%	(27.6, 53.8)

6.1.2 Sample size calculations for immunogenicity

The primary immunogenicity evaluation of each vaccine regimen will be an assessment of whether the regimen meets the 'adequate take/potency' (ATP) conditions, which are based on the assessment of the primary immunogenicity endpoints (section 5.1). For purposes of power and type 1 error calculations we use ATP conditions as defined in Table 6-3 based upon 11 endpoints from 4 immunogenicity assays. The assessment of the group 1 and 4 vaccine regimens, which use the MF59 adjuvant, will be based on the pooled HVTN 108 and HVTN 111 data. Since our knowledge of the importance of immunologic endpoints is evolving with time, changes may be made to the ATP definition prior to ATP assessment. The final criteria used to assess the ATP condition, once the trial is complete, and before analysis has commenced, will be defined in the HVTN 108 Statistical Analysis Plan (SAP).

Table 6-3 Adequate take/potency definition for power and type 1 error calculations (as of January 2015), ATP is established as meeting criterion (1) and at least 2 of criteria (2) - (4).

Immunogenicity endpoints	Criteria
(1) 1-3 endpoints: IgG binding antibodies to 3 vaccine-matched gp120 proteins	1. For ≥ 2 gp120s the LL of the 95% CI for vaccine response rate > 65%
(2) 4-6 endpoints: IgG binding to a panel of 3 scaffolded-V1V2 proteins	2. For ≥ 1 V1V2 variable the LL of the 95% CI for vaccine response rate > 30%
(3) 7-9 endpoints: Neutralizing antibodies to 3 vaccine-matched gp120 pseudoviruses	3. For ≥ 1 gp120 pseudoviruses the LL of the 95% CI for vaccine response rate > 30%
(4) 10-11 endpoints: CD4+ and CD8+ T-cell response to at least 1 peptide pool	4. The LL of the 95% CI for either CD4+ or CD8+ response rate to at least 1 peptide pool > 30%

Criteria for 'adequate take/potency' for each category of endpoints are presented in the right column of Table 6-3 based on comparing the lower limits (LL) of the 95% CIs for the crude positive response rates with the thresholds for the category. These criteria have largely been chosen to reflect the findings of the correlates analysis performed in the wake of the RV 144 trial (see Section 4.1.2). Future phase 2b efficacy testing will target vaccine efficacy (VE) of at least 40%; therefore, we would like to screen out regimens that have an immunogenicity profile that is unlikely to provide this level of efficacy. In particular:

1. The first category includes 3 variables measuring IgG binding antibodies to each of the 3 vaccine-matched gp120s. The criterion will be satisfied for this category if for ≥2 gp120s the LL of the 95% CI for vaccine response rate is greater than 65%. The rationale for choosing this 65% threshold is the belief that high rates of homologous IgG binding antibodies are likely to be necessary for a vaccine to prevent HIV infection with VE of at least 40%.
2. The second category includes IgG binding antibodies to a panel of scaffolded-V1V2 proteins. IgG binding antibodies to V1V2 proteins have been identified as an immune correlate of risk in RV144 with a response rate of 64% (LL 58%). The criterion will be satisfied for this category if for ≥1 V1V2 endpoint the LL of the 95% CI for vaccine response rate is greater than 30%. The rationale for choosing this 30% threshold is to screen out regimens that have very low chance of achieving 40%VE; note that a perfect binary correlate of protection would require a 40% response rate to achieve 40% VE.
3. The third category includes neutralizing antibody (Nab) to vaccine-matched gp120s. Nab are generally believed to be important for preventing infectious diseases. The criterion will be satisfied for this category if for ≥1 gp120s pseudoviruses the LL of the 95% CI for vaccine response rate is greater than 30%. The rationale for choosing this 30% % threshold is to screen out regimens that have very low chance of achieving 40%VE.
4. The fourth category includes CD4+ and CD8+ T-cell responses to vaccine-matched peptide pools. Adequate T-cell responses may be necessary for an efficacious vaccine. The estimated CD4+ T-cell response rate was 74% (LL 68%) in RV144.The criterion will be satisfied for this category if the LL of the 95% CI for either CD4+ or CD8+ T-cell response rates to at least 1 peptide pool is greater than 30%. The rationale for choosing this 30% threshold is to screen out regimens that have very low chance of achieving 40%VE.

A vaccine group satisfies the ATP condition if it meets the criteria for the first category IgG binding antibodies to vaccine-matched gp120s and satisfies the criteria for at least 2 out of categories 2-4.

Figure 6-1 addresses the power and type 1 error for passing ATP for a regimen of size 43, which allows for missing assay data from 15% of participants in a group with 50 participants enrolled. Among all possible configurations of the 11 endpoints in Table 6-3 (based on the endpoint either passing or failing it's individual pre-specified lower confidence limit threshold criteria), the minimum probability that a vaccine regimen passes the ATP condition given that it truly meets the condition and the maximum probability that a vaccine regimen passes the ATP condition given that it does not truly meet the condition are shown in Figure 6-1 for varying values of δ, where δ is the difference between the true response rate of an endpoint that passes the individual criteria and the lower confidence limit threshold. These calculations assume each individual endpoint either has positive response rate = lower confidence limit threshold (fails threshold criteria) or has positive response rate = threshold + δ (passes threshold criteria). The threshold value is used to define failure of the endpoint so that calculations provide the minimum power [P(Pass ATP|ATP)] and maximum Type 1 error [P(Pass ATP|Not ATP)]. With 43 participants, the probability of correctly identifying ATP for a regimen is at least 80% when δ=24%; and the probability of incorrectly identifying ATP for a vaccine regimen is at most 18% for $\delta \leq 30\%$ (Figure 6-1) (note that a vaccine does not satisfy the adequate take/potency condition if ≥ 2 endpoints in the first category fail or all endpoints in 2 or more of categories 2-4 fail). The group 1 and 4 regimens (HVTN 108 and 111 pooled data) will have slightly better properties with anticipated sample size of 51 participants evaluable for ATP. These simulations are based on 10,000 simulated datasets and use an exchangeable correlation structure between responses with pairwise correlations of 0.3 (a modest correlation based on RV144 data).

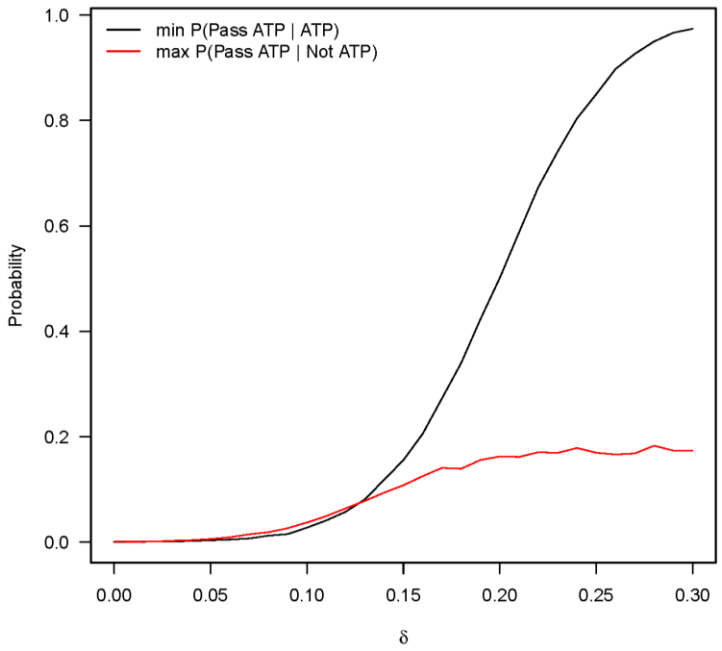

Figure 6-1 Power and type-I error for passing the individual regimen (n=43) adequate take/potency condition based on comparing positive response rates with prespecified thresholds (65% for IgG binding antibodies to vaccine-matched gp120s and 30% for other endpoints). The calculations assume an endpoint that passes its threshold criteria has positive response rate = δ% + threshold and an endpoint that fails its threshold criteria has positive response rate equal to the prespecified threshold.

The correspondence between response rate point estimates and CIs are shown in Table 6-4 for a range of observed response rates. Calculations are done using the score test method [73]. For example with an n of 43, a lower limit > 30% for the response rate corresponds to a point estimate ≥ 44.2%, and a lower limit > 65% for the response rate corresponds to a point estimate ≥ 81.4%.

Table 6-4 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses and calculated by the score method (n=43). Highlighted are the minimum number of responses necessary to achieve the 30% and 65% lower confidence limit thresholds.

No. of responses	Observed response rate (%)	95% Confidence interval (%)
5	11.6	(5.1, 24.5)
9	20.9	(11.4, 35.2)
13	30.2	(18.6, 45.1)
18	41.9	(28.4, 56.7)
19	44.2	(30.4, 58.9)
22	51.2	(36.8, 65.4)
26	60.5	(45.6, 73.6)
31	72.1	(57.3, 83.3)
35	81.4	(67.4, 90.3)
39	90.7	(78.4, 96.3)

6.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through a 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 (except in emergency situations as specified in the HVTN MOP). Randomization will be stratified by geographical region (Southern Africa and US).

6.3 Blinding

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

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

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 108 Protocol Safety Review Team (PSRT) should be consulted before emergency unblinding occurs.

6.4 Statistical analyses

This section describes the final study analyses, unblinded as to treatment group assignment. Data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan (SAP) will address how to analyze the participant's safety data. Analyses are modified intent-to-treat 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 specified in the SAP, when the assay is viewed as a collection of hypotheses (eg, testing multiple peptide pools to determine a positive response).

Data from HVTN 108 and 111 will be combined to assess the safety and immunogenicity of the MF59 regimens. Data from these studies may be evaluated with data from other phase 1/2a studies within the P5 partnership HIV vaccine 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.

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 J. These listings will be submitted to the FDA in all annual reports and clinical trial reports.

6.4.3.3 Local laboratory values

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

For each local laboratory measure, summary statistics will be presented by treatment group and timepoint, as well as changes from baseline for postenrollment values. In addition, the numbers (percentages) 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 postinfection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

For each of the 11 primary endpoints listed in Table 6-3, response rates will be analyzed by tabulating the frequency of positive response for each endpoint and treatment group at each timepoint for which an assessment is performed. For CD4+ and CD8+ T-cell response, response rates to the individual peptide pools will also be calculated. Crude response rates will be presented with their corresponding 95% confidence interval estimate calculated using the score test method [73]. For secondary and exploratory assay endpoints, response rates and 95% confidence intervals will be calculated if appropriate for the endpoint. Criteria for a positive response for an assay will be described in the SAP.

All of the primary assays, and likely some of the secondary assays, will have quantitative assay data, including IgG binding antibody magnitude, 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 as graphical and tabular summaries of the distributions by antigen, treatment group, and timepoint. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be presented by group.

Formal statistical comparisons between individual arms is not the primary objective of the trial, although these are likely to be made to better understand the effect of adjuvants on immune response and the effect of dose for arms adjuvanted with $AS01_B$. For comparisons in which the response rate for 1 of the groups is low (eg, $\leq$ 20% for the class), statistical testing will use Fisher's exact test comparing the 2 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 of response rates and magnitudes, then Lachenbruch's test statistic [74] 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 HVTN trials, missing 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 PBMCs. To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically, if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates.

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 [75] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [76] 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, as defined in Section 11.1.2, for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 108 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 6.5 month primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Analysis of secondary endpoints (ie, additional immunogenicity assays) will occur after the unblinded primary immunogenicity analysis since selection of secondary assays is dependent upon the primary analysis. Analysis of exploratory objectives (including the month 12 timepoint) will take place after the primary analysis. 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; completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly

5. **Agrees not to enroll in another study** of an investigational research agent before the last required protocol clinic visit

6. **Willing to be contacted by phone**, text message, or e-mail 6 months after completion of the scheduled clinic visits

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

HIV-Related Criteria:

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

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

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

Laboratory Inclusion Values

Hemogram/CBC

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

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

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

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

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

Chemistry

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

Virology

17. **Negative HIV-1 and -2 blood test**: US volunteers must have a negative FDA-approved enzyme immunoassay (EIA). Non-US sites may use locally available assays that have been approved by HVTN Laboratory Operations.

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

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

Urine

20. **Normal urine**:

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

Reproductive Status

21. **Volunteers who were born female**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination. Persons who are NOT of reproductive potential due to having undergone

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

22. **Reproductive status**:

United States

A volunteer who was born female must:

- Agree to consistently use effective contraception (see Appendix B) for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment through the last required protocol clinic visit. Effective contraception for participants in the United States is defined as using any 1 or more of the following methods:
  - Condoms (male or female) with or without a spermicide,
  - Diaphragm or cervical cap with spermicide,
  - IUD,
  - Hormonal contraception, or
  - Successful vasectomy in the male partner (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity postvasectomy), or
  - Any other contraceptive method approved by the HVTN 108 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.

Southern Africa

A volunteer who was born female must:

- Agree to consistently use effective contraception (see Appendix C) 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 for participants in Southern Africa is defined as using 2 methods of birth control. These include 1 of the following methods:
  - Condoms (male or female)
  - Diaphragm or cervical cap

  PLUS 1 of the following methods:
  - Intrauterine device (IUD),
  - Hormonal contraception (in accordance with applicable national contraception guidelines), or
  - Successful vasectomy in the male partner (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy,

or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity postvasectomy), or

- Any other contraceptive method approved by the HVTN 108 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.

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

Other

24. **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), OR
        - the 5 years prior to enrollment, with the latest result reported as normal, or ASCUS with no evidence of high risk HPV.

    - If no pap smear was done within the last 3 years (or within the last 5 years, if high risk HPV testing was performed), the volunteer 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 or any other study that requires non-HVTN HIV antibody testing during the planned duration of the HVTN 108 study

5. **Pregnant or breastfeeding**

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

Vaccines and other Injections

7. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 108 PSRT will determine eligibility on a case-by-case basis.

8. **Non-HIV experimental vaccine(s) received within the last 5 yea**rs in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 108 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 108 PSRT on a case-by-case basis.

9. **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)

10. **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)

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

Immune System

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

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

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

15. **Autoimmune disease**

16. **Immunodeficiency**

Clinically significant medical conditions

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

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

    - A process that would affect the immune response,

    - A process that would require medication that affects the immune response,

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

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

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

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

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

    Exclude a volunteer who:

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

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

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

25. **Hypertension**:

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

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

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

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

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

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

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

7.3 Participant departure from vaccination schedule or withdrawal

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

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 2 negative urine pregnancy tests taken from 2 different urine samples that may be collected on the same day; in this circumstance, the HVTN 108 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 108 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 108 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 108 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 108 Study Specific 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 9.8.1).

7.3.4 Participant termination from the study

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

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

Treatment 1 (T1): 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 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 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Group 2

Treatment 2 (T2): DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 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

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 3 and 6

AND

Bivalent Subtype C gp120 / $AS01_B$ (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and $AS01_B$) to be administered as 0.75 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Group 3

Treatment 3 (T3): DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 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

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 3 and 6

AND

Bivalent Subtype C gp120 / $AS01_B$ (an admixture of 20 mcg of TV1.C gp120, 20 mcg of 1086.C gp120, and $AS01_B$) to be administered as 0.75 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 3 and 6.

Group 4

Treatment 4 (T4): DNA-HIV-PT123 (4 mg/mL) 4 mg to be administered as 1 mL 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 0.5 mL IM in the RIGHT deltoid (unless medically contraindicated) at Months 0, 1 and 6

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 mL IM in the 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 mL IM in the RIGHT deltoid (unless medically contraindicated) at Month 3.

Group 5

Treatment 5 (T5): 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

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 and 6

AND

Bivalent Subtype C gp120 / $AS01_B$ (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and $AS01_B$) to be administered as 0.75 mL IM in the 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 mL IM in the RIGHT deltoid (unless medically contraindicated) at Month 3.

Group 6

Treatment 6 (T6): 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

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 and 6

AND

Bivalent Subtype C gp120 / $AS01_B$ (an admixture of 20 mcg of TV1.C gp120, 20 mcg of 1086.C gp120, and $AS01_B$) to be administered as 0.75 mL IM in the 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 mL IM in the RIGHT deltoid (unless medically contraindicated) at Month 3.

Group 7

Treatment 7 (T7): 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 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 and 6

AND

Bivalent Subtype C gp120 / $AS01_B$ (an admixture of 20 mcg of TV1.C gp120, 20 mcg of 1086.C gp120, and $AS01_B$) to be administered as 0.75 mL IM in the 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 mL IM in the RIGHT deltoid (unless medically contraindicated) at Month 3.

Group 8

Placebo 1 (P1): 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

AND

Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%) to be administered as 0.75 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 -20^0C +/-5^0C.

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 glass vial containing approximately 0.58 mL (800 mcg/mL) of protein in buffer. The protein is a clear colorless to slightly yellow liquid when thawed. The product must be stored frozen at -61°C or colder. Once thawed, the study product should be used immediately or stored in refrigerator at 2 to 8°C, for no longer than 24 hours. Unused vials should be quarantined after this time.

1086.C gp120 protein [labeled as 1086.C gp120]: The 1086.C gp120 protein will be provided in a glass vial containing approximately 0.58 mL (800 mcg/mL) of protein in buffer. The protein is a clear colorless to slightly yellow liquid when thawed. The product must be stored frozen at -61°C or colder. Once thawed, the study product should be used immediately or stored in refrigerator at 2 to 8°C, for no longer than 24 hours. Unused vials should be quarantined after this time.

The study product is described in further detail in the 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 Investigator's Brochure (IB).

$AS01_B$ is produced as a liposomal formulation containing MPL and QS-21. It is provided as an opalescent colorless to yellowish liquid in pre-filled vials. Each vial contains a volume to deliver 0.5 mL. The product must be stored refrigerated at 2 to 8° C.

The study product is described in further detail in the 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.

Placebo for Bivalent Subtype C gp120 / AS01$_B$ (Sodium Chloride for Injection, 0.9%)

Sodium Chloride for Injection, 0.9%, will be used as the placebo for Bivalent Subtype C gp120 / AS01$_B$. Product must be stored as directed by the manufacturer.

8.3 Preparation of study products

Pharmacists should refer to USP 38 General Chapter Physical Tests / <797> Pharmaceutical Compounding - Sterile, and should follow the requirements of their country, their institution, and their pharmacy regulatory authority regarding these procedures. At a minimum, study products must be prepared in a biological safety cabinet/isolator by appropriately trained/qualified pharmacy personnel using aseptic technique.

8.3.1 DNA-HIV-PT123

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

Once thawed, the pharmacist, using aseptic technique, will gently swirl the vial and then withdraw 1 mL into a 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

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 Bivalent Subtype C gp120 / MF59 (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and MF59C.1)

One vial of TV1.C gp120 protein, 1 vial of 1086.C gp120 protein, and 1 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 allowed 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 3 mL syringe or smaller. The needle should be removed and the syringe capped.

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. The syringe containing study product should be bagged for transport to the clinic where it will be administered. This study product should be administered immediately, defined as within 30 minutes as per IAC recommendations. If this is not possible, the site pharmacist may be permitted to label the syringe with a DO NOT USE AFTER 2 hour date and time from preparation. In this case, the vaccine should be stored at 2°C - 8°C until administration and if not used within 2 hours, it should be discarded.

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

8.3.4 Placebo for Bivalent Subtype C gp120 / MF59

Using aseptic technique, the pharmacist will withdraw 0.5 mL of Sodium Chloride for Injection, 0.9% into a 3 mL or smaller syringe. The needle should be removed and the syringe capped.

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. The syringe containing study product should be bagged for transport to the clinic where it will be administered. This study product should be administered immediately, defined as within 30 minutes as per IAC recommendations. If this is not possible, the site pharmacist may be permitted to label the syringe with a DO NOT USE AFTER 2 hour date and time from preparation. In this case, the vaccine should be stored at 2°C - 8°C until administration and if not used within 2 hours, it should be discarded.

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

8.3.5 Bivalent Subtype C gp120 / $AS01_B$ (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and $AS01_B$)

One vial of TV1.C gp120 protein, one vial of 1086.C gp120 protein, two vials of $AS01_B$, and one empty sterilized vial (for admixing) will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the TV1.C gp120 and 1086.C gp120 vials from the freezer and allow them to thaw at room temperature. The pharmacist will also remove the $AS01_B$ vials from the refrigerator.

Using aseptic technique, the pharmacist will withdraw 0.5 mL into a 1 mL syringe from the first vial of $AS01_B$. This volume will be transferred into the empty sterilized vial ("preparation vial"). The pharmacist will then repeat this process using the second vial of $AS01_B$. The preparation vial now contains 1 mL of $AS01_B$.

Using aseptic technique, the pharmacist will gently swirl the contents of the vial containing TV1.C gp120 and then withdraw 0.25 mL of TV1.C gp120 from the correct vial and inject it into the preparation vial containing $AS01_B$. The pharmacist will then gently swirl the vial containing 1086.C gp120 after which, using aseptic technique, the pharmacist will withdraw 0.25 mL of 1086.C gp120 from the correct vial and inject it into the preparation vial (which contains TV1.C gp120 and $AS01_B$). After gentle swirling and inversion (do not shake vigorously) the pharmacist, using aseptic technique, will withdraw 0.75 mL of the mixed preparation (100 mcg of each protein mixed with $AS01_B$ Adjuvant System) for dosing into a 3 mL syringe or smaller. The needle should be removed and the syringe capped.

The syringe should be labeled as "Bivalent Subtype C gp120 / $AS01_B$ or Placebo 0.75 mL" and have an overlay to maintain blinding. The syringe must also be labeled for administration in the deltoid of the right arm. The syringe containing study product should be bagged for transport to the clinic where it will be administered. This study product should be administered immediately, defined as within 30 minutes as per IAC recommendations. If this is not possible, the site pharmacist may be permitted to label the syringe with a DO NOT USE AFTER 2 hour date and time from preparation. In this case, the vaccine should be stored at 2°C - 8°C until administration and if not used within 2 hours, it should be discarded.

Any unused portion of vials, preparation vials, or expired prefilled syringes should be disposed of in compliance with local health, safety and environmental requirements.

8.3.6 Bivalent Subtype C gp120 / $AS01_B$ (an admixture of 20 mcg of TV1.C gp120, 20 mcg of 1086.C gp120, and $AS01_B$)

One vial of TV1.C gp120 protein, one vial of 1086.C gp120 protein, two vials of $AS01_B$, one vial/IV bag/ampule of Sodium Chloride for Injection, 0.9% , and one empty sterilized vial (for admixing) 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 them to thaw at room temperature. The pharmacist will also remove the $AS01_B$ vial from the refrigerator.

Using aseptic technique, the pharmacist will withdraw 0.5 mL into a 1 mL syringe from the first vial of $AS01_B$. This volume will be transferred into the empty sterilized vial ("preparation vial"). The pharmacist will then repeat this process using the second vial of $AS01_B$. The preparation vial now contains 1 mL of $AS01_B$.

Using aseptic technique, the pharmacist will gently swirl the contents of the vial containing TV1.C gp120 and then withdraw 0.05 mL of TV1.C gp120 from the correct vial and inject it into the preparation vial containing $AS01_B$. The pharmacist will then gently swirl the vial containing 1086.C gp120 after which, using aseptic technique, the pharmacist will withdraw 0.05 mL of 1086.C gp120 from the correct vial and inject it into the preparation vial (which contains TV1.C gp120 and $AS01_B$). Using a new syringe, the pharmacist will then withdraw 0.4 mL of Sodium Chloride for Injection, 0.9% and transfer it into the preparation vial (containing TV1.C gp120, 1086.C gp120 and $AS01_B$). After gentle swirling and inversion (do not shake vigorously) the pharmacist, using aseptic technique, will withdraw 0.75 mL of the mixed preparation (20 mcg of each

protein mixed with $AS01_B$ and 0.9% NaCl) for dosing into a 3 mL syringe or smaller. The needle should be removed and the syringe capped.

The syringe should be labeled as "Bivalent Subtype C gp120 / $AS01_B$ or Placebo 0.75 mL" and have an overlay to maintain blinding. The syringe must also be labeled for administration in the deltoid of the right arm. The syringe containing study product should be bagged for transport to the clinic where it will be administered. This study product should be administered immediately, defined as within 30 minutes as per IAC recommendations. If this is not possible, the site pharmacist may be permitted to label the syringe with a DO NOT USE AFTER 2 hour date and time from preparation. In this case, the vaccine should be stored at 2°C - 8°C until administration and if not used within 2 hours, it should be discarded.

Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in compliance with local health, safety and environmental requirements.

8.3.7 Placebo for Bivalent Subtype C gp120 / $AS01_B$ (Sodium Chloride for Injection, 0.9%)

Using aseptic technique, the pharmacist will withdraw 0.75 mL of Sodium Chloride for Injection, 0.9% into a 3 mL syringe or smaller. The needle should be removed and the syringe capped.

The syringe should be labeled as "Bivalent Subtype C gp120 / $AS01_B$ or Placebo 0.75 mL" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid. The syringe containing study product should be bagged for transport to the clinic where it will be administered. The study product should be administered immediately, defined as within 30 minutes as per IAC recommendations. If this is not possible, the site pharmacist may be permitted to label the syringe with a DO NOT USE AFTER 2 hour date and time from preparation. In this case, the vaccine should be stored at 2°C - 8°C until administration and if not used within 2 hours, it should be discarded.

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

8.3.8 Procedures to preserve blinding

The pharmacist will prepare all doses for administration and dispense to the clinic. In order to preserve blinding, the pharmacist will place an overlay on ALL the syringes.

8.4 Administration

All injections are to be given IM in the deltoid indicated. At sites where registered pharmacists are legally authorized to administer injections, the HVTN CRS may choose to have the pharmacist administer vaccinations.

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

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

For all syringes containing Bivalent Subtype C gp120/MF59 or placebo or Bivalent Subtype C gp120/$AS01_B$ or placebo, the person administering the injection should gently roll the syringe prior to administration of the study product.

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

8.5 Acquisition of study products

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

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

$AS01_B$ will be provided by GlaxoSmithKline Biologics.

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 from the NIAID Clinical Research Products Management Center (CRPMC) by following the ordering procedures given in Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

8.6 Pharmacy records

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

8.7 Final disposition of study products

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

9 Clinical procedures

The schedule of clinical procedures is shown in Appendix H.

9.1 Informed consent

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

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

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

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

9.1.1 Screening consent form

Without a general screening consent, screening for a specific study cannot take place until the site receives protocol registration from the DAIDS RSC Protocol Registration Office.

Some HVTN CRSs have approval from their IRB/EC and any applicable RE to use a general screening consent form that allows screening for an unspecified HIV vaccine trial. In this way, HVTN CRS staff can continually screen potential participants and, when needed, proceed quickly to obtain protocol-specific enrollment consent. Sites conducting general screening or prescreening approved by their IRB/EC and any applicable RE may use the results from this screening to determine eligibility for this protocol, provided the tests are conducted within the time periods specified in the eligibility criteria.

9.1.2 Protocol-specific consent forms

The protocol-specific consent forms describe the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. 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 the 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;
- 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 9.10),
  - HBsAg,
  - Anti-HCV Ab,
  - Syphilis test,
  - Urine or serum pregnancy test (volunteers who were born female);
  - Pap smear (only for volunteers 21 years or older who were born female and who agree to provide cervical samples; for specific requirements see Section 7.1, Bullet 24);
- Administration of behavioral risk assessment questionnaire;
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html);
- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.8; and

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

9.2.1 Use of screening results from another HVTN study

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

9.3 Enrollment and vaccination visits

Enrollment is simultaneous with first vaccination. The time interval between randomization and enrollment should not exceed 4 working days. The HVTN CRS randomizes the participant via the Web-based randomization system as described in the HVTN 108 *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 will be

given the 7 day postvaccination symptom log and is instructed on how to complete it. Contacts between the participant and the site staff should take place daily during the first 3 days of the assessment period. Site staff will review the rest of the postvaccination symptom log at the first follow up visit (as described in Section 9.11).

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

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

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

- 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;
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;
- Specimen collection (blood and/or mucosal and/or stool) should be completed prior to vaccination;
- For participants who agree to mucosal sampling collection (see Appendix H and Section 9.6):
    - Urine test or swab 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 9.8);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.9); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation);
- Assessment of new or continuing concomitant medications (as described in Section 9.2); and
- Assessment of new or unresolved AEs/intercurrent illnesses.

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

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

- 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 9.6):
  - Urine test or swab 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 9.6 and 9.14); and
  - Mucosal specimen collection.

9.5 AESI contact

Participants will be contacted 6 months after the last scheduled visit (see Appendix I). This contact may be accomplished by phone, text message, or email. At this contact, CRS staff will collect information about their health, specifically related to potential AESIs. If indicated, the participant may be asked to come in for a clinical assessment which may also include referrals for AESI assessment. AESIs are described further in Appendix J.

9.6 Mucosal sampling

Mucosal samples will be collected at the timepoints indicated in Appendix H 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. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Rectal secretion sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, if still within the visit window. In addition, rectal sampling will not be performed (or may be deferred to a later date if still within the visit window) if there is a contraindication to rectal 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).

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

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

Cervical fluid 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 3-5 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 cervical sampling with a sponge. For sampling with Softcup, the pregnancy test can be performed after collection has taken place, but should be performed on the same day as the collection. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Cervical sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, if still within the visit window. In addition, cervical sampling will not be performed (or may be deferred to a later date if still 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 if still within the visit window) if a participant is known to have an active GTI at the scheduled timepoint.

9.7 Stool sampling

Two stool samples will be collected from the study participants who agree to this procedure: 1 prior to enrollment (before the injection of the vaccine) and 1 at the Month 6.5 timepoint. These samples will be collected using swabs, either via rectal swabs or by taking swabs from stool.

9.8 HIV counseling and testing

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

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

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

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

9.8.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

The study product may elicit an antibody response to HIV proteins. Therefore, vaccine-induced positive serology (VISP) 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 H. 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 H). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the Laboratory Manual of Operations), which is able to distinguish vaccine-induced antibody responses from actual HIV infections.

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

- All participants who received vaccine product and who have vaccine-induced positive or indeterminate HIV-1 serology (as measured by the standard anti-HIV antibody screening tests) at or after the study is unblinded will be offered poststudy HIV-1 diagnostic testing (per the HVTN poststudy HIV-1 testing algorithm) periodically and free of charge as medically/socially indicated (approximately every 6 months) unless or until HIV Ab testing is no longer the standard test in clinical settings.

9.8.2 VISP registry

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

9.9 Contraception status

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

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

9.10 Urinalysis

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

If the screening dipstick is transiently abnormal due to menses or infection, document this issue in the participant's source documentation. For infection, provide appropriate

treatment and/or referral. Following resolution, repeat the dipstick and, if within the eligibility limits specified in the protocol, the participant may be enrolled.

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

9.11 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014, except as noted in Section 11.2.2.

The reactogenicity assessment period is 7 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants will be given a tool on which to record reactogenicity symptoms. Contacts between the participant and the site staff should take place daily during the first 3 days of the assessment period. Participants are instructed to record the resolution date of new or unresolved reactogenicity symptoms present at day 7 to resolution. Participants are instructed to contact the clinic for events that arise during the period between vaccination and the next scheduled visit. In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved.

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

Table 9-1 Schedule of reactogenicity assessments

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

[a] Day of vaccination

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

9.11.1 Assessment of systemic and local symptoms

Systemic symptoms include increased body temperature, malaise and/or fatigue, myalgia, headache, chills, arthralgia, nausea, and vomiting. Local symptoms include pain and/or

tenderness proximal to the injection site. The daily maximum severity reached for each symptom during the assessment period is reported.

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

9.11.2 Assessment of injection site

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

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

9.11.3 Assessment of lymph nodes

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

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

9.12 Visit windows and missed visits

Visit windows are defined in HVTN 108 Study Specific Procedures. For a visit not performed within the window period, a Missed Visit form is completed. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

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

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

9.13 Early termination visit

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

9.14 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given 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. In case of required termination from the study, enrollment in an observational study should be offered to the participant. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome.

10 Laboratory

10.1 HVTN CRS laboratory procedures

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

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

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

10.2 Total blood volume

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

10.3 Primary immunogenicity timepoint

The primary immunogenicity timepoint in this study is at visit 10 (day 182) (ie, 2 weeks after the 4th 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 G.

10.4 Endpoint assays: humoral

10.4.1 HIV-1 multiplex antibody assay

Total binding IgG antibodies to HIV-1 Env proteins (including V2 regions of interest) will be assessed on 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 antibody (nAb) assay

HIV-1–specific nAb assays will 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 the vaccine strains (96ZM651, TV1.C, 1086.C) and 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 [77,78].

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.

10.8.1 Microbiome analysis

Swabs of stool will be shipped to a central laboratory. Specimens are processed to enable nucleic acid sequencing. 16s rRNA sequences will then be determined using pyro-sequencing approaches or other 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 108 PSRT

The HVTN 108 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)
- Regional medical liaison (RML), 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 HVTN 108 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 108 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. 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 108 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 108 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 108 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 108 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, concomitant medications) before the end of the next business day after receiving the information. The forms should not be held in anticipation of additional information at a later date. If additional information is received at a later date, the forms should be updated and resubmitted before the end of the next business day after receiving the new information.

11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014, available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/, except:

- Weight loss is required to be reported as an AE only if it is considered to be deleterious to the participant's health (see *HVTN 108 Study Specific Procedures*);

- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:
  - Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area;
  - Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area;
  - Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage;

  - Grade 4 is: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue);

- The grading of Insomnia events will consider the criteria within the Insomnia parameter as well as the general AE functional table such that:
  - Grade 1 Insomnia is defined as: Mild difficulty falling asleep, staying asleep, or waking up early causing no or minimal interference with usual social and functional activities with intervention not indicated;
  - Grade 2 Insomnia is defined as: Moderate difficulty falling asleep, staying asleep, or waking up early, causing greater than minimal interference with usual social and functional activities with intervention indicated;
  - Grade 3 Insomnia is defined as: Severe difficulty falling asleep, staying asleep, or waking up early, causing inability to perform usual social & functional activities with intervention or hospitalization indicated.

  If a definition of insomnia falls between 2 grades, the final grading will be selected based on the degree of interference with usual social and functional activities caused by the symptoms.

Unsolicited AEs will be collected over a period of 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 J.

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

- SAEs/EAEs,
- AESIs,
- 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 or RML 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/hvtn108). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

In the case of email notification the CSS or RML 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 or RML 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 adverse events to DAIDS

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

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

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

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 1 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.

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

If the participant has completed the main study and has not yet completed the AESI contact (Section 9.5) the SUSAR reporting category will be used.

After the participant has completed the AESI contact and is off study, sites must report SUSARs if the study site staff become aware of the events on a passive basis (eg, from publicly available information).

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
- Bivalent Subtype C gp120/AS01B/placebo

The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the FDA in accordance with 21 CFR 312.32 (IND Safety Reports). All AESIs will be considered "unexpected", and, if deemed related to the study products, will be reported to FDA as SUSARs, if applicable. However, because safety is a primary study endpoint, the Sponsor Medical Officer will not be unblinded to study treatment assignment when there is an assessment of relatedness of the SAE with the study product(s); and the safety report will be sent to the FDA based on the blinded attribution assessment.

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 108 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 108 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

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

Event and relationship to study products	Severity	HVTN CRS action[a]	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and submit forms immediately	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately	Immediate HVTN 108 PSRT notification
SAE, related	Grade 3	Email and submit forms immediately	Prompt HVTN 108 PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and submit forms immediately	Prompt HVTN 108 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/hvtn108).

[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 108 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 108 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 108 PSRT assessment, the trial sponsor or designee(s) notifies pertinent national regulatory authorities as needed.

If an immediate HVTN 108 PSRT notification or prompt HVTN 108 PSRT AE review is triggered, HVTN Core notifies the HVTN 108 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 108 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 108 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 108 PSRT AE review criteria.

11.4.2 Weekly review

During the injection phase of the trial, the HVTN 108 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 108 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.

11.4.3 AESI contact

After the main study period, a monitoring team reviews safety reports after the AESI contact. This monitoring team comprises at least a DAIDS Medical Officer, Core medical monitor, and a CSS.

11.5 Study termination

This study may be terminated early by the determination of the HVTN 108 PSRT, FDA, 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 108 *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 or Synthetic Nucleic Acid 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 per NIH guideline *section IV-B07-a-(1).* 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. If required, the Registrar of GMO shall be consulted on all formal developments relating to this protocol and clinical trial, and a formal application will be made to the Registrar of GMO to review the HVTN 108 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 108 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 108 are described below.

Protocol history and modifications

Date: August 8, 2016

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

Item 1 Added to cover page: BB IND number

Item 2 Updated in Section 3.1, *Protocol Team*: Protocol leadership

Item 3 Revised in Section 5.1, *Primary objectives and endpoints*, Section 9.3, *Enrollment and vaccination visits*, Section 9.11, *Assessments of reactogenicity*, Appendix A, *SICF*, Item 12, and in Appendix H, *Procedures at HVTN CRS*: Reactogenicity assessment period extended from 3 days to 7 days

Item 4 Revised in Section 6.4.3.2, *AEs and SAEs*, Section 11.2.3, *Expedited reporting of adverse events to DAIDS*, and Appendix J, *Adverse events of special interest*: AESI reporting requirements

Item 5 Updated Section 8.3, P*reparation of study products*: instructions for preparing and handling of study products.

Item 6 Revised Section 9.2, *Pre-enrollment procedures*: Coding of concomitant medications

Item 7 Revised Section 9.6, *Mucosal sampling* and Appendix A, *SICF*, Item 14: Cervicovaginal secretion collection

Item 8 Clarified in Appendix A, *SICF*, Item 14: Rectal fluid collection

Item 9 Revised in Appendix A, *SICF*: Item 16 regarding sample testing

Item 10 Revised in Appendix A, *SICF*, Item 26: Study-related injury language

Item 11 Added to Appendix A, *SICF*, Item 28: MCC contact information for South African sites

Item 12 Corrected in Appendix A, *SICF*, Item 29 and Item 30: Placement of prompt to delete check-boxes

Item 13 Clarified in footnotes in Appendix G, *Laboratory procedures* and Appendix H, *Procedures at HVTN CRS*: Pregnancy testing and baseline specimen collection prior to initial vaccination

Item 14 Updated: Section 13, Version history

Date: May 8, 2015

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 2.0, November 2014. 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 108 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 108 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN Laboratory Manual of Operations. Accessible through the HVTN website.
- HVTN Manual of Operations. Accessible through the HVTN website.
- 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 *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Available at http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

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

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

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

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

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

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

15 Acronyms and abbreviations

Ab	antibody
Ad	adenovirus
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANOVA	analysis of variance
ART	antiretroviral therapy
AS	adjuvant system
AST	aspartate aminotransferase
ATP	adequate take/potency
AVEG	AIDS Vaccine Evaluation Group
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CI	confidence intervals
CoR	correlate of risk
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
CTL	cytotoxic T lymphocyte
DAERS	DAIDS Adverse Event Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
FDA	US Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure

IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IFN-γ	interferon gamma
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	intrauterine device
MAR	missing at random
MCAR	missing completely at random
MMR	measles, mumps, and rubella
nAb	neutralizing antibody
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NICD	National Institute for Communicable Diseases (Johannesburg, South Africa)
NIH	US National Institutes of Health
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
PI	Principal Investigator
PSRT	Protocol Safety Review Team
RAB	DAIDS Regulatory Affairs Branch
RE	regulatory entity
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
TB	tuberculosis
UW-VSL	University of Washington Virology Specialty Laboratory
VISP	Vaccine induced seropositivity
VRC	Vaccine Research Center (NIAID)

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

16 Literature cited

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Gorny MK, Zolla-Pazner S, LaBranche CC, Mascola JR, Korber BT, Montefiori DC. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. J Virol **2014**;88:2489-507.

Appendix A Sample informed consent form

Title: A phase 1/2a clinical trial to evaluate the safety and immunogenicity of HIV clade C DNA, and of MF59®- or AS01$_B$-adjuvanted clade C Env protein, in various combinations, in healthy, HIV-uninfected adult participants
HVTN protocol number: HVTN 108
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 334 people will take part in this study at multiple sites in Southern Africa and the US. At least 40 percent will be men and at least 40 percent will be women. 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 different combinations and doses of the study vaccines? (Your immune system protects you from disease.)

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

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

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

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

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

got the vaccine seemed to have a *lower* risk of getting HIV than people who did not get the vaccine. In studies with a different vaccine, some people who got the vaccine had a *higher* risk of getting HIV than people who did not get the vaccine.

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

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

4. These study vaccines are experimental.

There are 3 study vaccines being tested in this study. They are all experimental vaccines. That means we do not know whether the vaccines will be safe to use in people, or whether they will work to prevent HIV infection. These vaccines are used only in research studies. The study vaccines are called DNA-HIV-PT123, Bivalent Subtype C gp120/MF59, and Bivalent Subtype C gp120/AS01$_B$. From here on, we will call them the DNA vaccine, the Protein/MF59 vaccine, and the Protein/AS01$_B$ vaccine, or the study vaccines.

The DNA vaccine:

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

The DNA vaccine is being tested in people in 2 other studies in the US and in Switzerland. These studies are giving the DNA vaccine in combination 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/MF59 vaccine:

The Protein/MF59 vaccine was developed by Novartis Vaccines (Cambridge, MA, USA).

The Protein/MF59 vaccine has man-made pieces of a protein found on the outside of HIV. These proteins 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 vaccine the adjuvant is called MF59. MF59 is commonly used in licensed flu vaccines in many European countries. It has also been used in other vaccines that have been given to over 50,000 people in research studies without causing any serious health problems.

The Protein/MF59 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 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.

The Protein/AS01$_B$ vaccine:

The Protein/AS01$_B$ vaccine was developed by GlaxoSmithKline Biologicals (Rixensart, Belgium).

The Protein /$AS01_B$ vaccine has the same proteins used in the Protein/MF59 vaccine. However, these protein pieces are mixed with a different adjuvant called $AS01_B$. The Protein/$AS01_B$ has not been given to people before either. Similar vaccines have been given to over 1400 people in past studies. In these studies, the vaccines did not cause serious health problems.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Vaccines can also cause pain, redness, swelling, or itching at the injection site. 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.

Rarely, people who have received vaccines with adjuvants have developed illnesses called autoimmune diseases. Autoimmune diseases have also occurred in people who have not been vaccinated. These diseases develop when immune cells that normally protect you from illness attack your organs instead. Autoimmune diseases can be serious and can also be lifelong. They can involve, for example, your liver, kidneys, skin, joints, eyes, brain, as well as other parts of the body. Since no one knows for sure if vaccines with adjuvants might cause autoimmune diseases, we continue to monitor this situation closely.

Risks of the study vaccines:

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

The 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. Some people felt flu-like symptoms, nausea, and rash. These problems usually happened within a couple of days after injection(s) and usually went away on their own within a few days.

The Protein/MF59 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 headaches. 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 a stomach ache. Most people who had symptoms only had some of these symptoms, not all of them.

The Protein/$AS01_B$ vaccine:

This study vaccine has not been tested in people before. In studies with similar products some people had redness, swelling, pain, tenderness, warmth, or itching in the area where they got the injection. Some people had headache, fever, tiredness or weakness. A small

number of people had flu-like symptoms, nausea, rash, vomiting, diarrhea, or swollen lymph nodes after getting an injection. Most people who had symptoms only had some of these symptoms, not all of them.

Joining the study

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

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

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

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

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

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

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

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

We will also do blood and urine tests. These tests tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for: hepatitis B, hepatitis C, and syphilis. We will ask you about medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were born female, we will test you for pregnancy. People who have had a complete hysterectomy (removal of the uterus and ovaries, verified by medical records), are not required to have a pregnancy test.

We will review the screening results with you. The screening results may show you are not eligible to join the study, even if you want to. Also, you might not be able to join if we have already enrolled enough people of your same sex.

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

7. If we find that you have a health problem during screening or during the study.

- We will tell you about the care that we can give here for free.

- For the care that we cannot give, we will explain how we will help you get care elsewhere.

We will not be able to pay for care for health problems that are unrelated to this study.

8. **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 for your country here if you do not want to hand out the separate Approved Birth Control Methods sheet (Appendix B or Appendix C).

   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 approved birth control methods for your country from Appendix B or Appendix C 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:

9. **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).

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

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

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

    US sites only:

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

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

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

Some people will get the study vaccines and the placebo. Some people will get only the placebo. A placebo is a substance that does not contain vaccine. The placebo in this study is saline solution. We do not expect the placebo to cause any health problems in people. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.* Whether you get the study vaccines or the placebo is completely random, like flipping a coin.

You have about a 1 out of 14 chance of getting only the placebo. You have about an 13 out of 14 chance of getting some combination of study vaccines and placebo. We will compare the results from people who got only the placebo with results from people who got different combinations of the study vaccines and placebo.

The clinic staff 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.

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

There are 8 groups in this study. Each group will get a different combination of study products. Also, different groups will get different doses (high or low) of the protein used in the protein vaccines. Each group will get 12 injections during the study. You will get the injections into your upper arms. You will get 3 injections at every injection visit.

The DNA vaccine will go into the left arm. The Protein/MF59 vaccine and the Protein/AS01$_B$ vaccine will go into the right arm. The placebo can be given in both arms.

Site: A picture version of the injection schedule has been provided in Appendix E. You may insert it below in place of (or in addition to) the text version or give it as a separate document to your participants. You are not required to do either.

Group	Number of people	Protein dose	Arm	First injection visit	Time after first injection visit		
					1 month	3 months	6 months
1	30	High dose	Left	DNA	DNA	DNA	DNA
			Right	Placebo + Placebo	Placebo + Placebo	Protein/MF59 + Placebo	Protein/MF59 + Placebo
2	50	High dose	Left	DNA	DNA	DNA	DNA
			Right	Placebo + Placebo	Placebo + Placebo	Protein/$AS01_B$ + Placebo	Protein/$AS01_B$ + Placebo
3	50	Low dose	Left	DNA	DNA	DNA	DNA
			Right	Placebo + Placebo	Placebo + Placebo	Protein/$AS01_B$ + Placebo	Protein/$AS01_B$ + Placebo
4	30	High dose	Left	DNA	DNA	Placebo	DNA
			Right	Protein/MF59 + Placebo	Protein/MF59 + Placebo	Placebo + Placebo	Protein/MF59 + Placebo
5	50	High dose	Left	DNA	DNA	Placebo	DNA
			Right	Protein/$AS01_B$ + Placebo	Protein/$AS01_B$ + Placebo	Placebo + Placebo	Protein/$AS01_B$ + Placebo
6	50	Low dose	Left	DNA	DNA	Placebo	DNA
			Right	Protein/$AS01_B$ + Placebo	Protein/$AS01_B$ + Placebo	Placebo + Placebo	Protein/$AS01_B$ + Placebo
7	50	Low dose	Left	Placebo	Placebo	Placebo	Placebo
			Right	Protein/$AS01_B$ + Placebo	Protein/$AS01_B$ + Placebo	Placebo + Placebo	Protein/$AS01_B$ + Placebo
8	24	N/A	Left	Placebo	Placebo	Placebo	Placebo
			Right	Placebo + Placebo	Placebo + Placebo	Placebo + Placebo	Placebo + Placebo

At each injection visit, you will have to wait in the clinic for about a half hour after getting the injections to see if there are any problems. You will need to record how you feel each day for a total of 7 days after each injection visit. To help you do this, we will give you tools and show you how to use them. For 3-4 days after each injection visit, you will need to tell the clinic staff how you have been feeling. We will ask you the ways we can contact you. If you do not contact us, we will contact you. For the rest of the 7 days, you only need to contact us if you have any symptoms that prevent you from doing your usual activities. You should also contact us if you have any questions.

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.

13. 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.
- Take urine and blood samples.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 200 mL (2 teaspoons to a little more than ¾ cup). Your body will make new blood to replace the blood we take out.

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

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

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

14. If you agree, we will also collect stool, rectal fluid and cervical fluid or semen.

At the end of this form we will ask if you allow us to collect stool, 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.

Stool

We would like to collect a small sample of your stool to look at the bacteria living in your stomach. We want to learn if your immune response to the study vaccines is influenced by these bacteria. We will do this twice during this study. If you agree to give the rectal fluid sample described below, we can collect the stool sample at the same time, or you may provide a stool sample at home or at the clinic. The clinic must receive the stool sample within 24 hours after it is collected.

Rectal fluid, 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.

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 2 days before we collect these samples. This will help make sure your samples give accurate lab readings.

Rectal fluid

Site: You may delete the units of measure that are not used at your site in the next sentence. We will collect rectal fluid by first inserting a plastic tube into your rectum that is about 10 cm (4 inches) long and a little less than 2.5 cm (1 inch) wide. The tube will go in about 7 cm (3 inches). Then we will insert up to 3 small absorbent sponges through the tube into the rectum. The sponges will be left in place for 5 minutes and then removed.

For the 2 days before we collect your rectal fluid, we will ask you not to 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 infection. You should tell us if your rectal area is sore.

Cervical fluid

If you are 21 years or older you must have had a Pap smear within the last 3-5 years with the most recent result being normal. If you haven't had a Pap smear within the last 3-5 years and would like to get one, we will help you get one at no cost to you.

We will collect cervical fluids by using either a soft sponge inserted into the opening of your cervix, or by using something called 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 start the collection of cervical fluid if we learn that 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.

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.

16. We will test your samples for this study.

We will send your samples (without your name) to labs approved by the HVTN for this study, which are located in the United States and South Africa. Researchers at these labs will test your samples to see how your immune system responds to the study products. In rare cases, some of your samples may be sent to labs approved by the HVTN in other countries for research related to this study.

Researchers may also do genetic testing related to this study on your samples. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. These types of genetic tests involve some of your genes, not all of your genes (your genome). The researchers will study the genes related to the immune system and HIV and those that affect how people get HIV.

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

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

Tests done on your samples are for research purposes only. The labs will not give the results to you or this clinic, and the results will not become part of your study record.

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

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

17. 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 by HVTN or other researchers. 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 place called a repository. *[Site: choose one of the following two sentences. African sites should choose the sentence referencing the repository in South Africa. All other sites should choose the sentence referencing the repository in the United States.]* Your samples will be stored in the HVTN repository in South Africa. Your samples will be stored in the HVTN repository in the United States.

How long will the samples be stored? There is no limit on how long your extra samples will be stored. *[Site: 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. If the research plan is approved, the HVTN will send your samples to the researcher's location.

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.

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

US sites: Check HIPAA authorization for conflicts with this section.

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

Sites: Any change to the following highlighted text requires approval from HVTN Regulatory Affairs

- We do need to share your name with the HVTN in case you need proof in the future that you participated in an HIV vaccine study. The HVTN will keep your name in a secure file with these items:
  - The name of your study
  - Your age or date of birth
  - Your study ID number
  - What study product(s) you received

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

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

- The US NIH, its study monitors, and its chosen Southern African representatives,
- The US Food and Drug Administration,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- The South African Medicines Control Council,
- IPPOX Foundation, Novartis Vaccines, and GlaxoSmithKline 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]

US sites: Include the following boxed text. You can remove the box.

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

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

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

Sites: The text below may not be deleted or changed, per FDA requirement. It's OK to remove the box around it.

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

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

20. 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.

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

You will not be able to stay in this study. We will counsel you about your HIV infection and about telling your partner(s). We will tell you where you can get support and medical care, 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

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

This section describes the other risks and restrictions we know about. There may also be unknown risks, even serious ones. We will tell you if we learn anything new that may affect your willingness to stay in the study.

Risks of routine medical procedures:

In this study, we will do some routine medical procedures. These are taking blood and giving injections. These procedures can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore, bleeding, and (rarely) muscle damage or infection at the

injection site. Taking blood can cause a low blood cell count (anemia), making you feel tired.

Risks of collecting rectal and cervical fluids:

You may have some discomfort and minor bleeding during these procedures. This does not usually last very long.

Personal problems/discrimination/testing HIV antibody positive:

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

The body makes antibodies to fight or prevent infection. Most vaccines cause the body to make antibodies as a way of preventing infection. Your body may make antibodies to HIV because you received an HIV study vaccine. The study 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.

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

Unknown risks:

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

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

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

Benefits

23. The study may not benefit you.

We do not know whether getting the study vaccines might benefit you in any way. However, being in the study might still help you in some ways. The counseling that you get as part of the study may help you avoid getting HIV. The lab tests and physical exams that you get while in this study might detect health problems you don't yet know about.

This study may help in the search for a vaccine to prevent HIV. However, if the study vaccines later become approved and sold, there are no plans to share any money with you.

Your rights and responsibilities

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

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

Leaving the study

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

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

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

Injuries

Sites: Do not make changes to the following section without obtaining approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org

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

Next 4 paragraphs for US sites only

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

If you become sick or injured in this study, there is a process to decide if it is related to the study products and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met. The HVTN has limited funds to pay medical costs that it determines are reasonable. There may be funds from other groups as well. *(Sites: insert locale- appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

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

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

Next 6 paragraphs for South African sites only

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

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

(Sites: adjust the language in this paragraph so it is applicable to your site) In this study, our clinic has insurance to cover your medical treatment in the case of a study-related injury. The insurance will follow the Association of the British Pharmaceutical Industry guidelines for payment of study-related injury. We can give you a copy of these guidelines. In rare cases, the insurance funds may not be enough. There may be other ways that study-related injuries can be funded. We can give you more information, if you would like.

For study-related injuries that cannot be funded as described above, the HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale-appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

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

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

After-study contact

27. After your clinic visits end, we will contact you once, 6 months after your last scheduled study visit.

As we explained in Section 4 of this form, people who have received vaccines with adjuvants have rarely developed illnesses called autoimmune diseases. Since no one knows for sure if vaccines with adjuvants might cause autoimmune diseases, we continue to monitor this situation closely.

We will contact you by phone, email or text message *[Site: Modify mode of contact as appropriate]* once, 6 months after your last scheduled study visit, to ask questions about

your health and if you have been diagnosed with an autoimmune disease. If you prefer to answer these questions in person, you can come to the clinic to do this. If we have any concerns about your health, we may need to have more contact with you. You are also welcome to contact us at any time if you have concerns about your health related to being in the study.

Because we will want to contact you, please tell us if your contact information changes, or if you are moving away.

Questions

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

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

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

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

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

South African sites: Include the following:

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:

The Registrar of Medicines
Medicines Control Council
Department of Health
Private Bag X828
PRETORIA
0001

Fax: (012) 395 9201

e-mail: gouwsj@health.gov.za

Your permissions and signature

29. In section 14 of this form, we told you about collecting stool, 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 stool samples.

☐ I do not agree to provide stool samples.

☐ I agree to provide rectal fluid.

☐ I do not agree to provide rectal fluid.

For people born female:

☐ I agree to provide cervical fluid.

☐ I do not agree to provide cervical fluid.

☐ N/A

For people born male:

☐ I agree to provide semen.

☐ I do not agree to provide semen.

☐ N/A

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

30. In Section 17 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, 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.

31. 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) for US sites

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

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

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

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

You do not have to use birth control if:

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

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

Appendix C Approved birth control methods (for sample informed consent form) for Southern African sites

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

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

Effective birth control is defined as using 2 methods of birth control. These include 1 of the following methods:

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

PLUS 1 of the following methods:

- Birth control drugs that prevent pregnancy—given by pills, shots, 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 D Sample consent form for use of samples and information in other studies

Title: A phase 1/2a clinical trial to evaluate the safety and immunogenicity of HIV clade C DNA, and of MF59®- or $AS01_B$-adjuvanted clade C Env protein, in various combinations, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 108

Site: [Insert site name]

When samples are no longer needed for this study, the HVTN wants to keep them for use in other studies by HVTN or other researchers. 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 place called a repository. *[Site: choose one of the following two sentences. African sites should choose the sentence referencing the repository in South Africa. All other sites should choose the sentence referencing the repository in the United States.]* Your samples will be stored in the HVTN repository in South Africa. Your samples will be stored in the HVTN repository in the United States.

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. If the research plan is approved, the HVTN will send your samples to the researcher's location.

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.

U.S. Sites, include the following paragraph

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

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

People who may see your information are:

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

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

Questions

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

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

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

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

13. Please choose only one of the options below and write your initials or make your mark in the box next to it.

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

OR

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

OR

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

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

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

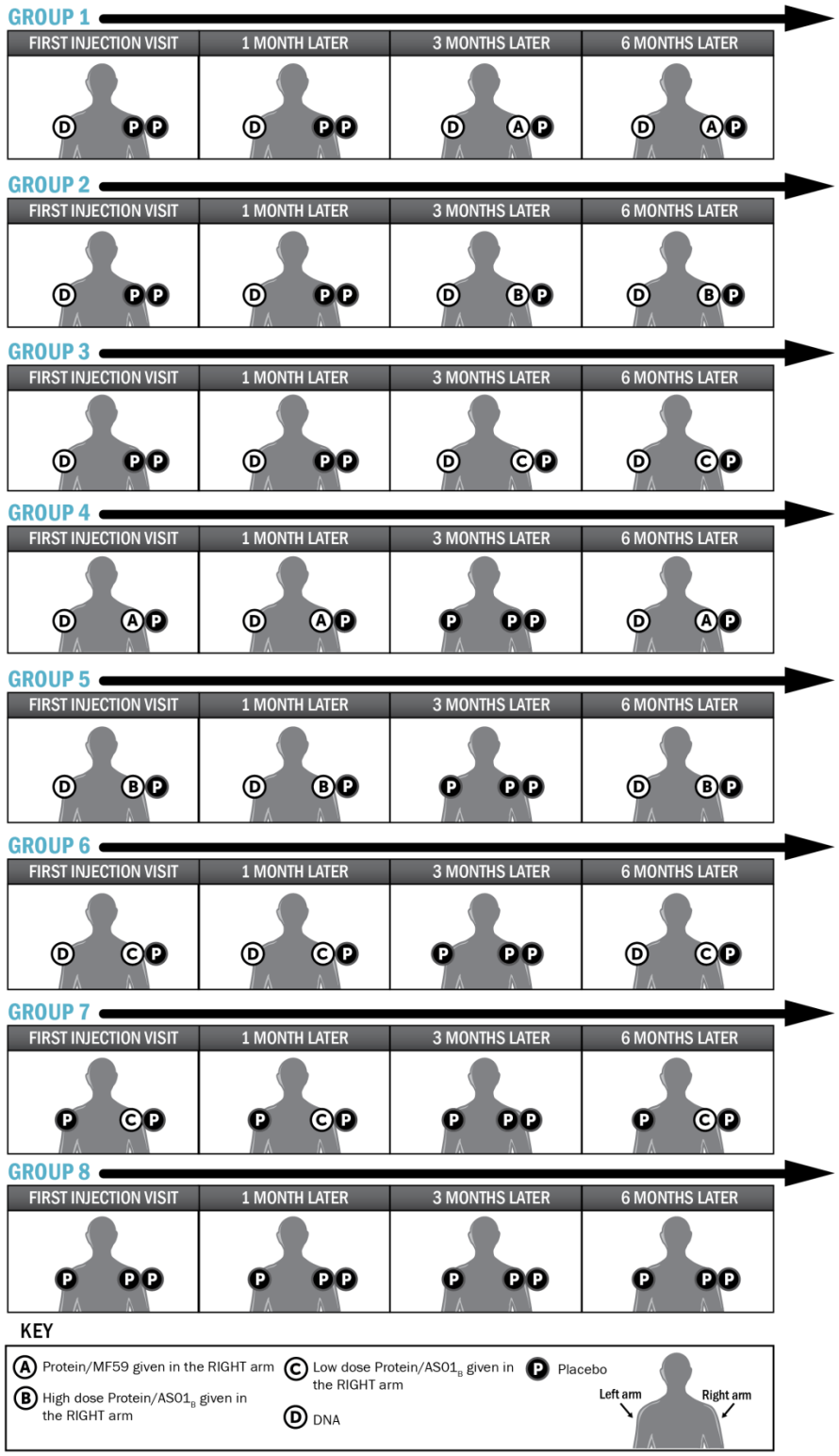
For participants who are unable to read or write, a witness should complete the signature block below:

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

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

Appendix E Injection schedule for sample informed consent form

This table shows the injections you will get while you are in the study. This table does not show all of your study visits.



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

Procedure	Screening visit(s)	First injection visit	Time after 1st injection visit									
			2 weeks	1 month	1½ months	3 months	3½ months	6 months	6 months + 1 week	6½ months	9 months	12 months
Injection		√		√		√		√				
Medical history	√											
Complete physical	√											√
Brief physical		√	√	√	√	√	√	√	√	√	√	
Urine test	√		√							√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√
Pregnancy test (participants born female)*	√	√		√		√		√		√**		√**
HIV testing and counseling	√					√		√			√	√
HIV risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√
Interview and/or questionnaire	√	√	√	√	√	√	√	√	√	√	√	√
Pap smear (if needed)	√											
Rectal fluids/cervical fluids/semen samples (optional)		√								√		√
Genital Tract Infection testing (urine, blood and swab for females) for people who agree to provide the optional samples		√								√		√
Stool Sample and/or swab (optional)***	(√)	(√)								√		

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.

Also not shown in this table is the after-study contact 6 months after your last scheduled study visit.

*People who are NOT of reproductive potential due to having had total hysterectomy or bilateral oophorectomy are not required to have pregnancy tests.

**Only for those who agree to provide cervical / rectal fluids samples.

***Stool sample collection at the Screening and First Injection visits indicates that only one stool sample collection will be performed at any point before the enrollment vaccination.

Appendix G Laboratory procedures

					Visit:	1	2	3	4	5	6	7	8	9	10	11	12	
					Day:	Screening visit[3]	D0	14	D28	D42	D84	D98	D168	D175	D182	D273	D364	
					Weeks		W0	W2	W4	W6	W12	W14	W24	W25	W26	W39	W52	
					Month		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.25	M6.5	M9	M12	
							VAC1		**VAC2**		**VAC3**		**VAC4**					
							DNA + Placebo OR DNA + Prot + MF59 OR DNA + Prot + AS01B OR Prot + AS01B OR Placebo		**DNA + Placebo OR DNA + Prot + MF59 OR DNA + Prot + AS01B OR Prot + AS01B OR Placebo**		**DNA + Prot + MF59 OR DNA + Prot + AS01B OR Prot + AS01B OR Placebo**		**DNA + Prot + MF59 OR DNA + Prot + AS01B OR Prot + AS01B 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 diagnostics[9]	UW-VSL / HSML-NICD	UW-VSL / 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	Local lab	Local lab	SST	5mL		5	5 [11]	—	—	—	—	—	—	—	5 [11]	—	5 [11]	**20**
Immunogenicity & Virologic assays[6]																		
HLA host genetics[7]	CSR	FHCRC	ACD	8.5mL		—	17	—	—	—	—	—	—	—	—	—	—	**17**
Cellular assays																		
ICS	CSR	FHCRC / CHIL	ACD	8.5mL		—	68	—	—	—	—	—	—	—	***68***	—	68	**204**
Humoral assays																		
Binding Ab Assay	CSR	Duke	SST	8.5mL		—	8.5	—	—	—	—	—	—	—	***8.5***	—	8.5	**25.5**
Neutralizing Ab Assay	CSR	Duke / SAIL-NICD	SST	8.5mL		—	8.5	—	—	—	—	—	—	—	***8.5***	—	8.5	**25.5**
Ab Avidity	CSR	Duke	SST	8.5mL		—	y	—	—	—	—	—	—	—	***y***	—	y	**0**
ADCC	CSR	Duke	SST	8.5mL		—	y	—	—	—	—	—	—	—	***y***	—	y	**0**
STORAGE																		
Serum storage	CSR	—	SST	8.5mL		—	17	—	—	—	—	—	—	8.5	8.5	17	8.5	**60**
PBMC storage	CSR	—	ACD	8.5mL		—	51	—	—	—	—	—	—	34	17	—	17	**119**
Visit total						**25**	**175**	**10**	**0**	**10**	**10**	**10**	**10**	**43**	**126**	**27**	**136**	**581**
56-Day total						**25**	**200**	**210**	**210**	**220**	**20**	**30**	**10**	**53**	**178**	**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	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)																		
Semen	CSR	Duke				—	X[13]	—	—	—	—	—	—	—	X	—	X	
Cervical Secretions	CSR	Duke				—	X[13]	—	—	—	—	—	—	—	X	—	X	
Rectal Secretions	CSR	Duke				—	X[13]	—	—	—	—	—	—	—	X	—	X	
STOOL COLLECTION (OPTIONAL)																		
Stool	CSR	FHCRC				—	X[14]	—	—	—	—	—	—	—	X	—	—	

[1]CSR = central specimen repository

[2]HVTN Laboratory Program includes laboratories at UW-VSL, Duke, FHCRC, HSML-NICD and SAIL-NICD, UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA); Duke = Duke University Medical Center (Durham, North Carolina, USA); Duke-ADCC = Antibody-Dependent Cellular Cytotoxicity Assay Laboratory, Duke University Medical Center (Durham, NC, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); CHIL = Cape Town HVTN Immunology Laboratory (Cape Town, South Africa); SAIL-NICD = South African Immunology, Laboratory–National Institute for Communicable Diseases (Johannesburg, South Africa); HSML-NICD = HIV Sero-Molecular 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 9.4 (postenrollment).

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

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

[8]For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination. Persons who have 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 9.13), 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 or swab 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 bacterial vaginosis, trichomonas vaginalis, and (if clinically indicated) yeast.

[13] Optional mucosal specimens must be collected prior to first vaccination once the participant has been found to have met mucosal specimen collection criteria specified in section 9.6.

[14] Optional stool specimens must be collected prior to first vaccination.

y = 17mL of SST blood collected for binding and neutralizing Ab assays will also cover specimen needs for Ab avidity and ADCC assays; no separate blood draw is needed.

Appendix H Procedures at HVTN CRS

Visit:	01[a]	02[n]	03	04	05	06	07	08	09	10	11	12	Post
Day:		D0	D14	D28	D42	D84	D98	D168	D175	D182	D273	D364	
Month:		M0	M0.5	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	—
Screening for low risk of HIV infection	X	—	—	—	—	—	—	—	—	—	—	—	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	—	—
Stool collection - optional[c]	—	X	—	—	—	—	—	—	—	X	—	—	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	X	X	X	—
Pregnancy prevention assessment[d]	X	X	X	X	X	X	X	X	X	X	X	X	—
Behavioral risk assessment questionnaire	X	—	—	—	—	X	—	X	—	—	X	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	—	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[e]	—	—	—	—	—	X	—	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	X	—	X	—	—	X	X
Local lab assessment													
Screening HIV test	X	—	—	—	—	—	—	—	—	—	—	—	—
Urine dipstick	X	—	X	—	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	X	—	X	—	X	—	X[g]	—	X[g]	—
CBC, differential, platelet	X	—	X	—	X	—	X	—	—	X	—	—	—
Chemistry panel	X	—	X	—	X	—	X	—	—	X	—	—	—
Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—
Syphilis	X	X[h]	—	—	—	—	—	—	—	X[h]	—	X[h]	—
Chlamydia/Gonorrhea[h]	—	X	—	—	—	—	—	—	—	X	—	X	—
Trichomonas vaginalis(cervical/vaginal swab)[i]	—	X	—	—	—	—	—	—	—	X	—	X	—

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

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

[c] Optional stool specimens must be collected prior to first vaccination.

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

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

[g] 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.

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

Bacterial vaginosis (cervical/vaginal swab)[i]	—	X	—	—	—	—	—	—	—	X	—	X	—
Yeast, if indicated (cervical/vaginal swab)[i]	—	X	—	—	—	—	—	—	—	X	—	X	—
Pap smear[j]	X	—	—	—	—	—	—	—	—	—	—	—	—
Mucosal secretion collection (optional)[k]													
Cervical, rectal, semen	—	X	—	—	—	—	—	—	—	X	—	X	—
Vaccination procedures													
Vaccination[l]	—	X	—	X	—	X	—	X	—	—	—	—	—
Reactogenicity assessments[m]	—	X	—	X	—	X	—	X	X	—	—	—	—
Poststudy													
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	X

[i] Cervical/vaginal swabs will only be collected from participants who agree to provide a cervical secretion sample; and, testing for Trichomonas vaginalis, bacterial vaginosis, and (if clinically indicated) yeast.

[j] Only for volunteers born female who consent to provide cervical secretion samples, per Sections 7.1 and 9.6. If collection of a pap smear is required, this may be done at any time provided the results are available prior to the collection of the cervical secretion samples.

[k] Optional mucosal specimens must be collected prior to first vaccination once the participant has been found to have met mucosal specimen collection criteria specified in in section 9.6.

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

[m] Reactogenicity assessments performed or recorded in the postvaccination symptom log for at least 7 days postvaccination (see Section 9.11).

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

Appendix I AESI contact

	Contact[a] Day	545
	Month	18
Procedures		
AESI assessment[b]		X

[a] Clinic visits are not required unless a participant indicates symptoms that require further assessment. See Section 9.5.
[b] See Appendix J.

Appendix J 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 108 Study Specific Procedures.*

<table>
<tr><th>Neuroinflammatory disorders</th><th>Musculoskeletal disorders</th><th>Skin disorders</th></tr>
<tr>
<td>
• 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
</td>
<td>
• 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
</td>
<td>
• Psoriasis
• Vitiligo
• Erythema nodosum
• Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)
• Alopecia areata
• Lichen planus
• Sweet’s syndrome
• Localized Scleroderma (Morphea)
• Cutaneous lupus erythematosus
Metabolic disorders
• Addison’s disease
• Autoimmune thyroiditis (including Hashimoto thyroiditis)
• Diabetes mellitus type I
• Grave's or Basedow’s disease
</td>
</tr>
<tr><th>Vasculitides</th><th>Blood disorders</th><th>Others</th></tr>
<tr>
<td>
• 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.
</td>
<td>
• Autoimmune hemolytic anemia
• Autoimmune thrombocytopenia
• Antiphospholipid syndrome
• Pernicious anemia
• Autoimmune aplastic anemia
• Autoimmune neutropenia
• Autoimmune pancytopenia
Gastrointestinal disorders
• Celiac disease
• Crohn’s disease
• Ulcerative colitis
• Ulcerative proctitis
Liver disorders
• Autoimmune cholangitis
• Autoimmune hepatitis
• Primary biliary cirrhosis
• Primary sclerosing cholangitis
</td>
<td>
• 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
</td>
</tr>
</table>