HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 120

A phase 1/2a clinical trial to evaluate the safety and immunogenicity of ALVAC-HIV (vCP2438) and of MF59®- or AS01$_B$-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

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CLINICAL TRIAL SPONSORED BY

Division of AIDS (DAIDS)
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GlaxoSmithKline Biologicals S.A.
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Contents

1 Ethical considerations

Multiple candidate HIV vaccines will need to be studied simultaneously in different populations around the world before a successful HIV preventive vaccine is found. It is critical that universally accepted ethical guidelines are followed at all sites involved in the conduct of these clinical trials. The HIV Vaccine Trials Network (HVTN) has addressed ethical concerns in the following ways:

- HVTN trials are designed and conducted to enhance the knowledge base necessary to find a preventive vaccine, using methods that are scientifically rigorous and valid, and in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and/or 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.

- Participants who become HIV-infected during the trial are referred to medical practitioners to manage their HIV infection and to identify potential clinical trials they may want to join. If a program for antiretroviral therapy (ART) provision is not available at a site and ART is needed, a privately established fund will be used to pay for access to treatment to the fullest extent possible.

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

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

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

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

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

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

2 IRB/EC review considerations

US Food and Drug Administration (FDA) and other US federal regulations require IRBs/ECs 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 Africa, with funding from the US NIH among others. 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. These research regulations and guidelines are based on ethical principles of respect for persons, beneficence, and justice. 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) ICH and/or GCP guidelines, each research location has a locally based Principal Investigator (PI) who is qualified to conduct (and supervise the conduct of) the research. 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.

2.1 Minimized risks to participants

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

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

2.2 Reasonable risk/benefit balance

45 CFR 46.111(a) 2 and 21 CFR 56.111(a) 2: Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and

the importance of the knowledge that may reasonably be expected to result.

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

2.3 Equitable participant selection

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

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

2.4 Appropriate informed consent

45 CFR 46.111 (a) 4 & 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 (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 (Section 11). Safety is monitored daily by HVTN Core and routinely by the HVTN 120 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 on Confidentiality and Use of Data and Specimens with the HVTN. In some cases, a comparable confidentiality agreement process may be acceptable. 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 ALVAC-HIV (vCP2438) and of MF59®- or AS01B-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

Primary objectives

Primary objective 1

- To evaluate the safety and tolerability of ALVAC-HIV and bivalent gp120 protein/MF59 or bivalent gp120 protein/$AS01_B$

Primary objective 2

- To compare HIV-specific CD4+ T-cell response rates at the month 6.5 timepoint (2 weeks after the fourth vaccination) of ALVAC-HIV and bivalent gp120 protein/MF59 to each of the bivalent gp120 protein/$AS01_B$ vaccine regimens.

Primary objective 3

- To compare HIV-specific Env-gp120 binding antibody response magnitudes at the month 12 timepoint (6 months after the fourth vaccination) of ALVAC-HIV and bivalent gp120 protein/MF59 to each of the bivalent gp120 protein/$AS01_B$ vaccine regimens.

Study products and routes of administration

- **ALVAC-HIV (vCP2438)** expresses the gene products 96ZM651 *gp120* (clade C strain) linked to the sequences encoding the HIV-1 transmembrane anchor (TM) sequence of *gp41* (28 amino acids clade B LAI strain) and *gag* and *pro* (clade B LAI strain). It is formulated as a lyophilized vaccine for injection at a viral titer $\geq 1 \times 10^6$ cell culture infectious dose $(CCID)_{50}$ and $< 1 \times 10^8$ $CCID_{50}$ (nominal dose of 10^7 $CCID_{50}$) and is reconstituted with 1 mL of sterile sodium chloride solution (NaCl 0.4%), 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.

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	50	100 mcg	Left	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV
			Right	-	-	Protein/MF59 + Placebo*	Protein/MF59 + Placebo*
2	50	100 mcg	Left	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV
			Right	-	-	Protein/AS01$_B$ + Placebo*	Protein/AS01$_B$ + Placebo*
3	50	20 mcg	Left	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV
			Right	-	-	Protein/AS01$_B$ + Placebo*	Protein/AS01$_B$ + Placebo*
4	10	N/A	Left	Placebo	Placebo	Placebo	Placebo
			Right	-	-	Placebo + Placebo*	Placebo + Placebo*

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

Participants

160 healthy, HIV-uninfected volunteers aged 18 to 40 years; 150 vaccinees, 10 placebo recipients

Design

Multicenter, randomized, controlled, double-blind trial

Duration per participant

12 months of scheduled clinic visits

Estimated total study duration

18 months (includes enrollment and follow-up)

Investigational New Drug (IND) study sponsor

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

Study product providers

- ALVAC-HIV (vCP2438) and diluent (NaCl 0.4%): Sanofi Pasteur (Swiftwater, PA, USA)
- Bivalent Subtype C gp120: GlaxoSmithKline Biologicals S.A. (GSK Vaccines) (Rixensart, Belgium)
- MF59: GSK Vaccines (Rixensart, Belgium)
- $AS01_B$: GSK Vaccines (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)

HIV diagnostic laboratories

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

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

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 Africa and US to be specified in the Site Announcement Memo

Safety monitoring

HVTN 120 PSRT; HVTN SMB

3.1 Protocol Team

Protocol leadership

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	Mark Lungu CIDRZ CTU CAB	*Community engagement unit representatives*	Nandi Luthuli HVTN Core, FHCRC
Regional medical liaison	Simba Takuva HVTN Core, PHRU	*Community educator/recruiter*	Charles Chasakara UZ-UCSF Collaborative Research Programme
Regulatory affairs	Meg Brandon HVTN Core, FHCRC	*Technical editor*	Richa Chaturvedi HVTN Core, FHCRC
DAIDS protocol pharmacist	Irene Rwakazina DAIDS, NIAID	*Laboratory Program representative*	On Ho HVTN Lab, FHCRC

4 Background

4.1 Building on RV144

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

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.

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 parts of 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 (number of infections $n = 51$ 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 post

vaccination (estimated 60.5%, 95% CI 22-80) with the vaccine effect waning over time to cumulative 31% 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 RV144 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 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 UNAIDS 2016 Fact Sheet, there were an estimated 36.7 million people living with HIV in 2015 globally. There were 2.1 million new HIV infections globally and 1.1 million AIDS deaths in 2015 [10]. Therefore, despite the well-recognized benefits from scaling-up Antiretroviral Therapy (ART) and certain HIV prevention strategies, the global burden of HIV remains enormous.

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.

Eastern and Southern Africa bears the preponderant burden of the HIV epidemic. With only 6.4% of the world's population, this region harbors half of the world's people living with HIV/AIDS and 46% of the world's new infections occurred here in 2015. The vast majority of newly acquired infections in this region occur during unprotected heterosexual intercourse and subsequent transmission to newborns and breastfed babies. Approximately 910,000 new HIV infections occurred in Eastern and Southern Africa in 2015 [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 1.1 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. There were an estimated 37,600 new infections in 2014, 45% of these in African Americans, and 24% in Hispanics/Latinos. 70% of new infections were estimated to be among men who have sex with men. 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.

4.3 Rationale for HVTN 120

The P5 partnership is investigating 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.

Based in large part on the success seen in the RV144 trial, the HVTN is conducting HIV vaccine studies in South Africa using a vaccine regimen that is similar to that tested in RV144. Two trials, designated HVTN 100 and HVTN 702, are evaluating ALVAC-HIV (vCP2438) prime at months 0 and 1 followed with three boosts of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59 at months 3, 6, and 12. Based on HVTN 100 interim safety and immunogenicity from two weeks after the second boost a decision has been made to advance the same vaccine regimen into a pivotal efficacy trial. This pivotal trial, HVTN 702, opened to enrollment on 21-October-2016 in the Republic of South Africa.

The ALVAC-HIV vector backbone in these trials is the same as was tested in RV144 but encodes a subtype C envelope, in lieu of the subtype E envelope, to better match the HIV subtype most prevalent in Southern Africa.

HVTN 120 will compare the HVTN100/HVTN702 regimen (without a boost at Month 12) with two corresponding regimens containing the $AS01_B$ adjuvant, one at the same protein dose (100 mcg), and the other at a lower protein dose (20 mcg). As such, HVTN 120 will generate supporting safety and immunological data regarding protein dose and adjuvant type in context of the HVTN 702 regimen. Systematic evaluations of well-characterized adjuvant/immunogen formulations, such as proposed in HVTN 120, will aid in developing an HIV vaccine that can elicit and drive effective and durable functional immune responses against HIV. With that, results can help guide the way forward in the development of an efficacious preventative HIV vaccine regimen. Once the optimal dose and adjuvant have been determined, further trials, in a sequential manner, can be used to improve upon the current regimen.

4.3.1 Rationale for MF59 and $AS01_B$ adjuvantation

A unique and scientifically compelling aspect of this trial design is the use of 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 [13], and the availability of novel commercial adjuvants that are potent and safe has been heralded as an important contribution that may advance HIV vaccines [14,15].

The MF59 adjuvant, an oil-in-water emulsion, is licensed for several flu vaccines in multiple countries, and in pre-clinical models [16] has demonstrated recruitment of antigen presenting cells and up-regulation of cytokines, chemokines, and receptors. The adjuvant has improved antibody affinity maturation [17], 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 [18]. 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.

The Adjuvant System AS01 is a liposome-based class of adjuvants which contains 2 immunostimulants, 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and QS-21 [19]. MPL is a non-toxic derivative of the lipopolysaccharide from *Salmonella minnesota* and is a TLR4 agonist [20]. It can stimulate NF-κB transcriptional activity and subsequent cytokine production [20]. MPL directly activates APCs such as dendritic cells (DCs) to produce cytokines and elevated levels of costimulatory molecules [21-23]. QS-21 is a natural saponin molecule extracted from the bark of the South American tree Quillaja saponaria Molina [24] (reviewed in [19,25]). 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 [26,27] and high Ag-specific Ab responses in humans [28]. 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 [29]. 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 [19,30-38] and recently with protection against malaria in African children [30,31]. 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 [39].

GSK PRO HIV-002 evaluated the gp120W6.1D 20 mcg / NefTat 20 mcg candidate HIV vaccine (clade B) 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 4-1 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 4-1 C showing $AS01_B$ responses).

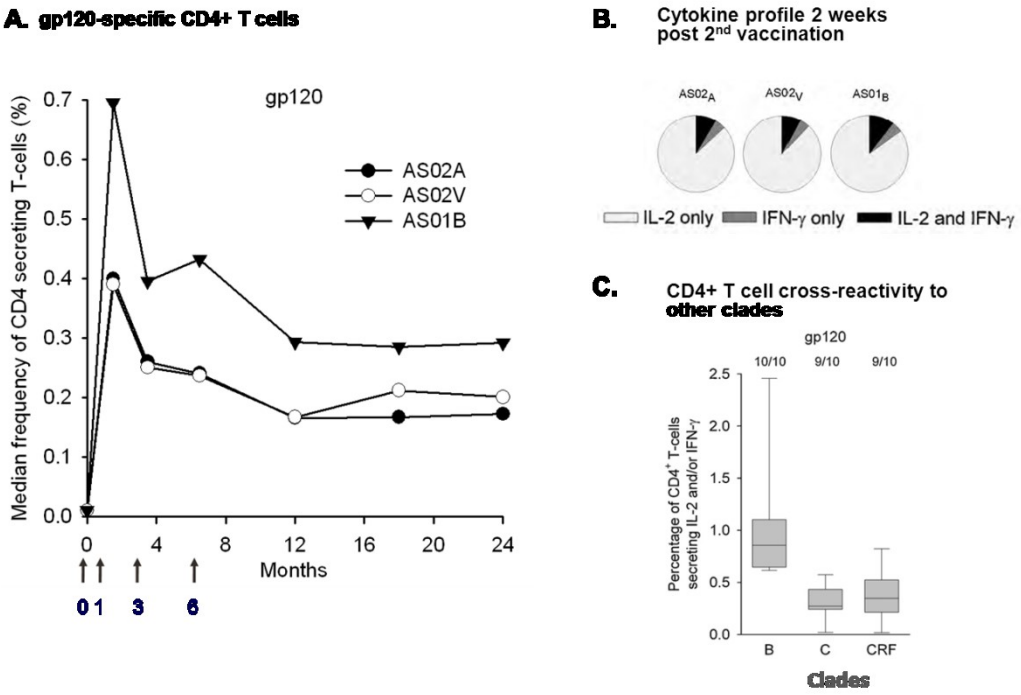

Figure 4-1 CD4+ T cell responses following vaccination with the gp120/NefTat vaccine candidate formulated with 1 of 3 different Adjuvant Systems ($AS02_A$, $AS02_V$ and $AS01_B$) in study PRO HIV-002

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 [38]. CD4+ T-cell response rate and magnitude are widely applied, validated measures of HIV vaccine induced immunogenicity. CD4+ T-cell magnitude is also frequently correlated with the polyfunctionality score proposed by Lin et al, which has been identified as a correlate of risk in RV144 [40].

Retrospective testing of stored specimens from the GSK PRO HIV-002 clinical study that evaluated gp120W6.1D/NefTat in different Adjuvant Systems was performed in Dr. Georgia Tomaras' lab, which had contributed to 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, after the vaccination all subjects had detectable IgG1 responses, and had detectable IgA against gp120 vaccine protein [41].

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

participants. 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 4-2) [42].

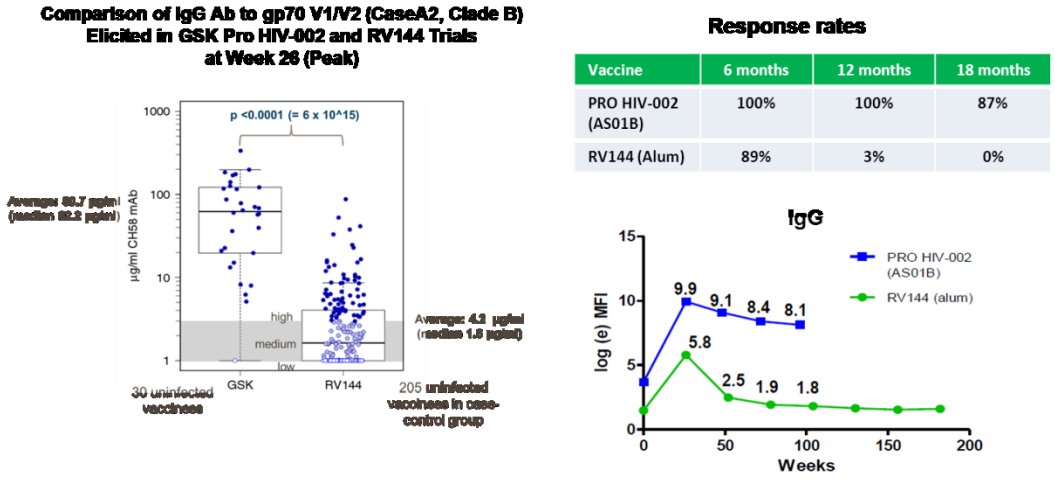

Figure 4-2 Total IgG Ab to gp70 V1V2 (Case A2, Clade B) elicited in GSK Pro HIV-002 and RV144 trials

Vaccination with gp120W6.1D (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 4-3) [42].

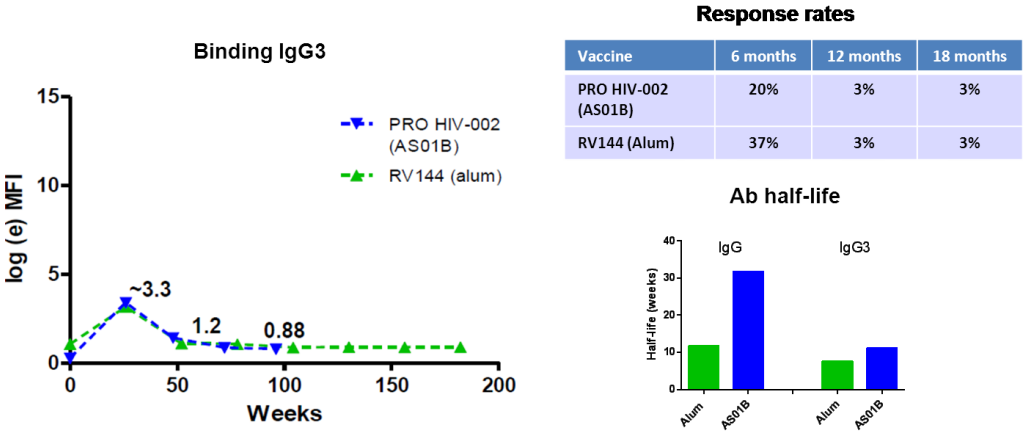

Figure 4-3 IgG3 Ab to gp70 V1V2 (Case A2, Clade B) elicited in GSK Pro HIV-002 and RV144 trials

A subset of GSK PRO HIV-005 (Section 4.3.2) volunteers primed 3 years before with 2 doses of 10 mcg F4, which is a recombinant fusion protein containing 4

HIV-1 clade B antigens (Gag p24, Pol-RT, Nef, and Gag p17) adjuvanted with $AS01_B$, 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 and 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 4-4) [33].

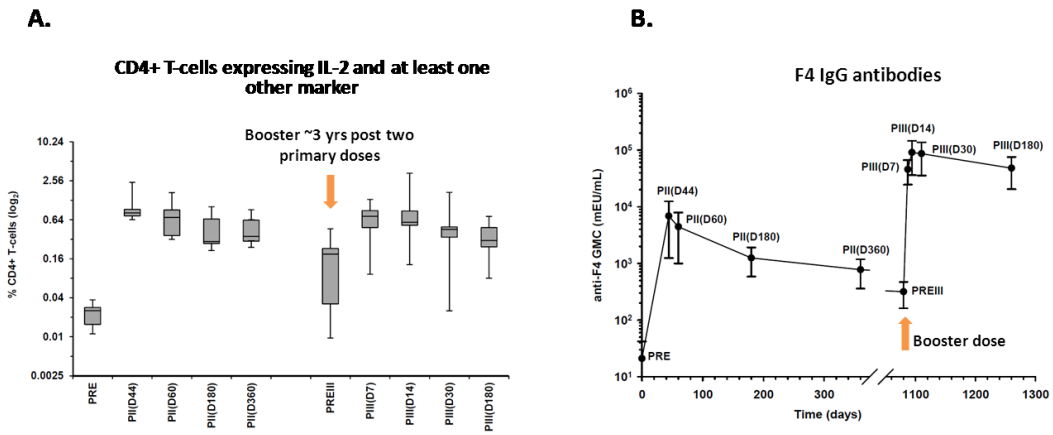

Figure 4-4 CD4+ T cell and antibody responses following primary and booster doses of the F4 / $AS01_B$ vaccine candidate in studies PRO HIV-005 and ECR-004.

4.3.2 Rationale for 2 protein doses with $AS01_B$ (20 mcg vs. 100 mcg)

There are only limited data available evaluating differences in immune responses with varying doses of Env proteins in the context of a common adjuvant and co-administration with a common ALVAC-HIV. Study RV135 evaluated the immune responses to a regimen identical to the one used in RV144 but utilizing two different doses of Env proteins (either 200 mcg or 600 mcg total [100 or 300 each of MN and A244 proteins]), both adjuvanted with Alum and given in combination with ALVAC-HIV (vCP1521) as a boost.

In RV135, humoral immune response assessments included Env binding and neutralizing antibody titers. Study participants receiving 200 mcg of AIDSVAX B/E had lower anti-MN and anti-A244 antibody response rates (95% and 86%, respectively) at peak immunogenicity (2 weeks after the fourth immunization) compared to participants receiving 600 mcg of AIDSVAX B/E (100% and 96%, respectively). Geometric mean titers (GMT) of antibodies to MN and A244 were also lower overall for participants in the lower dose group (3744 and 596, respectively) compared to the higher dose group (7730 and 1691, respectively). Similarly, neutralization titers indicated a dose-dependent humoral response to AIDSVAX B/E, with 47% of lower dose recipients and 71% of higher dose recipients showing neutralizing antibodies to either of two CRF01_AE targets (NP03/H9 or CM244/A3R5).

There are no studies evaluating differences in immune responses with varying doses of Env proteins in the context of ALVAC prime-boost and protein adjuvantation with MF59, AS01 or AS02 (oil-in-water emulsion of a combination of MPL and QS-21). However, there are trials evaluating different doses of AS01 adjuvanted proteins without viral vectors.

Clinical trials assessing gp120 with a 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 gp120W6.1D) formulated with $AS02_A$ 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 4-5).

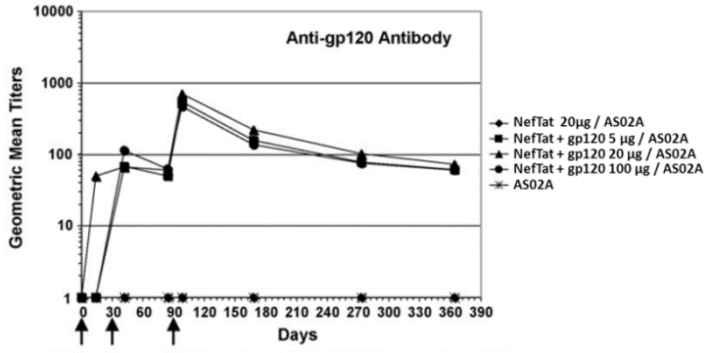

Figure 4-5 Kinetic of binding IgG antibodies induced by the NefTat/gp120/$AS02_A$ vaccine candidate in study HVTN 041

Antibodies able to neutralize the T-cell laboratory-adapted strain of HIV-1gp120W6.1D 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 (100 mcg) measured by lymphoproliferative and ICS assays [36] (see Figure 4-6).

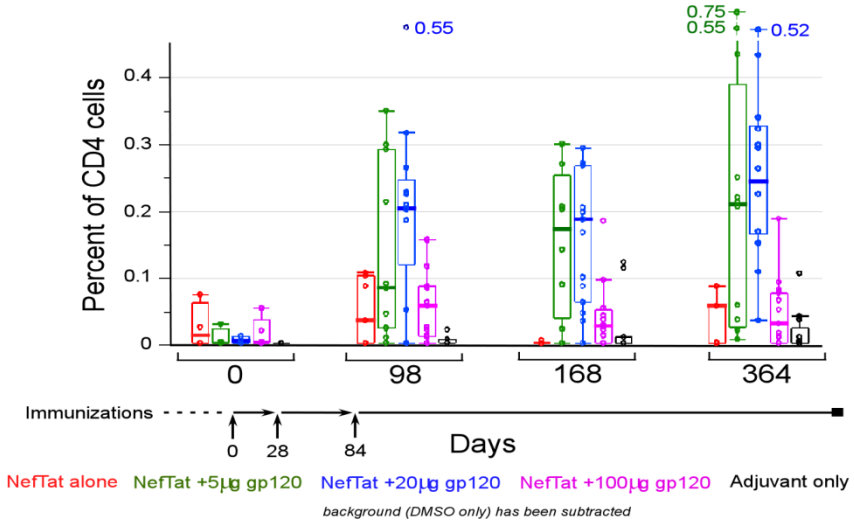

Figure 4-6 gp120-specific CD4+ T cell responses (IFN-γ and/or IL-2) induced by the NefTat/gp120/AS02$_A$ vaccine candidate in study HVTN 041

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 [43].

Combined together, data from HVTN 041 and GSK PRO HIV-005 have been used to generate the hypothesis that a higher dose of gp120 may dampen resulting CD4+ T-cell responses. However, as stated above, there are no data evaluating differences in immune responses with varying doses of Env proteins in the context of ALVAC prime and a boost of ALVAC co-administered with protein adjuvanted with MF59 or AS01. HVTN 120 will provide the direct comparison of different doses of gp120 adjuvanted with AS01B in the context of ALVAC prime-boost and another study (HVTN 108) will provide this direct comparison in the context of DNA prime-boost, DNA co-administration, and gp120 alone.

4.4 Study product descriptions

4.4.1 ALVAC-HIV (vCP2438)

ALVAC-HIV (vCP2438) is a preparation of live, attenuated recombinant canarypox-derived virus expressing products from the HIV-1 *env gp120* (clade

C), *env gp41 TM* (clade B), *gag* (clade B), and *protease* (clade B) coding sequences and cultured in primary chicken embryo fibroblasts (CEFs).

4.4.1.1 Constructs

The original strain of canarypox virus (Rentschler strain) was attenuated by serial passages on CEFs. The attenuated virus was plaque isolated and designated as ALVAC. Details of the manufacturing process are provided in the ALVAC-HIV (vCP2438) Investigator's Brochure (IB).

The inserted HIV-1 gene sequences are:

The region of the *env* gene encoding the extracellular Env gp120 moiety of the 96ZM651 strain of HIV-1 linked to the sequence encoding the HIV-1 TM anchor sequence of gp41 (28 amino acids) from HIV-1 strain LAI. The *env* gene sequence is under the control of the vaccinia virus H6 promoter.

The *gag* gene encoding the entire Gag protein and a portion of the *pol* sequences of the LAI strain of HIV-1 sufficient to encode the protease function. The *gag/protease* gene sequences are under the control of the same vaccinia virus I3L promoter.

Table 4-1 ALVAC-HIV (vCP2438) Construct Summary Table

Inserted gene	Strain	Promoter	Insertion Locus
env (gp120 + gp41 TM)	96ZM651 (gp120) LAI (gp41 TM)	H6 (vaccinia)	C6
gag + *pro*	LAI	I3L (vaccinia)	C6

4.4.1.2 Formulation characteristics

ALVAC-HIV (vCP2438) is formulated as a lyophilized vaccine for injection and is reconstituted with 1 mL of sterile sodium chloride solution (NaCl 0.4%) for injection of 1 mL as a single dose. The composition of 1 dose of ALVAC-HIV (vCP2438) is provided in Table 4-2.

Table 4-2 Composition of ALVAC-HIV (vCP2438)

Ingredient	Amount in 1 dose	Function
ALVAC-HIV (vCP2438)	≥ 1 x 10^6 $CCID_{50}$ and < 1 × 10^8 $CCID_{50}$	Immunogen
Tris-HCl	0.3 mg	Buffer
Lactose-monohydrate	26.325 mg	Component of lactoglutamate stabilizer
L-Glutamic acid	0.278 mg	Component of lactoglutamate stabilizer
$NaH_2PO_4.2H_2O$	0.15 mg	Component of lactoglutamate stabilizer
K_2HPO_4	0.55 mg	Component of lactoglutamate stabilizer
KOH	0.1 mg	Component of lactoglutamate stabilizer
Sucrose	50 mg	Component of freeze-drying stabilizer
Sodium glutamate monohydrate	5.5325 mg	Component of freeze-drying stabilizer
HCl	1.8 mg	Component of freeze-drying stabilizer
Non-essential amino acids	1.628 mg	Component of freeze-drying stabilizer
Essential amino acids	4.46 mg	Component of freeze-drying stabilizer
HCl	1.75 mg	Component of freeze-drying stabilizer

4.4.1.3 Manufacturing

ALVAC-HIV (vCP2438) Bulk Drug Substance is manufactured by IDT Biologika GmbH, Am Pharmapark, Dessau-Rosslau, Germany, under contract to Sanofi Pasteur. ALVAC-HIV (vCP2438) Drug Product is manufactured at the Sanofi Pasteur SA, facility located in Marcy l'Etoile, France. The diluent used for reconstitution is manufactured at the Sanofi Pasteur Inc. facility located in Swiftwater, Pennsylvania (USA).

ALVAC-HIV (vCP2438) is produced by inoculating the virus seed into cultured primary CEFs derived from eggs produced by specific pathogen free (SPF) flocks.

The manufacturing process for ALVAC-HIV (vCP2438) is similar to the manufacturing process for ALVAC-HIV (vCP1521) used in RV144.

4.4.2 Bivalent Subtype C gp120

4.4.2.1 Constructs

Bivalent Subtype C gp120, manufactured by GSK Vaccines at 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 HIV Env surface glycoprotein containing the receptor-binding domain. Each gp120 is modified from its wild type full-length form (gp160) by replacement of the native signal sequence and deletion of the entire gp41 C-terminal portion of the glycoprotein containing the TM and cytoplasmic domains.

4.4.2.2 Manufacturing and formulation

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

Following clone selection, a fed batch cell culture at 500 L or 1000 L 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 -61°C or colder. 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 qualitative composition per dose of each subtype C gp120 vaccine protein is provided in Table 4-3.

Table 4-3 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 Bivalent Subtype C gp120/MF59 IB.

4.4.3 Bivalent Subtype C gp120/MF59 for injection

The combination of the 2 subtype C gp120 proteins and the MF59 adjuvant is referred to as Bivalent Subtype C gp120/MF59. A final dose of 100 mcg of each recombinant Env protein will be mixed with MF59 adjuvant. The composition of 1 dose of the resulting vaccine is shown in Table 4-4.

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

Ingredient	Amount in1 dose	Function
Drug Substances		
TV1.C gp120 protein 1086.C gp120 protein	100 mcg 100 mcg	active active
Adjuvant (MF59)		
Squalene	9.75 mg	oil phase
Polysorbate	1.175 mg	surfactant
Sorbitan Trioleate	1.175 mg	surfactant
Excipients		
Sodium Citrate, Dihydrate Citric Acid, Monohydrate Sodium Chloride Water for injections	1.39 mg 0.051 mg 4.38 mg qs to 0.5 mL	buffer buffer tonicity modifying agent solvent

4.4.4 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 compositions of 1 dose of each of the resulting vaccines are shown in Table 4-5 and Table 4-6.

Table 4-5 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 one 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 4-6 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 one 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

HVTN 120 has 3 active groups and 1 placebo group. The study will compare ALVAC priming at months 0 and 1 followed by ALVAC + Protein boosting at months 3 and 6. Group 1 will evaluate a protein dose of 100 mcg, adjuvanted with MF59, which represents the regimen applied in HVTN 100 and HVTN 702. Group 2 will evaluate the same protein dose, with the AS01$_B$ adjuvant. Group 3 will evaluate the proteins at a lower dose of 20 mcg, adjuvanted with AS01$_B$. This approach can help to determine the protein dose and the adjuvant that can elicit the most promising immune responses in the context of the ALVAC + protein regimen, and as such can pave the way to potentially improve upon this already promising vaccine regimen. The placebo group is primarily included to provide controls for immunological endpoints.

4.5.1 Dose rationale

4.5.1.1 ALVAC-HIV (vCP2438) dose

ALVAC-HIV (vCP2438): Viral titer $\geq 1 \times 10^6$ CCID$_{50}$ and $< 1 \times 10^8$ CCID$_{50}$ (nominal dose of 10^7 CCID$_{50}$) lyophilized vaccine to be reconstituted for IM injection.

This study will utilize the same ALVAC-HIV dose that was used for the RV144 study. The ALVAC-HIV (vCP1521) dose targeted for study RV144 was $> 10^6$ CCID$_{50}$. The actual ALVAC-HIV titers from the 12 vaccine lots used in the RV144 study ranged from $10^{7.06}$ CCID$_{50}$ to $10^{7.41}$ CCID$_{50}$.

Titers of the ALVAC-HIV (vCP205) construct used in studies ranged from $10^{5.6}$ $CCID_{50}$ to $10^{6.85}$ $CCID_{50}$. A dose response analysis was conducted with samples collected on days 98 and 182 in the AIDS Vaccine Evaluation Group (AVEG) 022, 022A, 027, 032, 033, 034 and 034A studies. In summary, these data indicate that while there is not a positive dose-response relationship between ALVAC-HIV and cytotoxic T lymphocyte (CTL) responses, use of the lower titer is not optimal for induction of nAb responses. Therefore, many clinical studies in humans have targeted an ALVAC dose > 10^6 $CCID_{50}$.

HVTN 039 is the only study that has compared the safety and immunogenicity of ALVAC-HIV (vCP1452) given at the standard dose ($10^{7.25}$ $CCID_{50}$) to a dose 5.6 times higher (10^8 $CCID_{50}$), and placebo [44]. The high-dose ALVAC-HIV (vCP1452) resulted in unacceptable levels of reactogenicity, without evidence of improved immunogenicity. Although extrapolation of these findings to other ALVAC-HIV vaccines requires caution, the study suggested that an ALVAC-HIV dose < 10^8 $CCID_{50}$ is desirable.

4.5.1.2 Bivalent Subtype C gp120/MF59 dose

For the Bivalent Subtype C gp120/MF59 vaccine component, 100 mcg each of the 2 gp120 subtype C proteins (TV1 gp120 and 1086 gp120) will be mixed with the oil-in-water emulsion MF59 (9.75 mg squalene) by the Pharmacist at each CRS prior to IM administration.

The 200 mcg total dose was selected based on previous clinical experience with similar proteins. Limited dose range studies performed with Novartis (formerly Chiron) subtype B SF2 gp120 and subtype E gp120 protein candidates indicated that 50 mcg and 100 mcg, totaling 150 mcg, doses with MF59 adjuvant were immunogenic and well tolerated [45]. Bivalent Subtype C gp120/MF59 vaccine has been administered to humans in HVTN 100 and interim data are summarized in 4.7.2. For a detailed overview on previous human experience with MF59 and similar protein vaccines, see Section 4.7.6.

4.5.1.3 Bivalent Subtype C gp120/$AS01_B$ dose

For the Bivalent Subtype C gp120/$AS01_B$ vaccine component, 100 mcg or 20 mcg each of the 2 gp120 subtype C proteins (TV1 gp120 and 1086 gp120) will be mixed with the adjuvant by the Pharmacist at each CRS prior to IM administration. This dose comparison is supported by previous data obtained by GSK showing that protein doses that are too high may lead to suboptimal vaccine responses. This specific combination of Bivalent Subtype C gp120/$AS01_B$ at both doses will also be evaluated in combination with an HIV clade C DNA vaccine in another trial, HVTN 108. As of 09 December 2017, HVTN 108 has enrolled 246 participants. The study is still blinded, but approximately 78% of participants will receive Bivalent Subtype C gp120/$AS01_B$. So far, there have been no related serious adverse events. 13 adverse events have been deemed related to study product, 11 mild and 2 moderate. There have been 2 cases of grade 3 fever, and 3

cases of grade 4 fever, and 12 cases of other grade 3 systemic reactions, all self-limiting, as well as 5 cases of grade 3 erythema/induration, all self-limiting.

4.5.2 Schedule

The vaccination schedule of the regimens in this study is based upon the schedules used in RV144, HVTN 100, and HVTN 702.

4.5.3 Rationale for the adaptive and mucosal sampling timepoints

Examining the effects that the two adjuvants have on a variety of immune responses relative to each other is an important goal of this study. Adjuvants may influence antigen delivery, presentation, and immune responses and the constitution of the microbiome may influence immune responses in the context of either adjuvant.

Systemic adaptive immune responses will be measured in samples collected at day 0 prior to vaccination, month 3.5 (2 weeks following the third vaccination), month 6.25 (1 week following the fourth vaccination), and month 6.5 (2 weeks following the fourth vaccination). These samples will provide both baseline and postvaccine information.

Mucosal samples collected at day 0 provide the baseline values prior to vaccination while those collected at month 6.5 (2 weeks following the final vaccination), provide data on peak vaccine-induced systemic and mucosal adaptive immune responses.

Finally, collection at month 12 will provide the opportunity to assess the durability of systemic and mucosal immune responses 6 months after the last vaccination.

4.5.4 Choice of placebo

Sodium Chloride for Injection, 0.9% will be administered as a placebo for ALVAC-HIV.

Sodium Chloride for Injection, 0.9% will also serve as a placebo for the Bivalent Subtype C gp120/MF59 and Bivalent Subtype C gp120/AS01$_B$ vaccines. These placebos are necessary to maintain blinding as Protein/AS01$_B$ and Protein/MF59 consist of different injection volumes.

4.6 Summary of preclinical safety studies

4.6.1 ALVAC-HIV (vCP2438)

The toxicity studies described in Sections 4.6.1.1 and 4.6.1.2 were originally designed to match a different clinical trial protocol that included additional

vaccine regimens with the DNA-HIV-PT123 study product. However, the vaccine regimens relevant to HVTN 120 were also evaluated, therefore, the resulting data summaries from these studies have been included.

4.6.1.1 Intramuscular local tolerability and systemic toxicity study in New Zealand White Rabbits (Study AB20670)

The objective of the study was to determine the local tolerability and systemic toxicity of ALVAC-HIV (vCP2438)/ALVAC-HIV (vCP2438) with gp120+MF59 vaccines and DNA-HIV-PT123 with gp120+MF59 vaccines administered by the intramuscular route to New Zealand White Rabbits 7 or six times, respectively, at two-week intervals, followed by a two-week recovery period.

There were no deaths during the study. No treatment-related clinical signs were reported during the study and treatment was locally well tolerated. Body temperature was slightly increased mostly after the first injection but returned to normal with 48 hours. There were no effects of treatment on body weight or food consumption. No treatment-related ophthalmological findings were observed at the end of the treatment period.

When compared to the control group, a transient increase in C-reactive protein, globulin, fibrinogen or neutrophil count was observed after one or more of the immunizations. These effects correlated with the inflammatory findings observed histopathologically at the injection sites. There were no other treatment-related differences from the controls amongst the biochemistry or haematological parameters.

At necropsy, at the end of treatment, the only histologic changes due to the test items were in the injection sites and iliolumbar and sacral lymph nodes. In the sites injected with ALVAC or gp120s+MF59, the changes comprised inflammatory cell infiltrates, necrosis, fibrosis, hemorrhage, acellular material and mineralization. These findings were often only minimal or slight. In the lymph nodes that drained the injected sites, there was minimal or slight increased lymphoid follicle development, increased paracortex and granulocyte infiltrate. There was evidence of partial resolution of the described changes at both injection site and lymph nodes, based on necropsy observations after the recovery period.

In conclusion, under the defined study conditions, seven intramuscular administrations of ALVAC-HIV (vCP2438) vaccine associated with gp120s/MF59 (last 4 injections) to the New Zealand White Rabbit at two-week intervals were clinically and locally well tolerated.

4.6.1.2 DNA-HIV-PT123 + ALVAC/Bivalent Subtype C gp120 with MF59C.1 or $AS01_B$ and ALVAC/Bivalent Subtype C gp120 with $AS01_B$ – Intramuscular local tolerability and systemic toxicity (repeated dose, total of 7 doses) study in the New Zealand White rabbit (Study No. AB20850)

A GLP local tolerance and toxicity study in rabbits was conducted to evaluate the potential toxicity of vaccine regimens consisting of the combination of DNA-HIV-PT123, ALVAC-HIV (vCP2438), and Bivalent Subtype C gp120 with MF59 or $AS01_B$.

The test formulations were administered every other week (i.e., days 1, 15, 29, 43, 57, 71 and 85) during the study via intramuscular injection into the quadriceps muscle for all groups.

Animals were subjected to a full macroscopic examination at necropsy (5/group/sex) on day 87 and on day 99 following a two week no-treatment recovery period.

Parameters evaluated during the study period included mortality, clinical and cage-side observations, dermal injection site observations, body weights, body temperatures, food consumption, ophthalmology, clinical pathology, immunogenicity, macroscopic pathology, absolute and relative organ weights, and histopathology.

All animals survived to their scheduled necropsies. Treatment with DNA-HIV-PT123 + ALVAC/Bivalent Subtype C gp120 administered with MF59C.1 or $AS01_B$ adjuvant, as well as ALVAC/Bivalent Subtype C gp120 administered with $AS01_B$ adjuvant was well tolerated and there were no effects on systemic clinical observations or local reactions, body weight, food consumption, and ophthalmological evaluations.

Limited and transient increases in body temperature were noted in some animals mainly following ALVAC administration. Reversible treatment-related clinical pathology changes indicative of inflammatory reaction consisted of increased neutrophil counts, slightly elevated fibrinogen concentration, increased CRP and globulin concentrations. Consistent with these changes, and the immune response to vaccination, findings were observed histopathologically at the injection sites and in the spleen and draining iliolumbar lymph nodes.

At the injection sites, histopathological changes consisted of hemorrhage, inflammatory changes (mixed inflammatory cell infiltration) and myofiber degeneration in the muscular tissue in almost all treated rabbits, which accounted for the dark appearance of the injection sites noted in some treated rabbits. Additional test item-related histopathological changes, such as granulomatous changes and presence of foreign material in the injected muscle, were at a low incidence. In the draining iliolumbar lymph nodes, there was a test item-related increase in development of the lymphoid follicles and paracortical/medullary region. In the spleen, there was a test item-related increase in development of

lymphoid follicles, which accounted for the increased spleen weight. These findings were partially reversible on day 99, two weeks after the last administration. There were no relevant differences in the incidence and severity of the test item-related changes between the left and right quadriceps muscles and between the corresponding draining lymph nodes and between the treatment regimens.

Results of immunogenicity testing confirmed that all sera analyzed from animals receiving the DNA vaccines with gp120/MF59C.1 or gp120/AS01$_B$ and the ALVAC vaccine with gp120/AS01$_B$ contained specific antibodies to each 1086.C and TV1.C gp120 protein.

In conclusion, three intramuscular administrations of DNA-HIV PT123 vaccine with gp120s/MF59C.1 (group 2) or with gp120s/AS01$_B$ (group 4) or of ALVAC vaccine alone (group 3) at two-week intervals followed by four intramuscular administrations of ALVAC associated with gp120s/MF59C.1 (group 2) or with gp120s/AS01$_B$ (groups 3 and 4), at two-week intervals, to the NZW Rabbit were clinically and locally well tolerated with only slight and partially reversible microscopic inflammatory findings at the injection sites. Changes in draining lymph node and spleen that correlated with inflammation and/or the immune response to vaccination. There were no treatment related effects on body weight or food consumption. Limited and transient increases in body temperature were noted in some animals mainly following ALVAC administration. Clinical pathology findings were limited to signs of inflammation, such as increased neutrophil counts, CRP, globulin levels, and fibrinogen.

The sera of all treated animals contained specific antibodies to each protein (1086.C and TV1.C gp120) with no obvious differences between groups.

4.6.1.3 Other supportive nonclinical safety studies

In addition to the nonclinical safety study summarized above, the nonclinical safety data from a variety of ALVAC constructs further inform the safety profile of ALVAC-HIV (vCP2438). These studies include the following:

- Platform biodistribution study of ALVAC-HIV in rats;
- ALVAC viral replication in different cell lines;
- Virulence of the ALVAC vector versus Vaccinia strains;
- Single dose toxicity studies by intravenous route with various ALVAC recombinants in mice and rats;
- Repeated dose toxicity studies using several routes of administration (including IM) with various ALVAC recombinants in cynomolgus and rhesus monkeys;
- Local tolerance and sensitization studies with various ALVAC recombinants in rabbits; and
- Hypersensitivity study with ALVAC-HIV (vCP125) in guinea pigs.

The results of these studies show a satisfactory nonclinical safety profile and support the administration of the ALVAC-HIV (vCP2438) construct to humans. For additional information, see the ALVAC-HIV (vCP2438) IB.

4.6.2 Toxicity studies of HIV Env vaccines

The nonclinical safety of 4 doses of the gp120 proteins included in HVTN 120 when co-administered with ALVAC-HIV (vCP2438) as a boost after 3 doses of ALVAC-HIV (vCP2438) given alone (prime), was evaluated in study AB20670 in New Zealand White Rabbits, as mentioned in section 4.6.1.1. Overall, the immunizations were clinically and locally well tolerated.

The nonclinical safety of 6 doses of the gp120 proteins included in HVTN 120 when co-administered with DNA-HIV-PT123, was also evaluated in study AB20670 in New Zealand White Rabbits. There were no deaths during the study. No treatment-related clinical signs were reported during the study and treatment was locally well tolerated. There was no obvious effect on body temperature. A lower body weight gain was noted in males only over the study period. However, this was not related with lower food consumption. No treatment-related ophthalmological findings were observed at the end of the treatment period. When compared to the control group, a slight transient increase in CRP concentration was noted mainly after the first administration. These effects correlated with the inflammatory findings observed histopathologically at the injection sites. An increase in creatine kinase was noted after the first administration only. At necropsy, at the end of treatment, the only histologic changes due to the test items were in the injection sites and iliolumbar lymph nodes. In the sites injected with DNA-HIV-PT123 or gp120s+MF59, changes comprised fibrosis, hemorrhage, acellular material and inflammatory cell infiltrate usually minimal or slight, but occasionally more severe. In the lymph nodes that drained the injected sites, there was minimal to moderate increased paracortex and increased lymphoid follicle development and minimal granulocyte infiltration. There was evidence of partial resolution of the described changes at both injection site and lymph nodes, based on necropsy observations after the recovery period. In conclusion, under the defined study conditions, 6 intramuscular administrations of DNA-HIV-PT123 vaccine associated with gp120 proteins adjuvanted with MF59 to New Zealand White Rabbits at two-week intervals were clinically and locally well tolerated.

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 1 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

The nonclinical safety of MF59 co-administered with the gp120 proteins included in HVTN 120 in the context of ALVAC-HIV (vCP2438) or DNA-HIV-PT123 was evaluated in study AB20670 as described in sections 4.6.1.1 and 4.6.1.2 and 4.6.2. Overall, the immunizations were clinically and locally well tolerated in New Zealand White Rabbits.

MF59 is not associated with any potential for systemic toxicity and it has a low order of local reactogenicity. In repeat-dose rabbit studies, clinical pathology findings of increased fibrinogen and minor inflammatory and degenerative changes at the injection site are consistent with the effects of IM injections of an immunological adjuvant. These findings are readily reversible within days to 1 - 2 weeks. In repeat-dose toxicology studies in dogs, there were no effects on cardiovascular or central nervous system (safety pharmacology) parameters. MF59 is not genotoxic (Ames test) or clastogenic (mouse micronucleus), is not a dermal sensitizer (Guinea pig), and was not teratogenic (rat and rabbit) or a developmental toxicant (rat) [46].

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 postnatal 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.

4.6.5 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 $AS01_B$, 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 Subtype C gp120/$AS01_B$ 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 4-7-Panel A). This was not observed when measuring the TV1.C-specific CD4+ T cell responses (Figure 4-7-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 Subtype 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 4-7-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 4-7-Panel D).

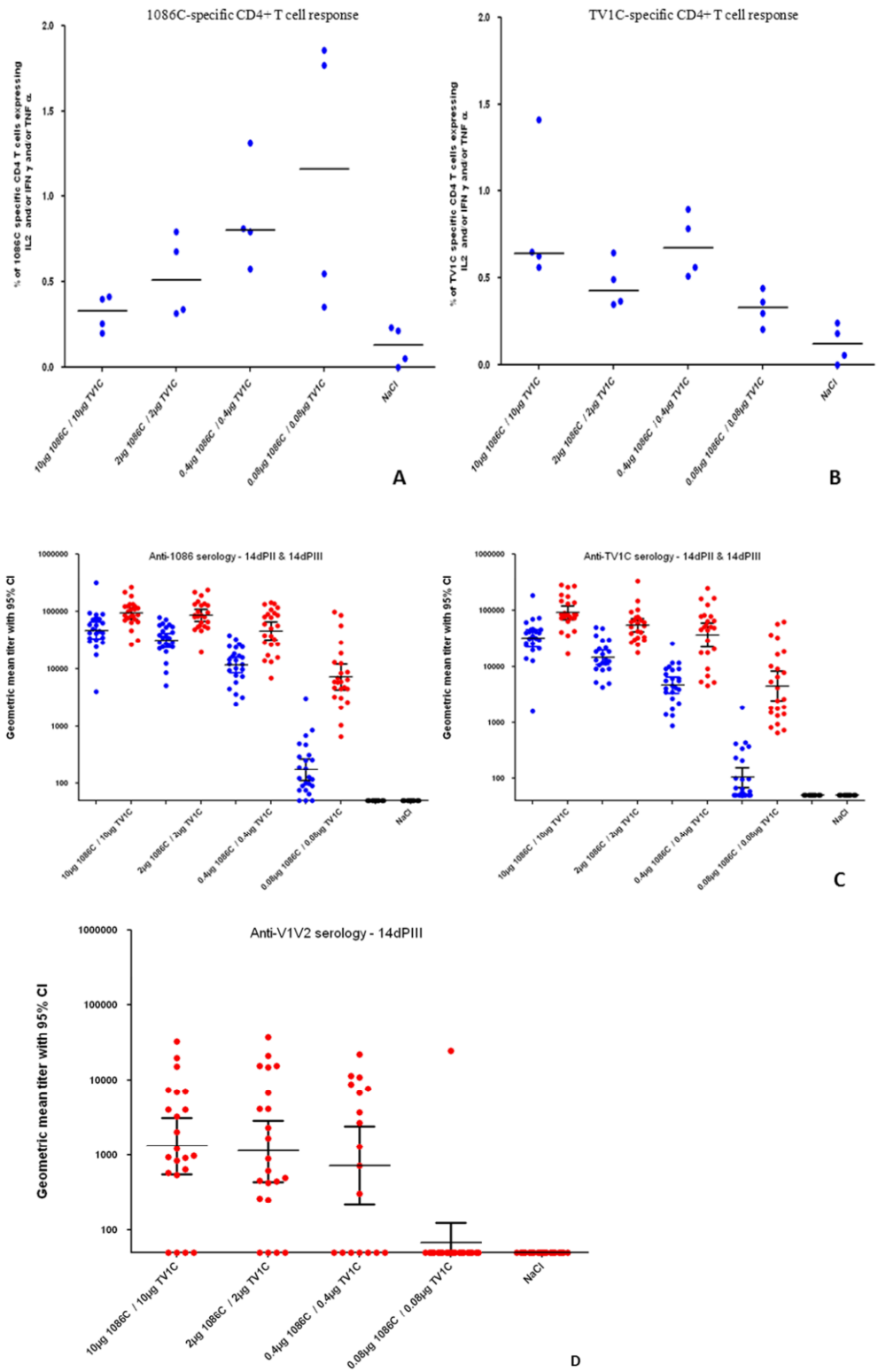

Figure 4-7: Immunogenicity of Bivalent Subtype C gp120 (TV1.C and 1086.C)/AS01B in CB6F1 mice.

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-g 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).

4.6.6 Evaluation of the need for using an adjuvant system to induce potent bivalent Subtype C gp120 (1086.C & TV1.C)-specific binding antibodies and cellular responses in CB6F1 mice (20150024)

To assess the need for an adjuvant system to elicit potent binding antibody and CD4+ T cell responses, the immunogenicity of the bivalent 1086.C & TV1.C gp120 antigens was characterized following immunization of CB6F1 mice (hybrid of C57Bl/6 and BALB/C mice) with 2 µg of each gp120 antigens, without adjuvant or formulated with 50 µg Al(OH)3 or 50 µL $AS01_B$ (containing 5 µg MPL and 5 µg QS-21 Stimulon® in a liposome-based formulation). Animals received IM injections at days 0, 14 and 28, and the T cell and antibody responses were characterized at 14 days post-second and third dose. The non-adjuvanted bivalent 1086.C & TV1.C gp120 antigens elicited detectable but low levels of binding antibodies with geometric mean titers (GMT) of 1973 and 1145, respectively, at 14 days post-third dose (Figure 4-8, A and B). Al(OH)3 formulation significantly increased the binding antibody titers up to 8807 (anti-1086.C GMT) and 4698 (anti-TV1.C GMT) and the $AS01_B$-based formulation elicited the highest binding antibody responses reaching anti-1086.C and anti-TV1.C binding antibody titers of 32936 and 31860 respectively (GMTs) (Figure 4-8, A and B). Post-third immunization, cross-reactive anti-V1V2 binding antibody responses (gp70- V1V2 scaffold Subtype B/Case A2) were detected with the strongest titers measured when the bivalent Subtype C gp120 antigens were formulated with $AS01_B$, although some animals remained negative (Figure 4-8, C).

Very low to undetectable 1086.C- and TV1.C-specific CD4+ T cell responses were measured at 14 days post-third immunization with the bivalent Subtype C gp120 antigens alone or formulated with Al(OH)3. In contrast, the bivalent Subtype C gp120/$AS01_B$ formulation elicited robust 1086.C- and TV1.C-specific CD4+ T cell responses (medians of 1% and 0.75% respectively) 14 days post-third dose (Figure 4-9). All together these data show that the bivalent Subtype C gp120 antigens (1086.C & TV1.C, 2 µg each) formulated with $AS01_B$ elicit potent 1086.C & TV1.C gp120-specific antibody and CD4+ T cell responses in CB6F1 mice, with higher intensities as compared to Al(OH)3 or non-adjuvanted formulations, supporting the use of $AS01_B$ for further clinical development.

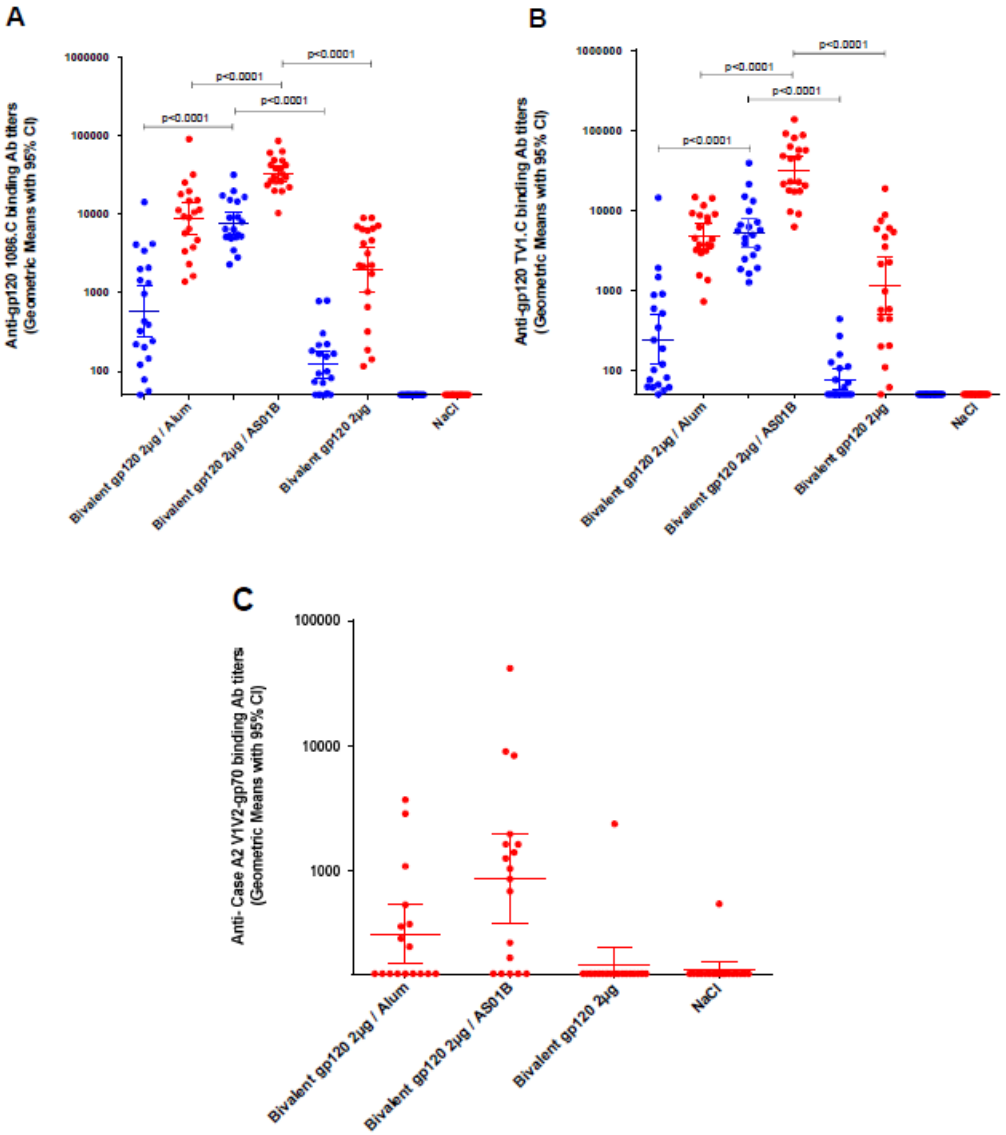

Figure 4-8 Antibody responses induced by Bivalent Subtype C gp120 (1086.C and TV1.C) with or without Aluminum hydroxide or AS01B in CB6F1 mice.

Animals were immunized intramuscularly on day 0, 14 and 28 with 2 µg of each gp120 (1086.C & TV1.C) alone or formulated with 50 µg of Al(OH)3 or 50 µL $AS01_B$. (A) anti-1086.C and (B) anti-TV1.C IgG binding antibody titers measured by ELISA 14 days post-second (blue) and third (red) immunization. (C) Anti-gp70-V1V2 (Subtype B/Case A2) binding antibodies measured by ELISA at 14 days post-third dose. Each dot corresponds to individual animals. Statistical analysis: Analysis of variance (ANOVA), with multiplicity adjustment.

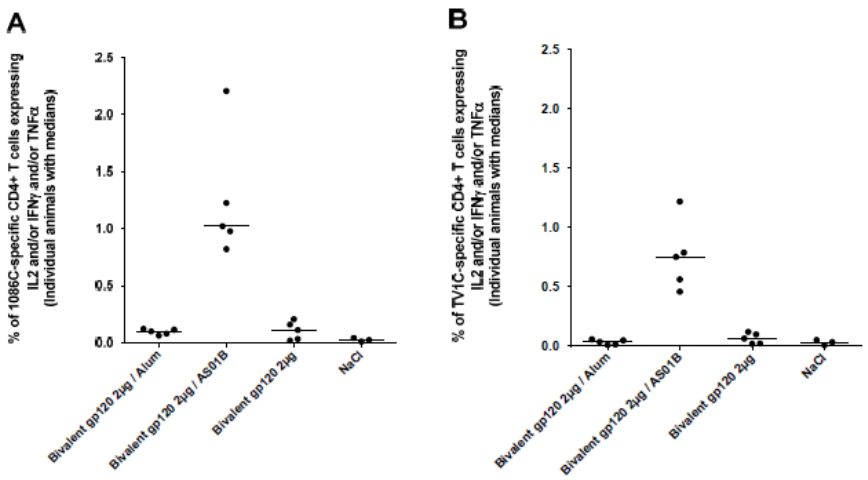

Figure 4-9 CD4+ T cell responses induced by Bivalent Subtype C gp120 (1086.C and TV1.C) with or without Aluminum hydroxide or AS01$_B$ in CB6F1 mice.

Animals were immunized intramuscularly on day 0, 14 and 28 with 2 µg of each gp120 (1086.C & TV1.C) alone or formulated with 50 µg of Al(OH)3 or 50 µL AS01$_B$. Percentage of (A) 1086.C- and (B) TV1.C-specific CD4+ T cells secreting IFN- γ and/or IL-2 and/or TNF-α were measured at 14 days post-third immunization. Intracellular staining was performed on splenocytes after a 6-hour re-stimulation with 1086 and TV1 Subtype C gp120 antigens. 5 individual animals with medians are represented.

4.6.7 Head to head immunogenicity comparison of the MF59 and AS01$_B$ adjuvant systems to formulate the bivalent Subtype C gp120 antigens in CB6 F1 mice: Acute and persistent responses (20150168)

The immunogenicity of the bivalent 1086.C & TV1.C gp120 Tox lots was characterized following immunization of CB6F1 mice (hybrid of C57Bl/6 and BALB/C mice) with a dose range of gp120 antigens (0.4 µg, 2 µg or 10 µg each), formulated with either 50 µL MF59 or 50 µL AS01$_B$. As a benchmark, mice were immunized with 2 µg of the consolidation lots of the bivalent Subtype C gp120 formulated with 50 µL AS01$_B$. Animals received IM injections at days 0, 14 and 28, and the T cell and antibody responses were monitored at 7 days or 14 days post-third dose respectively. The bivalent Subtype C gp120 formulated in AS01$_B$ elicited potent 1086.C- and TV1.C-specific CD4+ T cell responses according to an inverse dose-range, suggesting that a high amount of antigen may trigger regulatory mechanisms leading to a decreased intensity of the induced-CD4+ T cell responses (Figure 4-10, A and B). In contrast, very low to undetectable gp120-specific CD4+ T cell responses were measured following immunizations with the bivalent Subtype C gp120 formulated in MF59. At 2.5 months post immunization, bivalent Subtype C gp120s-specific CD4+ T cells were still detectable (frequencies of ~0.5%) in animals immunized with the AS01$_B$-based formulations (Figure 11, A and B).

The bivalent Subtype C gp120 antigens formulated in AS01$_B$ or MF59 induced dose dependent high levels of anti-1086.C and TV1.C antibody responses at 14 days post third immunization (Figure 4-10, C and D). The intensities of both anti-1086.C and TV1.C antibody responses were statistically significantly higher after immunization with the AS01$_B$-based formulations as compared to MF59-based

formulations at all gp120 doses tested. Interestingly, very good persistence was observed for the anti-1086.C and TV1.C binding antibody responses at 2 months post-last immunization, with both adjuvant systems tested (MF59 and $AS01_B$) and these responses were still statistically significantly higher for the $AS01_B$ formulations as compared to the MF59 formulations (Figure 4-11). All together, these data show that the bivalent Subtype C gp120 antigens (1086.C & TV1.C) formulated with $AS01_B$ elicit potent 1086.C & TV1.C gp120-specific antibody and CD4+ T cell responses with higher intensities than MF59-based formulations in CB6F1mice.

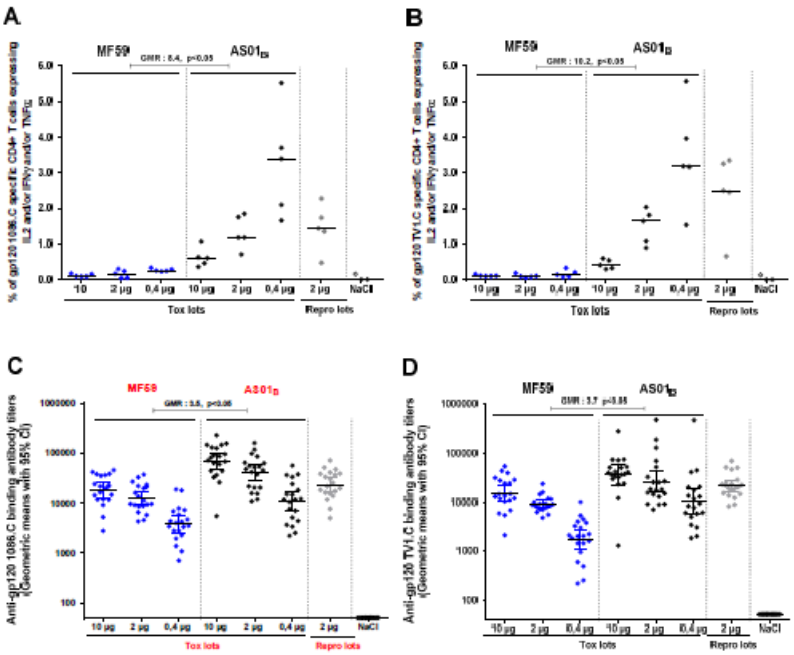

Figure 4-10 Immunogenicity of Bivalent Subtype C gp120 (TV1.C and 1086.C) formulated in $AS01_B$ or MF59 in CB6F1 mice.

Animals were intramuscularly immunized with 10 µg, 2 µg, or 0.4 µg of bivalent (1086.C & TV1.C) gp120 antigens (Tox lots 1023719 and 1023444) formulated in 50 µL of $AS01_B$ or 50 µL MF59 at days 0, 14 and 28. An additional group of mice was immunized with 2 µg of the gp120 consolidation lots (1086.C CR02 and TV1.C CR04) formulated in 50 µL $AS01_B$ as a control. Percentage of 1086.C- (A) and TV1.C- (B) specific CD4+ T cells secreting IFN-g and/or IL-2 and/or TNF-α was measured at 7 days post-third immunization. Intracellular staining performed on peripheral blood lymphocytes after a 6-hour re-stimulation with 1086.C and TV1.C gp120 antigens. 5 pools of 7 mice with medians are represented. Anti-1086.C (C) and TV1.C (D) binding antibody titers measured by ELISA 14 days post-third immunization. Each dot corresponds to individual animals for antibodies. Statistical analysis: Analysis of variance (ANOVA), with multiplicity adjustment.

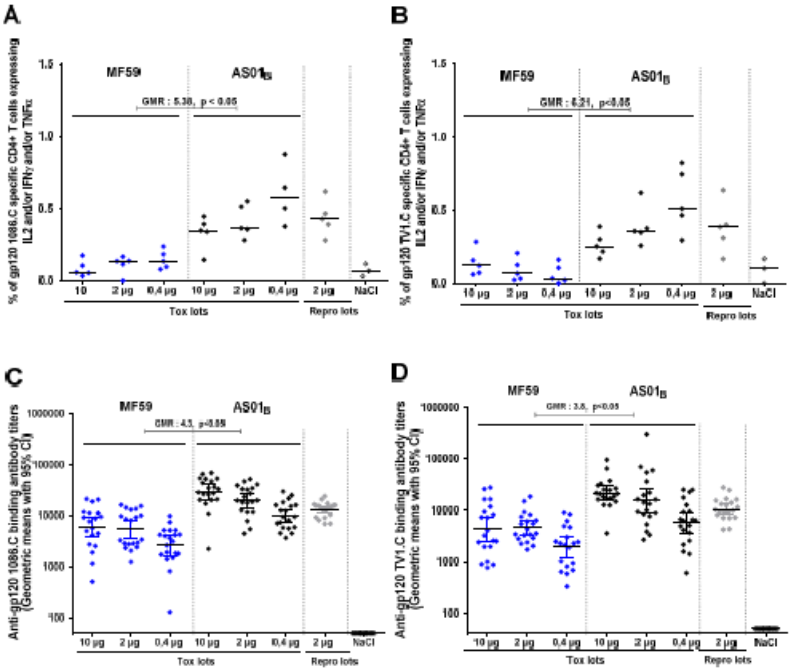

Figure 4-11 Persistence of the CD4+ T cell and binding antibody responses induced by the Bivalent Subtype C gp120s (TV1.C and 1086.C) formulated in AS01B or MF59 in CB6F1 mice, 2 months post-last dose.

Percentage of 1086.C- (A) and TV1.C- (B) specific CD4+ T cells secreting IFN-γ and/or IL-2 and/or TNFα was measured at 77 days post-third immunization. Intracellular staining performed on PBLs after a 6-hour re-stimulation with 1086.C and TV1.C gp120 antigens. 5 pools of 7 mice with medians are represented. Anti-1086.C (C) and TV1.C (D) binding antibody titers measured by ELISA 77 days post-third immunization. Each dot corresponds to individual animals (N=20/group) and geometric means with 95% CI are represented. Statistical analysis: Analysis of variance (ANOVA), with multiplicity adjustment.

4.7 Clinical studies

4.7.1 Clinical studies with ALVAC-HIV

The proposed ALVAC-HIV vaccine candidate specific for Southern Africa is ALVAC-HIV (vCP2438). The vaccine is very similar to ALVAC-HIV (vCP1521) (the ALVAC-HIV used in the RV144 trial) since it contains the same *gag, pro* and *gp41 env TM* components. However, it has been adapted to include 96ZM651 gp120 *env* insert (subtype C) (rather than the TH023 gp120 *env* insert [subtype E] used for the RV144 study regimen in Thailand).

ALVAC-HIV (vCP2438) is currently being investigated in the ongoing trial HVTN 100.

4.7.2 HVTN 100

HVTN 100 enrolled 252 participants (210 received vaccine and 42 placebo) between February and May 2015. All month 12 injections were completed by 2 June 2016.

4.7.2.1 Interim summary of blinded safety and tolerability data

Blinded safety data reported as of July 7, 2016 are summarized here. A total of 1198 ALVAC or placebo injections were given to participants in the left deltoid and 703 Bivalent Subtype C gp120/MF59 or placebo injections were given to participants in the right deltoid.

The vaccine regimen is very well tolerated thus far with the vast majority experiencing no or mild local reactogenicity symptoms. Mild pain and/or tenderness were reported by 58% of participants for injections in the left deltoid and by 48% for injections in the right deltoid. Moderate pain and/or tenderness were reported by 18% of participants for injections in the left deltoid and by 11% for injections in the right deltoid. Severe pain and/or tenderness was reported from a left deltoid injection by 1 participant (at injection timepoint #1) and for a right deltoid injection by 2 participants (1 at injection timepoint #3 and 1 at injection timepoint #5).

For the left deltoid, grade 1 (mild) erythema and/or induration injection site reactions were reported by 6% of participants and grade 2 (moderate) reactions by 5% of participants. For the right deltoid, grade 1 erythema and/or induration injection site reactions were reported by 4% of participants and grade 2 reactions by 2% of participants. Three participants have reported erythema and/or induration reactions meeting grade 3 criteria (severe) based on size ($\geq$ 10cm diameter or $\geq 100cm^2$ surface area) with no complications (such as ulceration, secondary infection, phlebitis, sterile abscess or drainage). One person reported a grade 3 right deltoid erythema reaction (> 100 cm2) occurring on day 2 of the fourth injection timepoint, which resolved within 4 days. This participant also self-reported severe induration on day 3, which resolved within 1 day. The participant returned to clinic on day 4 for examination, and clinic staff observed severe erythema alone without induration or swelling. Antibiotics, analgesics, and antihistamines were prescribed and the participant was discontinued from further vaccinations but continued in follow-up. Another person reported grade 3 induration and erythema in the right deltoid on day 3 after the fifth vaccination timepoint, resolving by day 5. Antibiotics, anti-inflammatory and analgesic medications were prescribed and were taken for 3 days. Another person reported severe erythema and induration reactions in the left deltoid occurring on day 0 after the fifth vaccination timepoint and resolving by day 3. Antihistamine, oral steroid and analgesic/anti-inflammatory medications were taken. In all 3 participants, the needle used for injection was < 1.5 inches long, consistent with weight-based guidance for needle length choice provided to sites in the SSPs [47,48].

Systemic reactions have been reported in 69% of participants thus far, with the vast majority of those reactions being mild in intensity. Malaise and/or fatigue, headache, myalgia and arthralgia appear to be the most common reactions, occurring in 42%, 41%, 37% and 30% of participants, respectively thus far. Other systemic reactogenicity symptoms have included nausea (15%), chills (10%), fever (8%), and vomiting (4%). Maximum severity of systemic symptoms of

moderate intensity has been reported in a total of 19% of participants; 7% reported systemic symptoms of moderate intensity after the first injection, 3% after the second, 5% after the third, 2% after the fourth and 5% after the fifth. Severe systemic reactions, have occurred in 2% of participants (4 participants): 2 participants with severe arthralgia occurring after the first injection, 1 person with severe malaise and/or fatigue and 1 with severe headache, each after the fifth injection.

4.7.2.2 Interim summary of Adverse Events (AEs) and Serious Adverse Events (SAEs)

As of July 7, 2016, 468 adverse events (AEs) have occurred in 180 participants (71.4% of participants), of which 288 AEs (61.5%) were mild, 163 were moderate, (34.8%), 13 (2.8%) were classified as severe, 3 (0.6%) were classified as potentially life-threatening, and 1 (0.2%) was fatal (see details in the next paragraph). AEs were reported by participants most frequently in the Systems Organ Class (SOC) Infections and infestations (107 participants [42.4% of enrolled participants]), followed by the SOC Investigations (55 participants [21.8%]). Fifteen AEs occurring in 11 participants have been assessed by the site investigator as being related to study product; 12 were mild, 3 were moderate and none were severe. These include injection site pruritus in 3 individuals (mild in 2, moderate in 1), lymphadenopathy in 2 individuals (both mild), abdominal pain (moderate), generalized pruritus (moderate), and mild events of diarrhea, injection site nodule, gastritis, dizziness, headache, neutrophil count decreased, and oral paresthesia in 1 individual each.

Seven SAEs have occurred in 5 participants during the trial, all unrelated to study product. One participant experienced 3 separate SAE events resulting from 3 separate assault attacks: severe soft tissue injuries due to assault, potentially life-threatening subdural hematoma, and then multiple injuries to the head and chest that were fatal. SAEs in other participants were gastrointestinal infection, bi-polar mood disorder, acute rheumatic fever, and transient ischemic attack.

4.7.2.3 Discontinued vaccinations and early terminations

As of July 14, 2016, 17 participants have terminated the study prematurely and 16 participants have discontinued vaccinations. Clinical events leading to early terminations and discontinuation of vaccinations (DOV) occurred in 5 participants: death from multiple injuries (unrelated to study product); severe local reactogenicity (DOV); hypertension (DOV for AE unrelated to study product); mild vomiting occurring on the day of first vaccination only (participant declined further study participation); psychiatric diagnosis (DOV and early termination for investigator discretion). Six other participants have terminated the study due to HIV infection. Reasons for DOV or early termination in other participants included participant refusal, unable to contact, unable to schedule within visit windows, unable to adhere to study schedule, relocation, desiring to fall pregnant, desiring to donate eggs, unwilling to use contraception, and

pregnancy. One pregnancy has been reported to date and this participant is continuing in follow-up.

4.7.2.4 Summary of Interim Immunogenicity data from HVTN 100

The immunological criteria guiding the decision whether to advance development of the ALVAC-HIV (vCP2438), Bivalent Subtype C gp120/MF59 regimen into efficacy testing in adults in South Africa (HVTN 702) are described in Table 4-7. Immunogenicity data from samples collected from HVTN 100 participants at the month 6.5 timepoint (2 weeks post month 6 vaccination) were compared to data from a new, randomly selected subset of stored samples from RV144 vaccine recipients who were HIV-1 uninfected upon completion of follow-up (the RV144 "comparator arm"). Samples from the RV144 comparator arm and HVTN 100 participants were analyzed contemporaneously using qualified assays in the same laboratories (along with placebo samples for blinding).

All four immunogenicity Go criteria were met.

Table 4-7 Go/No-Go criteria for advancement of the HVTN 100 vaccine regimen to efficacy testing

Variable Measured at month 6.5	Rationale	Go Criteria Threshold (LL of 95% CI)
1. Env Ab Response Rate (≥ 2 of 3 antigens)	Adequate Ab take to vaccine Env	≥75%
2. Env Ab Magnitude(≥ 2 of 3 antigens)	*Non-inferior* Ab magnitude vs. RV144	GM ratio (new/RV144) ≥50%*
3. Env CD4 Response Rate (1 of 1 antigen)	*Non-inferior* CD4 T-cell take vs. RV144	Difference within 30%*
4. Env V1V2 Response Rate (≥ 1 of 3 antigens)	Adequate to predict achieving estimated VE=50% for 2 years if V1V2 Ab is a predictive immune correlate	≥ 56%

*Non-inferior to RV144 response based on contemporaneous assessment of clade C vaccine samples vs. RV144 vaccinee samples by the same lab.

Binding antibody responses to Env (criteria 1 and 2):

At the month 6.5 timepoint, 100% of vaccinees in HVTN 100 developed binding antibodies to the gp120 Clade C strain Env antigens in the ALVAC vector, as well as the two Clade C strains in the bivalent gp120 protein boost (Figure 4-12). Antibody magnitude values measured by geometric mean titers were 3.6-8.8 fold greater than IgG binding antibody to vaccine-matched responses to Env antigens included in RV144 (Table 4-8). Hence, criteria 1 and 2 were met.

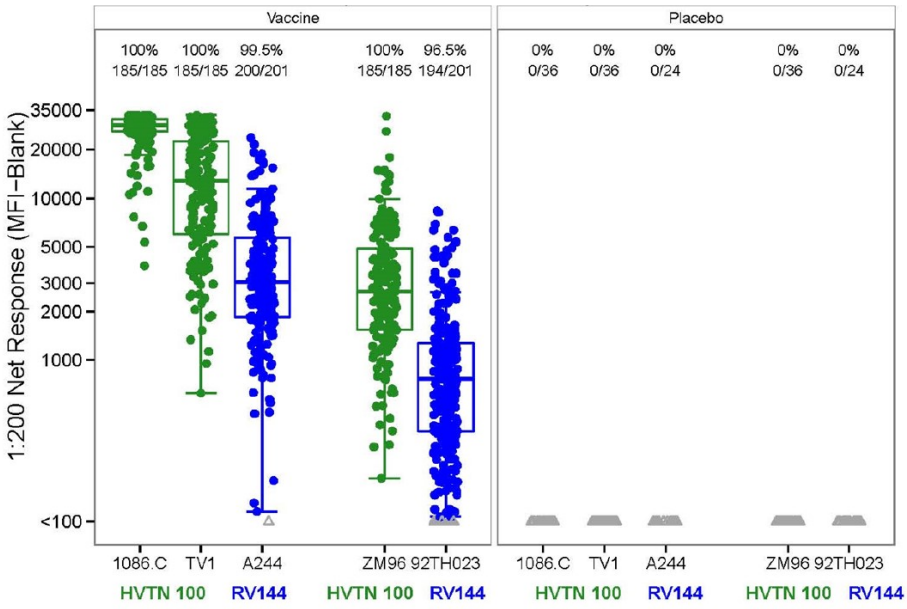

Figure 4-12 Box plots of the binding antibody titers to the vaccine antigens used in RV144 and HVTN 100. The midline of the box plot indicates the median, the ends of the box indicate the 25th and 75th percentiles. Closed dots represent positive responses, open triangles represent negative responses.

Table 4-8 HVTN 100 vs. RV144 Peak bAb magnitudes to gp120 among vaccinees

Protocol	Antigen	n	Geometric Mean Titer	GMR (100/RV144)	GMR 95% CI
HVTN 100	1086	185	26,257.5	8.8	(7.64, 10.2)
RV144	A244	201	2,968.9		
HVTN 100	TV1	185	10,726.7	3.6	(3.01, 4.34)
RV144	A244	201	2,968.9		
HVTN 100	96ZM651	185	2,685.4	3.6	(2.95, 4.39)
RV144	92TH023	201	746.1		

CD4 responses (criterion 3)

The CD4+ T cell response rate to vaccine-matched Env sequences in ALVAC (vCP2438), (Env peptide pool 96ZM651) in HVTN 100 participants was 58%. This was compared to the CD4+ T cell response rate to vaccine-matched Env sequences in ALVAC (vCP1521), (Env peptide pool 92TH023) in RV144 participants of 41%. The response rate difference (HVTN 100-RV144) was 16% (95% CI: 6, 16%; p-value 0.0019). This exceeded the LL response rate difference of -30% and therefore met criterion #3.

V1V2 antibody response rates (criterion 4)

The binding antibody response rate to the vaccine-matched 1086.C V1V2 antigen was 71% (95% CI 64% - 77%), thereby meeting criterion #4 that the prevalence of IgG antibodies to the Clade C V1V2 loop in at least one vaccine antigen must be at a lower limit threshold of > 56% (which equates to a response rate of 63%) (Table 4-7). The cumulative V1V2 response rate is 80%; well above the 63% threshold that was established for modeling a 50% efficacy if V1V2 was the sole correlate of protection.

Table 4-9 HVTN 100 Binding Antibody Env V1V2 Response Rates, Per-Protocol Cohort (Go/No-Go Criterion 4)

Antigen	Treatment	Response Rate	95% CI	LL of CI ≥56%	Criterion 4 Passed?
1086.C.V1V2 tags	T1 (n=183) P2 (n=35)	71% 0%	(64%, 77%) (0%, 10%)	Yes	Yes
gp70-V1V2CladeB CaseA2	T1 (n=183) P2 (n=35)	50% 0 %	(43%, 57%) (0.0%, 9.9%)	No	
gp70-V1V2.TV1	T1 (n=183) P2 (n=35)	62% 0%	(55%, 69%) (0%, 10%)	No	
1086.C.V1V2 tags or gp70-V1V2.TV1	T1 (n=183)	80%	(74%, 85%)	N/A	
1086.C.V1V2 tags or gp70-V1V2CladeB CaseA2 or gp70-V1V2.TV1	T1 (n=183)	80%	(74%, 85%)	N/A	

Based on the immunogenicity results of the month 6.5 timepoint in HVTN 100, the decision was made to proceed with HVTN 702.

4.7.3 Other clinical studies with related ALVAC-HIV® vaccines

Extensive previous experience with other ALVAC-HIV vaccines informs the expected safety, tolerability, and immunogenicity profile of the new vaccine (see Table 4-10). In all, more than 10,000 people have received ALVAC-HIV vaccines in clinical trials. The majority of these trials have been performed with ALVAC-HIV (vCP205), (vCP1452), or (vCP1521). These vaccines differ in the HIV gene inserts that have been introduced into the ALVAC vector.

Table 4-10 Recombinant ALVAC-HIV vaccine in human adult prevention trials

Candidate vaccine	# receiving ALVAC-HIV	Protocol	Status
ALVAC-HIV (vCP125)	20	ANRS VAC01	Completed
ALVAC-HIV (vCP125) (low and high dose)	92	AVEG 012A/012B	Completed
ALVAC-HIV (vCP205)	25	ANRS VAC03	Completed
ALVAC-HIV (vCP205) (low and high dose)	185	AVEG 022/022A	Completed
ALVAC-HIV (vCP205)	22	AVEG 029	Completed
ALVAC-HIV (vCP205)	56	AVEG 027	Completed
ALVAC-HIV (vCP205)	290	HVTN 203	Completed
ALVAC-HIV (vCP205)	56	AVEG 032	Completed
ALVAC-HIV (vCP205)	280	AVEG 202/ HIVNET 014	Completed
ALVAC-HIV (vCP205)	30	AVEG 033	Completed
ALVAC-HIV (vCP205)	20	HIVNET 007	Completed
ALVAC-HIV (vCP300)	20	ANRS VAC07	Completed
ALVAC-HIV (vCP300)	119	AVEG 026	Completed
ALVAC-HIV vector (vCP205, 1433, 1452)	20/35/35	AVEG 034	Completed
ALVAC-HIV (vCP205/1452)	15/40	AVEG 034A	Completed
ALVAC-HIV (vCP1452)	22 + 3	ANRS 010	Completed
ALVAC-HIV (vCP1452)	160	HVTN 203	Completed
ALVAC-HIV (vCP1452)	120	HIVNET/HVTN 026	Completed
ALVAC-HIV (vCP1452)	100	HVTN 039	Completed
ALVAC-HIV (vCP1521)	203	RV 132/135	Completed
ALVAC-HIV (vCP1521)	8197	RV 144	Completed
ALVAC-HIV (vCP1521)	135	RV 305	Completed
ALVAC-HIV (vCP1521)	327	RV 306	Ongoing
ALVAC-HIV (vCP1521)	80	HVTN 097	Completed
ALVAC-HIV (vCP2438)	210	HVTN 100	Ongoing
Total	**10,917**		

ALVAC-HIV (vCP205) is an ALVAC vector vaccine with genetic inserts of the HIV-1 *gag* gene (expressing the Gag p55-polyprotein of the HIV-1 LAI strain [clade B]), a fragment of the *pol* gene (that expresses the p15 Protease of the HIV-1 LAI strain), and a portion of the *env* gene (expressing the gp120 Env glycoprotein of the HIV-1 MN strain [clade B], and the anchoring TM region of gp41 of the HIV-1 LAI strain). The HIV genes are inserted in the C3 locus.

ALVAC-HIV (vCP1452) vaccine is an ALVAC vector vaccine expressing the products of the HIV-1 *env* (Env gp160 protein of the HIV-1 MN Strain [clade B]) and *gag* (HIV-1 LAI strain [clade B]) genes, the protease portion of the *pol* gene on a synthetic polynucleotide encompassing the known human CTL epitopes from the *nef* (BRU Strain) and the *pol* (LAI strain) gene products. The C3 locus was used for the insertion of the HIV-1 *env* and *gag* gene sequences and the C5 locus was used for the insertion of the sequences encoding the HIV-1 Nef and Pol CTL epitopes.

ALVAC-HIV (vCP1521) vaccine was generated by co-insertion of genes encoding HIV-1 gene products into the ALVAC genome in the C6 locus. The inserted HIV-1 gene sequences are: the region of the *env* gene encoding the

extracellular Env gp120 moiety of TH023 strain of HIV-1 (clade E) linked to the sequences encoding the HIV-1 TM anchor sequence of gp41 HIV-1 LAI strain (clade B); the *gag* gene encoding the entire Gag p55-polyprotein of the HIV-1 LAI strain; and a portion of the *pol* sequences of the LAI strain of HIV-1 sufficient to encode the protease function.

4.7.4 Clinical safety experience with related ALVAC-HIV vaccines

4.7.4.1 Summary of safety, reactogenicity, and tolerability from related human experience

The tolerability and safety of ALVAC-HIV (vCP1521) were evaluated initially in 2 phase 1-2 studies in Thailand [49,50]. The most relevant data, however, come from the RV144 efficacy study performed in Thailand [4,51], during which more than 8000 human subjects received the vaccine and ALVAC-HIV (vCP1521) was found to be safe and well tolerated. Vaccine recipients experienced local and/or systemic reactions significantly more frequently than placebo recipients; the frequencies of local reactions such as pain and tenderness were higher than those of systemic reactions such as headache, fatigue, arthralgia and myalgia; fever was rarely reported; ALVAC-HIV (vCP1521) was associated with a higher frequency of local reactions compared to the protein subunit used in the study (AIDSVAX B/E); the frequency of both local and systemic reactions gradually declined with subsequent vaccine administrations; most local and systemic reactogenicity symptoms were mild to moderate, resolving rapidly and spontaneously in the vast majority of cases; and the frequencies of adverse events (AEs) and Serious Adverse Events (SAEs) were not different between vaccine and placebo groups. Overall, results with ALVAC-HIV (vCP1521) are consistent with other ALVAC-HIV constructs, supporting the conclusion that the safety, reactogenicity and tolerability profile of ALVAC-HIV is determined in greater measure by the vector than by the HIV genetic material inserted into it.

Prior to the RV144 study, De Bruyn et al [52] characterized the tolerability and safety profile of ALVAC-HIV (vCP205) and ALVAC-HIV (vCP1452) (along with other ALVAC-vector vaccines) based on data from more than 1,000 clinical trial subjects. The authors concluded that:

- ALVAC-HIV vaccines were safe and well tolerated, with a reactogenicity profile comparable to that of existing vaccines licensed for use in adults; and

- Reactogenicity was similar for different ALVAC-HIV constructs, suggesting that reactogenicity is determined in greater measure by the vector than by the additional genetic material inserted into the vector.

Of interest as well was the observation that reactogenicity seemed to differ according to certain demographic variables: Black, non-Hispanic participants reported significantly less reactogenicity than did White, non-Hispanic participants; and males reported less pain than females.

Additional information is available in the ALVAC-HIV (vCP2438) IB.

4.7.4.2 Summary of pregnancy occurrence and outcomes

In study RV144, over a period of 3.5 years of follow-up, among women a total of 967 (30.6%) vaccine (vCP1521) and 955 (30.1%) placebo recipients reported a pregnancy during the study, with 139 vaccine and 116 placebo recipients reporting more than 1 pregnancy. Birth was reported for 1843 infants, 14 of them representing 7 twin pairs. Of these, 277 births (137 vaccine and 140 placebo recipients; 1 twin pair per treatment) occurred within 450 days of study entry. For these infants, birth weight, gestational age and Apgar scores were similar between the vaccine and placebo groups. Three congenital abnormalities (1 vaccine and 2 placebo recipients) were reported among these 277 births, the vaccine group abnormality being a respiratory distress syndrome with patent *ductus arteriosus*. Abnormal pregnancy outcomes were experienced in 165 out of 3,165 (5.2%) female vaccine recipients and 139 out of 3,169 (4.4%) female placebo recipients ($p = 0.13$), and in 17.1% and 14.6% ($p = 0.13$) of vaccine and placebo pregnancies, respectively [51].

A total of 15 of the 245 female subjects became pregnant during the AVEG studies. Twelve subjects received ALVAC-HIV (vCP205) and 3 received placebo. Of these 15 subjects aged 19 to 37 years, 9 subjects had live births, 3 subjects had elective abortions, 1 subject had a spontaneous abortion, and the outcome of 2 pregnancies remains unknown. Of the 9 live births, 3 were by caesarean section. Overall, no complications during pregnancy or congenital abnormalities at the time of birth were reported.

In study HVTN 203 [53] there were 2 participants with miscarriages among the total of 80 female study participants who received ALVAC-HIV (vCP1452). These events were classified as unrelated to the study product by the investigators.

4.7.4.3 Summary of safety and tolerability data in African studies that have used ALVAC-HIV

To date, 3 studies have been conducted and completed in Africa with ALVAC-HIV: HIVNET 007, HIV Prevention Trials Network (HPTN) 027, and HVTN 097.

HIVNET 007 [54] was a randomized, double-blind, placebo-controlled clinical trial conducted in Kampala, Uganda. In this study, 40 HIV-seronegative Ugandan volunteers were randomly assigned to receive ALVAC-HIV (vCP205) ($n = 20$), control ALVAC containing the rabies virus glycoprotein G gene ($n = 10$), or saline placebo ($n = 10$). Adverse reactions to immunizations were similar to those in previous trials with these vaccines in HIV-seronegative volunteers in the United States. No severe (grade 3 or 4) adverse reactions attributable to receipt of the vaccine were observed.

HPTN 027 [55] was a phase 1 randomized, single-center, double-blind, placebo-controlled trial that evaluated the safety and immunogenicity of ALVAC-HIV (vCP1521) in infants born to HIV-1 infected women in Uganda. Sixty infants were enrolled; 48 in the active group and 12 in the placebo group. Forty-seven infants received all 4 vaccinations and completed follow-up (38 in the vaccine arm and 9 in the placebo arm). There were 3 deaths in the HPTN 027 study (2 in the vaccine group and 1 in the placebo group); all were reported as unrelated to the vaccine. The deaths included pneumonia-like illness, cor pulmonale secondary to congenital heart disease complicated by pneumonia, and gastroenteritis complicated with electrolyte imbalance. The rate of SAEs was similar between groups (56% in the vaccine group and 50% in the placebo group). Thirteen infants in HPTN 027 experienced an AE that led to discontinuation of vaccinations: 10 subjects in the vaccine group and 3 in the placebo group. There were no severe or life-threatening reactogenicity events. Mild reactogenicity events were common in both study arms, with only 1 moderate event (irritability) in the placebo arm and 7 in the vaccine group (erythema, induration, pain, fever, and irritability).

HVTN 097 was designed to evaluate whether the same vaccine regimen (with a higher dose of ALVAC-HIV) that was used in Thailand in Study RV144 would be comparably safe and immunogenic in a South African population. The study enrolled 100 healthy, HIV-1–uninfected participants aged 18 to 40 years, 51 male and 49 female. One hundred percent were black, non-Hispanic. Ninety-one participants completed vaccinations and follow-up. The study was completed in December 2013. There were 4 participants who discontinued vaccinations, 2 due to pregnancy and 2 due to “other” reason; there were no discontinuations due to AEs or reactogenicity.

Local and systemic reactogenicity was assessed for the investigational ALVAC-HIV (vCP1521) and AIDSVAX vaccinations. Local injection site reactions of pain and/or tenderness were more common in participants receiving active HIV vaccinations versus placebo injections. Most pain and/or tenderness reactions to the HIV vaccinations were mild (48%), 28% were moderate (similar rates for ALVAC compared to the AIDSVAX vaccinations) and approximately 9% were severe (all but 1 severe pain/tenderness reactions were from ALVAC vaccination). In placebo recipients, the maximum pain and/or tenderness reactions were mild (52%). The majority of participants in both active (Group [G]1 and G2 combined) and placebo groups experienced no erythema and/or induration reactions (84% and 95%, respectively); with all but 1 reaction (> 9 cm erythema/induration from an ALVAC vaccination) being non-gradable by the Division of AIDS (DAIDS) AE Grading Table (0-25 cm2), occurring in G1. Moderate or severe systemic reactions associated with vaccine administration included malaise and/or fatigue (15% versus 5.3% in placebo), myalgia (12.5% versus 0% in placebo), headache (7.5% versus 21% in placebo), nausea (1.25% versus 0% in placebo), chills (3.75% versus. 5.3% in placebo), and arthralgia (6.25% versus 0% in placebo). The maximum temperature elevations were Grade 2, which occurred in 2 vaccinees compared to 0 in placebo. Overall 88.75% of vaccine recipients experienced at least 1 AE compared to 85% of placebo recipients. There were 2 SAEs and both were unrelated to treatment: thermal burn

in a vaccinee and substance-induced psychotic disorder in a placebo recipient. There were no Grade 4 (life threatening) or 5 (death) AEs. There were 5 Grade 3 AEs in vaccine recipients (6.25%) and 2 Grade 3 events in 1 placebo recipient (5.3%), all deemed unrelated to study treatment. These included alanine transaminase (ALT) increase, headache, hypertension, abnormal loss of weight, and thermal burn in vaccinees and substance-induced psychotic disorder and abnormal loss of weight in 1 placebo recipient. Moderate AEs were experienced by 61.25% of vaccinees and 50% of placebo recipients. AEs considered related to the vaccine included itching at the injection site, lymph node swelling, faster heartbeat, abdominal pain, flu-like illness, diarrhea, injection site skin lump, and muscle spasms. Each of these reactions were mild or moderate, only occurred in 1 person (except for the skin lump which occurred in 3 participants), and did not last long. All participants recovered without sequelae. A few participants had changes in their laboratory, blood, and urine test results that were considered related to the vaccinations and all returned to normal. Overall, the study indicated that the vaccine regimen used in RV144 appears safe and well-tolerated in South Africans.

4.7.4.4 Previous human experience with ALVAC-HIV used in combination with subunit protein boost adjuvanted with MF59

There has been meaningful previous human experience with the use of ALVAC-HIV vaccines in combination with recombinant gp120 proteins adjuvanted with MF59. In addition to the exposures that occurred in the context of study HVTN 100, 440 human subjects have received this combination across 7 clinical trials (Table 4-11). No safety signal of concern was identified in these studies.

Table 4-11 Clinical studies performed with ALVAC-HIV and gp120+MF59 before HVTN 100

Study (Country)	ALVAC-HIV	Protein	Subjects[a]
RV132 [50] (Thailand)	vCP1521	gp120 + MF59 clades B/E made in CHO cells	n = 45
AVEG 022A [56] (USA)	vCP205	gp120 + MF59 clade B made in CHO cells	n = 47
AVEG 029 [57] (USA)	vCP205	gp120 + MF59 clade B made in CHO cells	n = 22
AVEG 202/HIVNET 014 [58] (USA)	vCP205	gp120 + MF59 clade B made in CHO cells	n = 145
AVEG 032 [59] (USA)	vCP205	gp120 +/- p24 + MF59 clade B gp120 made in CHO cells p24 made in *S. cerevisiae*	n = 56
AVEG 026 [60] (USA)	vCP300[b]	gp120 + MF59 clade B made in CHO cells	n = 85
AVEG 012A 012B [61] (USA)	vCP125[c]	gp120 + MF59 clade B made in CHO cells	n = 40
Total			**N = 440**

[a]Number of subjects that received both the ALVAC-HIV prime and the gp120/MF59 boost.

[b]ALVAC-HIV (vCP300) is similar to vCP205 and contains additional sequences encoding Pol and Nef epitopes.
[c]ALVAC-HIV (vCP125) contains the gene for gp160 from clade B

4.7.4.5 Previous human experience with ALVAC-HIV used in combination with subunit protein boost adjuvanted with $AS01_B$

There is no previous human experience with this specific product combination.

4.7.5 Immunogenicity experience with related ALVAC-HIV vaccines

4.7.5.1 Summary of immunogenicity from related human experience

Immunogenicity measures in ALVAC-HIV studies have evolved over a period of more than 2 decades, informed by evolution in knowledge about relevant immune responses. Initial studies focused on the measurement of CTL activity, CD4+ T-cell lymphoproliferation, and nAb activity. Subsequent studies have focused on Ab binding to the Env glycoproteins and on intracellular cytokine staining (ICS) as well. Most recently, a large collaborative consortium performed a case-control study to evaluate immune CoR based on the RV144 study that used the prime-boost regimen of ALVAC-HIV (vCP1521) and the gp120 protein AIDSVAX B/E [5].

The development of assays for measuring immunogenicity has evolved during more than 20 years of testing in humans. Therefore, immunogenicity data cannot be fully integrated. However, extensive data from previous studies with ALVAC-HIV can inform many relevant immunogenicity-related issues as described below.

4.7.5.2 ALVAC-protein schedule and immunogenicity

The selection of a vaccination schedule for the large efficacy trial in Thailand (RV144) was based on existing scientific knowledge at the time [62]. In addition to safety, the key parameters taken into consideration were the CTL immune responses and the nAb responses. The HIV vaccine field has evolved significantly since then and the relevance of these immune measures is currently debated. However, the demonstration of vaccine protection in RV144 mandates the conservation of vaccination regimen features that are believed to have contributed to vaccine protection, even when the mechanism of protection has not been definitely established. The following paragraphs summarize the considerations that were taken into account in the selection of a vaccine regimen and schedule for the RV144 study.

Prior to RV144, several schedules of administration with ALVAC and protein boost were examined in multiple clinical trials [56,58,63-65]. While the studies were not designed or powered to discriminate statistically between the various vaccination schedules, an analysis of the data suggested that 4 doses of ALVAC induced better CTL responses than 3 or 2 doses. Specifically, net point prevalence CTL response rates on days 182 and 273 using 4-dose immunization regimens (months 0, 1, 3, and 6 or months 0, 1, 6, and 9) produced higher response rates than the 3-dose regimen (months 0, 1, and 6) on days 182 and 273. Regarding neutralization data, both ALVAC schedules (ALVAC alone and ALVAC plus subunit protein boost), showed significantly higher neutralization response rates compared to the control schedule.

The addition of a subunit protein boost to ALVAC did not appear to alter the CTL response rates [64]. In contrast, the protein boost had a significant effect on Ab responses. The ALVAC plus subunit protein boost schedule had significantly higher nAb response rates when compared to the ALVAC alone schedule.

On the basis of these observations, a 4-dose regimen (months 0, 1, 3, and 6) of ALVAC was proposed in order to maximize CTL responses. In addition, 2 doses of the protein boost were proposed at months 3 and 6 to maximize Ab responses. The RV144 study implemented this vaccination schedule.

4.7.5.3 Immunogenicity of ALVAC used in combination with protein boost plus MF59

The vaccine regimen proposed for development in South Africa combines ALVAC-HIV (vCP2438) with a bivalent recombinant gp120 protein (total of 200 mcg, 100 mcg of each protein) adjuvanted with MF59. Both vaccine components have been adapted to target the predominant HIV clade circulating in South

Africa (clade C). Interim immunogenicity data from HVTN 100 are summarized in Section 4.7.2.4. Studies prior to HVTN 100 with related vaccines provided useful preliminary information on whether peak immunogenicity is expected to be at least similar to that elicited by the RV144 regimen and whether the use of MF59 could be dose-sparing for the protein.

Study RV132 [50] used ALVAC-HIV (vCP1521) in the same dose and schedule as in study RV144 but 45 subjects in 1 of the study arms received a bivalent recombinant gp120 protein manufactured in CHO cells by Novartis Vaccines and adjuvanted with MF59 as a protein boost. The dose of the proteins was 150 mcg in total (100 mcg of the CM235 protein and 50 mcg of the SF2 protein). Study RV135 [49] used ALVAC-HIV (vCP1521) in the same dose and schedule as in study RV144, and 97 subjects in 2 study arms received a bivalent recombinant gp120 protein manufactured in CHO cells by VaxGen/Global Solutions for Infectious Diseases (GSID) and adjuvanted with alum as a protein boost. The study explored 2 doses of the proteins: a total dose of 200 mcg (100 mcg for the A244 protein and 100 mcg for the MN protein) and a total dose of 600 mcg (300 mcg each of the same proteins in the lower dose formulation). The vaccine regimen with ALVAC-HIV and 600 mcg of gp120 protein adjuvanted with alum was utilized in study RV144. Table 4-12 summarizes the nAb response rates for the pertinent study arms from these studies.

Table 4-12 nAb response rates for selected regimens in RV132 and RV135 studies

Study	gp120 dose	Adjuvant	N	NPO3 Strain	SF2 Strain	CM244 Strain	MN Strain	Any Clade E
RV132 [50]	100mcg CM235* 50 mcg SF2**	MF59	45	89%	61%	95%	19%	100%
RV135 [49]	100mcg A244* 100mcg MN**	alum	50	23%	--	44%	100%	47%
	300mcg A244* 300mcg MN**	alum	47	31%	--	64%	98%	71%

* clade E Strain
** clade B Strain

The geometric mean (GM) nAb titers were also reported in these studies for 2 of the clade E strains. Data are summarized in Table 4-13.

Table 4-13 nAb GM titers to clade E strains

Study	gp120 dose	Adjuvant	N	NPO3 Strain	CM244 Strain
RV132 [50]	100mcg CM235* 50 mcg SF2**	MF59	45	45	32.66
RV135 [49]	100mcg A244* 100mcg MN**	alum	50	12.3	7
	300mcg A244* 300mcg MN**	alum	47	14.8	5.4

* clade E Strain
** clade B Strain

Although the data should be interpreted with caution, they suggest that 100 mcg of gp120 protein adjuvanted with MF59 can induce Ab responses after ALVAC

prime at least comparable to and possibly greater than 300 mcg of gp120 protein adjuvanted with alum after ALVAC prime. These data suggest that MF59 allows for protein dose sparing compared with the less potent alum adjuvant.

4.7.6 Clinical studies with HIV-1 subunit protein vaccines

For description of interim safety/tolerability and immunogenicity results from HVTN 100, see Section 4.7.2.4.

In addition, other closely related recombinant monomeric (gp120) subunit vaccine formulations from GSK Vaccines (formerly Novartis) have been tested in clinical trials. In addition, recombinant oligomeric (o-gp140) Env proteins for subtypes B and C from Novartis have been or are currently in clinical trials. Overall, in these studies, recombinant HIV-Env proteins manufactured by Novartis were well tolerated and immunogenic. In most cases, recombinant HIV-Env proteins (either gp120 or gp140) were CHO-based and administered with MF59, Novartis' proprietary oil-in-water emulsion adjuvant [66]. MF59 safety has been established in clinical studies as well as in commercial products. A seasonal influenza vaccine adjuvanted with MF59 (Fluad®) is licensed in the EU and other countries for use in the elderly. MF59 is also used in a prepandemic H5N1 influenza vaccine (Aflunov®) licensed in the EU for use in adults, and in 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 [45,50,60,67-69]. 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 being conducted by the NIH-sponsored HVTN in the US and the RSA. Table 4-14 summarizes clinical trial experience with Novartis gp120 and gp140 recombinant vaccine candidates.

Table 4-14 Novartis recombinant gp120 and gp140 vaccines in human clinical trials [68]

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, aluminum hydroxide, MPL-A, liposome-encapsulate MPL-A, MTP-PE/MF59®	107	AVEG 015	Completed
SF2 gp120 (CHO)/MF59® and ALVAC	47	AVEG 022A	Completed

Candidate vaccine	# receiving Novartis protein	Protocol	Status
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	145	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) & ISS TAT	30	ISS P-002	Completed
Subtype C (TV1) gp140 (CHO)/MF59®	20	HVTN 088	Completed
Subtype C (TV1) gp140 (CHO)/MF59® and SAAVI DNA-C2 and SAAVI MVA-C	24	HVTN073E	Completed
Subtype C (TV1) gp140 (CHO)/MF59® and SAAVI DNA-C2 and SAAVI MVA-C	114	HVTN 086	Completed

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 [4,45,50,60,67-71].

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

For description of interim safety/tolerability results from HVTN 100, see Section 4.7.2.4

In addition, two other clinical trials have been conducted recently using Novartis CHO-based subtype B gp140 with MF59 and 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 years, 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, pharyngolaryngeal pain and rhinorrhea. The subtype B gp140 MF59 was well tolerated following IM boost.

Another completed study with Novartis subtype B gp140 MF59 was a multicenter, placebo-controlled trial (HVTN 049) conducted by the HVTN in the US[73]. 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 1000/1000 mcg) or placebo (5 to 1 ratio) as a single IM injection at 0, 1 and 2 months, followed by a boost of subtype B gp140 with MF59 (or placebo) at 6 and 9 months. An additional group of subjects received subtype B gp140 with MF59 without DNA prime, administered at 0, 3, and 9 months. Overall 96 healthy, HIV-1–uninfected adult subjects were enrolled and 86 subjects completed all planned vaccinations. There were no SAEs reported as related to study vaccine. There were 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 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 generally well tolerated.

A third study, HVTN 073E, was conducted in the US and the Republic of South Africa (RSA) as an extension to the previous HVTN 073/SAAVI03 study. This extension study examined the safety and immunogenicity of 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 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 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 US in order to evaluate the safety and immunogenicity of a long-interval, cross-clade subtype C gp140/MF59 boost in subjects previously administered subtype B gp120/MF59 or subtype B gp140/MF59 in previous trials. This includes subjects from the HVTN049 DNA/PLG prime, gp140/MF59 boost study already described. 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 HVTN 088 to receive a clade C protein boost in an open label phase 1

trial. There have been 3 SAEs reported in this trial, 1 involving traumatic injury, 1 instance of gastroenteritis, and 1 of appendicitis. All of these were assessed as unrelated to study agents.

The second 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.

4.7.6.2 Summary of immunogenicity from recent human experience

The immunogenicity of Novartis recombinant proteins has been demonstrated consistently in all clinical trials and in all 3 of the recently completed studies using Novartis CHO-based subtype B gp140 MF59. In the HVTN 049 DNA/PLG prime protein boost study, the primary cellular immunogenicity endpoints included interferon gamma (IFN-γ) enzyme-linked immunospot (ELISpot) and ICS responses. Immunogenicity was assessed 14 days after each vaccination. Env-specific IFN-γ ELISpot response rates did not increase substantially compared to baseline after the 3 DNA/PLG prime vaccinations, but did rise after the first protein/MF59 boost. nAb titers against the homologous SF162 isolate were detectable in 2 subjects after the third DNA/PLG priming vaccination and in 13 subjects after the first protein boost. Neutralization was boosted to high titers in all but 1 subject following the second protein boost. Similarly, in the group of subjects who received subtype B gp140/MF59 without a DNA/PLG prime, a nearly complete response to the SF162 isolate was observed at the second vaccination (all 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.

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.

HVTN 073E data are available from specimens collected 2 weeks after each protein administration. Boosting with protein enhanced levels of binding and neutralizing antibodies as well as CD4+ T-cell responses to HIV-1 envelope. The protein boost increased CD4+ T-cell response rates from 74% to 87% of the subjects. No neutralizing antibodies were detected with the DNA/MVA regimen before the protein boosts. After the first protein boost, neutralizing antibodies to tier 1 viruses were detected in up to 56% of participants. After the second protein boost, neutralizing antibody responses were seen in up to 100% of participants and persisted in 75% of those participants for at least 6 months [74].

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 gp140 were present in 64% (IgG) and 7% (IgA) of primed participants at baseline, and rose to 93% and 85%, respectively, after 1 dose of protein. Primed participants demonstrated (71%) 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%).

4.7.7 Clinical studies with HIV-1 subunit protein vaccines and $AS01_B$

Immunogenicity data for studies HVTN 041, PRO HIV-005, ECR-004 and PRO HIV-002 are presented in Section 4.3.1 and 4.3.2.

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 gp120W6.1D) formulated with AS02A (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) 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 gp120W6.1D 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 gp120W6.1D groups (5 mcg and 100 mcg of gp120). Severe tenderness was observed in 2/20 subjects of 1 NefTat and gp120W6.1D group (5 mcg of gp120). Severe malaise was observed in 2/20 subjects of 1 NefTat and gp120W6.1D group (20 mcg of gp120). All vaccine-related reactogenicities were transient and had either improved or resolved within 48 hours of onset. Grade 4 laboratory abnormalities were observed in 1 single participant who was administered the AS02A-adjuvanted NefTat 20 mcg / gp120W6.1D 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 gp120W6.1D 5 mcg (associated to limitation of limb movement in 1 case), 2 occurrences of severe malaise in 2 subjects of group NefTat 20 mcg / gp120W6.1D 20 mcg, a febrile illness (103.2 ˚F) in 1 subject of group NefTat 20 mcg / gp120W6.1D 20 mcg, and an episode of generalized urticaria in 1 subject from group NefTat 20 mcg / gp120W6.1D 100 mcg. All of these SAEs significantly improved within 24 hours of onset. It is important to note that none of these SAEs would be regarded as serious 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 gp120W6.1D 20 mcg / NefTat 20 mcg 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.

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/$AS01_B$ group the overall frequency of the general symptoms tended to be higher after the second, third, or fourth 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). Six 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. This study had no negative social impact (only 2.8% of the subjects reported any disturbance) and 19.4% of the subjects notified even a beneficial impact.

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 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 / $AS01_B$ (PRO HIV-005) received a (third) single booster dose of F4 10 mcg / $AS01_B$.

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.

4.8 Potential risks of study products and administration

Table 4-15 includes general risks of vaccine administration along with risks known from prior clinical studies of ALVAC-HIV products and of envelope protein vaccines adjuvanted with MF59 and with $AS01_B$.

Table 4-15 Summary of potential risks of study products and administration

Common	• Mild to moderate injection site pain, tenderness, erythema, or swelling/induration/edema • Malaise/fatigue/weakness, myalgia, arthralgia, nausea/vomiting, lymphadenopathy, asthenia, fever, chills, or headache in the first few days following injection • Arm movement limitation • A vaccine-induced positive HIV Ab test result
Less common	• Severe injection site pain or tenderness • Flu-like syndrome, diarrhea, rash, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, other transient lesions, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection site reaction, such as > 10 cm diameter erythema or induration, sterile abscess or secondary bacterial infection • Allergic reaction, including rash, urticaria, angioedema, eyelid swelling, bronchospasm, or anaphylaxis • Injection site pruritus, warmth, nodule or other non-specific injection site reaction • Generalized pruritis • Oral parasthesia • Syncope, insomnia • Abdominal pain, anorexia, gastritis, dysgeusia • Skin disorder, acne, muscle damage at the injection site
Theoretical risks	• Autoimmune disease • Effects on a participant's response to an approved HIV vaccine administered in the future • Effects on susceptibility to HIV, if the participant is exposed to HIV • Effects on the course of HIV infection/disease, if the participant is infected with HIV • Effects on the fetus and on pregnancy

5 Objectives and endpoints

5.1 Primary objectives and endpoints

Primary objective 1

- To evaluate the safety and tolerability of ALVAC-HIV and bivalent gp120 protein/MF59 or bivalent gp120 protein/$AS01_B$.

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 compare HIV-specific CD4+ T-cell response rates at the month 6.5 timepoint (2 weeks after the fourth vaccination) of ALVAC-HIV and bivalent gp120 protein/MF59 to each of the bivalent gp120 protein/$AS01_B$ vaccine regimens.

Primary endpoint 2

- HIV-specific CD4+ T-cell response rates as assessed by flow cytometry.

Primary objective 3

- To compare HIV-specific Env-gp120 binding antibody response magnitudes at the month 12 timepoint (6 months after the fourth vaccination) of ALVAC-HIV and bivalent gp120 protein/MF59 to each of the bivalent gp120 protein/$AS01_B$ vaccine regimens.

Primary endpoint 3

- HIV-specific Env-gp120 binding antibody response magnitude as assessed by multiplex assay.

5.2 Secondary objectives and endpoints

Secondary objective 1

- To further evaluate the systemic immune responses and the durability of immunogenicity of each vaccine regimen at the month 6.5 and month 12 timepoints.

Secondary endpoint 1

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

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

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

- Additional immunogenicity assays may be performed on blood samples based on the HVTN Laboratory Assay Algorithm

5.3 Exploratory objectives

Exploratory objective 1

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

Exploratory objective 2

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

Exploratory objective 3

- 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 160 healthy, HIV-uninfected adult participants ages 18-40 years in Africa and the United States. Participants will be randomized to 4 treatment groups (3 vaccine groups of size 50 each and a placebo group of size 10). The three vaccine groups (groups 1-3) will evaluate ALVAC priming followed by boosting with ALVAC and gp120 protein. Three different formulations of the protein will be evaluated: 1) a dose of 100 mcg of each protein formulated with MF59; 2) a dose of 100 mcg of each protein formulated with $AS01_{B;}$ and 3) a dose of 20 mcg of each protein formulated with $AS01_{B}$. To ensure that both participants assigned male and female at birth will be adequately represented in the trial, the trial will enroll at least approximately 40% of each sex. Hence, when approximately 96 participants of one 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 assay 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 account for 8 enrolled participants on each of the vaccine groups having missing data for the primary immunogenicity endpoints.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect SAEs can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each vaccine group of the study (n =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 0.1% 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(0/100)	Pr(1+/100)	Pr(2+/100)
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 (CI) for the true rate of an adverse event based on the observed data. Table 6-2 shows the 2-sided 95% CIs for the probability of an event based on a particular observed rate. Calculations are done using the score test method [75]. 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 vaccine 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 is to compare Groups 2 and 3 ($AS01_B$ groups) separately to Group 1 (MF59 group) based on the following two criteria:

1. Establish superiority of the $AS01_B$ regimen in CD4+ T-cell response rate at month 6.5 for either TV1 or 1086; AND

2. Establish superiority of the $AS01_B$ regimen in magnitude of anti-gp120 antibody response at month 12 for 1086.

Power is calculated for each group comparison separately and assumes that:

1. The CD4+ T-cell response rate for the $AS01_B$ regimen is at least 31% higher for both antigens; AND,

2. The true geometric mean anti-gp120 binding antibody response is 1.5-fold higher for the $AS01_B$ regimen.

Power is based on data simulations using HVTN 100 CD4+ T-cell data from the month 6.5 visit (2 weeks after the fourth vaccination) and predicted anti-gp120

1086 antibody binding magnitude at the month 12 visit. Simulated HVTN 120 data sets were generated by sampling with replacement from the HVTN 100 data and power is reported as the percentage of the 10,000 simulated HVTN 120 trials that reject the composite null hypothesis defined by the primary immunogenicity evaluation ("Overall power") and separately for the null hypotheses defined by the CD4+ T-cell and the anti-gp120 antibody criteria. CD4+ T-cell response calls were simulated for the MF59 group (Group 1) by dichotomizing the ICS IL2/IFNγ CD4+ T-cell response magnitude using a threshold defined by the observed response rates of 49% to the TV1 antigen and 40% to the 1086 antigen. For the AS01 $_B$ comparator group (Group 2 or 3), CD4+ T-cell response calls were simulated based on threshold corresponding to response rates of 80% for TV1 and 71% for 1086 antigens. The rationale for this approach is to maintain the between antigen correlation and allow simulation of a higher response rate in the AS01 $_B$ group. Predicted HVTN 100 Env-gp120 binding data at month 12 were used for the second primary immunogenicity criterion. Env-gp120 binding magnitudes in the MF59 group (Group 1) at month 12 were predicted using HVTN 100 month 6.5 data and assuming the same antibody decline between 6.5 and 12 months that was obtained from modeling the decline of the antibody response to the A244 gp120 antigen in RV144 [76]. AS01$_B$ (Group 2 or 3) IgG response magnitudes were simulated on a logit scale using a fold-change equivalent to a 1.5-fold change between group geometric means on the MFI scale. Specifically, the logit transformation of MFI is defined by logit(MFI) = log((M-L)/L) – log(MFI/(M-MFI)) where $M=2^{15}$ and L=100, the upper and lower limits of detection of the binding antibody multiplex assay (BAMA). The rationale for using the logit scale is to simulate MFI values within the range of the assay.

Overall power, accounting for both primary immunogenicity criteria defined above, depends on inter-correlations between the CD4+ T-cell and anti-gp120 readouts. The correlation coefficient between month 6.5 TV1 and 1086 CD4+ T-cell magnitudes in HVTN 100 is high (0.86, Spearman rank correlation). Correlation coefficients between month 6.5 CD4+ T-cell magnitude (TV1 or 1086) and month 12 anti-gp120 1086 antibody magnitude are low (0.35 and 0.33, Spearman rank correlation). Although these correlations are difficult to interpret since they are based on predicted month 12 antibody responses, they are consistent with correlations between week 26 cellular and week 52 antibody responses from HVTN 096 and HVTN 205 [76]. Therefore, the results for overall power shown in Table 6-3, are based on the correlation between month 6.5 CD4+ T-cell magnitude and predicted anti-gp120 1086 antibody magnitude. An alternative is to assume independence between the month 6.5 CD4+ T-cell responses and the month 12 anti-gp120 responses. Under this scenario the overall power is virtually the same (results not shown).

Power calculations are based on 1-sided Fisher's exact tests for the TV1 and 1086 CD4+ T-cell response rate comparisons and a 1-sided t-test comparing log(MFI) for the anti-gp120 responses comparison. Comparisons are based on an alpha level of 0.025.

Table 6-3 Power for each primary immunogenicity endpoint separately and overall

Sample Size		Power		
$ASO1_B$	MF59	CD4+ T-cell response	gp120 magnitude	Overall
43	43	0.892	0.913	0.819

6.2 Randomization

A participant's randomization assignment will be computer generated and provided to the HVTN CRS pharmacist through a Web-based randomization system. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments (except in emergency situations as specified in the HVTN MOP).

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participants' 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.

In some cases, the CRS, PSRT, or study sponsor may believe unblinding of the site PI and participant would be appropriate to facilitate the clinical management of an AE or SAE. The HVTN Unblinding MOP specifies procedures for emergency unblinding, and for early unblinding for medical reasons.

6.4 Statistical analysis

This section describes the final study analysis, unblinded as to treatment group assignment. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan will address how to analyze the participant's safety data. Analyses are modified intent-to-treat in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between

randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

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

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

Immunogenicity data from this study may be combined with other phase 1/2a studies within the 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 H. These listings will be submitted to the FDA in all annual reports and clinical trial reports.

6.4.3.3 Local laboratory values

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

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

6.4.3.4 Reasons for vaccination discontinuation and early study termination

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

6.4.4 Immunogenicity analysis

6.4.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Additional analyses may be

performed, limited to participants who received all scheduled injections per protocol. Assay results that are unreliable, from specimens collected outside of the visit window, or from HIV-infected participants after infection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample after enrollment, then all data from that participant may be excluded from the analysis.

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 [75]. For the primary endpoints of CD4+ T-cell response rates for TV1 and 1086, differences between groups will be tested with a 2-sided Barnard's or Fisher's exact test (as specified in the SAP) at an alpha level of 0.05. For secondary and exploratory assay endpoints, response rates and 95% confidence intervals will be calculated if appropriate for the endpoint. No adjustment will be made to the vaccine group estimates for the false positive rates in the placebo group.

The primary endpoint of anti-gp120 binding antibody response magnitude, and likely some of the secondary assays, will have quantitative assay data. Other quantitative measures include binding antibody magnitude from the multiplex assay, neutralizing antibody titers, area under the magnitude-breadth curve [AUC-MB] for the neutralizing antibody assay, and percentage of positive cells from the ICS assay. Quantitative data will be displayed as graphical and tabular summaries of the distributions by antigen, treatment group, and timepoint. For the primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be presented by group. For the primary endpoint, differences between groups will be tested with a nonparametric Wilcoxon rank sum test if the data are not normally distributed or with a 2-sample t-test if the data appear to be normally distributed, at an alpha level of 0.05.

Formal statistical comparisons between groups for secondary and exploratory immunogenicity endpoints are not objectives of the trial, although these are likely to be made to better understand the effect of each vaccine regimen. For comparisons in which the response rate for 1 of the groups is low (eg, $\leq 20\%$ for the class), statistical testing will use Barnard's or Fisher's exact tests (as specified in the SAP) 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. If treatment group differences for these assays are best summarized by a mixture model, then either Lachenbruch's test statistic [77] or an alternative two-part test [78] (as defined in the SAP) 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. 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.

6.4.4.2 Missing data considerations

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). 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 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 [79] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [80] 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 and data sharing 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 in accordance with Sections 6.4.5.1 and 6.4.5.2. Interim blinded safety and immunogenicity data should not be shared outside of the SMB, HVTN 120 PSRT, the protocol team leadership, the HVTN Executive Management Team, the study product developer, and the study sponsor and/or its designee(s) for their regulatory reporting unless approved by the protocol leadership and the HVTN leadership.

6.4.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the study, as defined in Section 11.1.2, for review by the HVTN SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 120 PSRT. Refer to the process described in the HVTN unblinding MOP any requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity analyses

The unblinded analysis of the primary endpoint of CD4+ T-cell response rates measured at month 6.5 will be conducted when all participants have completed the visit and all samples have been analyzed. Unblinded results will not be made publicly available until participants have completed their month 12 visit. Unblinded data analyses of secondary and exploratory endpoints (ie, additional immunogenicity assays) measured at month 6.5 will occur after the unblinded CD4+ T-cell immunogenicity analysis since selection of secondary assays is dependent upon the primary analysis. Analysis of secondary and exploratory objectives for month 6.5 will take place after the primary analysis and may be performed when assay data are available for analysis from at least 80% of participants.

Analysis of anti-gp120 binding antibody response magnitudes measured at month 12 will be conducted when all participants have completed the visit and all samples have been analyzed. Unblinded analysis of secondary and exploratory endpoints from the month 12 timepoint will take place after the primary analysis of anti-gp120 binding antibody and may be performed when assay data are available for analysis from at least 80% of participants.

The Laboratory Program will review analysis reports 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 decisions related to future trials. 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 information available at the time of enrollment, including results of screening laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions.

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

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

7.1 Inclusion criteria

General and Demographic Criteria

1. **Age** of 18 to 40 years

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

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

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

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

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

HIV-Related Criteria:

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

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

9. Assessed by the clinic staff as being at **"low risk" for HIV infection** and committed to maintaining behavior consistent with low risk of HIV exposure through the last required protocol clinic visit (see low risk guidelines in Appendix J and Appendix K).

Laboratory Inclusion Values

Hemogram/Complete blood count (CBC)

10. **Hemoglobin** ≥ 11.0 g/dL for volunteers who were assigned female sex at birth, ≥ 13.0 g/dL for volunteers who were assigned male sex at birth. For transgender participants who have been on hormone therapy for more than 6 consecutive months, determine hemoglobin eligibility based on the gender with which they identify (ie, a transgender female who has been on hormone therapy for more than 6 consecutive months should be assessed for eligibility using the hemoglobin parameters for persons assigned female sex at birth).

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

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

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

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

Chemistry

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

Virology

16. **Negative HIV-1 and -2 blood test**: 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.

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

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

Urine

19. **Normal urine**:

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

Reproductive Status

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

21. **Reproductive status**:

Africa

A volunteer who was assigned female sex at birth 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 Africa is defined as using 2 methods of birth control. These include 1 of the following methods:
  - Condoms (male or female), or
  - Diaphragm or cervical cap,

  PLUS 1 of the following methods:
  - Intrauterine device (IUD),
  - Hormonal contraception (in accordance with applicable national contraception guidelines),
  - Successful vasectomy in any partner assigned male at birth (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity after vasectomy); or
  - Any other contraceptive method approved by the HVTN 120 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.

United States

A volunteer who was assigned female sex at birth 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 the United States is defined as using any 1 or more of the following methods of birth control:
  - Condoms (male or female) with or without spermicide,
  - Diaphragm or cervical cap with spermicide,
  - IUD,
  - Hormonal contraception, or
  - Successful vasectomy in any partner assigned male at birth (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity after vasectomy); or
  - Any other contraceptive method approved by the HVTN 120 PSRT
- Or must 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 must be sexually abstinent.

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

Other

23. **Volunteers 21 years of age and older who were assigned female sex at birth 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 prior to enrollment (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 120 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 120 PSRT will determine eligibility on a case-by-case basis.

8. **Previous receipt of monoclonal antibodies (mAbs)**, whether licensed or investigational; the HVTN 120 PSRT will determine eligibility on a case-by-case basis.

9. **Non-HIV experimental vaccine(s) received within the last 5 years** in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 120 PSRT will determine eligibility on a case-by-case basis. For volunteers who have received an experimental vaccine(s) more than 5 years ago, eligibility for enrollment will be determined by the HVTN 120 PSRT on a case-by-case basis.

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

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

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

Immune System

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

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

15. **Immunoglobulin** received within 60 days before first study vaccination (for mAb see criterion 8 above)

16. **Autoimmune disease**

17. **Immunodeficiency**

Clinically significant medical conditions

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. (Not excluded: type 2 cases controlled with diet alone or a 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
  - Receipt of allergy treatment with antigen injections
- Within 14 days prior to any study injection
  - Receipt of any vaccines that are not live attenuated vaccines (eg, pneumococcal)
- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.
- 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 120 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 120 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 temporarily or 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 120 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);
  - HIV infection;
  - Any grade 4 local or systemic reactogenicity symptom, lab abnormality, or AE that is subsequently considered to be related to vaccination;

- Any grade 3 lab abnormality that is subsequently considered to be related to vaccination
- Other grade 3 clinical AE (exception: fever or vomiting and subjective local and systemic symptoms) that is subsequently considered to be related to vaccination. For grade 3 injection site erythema and/or induration, upon review, the PSRT may allow continuation of vaccination
- SAE that is subsequently considered to be related to vaccination
- Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 120 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination;

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

A study participant who misses a study vaccination is permitted to continue with subsequent vaccinations that can still be scheduled within the time intervals specified for those procedures in the *HVTN 120 Study Specific Procedures* (SSP), unless there is a protocol-mandated reason for discontinuation.

Participants discontinuing study product for reasons other than HIV infection 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 (see HVTN 120 SSP).

Participants diagnosed with HIV infection during the study should be encouraged to participate in follow-up visits as indicated in Section 9.14).

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,
- 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): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0 and 1;

THEN

ALVAC-HIV (vCP2438) to be administered as 1 mL IM in 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 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 RIGHT deltoid (unless medically contraindicated) at months 3 and 6.

Group 2

Treatment 2 (T2): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0 and 1;

THEN

ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT 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;

AND

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

Group 3

Treatment 3 (T3): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0 and 1;

THEN

ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT 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;

AND

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

Group 4

Placebo 4 (P4): Placebo for ALVAC-HIV (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0 and 1;

THEN

Placebo for ALVAC-HIV (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM in LEFT 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;

AND

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

8.2 Study product formulation

ALVAC-HIV (vCP2438) [Labeled as ALVAC-HIV (vCP2438)]

ALVAC-HIV (vCP2438) is provided as a lyophilized, white to beige product. It must be stored refrigerated (2-8°C). Once reconstituted with 1 mL of Diluent (0.4% NaCl), it appears as a clear to slightly opalescent solution, colorless with possible presence of particles or filaments.

The study product is described in further detail in the IB.

Diluent for ALVAC-HIV (vCP2438) (labeled as Diluent 0.4% NaCl)

The diluent is provided in a vial filled with a volume to deliver 0.5 mL of sterile sodium chloride solution (NaCl 0.4%). Two vials will be needed to reconstitute each vial of ALVAC-HIV (vCP2438). It must be stored refrigerated (2-8°C). DO NOT FREEZE.

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

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

Bivalent 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 (462 mcg) 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.

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 (462 mcg) 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.

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 is an opaque whitish suspension 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, protected from light. Do not freeze.

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.

AS01B is produced as a liposomal formulation containing MPL and QS-21 Stimulon®. 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 / AS01B (Sodium Chloride for Injection, 0.9%)

Sodium Chloride for Injection, 0.9%, will be used as the placebo for Bivalent Subtype C gp120 / AS01B. 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 ALVAC-HIV (vCP2438)

One vial of ALVAC-HIV (vCP2438) and 2 vials of diluent (NaCl 0.4%) are needed to prepare this dose.

Before reconstitution, the pharmacist will allow the vials to equilibrate to room temperature. The pharmacist, using aseptic technique, will withdraw a total of 1 mL from the 2 vials containing diluent (NaCl 0.4%) and slowly inject (the 1 mL of diluent) into the vial containing the lyophilized ALVAC-HIV. The pharmacist will then set the vial aside and allow the vial to sit for up to 3 minutes to allow for dissolution of the vaccine. The pharmacist will gently swirl the vial to assure the

contents are well dissolved. DO NOT SHAKE THE VIAL. (Note: Presence of particles or filaments in the dissolved solution is possible). The study product is stable in the vial for 6 hours after reconstitution. Using aseptic technique, the pharmacist will then withdraw the total contents of the ALVAC-HIV vial into a 2, 3 or 5 mL syringe. The pharmacist will apply an overlay to the syringe.

The syringe should be labeled as "ALVAC-HIV or placebo 1 mL" as well as "Administer in Left Deltoid". Once the dose is drawn up in a syringe, the study product should be administered as soon as possible within 30 minutes (per Immunization Action Coalition [IAC] and US Centers for Disease Control and Prevention [CDC] recommendations).

Any unused portion of reconstituted vials or prefilled expired syringes is disposed of in accordance with institutional or pharmacy policy for a biological safety level 1 product.

8.3.2 Placebo for ALVAC-HIV

Using aseptic technique, the pharmacist will withdraw 1 mL of Sodium Chloride for Injection, 0.9% into a 2, 3, or 5 mL syringe. The pharmacist will apply an overlay to the syringe.

The syringe should be labeled as "ALVAC-HIV or placebo 1 mL" as well as "Administer in Left Deltoid". Once the dose is drawn up in a syringe, the study product should be administered as soon as possible within 30 minutes per IAC and US CDC recommendations.

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 MF59)

One vial of TV1.C gp120 protein, one vial of 1086.C gp120 protein, and one vial of MF59C.1 will be needed to prepare the dose.

Prior to dispensing, the pharmacist will remove the TV1.C gp120 and 1086.C gp120 from the freezer and allow to thaw at room temperature. (Note: Once thawed, the 1086.C gp120 and/or TV1.C gp120 vials should be used immediately for preparation or stored in a refrigerator at 2°C – 8°C for no longer than 24 hours. Unused 1086.C gp120 and/or TV1.C gp120 protein vials should be quarantined for destruction after this time.) The pharmacist will also remove the MF59C.1 vial from the refrigerator and mix by repeated gentle swirling and inversion (do not shake vigorously).

Using aseptic technique, the pharmacist will gently swirl the contents of the vial containing TV1.C gp120 and then withdraw 0.35 mL of TV1.C gp120 from the correct vial and inject it into the vial containing MF59C.1. The pharmacist will

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

The syringe should be labeled as "Bivalent Subtype C gp120/MF59 or Placebo 0.5 mL", as well as "Administer 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 study product 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.

8.3.4 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 1 or 2 mL syringe. The pharmacist will apply an overlay to the syringe.

The syringe should be labeled as "Bivalent Subtype C gp120/MF59 or Placebo 0.5 mL", as well as "Administer 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 study product 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. (Note: Once thawed, the 1086.C gp120 and/or TV1.C gp120 vials should be used immediately for preparation or stored in a refrigerator at 2°C – 8°C for no longer than 24 hours. Unused 1086.C gp120 and/or TV1.C gp120 protein vials should be quarantined for destruction after this time.) 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) for dosing into a 3 mL syringe or smaller. The pharmacist will apply an overlay to the syringe.

The syringe should be labeled as "Bivalent Subtype C gp120 / $AS01_B$ or Placebo 0.75 mL", as well as "Administer 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 study product 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. (Note: Once thawed, the 1086.C gp120 and/or TV1.C gp120 vials should be used immediately for preparation or stored in a refrigerator at 2°C – 8°C for no longer than 24 hours. Unused 1086.C gp120 and/or TV1.C gp120 protein vials should be quarantined for destruction after this time.) 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 $AS01_B$). 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 pharmacist will apply an overlay to the syringe.

The syringe should be labeled as "Bivalent Subtype C gp120 / $AS01_B$ or Placebo 0.75 mL", as well as "Administer 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 study product 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 pharmacist will apply an overlay to the syringe.

The syringe should be labeled as "Bivalent Subtype C gp120 / $AS01_B$ or Placebo 0.75 mL", as well as "Administer 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. If this is not possible, the study product 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.

8.4 Administration

All injections are to be given IM in the deltoid indicated. Any administrator of study product will be blinded to the individual participant's treatment assignment.

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

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

The ALVAC-HIV (vCP2438) and diluent (0.4% NaCl) will be supplied by Sanofi Pasteur and will be available through the NIAID Clinical Research Products Management Center (CRPMC).

Bivalent Subtype C gp120, MF59®, and $AS01_B$ will be supplied by GSK Vaccines and will be available through the NIAID CRPMC.

Placebo for ALVAC-HIV (Sodium Chloride for Injection, 0.9%), Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0.9%), and Placebo for Bivalent Subtype C gp120/$AS01_B$ (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, the pharmacist can obtain study products from the CRPMC by following the ordering procedures outlined in the 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

For US clinical research sites, all unused study products must be returned to the CRPMC after the study is completed or terminated unless otherwise instructed by the study sponsor. For non-US clinical research sites, all unused study products must be destroyed after the study is completed or terminated unless otherwise instructed by the study sponsor. The procedures 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 G.

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 IRB/EC and any applicable Regulatory Entity (RE) for human subjects protection review and approval.

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

9.1.1 Screening consent form

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

Some HVTN CRSs have approval from their IRB/EC and any applicable RE to use a general screening consent form that allows screening for an unspecified HIV vaccine trial. In this way, HVTN CRS staff can continually screen potential participants and, when needed, proceed quickly to obtain protocol-specific

enrollment consent. Sites conducting general screening or prescreening approved by their IRB/EC and any applicable RE may use the results from this screening to determine eligibility for this protocol, provided the tests are conducted within the time periods specified in the eligibility criteria.

9.1.2 Protocol-specific consent forms

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

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

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 E. The consent form(s) must be developed in accordance with requirements of the following:

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

Study sites are strongly encouraged to have their local CABs review their site-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 forms include 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 (see low risk guidelines in Appendix J and Appendix K);
- 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, including:
  - Screening HIV test,
  - CBC with differential and platelet count,

  - Chemistry panel (ALT, AST, ALP, and creatinine),
  - Urine dipstick (urinalysis if indicated, see Section 9.9),
  - HBsAg,
  - Anti-HCV Ab,
  - Urine or serum pregnancy test (volunteers who were assigned female sex at birth);
  - Pap smear (Only for volunteers who were assigned female sex at birth and who agree to provide cervical samples; not required if volunteer has had Pap smear within previous 3-5 years with most recent result normal or ASCUS or less than 21 years old (see Section 7.1, Criterion 23;

- Syphilis test,
- 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 9.7; 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 assigned female sex at birth and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.

9.2.1 Use of screening results from another HVTN study

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

9.3 Enrollment and vaccination visits

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

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

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

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

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

Immediately following vaccination, the participant remains in the clinic for observation. An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes. Before leaving the clinic, the participant is given the Participant Diary and is instructed on how to complete it. The site will make arrangements to be in contact with the participant during the reactogenicity period (as described in Section 9.10).

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 9.7);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.8); 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 G:

- 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 G):
  - 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 9.7);

- Pregnancy prevention assessment (as described in Section 9.2 and 9.8); 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 G:

- Administration of behavioral risk assessment questionnaire;
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;
- HIV infection assessment including pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Specimen collection (blood and/or mucosal and/or stool);
- Clinical laboratory tests including:
  - CBC with differential,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.9); and

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

- For participants who agree to mucosal sampling collection (see Section 9.5):
  - Urine test 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; and

- Mucosal specimen collection.

9.5 Mucosal sampling

Mucosal samples will be collected at the timepoints indicated in Appendix G 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 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. Sample collection may not be performed or may be deferred to a later date within the visit window if a contraindication to sampling (eg, active GTI) is present (as indicated below).

Rectal fluid sampling (both sexes): For participants assigned female sex at birth, a pregnancy test must be performed and be negative prior to any rectal mucosal sampling. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Rectal 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 a herpes simplex

virus (HSV)-2 outbreak or inflamed hemorrhoids or colitis/diarrhea] or if the participant has any active genital tract infection (GTI).

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

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

Cervical fluid sampling (only for participants who were assigned female sex at birth): Participants who are 21 years of age and older must report having had a Pap smear within the 3-5 years prior to enrollment, with the latest result reported as normal or ASCUS (see Section 7.1). A pregnancy test must be performed on the day of cervical sampling. The pregnancy test can be performed after collection has taken place. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. 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 is known to have an active ulcerative genital lesion or 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 or believes to have an active GTI at the scheduled timepoint.

9.6 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.

9.7 HIV counseling and testing

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

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

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

Potential participants identified as being HIV-infected during screening are not enrolled. Potential and enrolled participants identified as HIV infected will be referred for medical treatment, counseling, and management of the HIV infection. Participants who are found to be HIV-infected after enrollment will not receive any additional study product but will continue to be followed in the study for safety assessments. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.7.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

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

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

- HIV testing will be performed at multiple timepoints throughout the study (see Appendix F). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the HVTN 120 SSP), which is able to distinguish vaccine-induced Ab responses from actual HIV infections.

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

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

9.7.2 VISP registry

Experimental HIV vaccines may induce Ab production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP) (see Section 9.7.1). In order to provide poststudy HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of poststudy testing and counseling services to HIV vaccine recipients. The registry contains the names of all study participants, unless they request that their names be removed.

9.8 Contraception status

Contraception status is assessed and documented at every scheduled clinic visit for a participant who was assigned female sex at birth 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 assigned female sex at birth 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.

9.9 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.

9.10 Assessments of reactogenicity

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

The reactogenicity assessment period is 7 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record

symptoms using a Participant Diary. Contacts between the participant and the site staff should take place at least once between 1-3 days postvaccination . In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 to resolution.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, vaccine-related lesions. 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 criteria for SAE/adverse events requiring expedited reporting to regulatory authorities, are recorded on an adverse experience log form.

Table 9-1 Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Baseline: before vaccination	HVTN CRS clinician
	Early: 25-60 minutes after vaccination	HVTN CRS clinician
	Between early assessment and 11:59 pm day 0	HVTN CRS clinician or participant
1-7[b]	Between 12:00 am and 11:59 pm on the respective day 1-7	HVTN CRS clinician or participant

[a] Day of vaccination

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

9.10.1 Assessment of systemic and local symptoms

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

Body temperature is measured by oral or infrared thermometry. All temperatures must be measured by non-axillary thermometry. This includes temperatures taken in the clinic, as well as temperatures taken by participants during the reactogenicity period.

Temperature is 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.

9.10.2 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 Visit windows and missed visits

Visit windows are defined in HVTN 120 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.

9.12 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, and chemistry panel), pregnancy testing, social impact assessment, and HIV test. For participants who have a confirmed diagnosis of HIV infection, see Section 9.14.

9.13 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 available. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome. Pregnancies and pregnancy outcomes will be reported.

9.14 HIV infection during the study

If a participant becomes HIV-infected during the course of the study, no additional study product will be administered. Participants will be encouraged to continue scheduled study visits for up to 24 weeks following their last study product administration. Follow-up duration for participants diagnosed with HIV infection may be adjusted in consultation with the CRS investigator and the HVTN 120 PSRT (eg, to avoid interference with participant initiation of HIV treatment). At post-infection follow-up visits, only specimens required for protocol-specified safety laboratory tests, urinalysis and pregnancy tests will be collected; in addition, some clinic procedures may be modified or discontinued (see Appendix F and Appendix G).

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN Site Lab Instructions and SSP provide further guidelines for operational issues concerning the clinical and processing laboratories. These documents include guidelines for general specimen collection, special considerations for phlebotomy, specimen labeling, whole blood processing, HIV screening/diagnostic testing, and general screening and safety testing.

Tube types for blood collection are specified in Appendix F. 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.

Of note, all assays described below are performed as research assays to evaluate the ability of the vaccine to induce immune responses in the context of the participants' genetic background, and are not approved for use in medical care. Results from these assays are not made available to participants or medical professionals to guide treatment decisions.

10.2 Total blood volume

Required blood volumes per visit are shown in Appendix F. 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 timepoints

The primary immunogenicity timepoints in this study are at month 6.5 and at month 12. Endpoint assays for humoral and cellular responses are performed on participants at the appropriate primary immunogenicity timepoints and may be performed on samples collected at baseline. Depending on the initial results, assays for humoral and cellular responses may be performed on samples collected from participants at other timepoints; the schedule is shown in Appendix F.

10.4 Endpoint assays: humoral

10.4.1 Binding antibody multiplex assay (BAMA)

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

10.4.2 Neutralizing Ab (nAb) assay

HIV-1–specific nAb assays may be performed on serum samples from study participants taken at the primary immunogenicity timepoint(s). Specimens from the baseline and other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoints. The TZM-bl assay will test neutralization of the vaccine strains (96ZM651, TV1.C, 1086.C) and a single highly neutralization-sensitive tier 1 virus as a positive control (MW965.26). The global panel and clade C panel may be used to assess tier 2 neutralization [81,82].

10.4.3 Antibody-dependent cell-mediated cytotoxicity (ADCC) assay

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

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. 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. 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 genes, including those associated with immune responses (eg, immunoglobulin, or T cell receptor genes), 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 at the FHCRC where they will be stored at -80ºC prior to DNA extraction. DNA extraction will be performed with bead-beating and chaotropic lysis of bacteria. Bacterial communities in stool samples will be assessed using broad-range 16S rRNA gene PCR followed by high throughput sequencing and phylogenetic/taxonomic analysis of the sequences. Associations identified by broad range PCR may be confirmed using taxon-directed quantitative PCR assays.

10.9 Specimen storage and other use of specimens

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

Other use of specimens is defined as studies not covered by the protocol or the informed consent form for the main study (see Appendix A).

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

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

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

10.10 Biohazard containment

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

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

11 Safety monitoring and safety review

11.1 Safety monitoring and oversight

11.1.1 HVTN 120 PSRT

The HVTN 120 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) /Regional Clinical Safety Liaison (RCSL), and
- A medical officer from an organization in Africa designated by the study sponsor will also participate in the PSRT.

The clinician members of the HVTN 120 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, clinical data manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 120 PSRT meetings.

11.1.2 HVTN SMB

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

The SMB reviews safety data, unblinded as to treatment arm, approximately every 4 months. The reviews consist of evaluations of cumulative reactogenicity events, AEs, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS and pertinent national regulatory authorities. To increase the sensitivity for detecting potential safety problems, the SMB will review safety data aggregated across multiple protocols that use the same or similar vaccine candidates. The SMB conducts additional special reviews at the request of the HVTN 120 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 120 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 120 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 120 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

Sites must submit all safety forms (eg, reactogenicity, adverse experience, urinalysis, local lab results, and concomitant medications) before the end of the next business day, excluding federal or bank holidays. 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. For the case of a longer site holiday closure, site staff must submit the data by the end of the 5th day (local time) after receiving the information even if this day is a holiday.

For example: If the site becomes aware of an AE on Thursday (Day 0), the site must submit the data by the end of the next business day, on Friday. If there is a longer site holiday closure, then this AE must be reported no later than the end of the fifth day, Monday (Day 4). If Monday is a holiday as well, all safety forms still need to be submitted by the end of Monday (Day 4).

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), Corrected Version 2.1, July 2017, available on the RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables, except:

- Unintentional weight loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health (see *HVTN 120 Study Specific Procedures*);

- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:
  - Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area;
  - Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area;
  - Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage;
  - Grade 4 is: Potentially life-threatening consequences (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue);

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 H.

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 HVTN clinical safety staff of any serious safety concern requiring their attention (see Table 11-1). Telephone numbers and email addresses are found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/HVTN 120). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

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

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

11.2.3 Expedited reporting of AEs to DAIDS

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

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

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

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

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

- results in death,
- is life-threatening (Note: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death, if it were more severe),
- requires patient hospitalization or prolongation of existing hospitalization,

- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect, or
- is a medically important event or reaction*

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

The expedited reporting period for this study comprises the entire study period for each individual participant (from study enrollment until study completion or discontinuation from the study). After the expedited reporting period for the study, unless otherwise noted, only Suspected, Unexpected Serious Adverse Reactions as defined in Version 2.0 of the DAIDS EAE Manual must be reported to DAIDS, if the study staff become aware of the events.

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

In some cases, the PSRT or CRS may believe unblinding of the site PI and participant would be appropriate to facilitate the clinical management of an AE or SAE. The HVTN MOP specifies procedures for emergency unblinding, and for early unblinding for medical reasons.

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

- ALVAC-HIV (vCP2438)/placebo
- Bivalent Subtype C gp120/MF59/placebo
- Bivalent Subtype C gp120/AS01$_B$/placebo

11.2.4 Expedited reporting of AEs to pertinent national regulatory authorities

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

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

11.3 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccination with the product related to the event that triggered the pause will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 120 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 120 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 120 PSRT notification
SAE, related	Grade 3, 2, or 1	Email and submit forms immediately	Immediate PSRT notification and prompt PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and submit forms immediately	Immediate PSRT notification and prompt 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/HVTN 120).

[b] Does not include the following Grade 3 subjective reactogenicity symptoms [injection site pain, tenderness, fatigue/malaise, myalgia, arthralgia, chills, headache, nausea (unless IV rehydration required)].

For all safety pauses, HVTN Core notifies the HVTN 120 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 120 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 120 PSRT assessment, the trial sponsor or designee(s) notifies FDA and other pertinent national regulatory authorities as needed.

If an immediate HVTN 120 PSRT notification or prompt HVTN 120 PSRT AE review is triggered, HVTN Core notifies the HVTN 120 PSRT as soon as possible during working hours (local time)—or, if the information was received during off hours, by the morning of the next work day. If a prompt HVTN 120 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

The HVTN requires that each CRS submit to its IRB/EC and any applicable RE protocol-related safety information (such as IND safety reports, notification of vaccine holds due to the pause rules, unanticipated problems involving risks to participants or others, 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 120 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 120 PSRT AE review criteria.

11.4.2 Weekly review

During the injection phase of the trial, the HVTN 120 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 120 PSRT. HVTN Core reviews reports of clinical and laboratory AEs. Events identified during the review that are considered questionable, inconsistent, or unexplained are referred to the HVTN CRS clinic coordinator for verification.

11.5 Study termination

This study may be terminated early by the determination of the HVTN 120 PSRT, 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;
- Exploratory and ancillary studies and sub-studies, and
- Destruction of specimens.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 120 *Study Specific Procedures.*

12.1 Social impacts

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

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

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

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

Because this study is evaluating products containing recombinant or synthetic DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (April 2016). Information about the study must be submitted to site Institutional Biosafety Committees (IBC). Investigators at each site are responsible for obtaining IBC approval per NIH guideline *section IV-B7-a-(1).* IBC review and approval must be documented by the investigator and submitted as part of DAIDS's initial protocol registration for this trial before participants are enrolled at the site. If this protocol is amended, investigators should follow the requirements of their IBC.

12.3 Regulatory considerations for African countries

Any regulations specific to African countries containing CRSs at which HVTN 120 will be implemented will 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 can contact the participant without IRB/EC approval if such communication is necessary to avoid imminent harm to the study participant. The CRS must 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 120 are described below.

Protocol history and modifications

Date: September 12, 2018

Protocol version: Version 3.0
Protocol modification: Full Protocol Amendment 2

Item 1	Revised in Sections 3 and 6 and Appendix A: Sample size reduced from 320 to 160
Item 2	Updated in Sections 1, 14, and 15: ICH full name
Item 3	Updated in Section 3.1, *Protocol Team*: Other contributors to the protocol
Item 4	Revised in Section 6.4.4.1, *General approach*: Tests for comparing response rates between arms
Item 5	Clarified in Sections 2.1, 7.1, 9.2, 9.3, 9.4, 9.5, 9.8, and Appendices A, B, C, F, and G: Assignment of sex at birth
Item 6	Revised in Sections 7.3.3, 7.3.4, 9.7, 9.12, 9.14, and Appendices A, F, and G: Follow-up for participants diagnosed with HIV infection during study participation
Item 7	Clarified in Section 7.3.3, *Discontinuing vaccination for a participant*: Specified time intervals apply to entire vaccination procedure
Item 8	Clarified in Section 9.1.2, *Protocol-specific consent forms*: Local use consent forms
Item 9	Clarified in Section 9.2, *Pre-enrollment procedures*: Required laboratory tests
Item 10	Revised in Section 9.7.1, *Distinguishing intercurrent HIV infection from vaccine-induced positive serology*: Location of HVTN HIV testing algorithm
Item 11	Revised in Section 10.4.1: Description of the BAMA assay
Item 12	Clarified in Section 11.2.3, *Expedited reporting of AEs to DAIDS*: DAIDS EAE Manual source and DAERS contact information
Item 13	Removed in Section 11.3, *Safety pause and prompt PSRT AE review*: Reactogenicity symptom duration
Item 14	Clarified in Section 11.3, *Safety pause and prompt PSRT AE review*: Reporting of protocol-related safety information
Item 15	Revised in Sections 2, 3, 6.1, 7.1, 11.1.1, 12.3 and Appendices A, E, and K: References to *Southern* Africa

Item 16	Updated in Appendix A, *Sample informed consent form*: Study related injury language
Item 17	Clarified in Appendices A, B, and C: Birth control requirements
Item 18	Clarified in Appendix I, *Injection schedule for sample informed consent*: Number of right-arm injections shown at Months 3 and 6
Item 19	Corrected: Typographical errors and stylistic deviations

Date: April 16, 2018

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 1

Item 1	Revised in Section 6.4.4.1: Tests for comparing response rates between arms
Item 2	Clarified in Appendix I: Injection schedule graphic for sample informed consent form

Date: January 3, 2018

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

Item 1	Added: enrollment in the United States
Item 2	Added in Section 3, *Study product providers*: diluent for ALVAC-HIV to list of products being provided in the study
Item 3	Updated in Section 3.1, *Protocol Team*: Protocol team leadership
Item 4	Updated in Section 4.5.1.3, *Bivalent Subtype C gp120/AS01$_B$ dose*: current safety data from HVTN 108, which is evaluating the same vaccine.
Item 5	Added in Section 6.4.5, *Analyses and data sharing prior to end of scheduled follow-up visits*: data sharing restrictions for interim blinded safety and immunogenicity data
Item 6	Clarified in Section 7.1, *Inclusion criterion, Criterion 10*: hemoglobin eligibility for transgender participants
Item 7	Revised in Section 7.2, *Exclusion criterion*
Item 8	Removed the eligibility requirement regarding untreated or incompletely treated syphilis infection
Item 9	Revised in Section 7.3.1, *Delaying vaccinations for a participant*: criteria for delaying any study vaccinations due to the timing of receipt of a live attenuated influenza vaccine
Item 10	Revised in Section 7.3.3, *Discontinuing vaccination for a participant*
Item 11	Updated in Section 8, *Study product preparation and administration*
Item 12	Revised in Section 9.3, *Enrollment and vaccination visits*: participant diary will now be used in place of the postvaccination memory tool
Item 13	Removed in Section 9.4, *Follow-up visits*: platelet counts from required clinical lab tests

Item 14 Updated in Section 9.10, *Assessments of reactogenicity*, 11.2.2, *AE reporting*, and Section 14, *Document references (other than literature citations)*: version number for the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events

Item 15 Deleted Section 9.10.3, *Assessment of lymph nodes*

Item 16 Updated in Section 11, *Safety monitoring and safety review*

Item 17 Updated: Section 13, *Version history*

Item 18 Revised in Appendix A, *Sample informed consent form*

Item 19 Revised in Appendix F, *Laboratory procedures*

Item 20 Added in Appendix G, *Procedures at HVTN CRS*

Item 21 Added Appendix J and Appendix K, *Low Risk Guidelines* for sites in US and sites in Southern Africa

Item 22 Added Appendix L, *Protocol Signature Page*

Item 23 Updated HVTN protocol template language throughout the protocol

Item 24 Replaced the terms "(*fe)male*" or "(*wo)man*" with "*participant who was assigned (fe)male at birth*" throughout the protocol.

Date: October 25, 2017

Protocol version: 1.0
Protocol modification: Clarification memo 1

Item 1 References to the DAIDS table for grading of adverse events updated to current version 2.1 and clarifications to the protocol to be consistent with new version of the grading table

Date: December 20, 2016

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.
  - Revised Guidelines for HIV Counseling, Testing, and Referral. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5019a1.htm
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures
- Division of AIDS Protocol Registration Manual. Available at https://www.niaid.nih.gov/sites/default/files/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Corrected Version 2.1, July 2017. Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 120 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 120 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN 120 Site Lab Instructions. Accessible through the HVTN protocol-specific website
- HVTN Manual of Operations. Accessible through the HVTN website.

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

- Lab assay algorithm (available upon request)

- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6, Guideline for Good Clinical Practice: Section 4.8, Informed consent of trial subjects. Available at http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html

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

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

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

- Title 45, Code of Federal Regulations, Part 46. Available at http://www.hhs.gov/ohrp/humansubjects/regulations-and-policy/regulations/45-cfr-46/index.html

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

15 Acronyms and abbreviations

Ab	antibody
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANOVA	analysis of variance
ART	antiretroviral therapy
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
CRP	C-reactive protein
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
CTL	cytotoxic T lymphocyte
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
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
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HVTN	HIV Vaccine Trials Network

IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
IFN-γ	interferon gamma
IND	Investigational New Drug
IoR	Investigator of Record
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
SHIV	simian-human 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	Dale and Betty Bumpers Vaccine Research Center (NIAID)

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

16 Literature cited

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Appendix A Sample informed consent form

A phase 1/2a clinical trial to evaluate the safety and immunogenicity of ALVAC-HIV (vCP2438) and of MF59®- or AS01B-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 120

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 160 people will take part in this study at multiple sites in Africa and the United States. 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 chance of becoming infected with HIV if you are exposed to the virus.

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

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

We do not know whether the vaccines in this study will affect your risk of getting HIV from another person. The risk could be higher, lower, or unchanged. It is 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 ALVAC-HIV (vCP2438), Bivalent Subtype C gp120/MF59, and Bivalent Subtype C gp120/AS01$_B$. From here on, we will call them the ALVAC vaccine, the protein/MF59 vaccine, and the protein/AS01$_B$ vaccine, or the study vaccines.

The ALVAC vaccine

The ALVAC vaccine is made out of canarypox virus. Canarypox virus infects birds but cannot infect humans. This virus has small bits of man-made DNA inserted into it. DNA is a natural substance found in all living things, including people and some viruses. The canarypox virus helps get the DNA into the body's cells. The DNA then tells those cells to make small amounts of proteins that look like some of the ones found in HIV.

The Protein/MF59 vaccine:

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 countries. It has also been in other vaccines that have been given to over 50,000 people in research studies without causing any serious health problems.

The ALVAC vaccine and the Protein/MF59 vaccine combination is currently being given in South Africa in 2 studies named HVTN 100 and HVTN 702. For HVTN 100, 210 participants have received this vaccine combination. It is also being given in South Africa and other African countries in a study named HVTN 107. In addition, the Protein/MF59 is being given in 2 other studies named HVTN 111 and HVTN 108. Similar Protein vaccines have been given to more than 10,000 people in research studies. In these studies, the protein vaccines did not cause serious health problems.

The Protein/AS01$_B$ vaccine:

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$_B$. This adjuvant is also added to this vaccine to help the immune systems respond better.

The Protein/AS01$_B$ is also being given in one of the studies mentioned above, named HVTN 108. Similar vaccines have been given to over 1400 people in past studies. In these studies, the vaccines did not cause serious health problems.

This study will be the first time the ALVAC vaccine and the Protein/AS01$_B$ vaccine combination will be given to humans.

General risks of vaccines:

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

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

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:

The ALVAC vaccine and the Protein/MF59 vaccine:

In HVTN 100, the study vaccines have not caused serious health problems. About 3 out of 4 participants had mild or medium pain or tenderness on the arm where they got the injections. Three participants had severe arm pain after one of their injections. About 1 out of 7 participants had a small area of redness or hardening of the skin on the arm where they got the injections. These side effects did not bother most people much and all went away within a few days. Three participants had larger areas of redness or swelling (more than 10 cm in diameter) where they got the injection that bothered them. They were treated with prescription medications and the symptoms went away within a few days.

About 2 out of 3 participants felt weak or tired, or had a headache or body aches after an injection. A small number had nausea, chills, fever, or vomiting. These symptoms were mostly mild but in 4 participants their daily activities were affected (joint aches in 2, weakness/tiredness in 1 and headache in another person). All of these symptoms went away within a few days.

A few participants had one of these side effects after an injection: lump where they got the injection, itching where they got the injection or all over, lymph node swelling, stomach pain, diarrhea, dizziness, brief tingling around the mouth. These symptoms were all mild or medium and all went away within a few days.

Most of the symptoms participants had are common side effects of vaccines or of getting injections. We do not know yet which participants got the study vaccines and which got the placebo because the study is still "blinded", so we do not know if the study vaccines caused these side effects.

The Protein/AS01$_B$ vaccine:

The Protein/AS01$_B$ is being given in HVTN 108 and we will give you any important information that may come out of that study that may affect your health or your participation.

In studies with similar products some people had redness, swelling, pain, muscle tenderness, or itching in the area where they got the injection. Some people had headache, weakness, increased heart rate, and increased sensitivity to stimulation at the site of injection. A small number of people had flu-like symptoms, nausea, rash, vomiting, diarrhea, or swollen lymph nodes after getting an injection. A very small number of people had tiredness and difficulty sleeping. People who have these symptoms may only have a few of them and they usually go away within a few days.

There may be other risks of the study vaccines that we do not yet know about. We will tell you if we learn anything new that may affect your participation in the study.

Joining the study

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

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

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

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

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

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

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

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

   We will also do blood and urine tests. These tests tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for Hepatitis B, Hepatitis C, and syphilis. We will ask you about medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy. People who have had a hysterectomy or oophorectomy (removal of the uterus or ovaries, verified by medical records), are not required to have a pregnancy test. We will also ask if you have ever been allergic to eggs, egg products, or the antibiotic Neomycin.

   We will review the screening results with you. 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 assigned female sex at birth and could become pregnant, you must agree to use birth control to join this study.**

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

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 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.

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 laboratory test result 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: Include the following paragraph:

Payments you receive for being in the study may be taxable. We may need to ask you for your Social Security number for tax reasons.

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

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

Everyone in this study will get some placebos. Placebos are substances that do not contain vaccine. Some people will get the study vaccines and also some placebos. Some people will get only placebos. We will compare the results from people who got the placebos with results from people who got the study vaccines.

The placebo for the study vaccines is saline solution. We do not expect the placebo to cause any health problems in people.

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

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

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

There are 4 groups in this study. Each group will get a different combination of study products. The group you get assigned to is completely random. Each group will get 8 injections during the study. You will get the injections into your upper arms. At some visits you will get one injection. At other visits, you will get 3 injections.

The ALVAC vaccine or its placebo will go into the left arm. The Protein/MF59 vaccine and Protein/AS01$_B$ vaccine and their placebos will go into the right arm.

Site: You may insert the picture version of the injection schedule (Appendix I) in place of (or in addition to) the text version or give it as a separate document to volunteers if you believe it will be helpful to them. You are not required to do either.

Group	Number of people	Protein dose	Arm	First injection visit	Time after first injection visit: 1 month	3 months	6 months
1	50	High dose	Left	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV
			Right			Protein/MF59 + Placebo	Protein/MF59 + Placebo
2	50	High dose	Left	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV
			Right			Protein/AS01$_B$ + Placebo	Protein/AS01$_B$ + Placebo
3	50	Low dose	Left	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV	ALVAC-HIV
			Right			Protein/AS01$_B$ + Placebo	Protein/AS01$_B$ + Placebo
4	10	N/A	Left	Placebo	Placebo	Placebo	Placebo
			Right			Placebo + Placebo	Placebo + Placebo

You will have to wait in the clinic for about a half hour after each set of injections to see if there are any problems. Then for that night and for 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. Within 3 days of vaccination, we will ask you to contact us or clinic staff will contact you to check on how you are doing. You should always contact us 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;
- Do physical exams;
- Do pregnancy tests if you were assigned female sex at birth;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study, and;
- Take blood and urine samples.

Each time we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 200 mL (2 teaspoons to a little less than 1 cup/ 14 tablespoons). Your body will make new blood to replace the blood we take out.

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

Site: Insert Appendix D, 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.

Site: in the following section, delete references to semen if your site is not participating in semen collection.

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 assigned female sex at birth) or semen (if you were assigned male at birth). 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 that 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 assigned female sex at birth, 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 the 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 inside your bum 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:

- Do not have receptive anal intercourse
- Do not put anything into your anus, including cleaning products (creams, gels, lotions, pads, etc.), lubricant, enemas or douches (even with water)
- Do not use any anti-inflammatory creams in or around your anus.

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

Cervical fluid

If you are 21 or older, you must have had a Pap smear within the last 3 to 5 years with the most recent result being normal. If you have not had a Pap smear within the last 3 years and would like to get one, we will tell you where you can get one. If you are younger than 21, you do not need a Pap smear because at that age it is not medically necessary.

To collect cervical fluid, we will give you a menstrual cup to insert into your vagina. The study staff will explain how to insert and remove the cup, or they can do it for you at the clinic. We will explain how many cups we will collect and how long you should wear them.

For the 2 days before we collect your cervical fluid,

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

Do not insert the menstrual cup:

- if you think you may be pregnant.
- if you think you may have a cervical or vaginal infection. We may ask you to collect this sample at a later date.

Semen

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

- **not** to have sex, including oral sex
- **not** to ejaculate
- **not** to use anything with lubricants
- **not** to put saliva on the penis

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

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

We will send your samples (without your name) to labs approved by the HVTN for this study, which are located in the United States 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. The genetic testing will only involve some of your genes, not all of your genes (your genome). The researchers will study only the genes related to the immune system and HIV and those that affect how people get HIV.

If you become HIV infected, the researchers may look at all of the genes of the virus found in your samples. If you give 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 product(s). 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 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, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions.

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

16. We will counsel you on avoiding HIV infection.

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

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

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

The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this form. If you have any questions, please ask.

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. *[Site: choose one of the following two sentences. African sites should choose the sentence referencing the repository in South Africa. All other sites should choose the sentence referencing the repository in the United States.]* Your samples will be stored in the HVTN repository in South Africa. Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

How do other researchers get my samples and information? When a researcher wants to use your samples and information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: If review by your institution's IRB/EC/RE is also required, insert a sentence stating this.]* IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

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

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

Researchers may also do genetic testing on your samples.

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

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, but your name and other personal information will not be included. 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. There may be other unknown risks.

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

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information

- Any regulatory agency that reviews the research using your extra samples and information

- The researcher's Institutional Review Board or Ethics Committee

- The people who work with the researcher

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

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

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 National Institutes of Health, its study monitors, and its chosen representatives,

- The US Food and Drug Administration,

- Any regulatory agency that reviews clinical trials,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- GlaxoSmithKline Biologicals S.A., Sanofi Pasteur 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 more injections.

This may happen if:

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

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

20. We will stop your injections if you become pregnant before your last injection.

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

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

21. If you get infected with HIV during the study, we will stop your injections, take fewer samples, and help you get care and support.

We will encourage you to stay in this study for up to 6 months after your last study product administration if you choose. We will discuss your study options with you. We will counsel you about your HIV infection and about telling your partner(s). We will tell you where you can get support and medical care. *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

Other Risks

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

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

Risks of routine medical procedures:

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

Risks of collecting 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 HIV study vaccines. The study vaccines are likely to cause you to test positive on some types of HIV tests, even if you are not infected with HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccines, a routine HIV test done outside this clinic is likely to say you have HIV, even if you don't. For this reason, you should plan to get HIV tests only at this clinic during the study. Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccines.

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

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

Site: Modify the following paragraph if applicable. If someone believes you are infected with HIV even if you are not, you could face discrimination and other problems. For example, 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. For most babies antibodies from the mother last for about six months.

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

Embarrassment/anxiety:

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

Risks of disclosure of your personal information:

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

Risks of genetic testing:

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

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. 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 change text in the following section (except as prompted) without obtaining prior approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org

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

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 you 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.

Next paragraph for African sites:

Sites: adjust the language in this paragraph so it is applicable to your site. Note: insurance is purchased for all African countries. In this study, our clinic has insurance to cover your medical treatment in the case of a study-related injury. In rare cases, the insurance funds may not be enough. The vaccine developers have agreed to pay medical costs for study-related injuries that are determined to be caused by their own study products.

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.

Questions

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

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

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

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

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

Your permissions and signature

28. In section 14 of this form, we told you about 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 assigned female sex at birth:

☐ I agree to provide cervical fluid.

☐ I do not agree to provide cervical fluid.

☐ N/A: assigned male sex at birth

Site: Delete the following if your site is not participating in semen collection

For people assigned male sex at birth:

☐ I agree to provide semen.

☐ I do not agree to provide semen.

☐ N/A: assigned female sex at birth

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

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

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

OR

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

OR

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

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

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

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

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

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

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

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

*Witness is impartial and was present for the entire discussion of this consent form.

Appendix B Approved birth control methods for persons assigned female sex at birth (for sample informed consent form) for African sites

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 study injection until 6 months after your last study injection.

Effective birth control for participants in Africa is defined as using 2 methods of birth control every time you have sex. 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, male and female condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

Appendix C Approved birth control methods for persons assigned female sex at birth (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.

You must agree to use effective birth control, from 3 weeks before your first study injection until 6 months after your last study injection.

Effective contraception for participants in the US 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, male and female condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

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

Procedure	Screening visit(s)	Time after first injection visit (in months)										
		First injection visit	½	1	1½	3	3½	6	6¼	6½	9	12
Injection		√		√		√		√				
Medical history	√											
Complete physical	√											√
Brief physical		√	√	√	√	√	√	√	√	√	√	
Urine test	√		√							√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√
Pregnancy test[a] (participants assigned female sex at birth)	√	√		√		√		√		√[c]	√	√[c]
HIV testing & pretest counseling	√					√		√			√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√
Pap smear[b]	√											
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.

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

[b] For participants who were assigned female sex at birth and who agree to provide cervical and/or rectal fluids samples and are ≥ 21 years of age.

[c] For participants who were assigned female sex at birth and who agree to provide cervical and/or rectal fluids sample

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

A phase 1/2a clinical trial to evaluate the safety and immunogenicity of ALVAC-HIV (vCP2438) and of MF59®- or AS01B-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 120

Site: [Insert site name]

When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers. The HVTN calls these samples "extra samples". The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this form. If you have any questions, please ask.

1. **Do I have to agree?**

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

2. **Where are the samples stored?**

   Extra samples are stored in a secure central place called a repository. *[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: Revise the previous sentence to insert limits if your regulatory authority imposes them.]*

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

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

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

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

6. Will the HVTN sell my samples and information?

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

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

When a researcher wants to use your samples and information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: If review by your institution's IRB/EC/RE is also required, insert a sentence stating this.]* IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

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

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

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

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

Researchers may also do genetic testing on your samples.

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

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, but your name and other personal information will not be included. 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. There may be other unknown risks.

10. What are the risks of genetic testing?

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

US 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 extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- Any regulatory agency that reviews the research using your extra samples and information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

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

Questions

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

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

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

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

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

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

OR

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

OR

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

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

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

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

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

*Witness is impartial and was present for the entire discussion of this consent form.

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Appendix F Laboratory procedures

Procedure	Ship to[1]	Assay location[2]	Tube Type[4]	Tube size (vol. capacity)[4]	Visit: 1	2	3	4	5	6	7	8	9	10	11	12	Total
					Day: Screening visit[3]	D0	D14	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**					
						ALVAC OR Placebo		**ALVAC OR Placebo**		**ALVAC + Protein w/ MF59 OR AS01B OR Placebo**		**ALVAC + Protein w/ MF59 OR AS01B OR Placebo**					
BLOOD COLLECTION																	
Screening/Diagnostic																	
Screening HIV test	Local lab	Local lab	EDTA	5mL	5	—	—	—	—	—	—	—	—	—	—	—	**5**
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	**5**
HIV diagnostics[9]	HSML-NICD / UW-VSL	HSML-NICD / UW-VSL	EDTA	10mL	—	—	—	—	—	10	—	10	—	—	10	20[9]	**50**
Safety labs[15]																	
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	5	—	5	—	5	—	—	5	—	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	5	—	5	—	5	—	—	5	—	—	**25**
STI Serology																	
Syphilis[11]	Local lab	Local lab	SST	5mL	5	5[11]	—	—	—	—	—	—	—	5[11]	—	5[11]	**20**
Immunogenicity & Virologic assays[6]																	
HLA host genetics[7]	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	—	—	—	—	—	—	—	**17**
Cellular assays																	
ICS	CSR	HVTN Labs	ACD	8.5mL	—	68	—	—	—	—	68	—	—	***68***	—	***68***	**272**
pTfh and Plasmablast	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	—	—	—	17	—	—	—	**34**
Humoral assays																	
Binding Ab Assay	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	8.5	—	—	***8.5***	—	***8.5***	**34.0**
Neutralizing Ab Assay	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	8.5	—	—	***8.5***	—	***8.5***	**34.0**
Ab Avidity	CSR	HVTN Labs	SST	8.5mL	—	y	—	—	—	—	—	—	—	***y***	—	***y***	**0**
ADCC	CSR	HVTN Labs	SST	8.5mL	—	y	—	—	—	—	—	—	—	***y***	—	***y***	**0**
STORAGE																	
Serum storage	CSR	—	SST	8.5mL	—	17	—	—	25.5	—	8.5	25.5	8.5	8.5	25.5	8.5	**128**
PBMC storage	CSR	—	ACD	8.5mL	—	42.5	—	—	25.5	—	17	—	34	17	—	17	**153**
Visit total					**25**	**184**	**10**	**0**	**61**	**10**	**121**	**36**	**60**	**126**	**36**	**136**	**802**
56-Day total					**25**	**209**	**219**	**219**	**280**	**71**	**192**	**36**	**95**	**221**	**36**	**136**	
URINE COLLECTION																	
Urinalysis[15]	Local lab	Local lab			X	—	X	—	—	—	—	—	—	X	—	—	
Pregnancy Test[8, 15]	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)[13]																	
Semen	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—	X	—	X	
Cervical Secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—	X	—	X	
Rectal Secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—	X	—	X	
STOOL COLLECTION (OPTIONAL)																	
Stool	CSR	HVTN Labs			—	X[14]	—	—	—	—	—	—	—	X	—	—	

Footnotes for Laboratory procedures

[1] CSR = Central Specimen Repository; HSML-NICD = HIV Sero-Molecular Laboratory-National Institute for Communicable Diseases (Johannesburg, South Africa); UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Cape Town HVTN Immunology Laboratory (CHIL, Cape Town, South Africa); South African Immunology Laboratory–National Institute for Communicable Diseases (SAIL-NICD, Johannesburg, South Africa); Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

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

[6] Immunogenicity assays will be performed at M0 (for binding Ab assay), M6.5, and M12. 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 assigned female sex at birth, pregnancy test must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to 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.

[9] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 12 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14).

[10] Pregnancy testing at the indicated visit is only required of participants who were assigned female sex at birth and are providing a cervical and/or rectal secretion sample.

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

[12] Cervical/vaginal swabs will only be collected from participants who agree to provide a cervical secretion sample and for yeast if clinically indicated.

[13] Optional mucosal specimens may be collected as part of screening and prior to the enrollment visit once the participant has been found to have met mucosal specimen collection criteria specified in Section 9.5.

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

[15] For participants with confirmed diagnosis of HIV infection, only specimens required for protocol-specified safety laboratory tests, urinalysis, and pregnancy tests will be collected.

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

Appendix G Procedures at HVTN CRS

Visit:	01[a]	02[m]	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	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	—	—
Risk reduction counseling[r]	X	X	X	X	X	X	X	X	X	X	X	X	—
Pregnancy prevention assessment[c]	X	X	X	X	X	X	X	X	X	X	X	X	—
Behavioral risk assessment[s]	X	—	—	—	—	X	—	X	—	—	X	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	X	—	X	—	—	—	X	—
Outside testing and belief questionnaire	—	—	—	—	—	—	—	X	—	—	—	X	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	—
Intercurrent illness/adverse experience[d]	—	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[e]	X	—	—	—	—	X	—	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	X	—	X	—	—	X	X
Local lab assessment[t]													
Urine dipstick	X	—	X	—	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	X	—	X	—	X	—	X[o]	X	X[o]	—
CBC, differential, platelet	X	—	X	—	X	—	X	—	—	X	—	—	—
Chemistry panel (see Section 9.2)	X	—	X	—	X	—	X	—	—	X	—	—	—
Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—
Syphilis	X	X[n]	—	—	—	—	—	—	—	X[n]	—	X[n]	—
Pap smear[g]	X	—	—	—	—	—	—	—	—	—	—	—	—
Chlamydia/gonorrhea (urine)[h]	—	X	—	—	—	—	—	—	—	X	—	X	—
Trichomonas vaginalis[i]	—	X	—	—	—	—	—	—	—	X	—	X	—
Bacterial vaginosisi	—	X	—	—	—	—	—	—	—	X	—	X	—
Yeast[j]	—	X	—	—	—	—	—	—	—	X	—	X	—
Mucosal and stool sample collection (optional)													
Rectal secretions, cervical secretions, semen	—	X	—	—	—	—	—	—	—	X	—	X	—
Stool[q]	—	X[p]	—	—	—	—	—	—	—	X	—	—	—
Vaccination procedures													
Vaccination[k]	—	X	—	X	—	X	—	X	—	—	—	—	—
Reactogenicity assessments[l]	—	X	—	X	—	X	—	X	—	—	—	—	—
Poststudy													
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	X

Footnotes for Procedures at HVTN CRS

[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 F.

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

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

[e] Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant. If a participant has a confirmed diagnosis of HIV infection, do not perform HIV infection assessment.

[f] For a participant who was assigned female sex at birth, pregnancy test must be performed on urine or blood specimens within 24 hours 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] Only for volunteers who were assigned female sex at birth and who agree to provide cervical samples. Per Section 7.1, Criterion 23; Pap smear not required if volunteer has had Pap smear within previous 3-5 years with most recent result normal or ASCUS or less than 21 years old.

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

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

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

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

[l] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.10).

[m] 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

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

[o] Pregnancy testing at the indicated visit is only required of participants who were assigned female sex at birth and are providing a cervical and/or rectal secretion sample.

[p] Optional stool specimen must be collected prior to first vaccination.

[q] Collect dietary, antibiotic use, and gastrointestinal symptom information from participants providing stool specimen.

[r] Includes transmission risk reduction counseling for HIV-infected participants.

[s] Not applicable to HIV-infected participants.

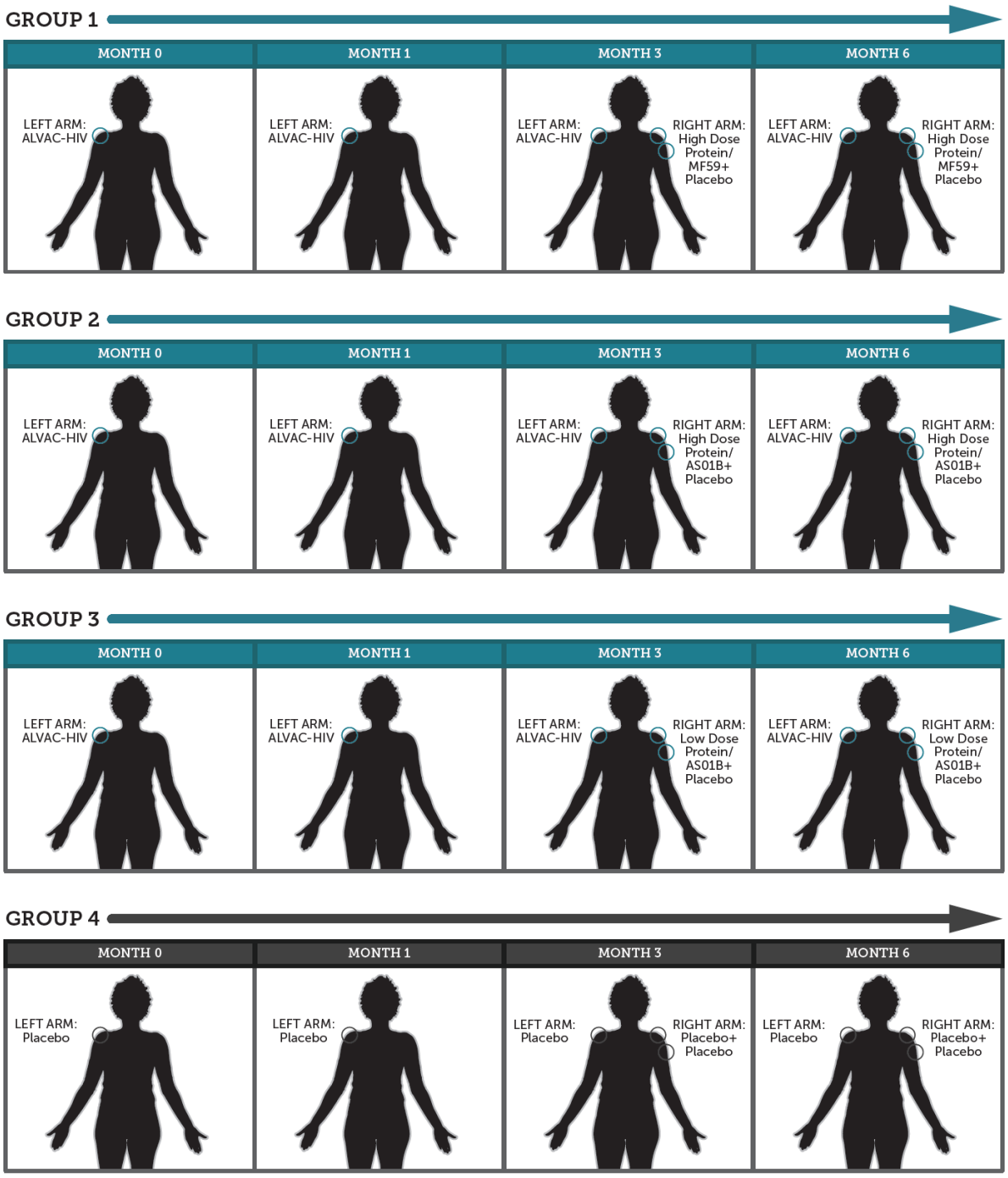
[t] For participants with a confirmed diagnosis of HIV infection, only specimens listed under “Safety labs” in Appendix F, urinalysis, and urine pregnancy tests will be collected per the protocol schedule.

Appendix H 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.

<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>

Appendix I Injection schedule for sample informed consent



Appendix J Low Risk Guidelines for the United States

The following are intended as guidelines for the investigator to help identify potential vaccine trial participants at "low risk" for HIV infection in the US. These guidelines are based on behaviors within the last 6-12 months prior to enrollment; however, it may be appropriate to consider a person's behavior over a longer period of time than specified to assess the person's likelihood of maintaining low risk behavior. *Some volunteers may not be appropriate for enrollment even if they meet these guidelines.* These guidelines should be supplemented and interpreted with local epidemiologic information about HIV prevalence in your area and community networks. The investigator may review the risk level of any volunteer with the site PI and/or protocol safety review team.

A volunteer may be appropriate for inclusion if he/she meets these guidelines:

1. SEXUAL BEHAVIORS

In the **last 12 months** did not:

- Have oral, vaginal or anal intercourse with an HIV-infected partner, or a partner who uses injection drugs
- Give or receive money, drugs, gifts or services in exchange for oral, vaginal or anal sex

AND

In the **last 6 months** has abstained from penile/anal or penile/vaginal intercourse, OR

In the **last 6 months**:

- Had 4 or fewer partners of the opposite birth sex for vaginal and/or anal intercourse, OR

Is an MSM (person born male with partner(s) born male) who, in the **last 12 months**:

- Had 2 or fewer MSM partners for anal intercourse and had no unprotected anal sex with MSM, OR
- Had unprotected anal intercourse with only 1 MSM partner, within a monogamous relationship lasting at least 12 months (during which neither partner had any other partners). If the monogamous relationship ended, the volunteer may then have had protected anal intercourse with 1 other MSM partner (total 2 or fewer partners in the last 12 months).

Is a transgender person, regardless of the point on the transition spectrum, having sex with men (born male) and/or other transgender persons, who **in the last 12 months**:

- Had 2 or fewer partners for anal or vaginal intercourse, and had no unprotected anal or vaginal sex, OR
- Had unprotected anal or vaginal intercourse sex with 1 partner only within a monogamous relationship lasting at least 12 months (during which neither partner had any other partners). If the monogamous relationship ended, may then have had protected anal or vaginal sex with 1 other partner (total 2 or fewer partners in the last 12 months).

AND

Uses or intends to use condoms in situations which may include penile/anal or penile/vaginal intercourse with new partners of unknown HIV status, occasional partners, partners outside a primary relationship, and/or partners known to have other partners.

2. NON-SEXUAL BEHAVIORS

In the **last 12 months** did not:

- Inject drugs or other substances without a prescription
- Use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment rendered the participant at greater than low risk for acquiring HIV infection. The investigator's judgment should consider local epidemiologic information about HIV prevalence in the area and community networks.

A volunteer is NOT appropriate for inclusion if he/she:

Acquired an STI (i.e. new infection) in the last 12 months:

- Syphilis
- Gonorrhea
- Non-gonococcal urethritis
- Herpes Simplex Virus type 2 (HSV2)
- Chlamydia
- Pelvic inflammatory disease (PID)
- Trichomonas
- Mucopurulent cervicitis
- Epididymitis
- Proctitis
- Lymphogranuloma venereum
- Chancroid
- Hepatitis B

Appendix K Low Risk Guidelines for Africa

The following are intended as guidelines for the investigator to help identify potential vaccine trial participants at "low risk" for HIV infection. These guidelines are based on behaviors within the last 12 months prior to enrollment; however, it may be appropriate to consider a person's behavior over a longer period of time than specified to assess the person's likelihood of maintaining low risk behavior. *Some volunteers may not be appropriate for enrollment even if they meet these guidelines*. These guidelines should be supplemented and interpreted with local epidemiologic information about HIV prevalence in your area and community networks. The investigator may review the risk level of any volunteer with the site PI and/or protocol safety review team.

ASSESSMENT OF SEXUAL BEHAVIORS

Consider whether a volunteer would be appropriate for inclusion if, within 12 months prior to enrollment, the person:

- Abstained from penile/vaginal and penile/anal intercourse, or
- Was in a mutually monogamous relationship with a partner with a known HIV-uninfected status, or
- Had one partner believed to be HIV-uninfected with whom he/she regularly used condoms for penile/vaginal and penile/anal intercourse.

Exclude a volunteer if:

Within the 12 months prior to enrollment: a history of newly acquired syphilis, gonorrhea, chlamydia, trichomoniasis, active HSV lesions, chancroid, pelvic inflammatory disease (PID), genital sores or ulcers, cervicitis, genital warts of the labia minora, vagina, or cervix, or any other symptomatic genital warts.

Appendix L Protocol Signature Page

A phase 1/2a clinical trial to evaluate the safety and immunogenicity of ALVAC-HIV (vCP2438) and of MF59®- or AS01B-adjuvanted clade C Env protein, in healthy, HIV-uninfected adult participants

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

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

DAIDS Protocol Number: HVTN 120

DAIDS Protocol Version: Version 3.0

Protocol Date: September 12, 2018