HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 107

A Phase 1/2a partially double-blinded, randomized clinical trial to characterize the safety and immunogenicity of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120 alone, with MF59® adjuvant, and with alum adjuvant in healthy, HIV-uninfected adult participants

DAIDS DOCUMENT ID 12006

CLINICAL TRIAL SPONSORED BY

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

STUDY PRODUCT(S) PROVIDED BY

Sanofi Pasteur
Swiftwater, Pennsylvania, USA

GlaxoSmithKline Biologicals, S.A.
Rixensart, Belgium

NIH, NIAID, Vaccine Research Center (VRC)
Bethesda, Maryland, USA

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Contents

1 Ethical considerations

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

- HVTN trials are designed and conducted to enhance the knowledge base necessary to find a preventive vaccine, using methods that are scientifically rigorous and valid, and in accordance with Good Clinical Practice (GCP) guidelines.

- HVTN scientists and operational staff incorporate the philosophies underlying major codes [1-3], declarations, and other guidance documents relevant to human subjects research into the design and conduct of HIV vaccine clinical trials.

- HVTN scientists and operational staff are committed to substantive community input—into the planning, conduct, and follow-up of its research—to help ensure that locally appropriate cultural and linguistic needs of study populations are met. Community Advisory Boards (CAB) are required by DAIDS and supported at all HVTN research sites to ensure community input, in accordance with Good Participatory Practices (GPP) and all local and national guidelines. Specifically, the HVTN leadership is aware of the *Guidelines for Good Clinical Practice in the Conduct of Clinical Trials with Human Participants in South Africa* Section 2.4 "Communication and Community Involvement" and the South African Medical Research Council's *Guidelines on Ethics for Medical Research: HIV Preventive Vaccine Research* (particularly section 5, Community Participation) and works to implement both guidelines generally and those sections specifically.

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

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

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

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

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

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

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

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

2 IRB/EC review considerations

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

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

In compliance with the Guidelines For Good Practice In The Conduct Of Clinical Trials In Human Participants In South Africa ("South African GCPs"), each research location in South Africa has a South African-based Principal Investigator (PI) who is qualified to conduct (and supervise the conduct of) the research; and the research addresses an important South African health need for an HIV vaccine in line with the national strategic plan for South Africa and the national South African HIV vaccine plan. In addition, the investigators take responsibility for the conduct of the study and the control of the study products, including obtaining all appropriate South African regulatory and ethical reviews of the research. Each participating site has a standard operating procedure for ensuring that participants have the necessary information to make a decision whether or not to consent to the research.

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

2.1 Minimized risks to participants

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

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

2.2 Reasonable risk/benefit balance

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

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

2.3 Equitable subject selection

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

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

2.4 Appropriate informed consent

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

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

2.5 Adequate safety monitoring

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

This protocol has extensive safety monitoring in place (see Section 11). Safety is monitored daily by HVTN Core and routinely by the HVTN 107 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

2.6 Protect privacy/confidentiality

45 CFR 46.111 (a) 7 and 21 CFR 56.111 (a) 7: There are adequate provisions to protect the privacy of subjects and maintain the confidentiality of data.

Privacy refers to an individual's right to be free from unauthorized or unreasonable intrusion into his/her private life and the right to control access to individually identifiable information about him/her. The term "privacy" concerns research participants or potential research participants as individuals whereas the term "confidentiality" is used to refer to the treatment of information about those individuals. This protocol respects the privacy of participants by informing them about who will have access to their personal information and study data (see Appendix A). The privacy of participants is protected by assigning unique identifiers in place of the participant's name on study data and specimens. In addition, each staff member at each study site in this protocol signs a Confidentiality Agreement with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A Phase 1/2a partially double-blinded, randomized clinical trial to characterize the safety and immunogenicity of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120 alone, with MF59® adjuvant, and with alum adjuvant in healthy, HIV-uninfected adult participants

Primary objective(s)

Primary objective 1

To compare the humoral immune responses induced by ALVAC-HIV (vCP2438) prime with 2 ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® boosts or 2 ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension boosts in HIV-uninfected participants

Primary objective 2:

To compare the humoral immune responses induced by ALVAC-HIV (vCP2438) prime with 2 ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® boosts to the regimen with 3 ALVAC-HIV (vCP2438) + Bivalent Subtype C /MF59® vaccinations in HIV-uninfected participants

Primary objective 3

To evaluate the safety and tolerability of each vaccine regimen through the Month 6.5 timepoint

Study products and routes of administration

- **ALVAC-HIV (vCP2438):** ALVAC-HIV (vCP2438) expresses the gene products ZM96 gp120 (clade C strain) linked to the sequences encoding the HIV-1 transmembrane (TM) anchor 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%) for intramuscular (IM) injection as a single dose.

- **Bivalent Subtype C gp120/MF59®:** Bivalent Subtype C gp120/MF59® consists of 2 subtype C recombinant monomeric proteins, TV1.C gp120 Env and 1086.C gp120 Env, each at a dose of 100 mcg, mixed with MF59® adjuvant (an oil-in-water emulsion) and delivered as a 0.5 mL IM injection.

- **Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension:** Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension consists of 2 subtype C recombinant monomeric proteins, TV1.C gp120 Env and 1086.C

gp120 Env, each at a dose of 100 mcg, admixed with Aluminum Hydroxide Suspension (~625 mcg aluminum content) and delivered as a 0.5 mL IM injection.

- **Bivalent Subtype C gp120:** Bivalent Subtype C gp120 consists of 2 subtype C recombinant monomeric proteins, TV1.C gp120 Env and 1086.C gp120 Env, each at a dose of 100 mcg, mixed with sodium chloride for injection, 0.9% and delivered as a 0.5 mL IM injection.

Table 3-1 Schema

Group	N (total)	N (innate/mucosal subset)	Dose of each protein component	Injection schedule in Months (Days)				
				M0 (D0)	M1 (D28)	M3 (D84)	M6 (D168)	M12 (D364)
1	36	18	100 mcg	ALVAC-HIV (vCP2438)	ALVAC-HIV (vCP2438)	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®
2	36	18	100 mcg	ALVAC-HIV (vCP2438)	ALVAC-HIV (vCP2438)	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension
3	36	18	100 mcg	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®	—	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®
4	24	18	100 mcg	ALVAC-HIV (vCP2438)	ALVAC-HIV (vCP2438)	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120
Total	132	72						

Participants

132 healthy, HIV-1–uninfected volunteers aged 18 to 40 years

Design

Multicenter, randomized, controlled, (partially) double-blind trial

Duration per participant

18 months of scheduled clinic visits

Estimated total study duration

21 months (includes enrollment and follow-up)

Study sponsor

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

Study product providers

- ALVAC-HIV (vCP2438): Sanofi Pasteur (Swiftwater, Pennsylvania, USA)
- Bivalent Subtype C gp120: GlaxoSmithKline Biologicals, S.A. (GSK Vaccines) (Rixensart, Belgium)
- MF59®:: GlaxoSmithKline Biologicals, S.A. (GSK Vaccines) (Rixensart, Belgium)
- Aluminum Hydroxide Suspension: NIH, NIAID, Vaccine Research Center (Frederick, Maryland, USA)

Core operations

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

Statistical and data management center (SDMC)

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

HIV diagnostic laboratory

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

Endpoint assay laboratories

Cape Town HVTN Immunology Laboratory (CHIL) (Cape Town, South Africa)

Duke Human Vaccine Institute (DHVI), Duke University Medical Center (Durham, North Carolina, USA)

Neutralizing Antibody (nAb) Assay Laboratory, Duke University Medical Center (Durham, North Carolina, USA)

FHCRC/University of Washington (Seattle, Washington, USA)

South Africa Immunology Laboratory and National Institute for Communicable Diseases (SAIL-NICD) (Johannesburg, South Africa)

Study sites

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

Safety monitoring

HVTN 107 PSRT; HVTN SMB

3.1 Protocol Team

Protocol leadership

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	Lindiwe Mbhele UKZN	*Technical editor*	Erik Schwab HVTN Core, FHCRC

4 Background

4.1 Rationale for trial concept

The RV144 HIV vaccine trial in Thailand, which evaluated a heterologous regimen of 2 doses of ALVAC-HIV (vCP1521) followed by the 2 doses of ALVAC-HIV plus AIDSVAX® clades B/E gp120 protein, was the first clinical HIV vaccine trial to demonstrate efficacy in the prevention of HIV acquisition [4].The RV144 trial results have triggered a suite of clinical trials designed to deepen understanding of the mechanism of protection and ultimately to develop a licensable product appropriate to regions with significant HIV burden. Specifically, several trials are planned in the clinical testing of an HIV vaccine regimen similar to that tested in RV144 but adapted for the Southern African region, that is, with a clade C viral vector vaccine insert and clade C subunit proteins. This trial will directly compare the differences in elicited immune response between aluminum hydroxide and MF59® as adjuvants in the RV144-like vaccine regimen modified for the Southern African region. It will also evaluate concurrent administration of the viral vector and clade C subunit proteins with MF59® from Month 0.

With approximately 6.1 million people living with HIV as of 2012 [5], South Africa's epidemic remains the largest in the world and Sub-Saharan Africa bears the preponderant burden of the HIV epidemic with almost 70% of all infections worldwide [6]. The vast majority of newly acquired infections in this region occur during unprotected heterosexual intercourse.

ART was introduced into the public sector in South Africa in 2003 and, as of 2012, approximately 2.2 million people were on treatment, representing 80% of people requiring ART [7]. While universal access to antiretroviral HIV treatment is a global ideal and progress toward this goal has been made [7], in many regions of Sub-Saharan Africa limited access and other barriers to care continue to undermine the prevention potential of widespread ART [8]. Moreover, the cost and health care burden of delivering ever-increasing amounts of treatment in resource constrained settings pose significant challenges and drives the quest for effective prevention of infection [9-12]. In addition, while studies conducted over the past few years have confirmed the promise of antiretroviral chemoprophylaxis, adherence to drug regimens, a critical factor in their effectiveness, has proved problematic in several recent clinical trials [13-15]. Clearly, effective methods for preventing the acquisition and transmission of HIV-1 are urgently needed for this region, and it remains well recognized that the way to eradicate a global viral epidemic is to design, mass-produce, and then systematically vaccinate the target population with an effective prophylactic vaccine [16].

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; results were published in 2009 [4]. This 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) ($>10^6$ $CCID_{50}$), expressing clade E *env* and clade B *gag* and *pro*, followed by the AIDSVAX® clades B/E gp120 protein boost (300 mcg of each protein [600 mcg total] formulated with 600mcg of alum adjuvant). These products were based on viruses commonly circulating in Thailand at the time. This vaccine regimen

demonstrated 31.2% efficacy when compared with placebo (51 and 74 cases in the vaccines and placebo recipients, 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 in the first year postvaccination (estimated 60.5%, 95% confidence interval [CI] 22%-80%) with the vaccine effect waning over time to 31% cumulative reduction in HIV acquisition through 3.5 years [17].

4.1.2 Correlates of risk (CoR) in RV144

To better understand how the RV144 vaccine regimen reduced the risk of HIV infection, a large consortium of independent laboratories worked together systematically to ensure maximal information could be derived from samples obtained from participants who were vaccinated but became infected compared with those who had been vaccinated but who were uninfected at the end of the trial. A case control study was performed on 41 infected vaccine recipients, 205 uninfected vaccine recipients (1:5) and 40 placebo recipients (20 infected and 20 uninfected) within the RV 144 clinical trial to identify CoR [18]. Among the 6 primary immunological variables selected for the correlates analysis (5 different antibody [Ab] responses and CD4+ T-cell cytokine production) that were measured at the 2 weeks after the final vaccination visit (ie, at or near peak immunogenicity), 2 immune CoR of HIV acquisition were identified among vaccine recipients in the RV144 case control study. The first was the presence of immunoglobulin G (IgG) Ab that bound to a scaffolded gp70 V1V2 recombinant protein; this variable correlated inversely with infection rate (ie, higher V1V2 Ab→lower infection rate). The second was plasma Env-specific binding immunoglobulin A (IgA), which correlated directly with infection rate (ie, higher IgA to Env→higher infection rate). The other 4 primary variables correlated inversely with infection rate, but only when the level of IgA binding was low. This observation engendered the hypothesis that IgA might interfere with protective IgG effector functions, as had been observed previously with some other pathogens. In support of this possibility, it was noted that vaccinees who produced serum IgA to the first conserved region of Env protein (C1) had a significantly higher risk of infection than vaccinees without these antibodies. It was further noted that the C1 region of gp120 contains an epitope that can be a target on the surface of virus-infected cells for antibodies that mediate antibody-dependent cellular cytotoxicity (ADCC). The researchers concluded that if the protection conferred by V1V2 IgG antibodies could be confirmed, then a vaccine that could induce high levels of those antibodies along with low levels of monomeric Env-specific IgA antibodies might be more efficacious. Notably, despite the positive correlation of Env-specific IgA with infection risk in the RV144 study, 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 [18].

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 [19]. These data further substantiate the importance of antibodies directed against this region in protecting against infection [18]. Yates and colleagues noted that Env V1V2-specific IgG3 was the immunoglobulin subclass showing the strongest correlation with prevention of HIV acquisition in RV144 [20]. 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 [21]. In addition, Tomaras and colleagues confirmed that IgA antibodies to the C1 region of Env did indeed block IgG

binding and effector functions in RV144 vaccinees [22]. In sum, these CoR studies point to the importance of functional Ab responses, directed against a specific region of Env, in mediating the differing rates of HIV acquisition observed in RV144. They lay the groundwork for directing immune analyses planned for future HIV vaccine clinical trials.

4.1.3 Rationale for the proposed study

Based in large part on the successes seen in the RV144 trial, the HVTN is extending the efficacy studies to Southern Africa using a vaccine regimen that is similar to that tested in RV144, with HVTN 100 being the first phase 1-2 trial evaluating ALVAC-HIV (vCP2438) prime with ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® boosts. The ALVAC-HIV vector in this trial is the same as was tested in RV144 but encodes a subtype C envelope protein to better match the HIV subtype most prevalent in Southern Africa. Similarly the protein boost will consist of bivalent recombinant subtype C gp120 monomers. Additionally, MF59® has been selected as the protein adjuvant because it has generated superior Ab responses in vaccines against influenza, hepatitis B, and HIV [23-28]. For HIV in particular, MF59®, given together with a gp120/HIV-1SF2 HIV protein vaccine candidate, elicited higher HIV specific immune responses than alum [23,24,27].

Vaccines adjuvanted with MF59® elicit higher magnitude systemic immune responses than do the same vaccines adjuvanted with alum and, as noted previously, the reduction in risk of HIV infection seen in RV144 was greatest when the systemic antibody responses were of highest magnitude (following the final dose of the vaccine up to around 6 months thereafter) [17]. For this reason, the HVTN is interested in eliciting antibody responses more rapidly and maintaining them for longer periods of time. Regarding the capacity to raise antibody responses rapidly, the HVTN 096 study demonstrated that a recombinant pox vector or plasmid DNA (pDNA) given concurrently with recombinant gp120 at all vaccination timepoints induced antibody responses more rapidly than priming with a pox vector or pDNA and waiting to boost with recombinant protein at months 3 and 6 [29]. Moreover, in HVTN 096 the lowest levels of vaccine-specific serum IgA responses were seen in participants who received an HIV recombinant NYVAC vector expressing gp120 concurrently with AIDSVAX® B/E at all vaccinations [29]. In contrast, those who were primed with recombinant NYVAC followed by a NYVAC/gp120 dual boost had higher serum IgA responses to vaccine-matched antigens. Whether such a finding is reproducible, whether it has functional consequences similar to those observed in the RV144 trial, and whether it extends to the ALVAC-HIV + Bivalent Clade C gp120/MF59® vaccine regimens remain to be determined.

Quicker production of higher magnitude antibody responses that may also be more durable would seem desirable, especially if potential interference from less helpful immune responses can be minimized. However, several recent studies have called into question whether higher magnitude systemic immune responses necessarily translate into superior vaccine efficacy.

In particular, a recent study by Franchini and colleagues, using the non-human primate (NHP) model of HIV infection, made an unexpected and relevant observation [30]. In this preclinical study, NHP were assigned to receive the RV144 vaccine regimen using simian immunodeficiency virus (SIV) instead of the HIV inserts. One group received alum (n = 27), as given in the RV144 study, while another group received MF59® in the place of alum (n = 27). A third group served as a placebo control (n = 47). As expected, the systemic Ab responses achieved greater magnitude in the MF59® group. NHP were

then challenged intra-rectally with $SIV_{mac}251$ four weeks after the final immunization. Surprisingly, the only protection seen was in the alum group, with an estimated vaccine efficacy or 44% in preventing SIV infection at each challenge, similar to the reduction in HIV infection seen among vaccinees in RV144. The MF59® group was not protected against infection, and similar to the RV144 Study, there was no effect on viral load in the infected primates. Dr. Franchini and colleagues have subsequently analyzed the mucosal immune responses and found that despite increased total envelope-specific IgG antibodies in the rectal mucosa of the MF59® NHP, the V2-specific antibodies were decreased compared to the alum group. They also analyzed the expression of the gut mucosal homing marker $\alpha 4\beta 7$ on plasmablasts in the peripheral blood and noted that the MF59® group had decreased expression of this marker compared to the alum group.

In another NHP study, Franchini and colleagues compared immune responses elicited by an RV144-like ALVAC-SIV prime and ALVAC-SIV plus gp120 with alum boost to the responses elicited by regimens with the RV144-like SIV vaccines primed by plasmid DNA and adenovirus serotype 26 (Ad26) SIV vaccines [31]. While the Ad26-primed regimen showed the highest magnitude Ab responses in blood and mucosal secretions (including V1V2 binding antibodies [the CoR in RV144]), superior neutralization of Tier-1–like SIV strains, and generally superior T-cell responses, this regimen performed poorly against serial $SIV_{mac}251$ intrarectal challenges begun 4 weeks after the last vaccination, showing no significant difference from the control group. The investigators concluded that increased immunogenicity does not necessarily translate into increased efficacy.

These surprising and unanticipated results in nonclinical studies call into question the blanket assumption that higher magnitude immune response equate with improved vaccine efficacy. However, there are several caveats that must be considered. First of all, NHP studies have not always translated to similar results in humans [32,33]. Furthermore, the first NHP study described above was not a single study but instead data compiled from 2 separate studies, neither of which was randomized. Moreover, the small number of studied monkeys makes broad conclusions difficult; as does the fact that a rectal challenge was used. The latter may not be pertinent in a population where the majority of infections are expected to occur as a result of heterosexual transmission. Finally, the NHP were challenged 4 weeks following the final vaccination, which likely does not adequately represent the timing that would occur in humans. A longer period between vaccination and challenge would allow for optimal maturation of the immune response the quality of which is likely to be different at 4 weeks than at 4-6 months following the final boost [34]. One of the best examples of this phenomenon is seen with the attenuated SIV vaccine where efficacy is not seen until several months following the final vaccination [35].

The previously cited analyses of clinical samples by Yates et al [20] and Chung et al [21] arrived at similar conclusions based on comparisons of Ab titers elicited by the modestly efficacious RV144 regimen and by repeated doses (up to 7) of the same AIDSVAX® vaccine in clinical trial VAX003, which was also conducted in Thailand, albeit among a higher risk cohort of persons who inject drugs. Both groups found that, in terms of most of the Ab responses measured, including binding Abs to V1V2, the VAX003 vaccine regimen outperformed the RV144 vaccine regimen. However, the apparently superior Ab responses in VAX003 did not translate into vaccine efficacy, as none was observed in that trial [36]. As noted, these studies direct attention toward specific Ab subtypes and subclasses associated with Fc-mediated effector responses.

In light of these preclinical and clinical findings, it is important for the HVTN to determine the extent to which they apply to the vaccine/adjuvant combinations being evaluated in this study. For instance, while the MF59® adjuvant increases overall Ab responses in the systemic circulation, how do the adjuvants differ in terms of specific systemic immune responses elicited (eg, IgG3) and also in terms of innate and mucosal responses? To help answer these questions, this study will evaluate both the magnitude and the quality of the immune responses elicited in association with these adjuvants, both systemically and in the mucosa.

While the mechanism for SIV protection in the NHP study in the alum arm remains unclear, a leading hypothesis is that the mucosal antibody responses were quantitatively and qualitatively different when comparing the two adjuvants. Although we are able to measure some surrogate markers of mucosal homing using samples derived from blood, these are indirect measurements, which may not correlate well with the actual mucosal levels of vaccine-induced antibody responses. Furthermore, the quality of the mucosal antibody response (specificity, isotype, protein glycosylation, etc.) can only be attained from samples taken at the mucosal site. Even though mucosal responses are more labor intensive to evaluate and collecting these samples requires more time and effort on the part of the participant, our judgment, based on this information, is that the potential scientific information gained from these samples outweighs these expressed concerns.

The mucosal compartment as a primary portal of entry for HIV-1 plays a critical role in HIV transmission and early events after HIV infection [37]. Hence, understanding vaccine-induced mucosal immune responses is an important goal of HIV vaccine research. As observed in some NHP studies, vaccine-induced systemic immune responses may not necessarily predict or correlate with responses at the mucosa, highlighting the importance of assessing immune responses in both compartments. In 1 NHP study, for example, protection from vaginal virus challenge was associated with vaccine induced Ab responses in the mucosal compartment that were not detected in blood [38]. For these reasons, this study will explore rectal, semen, and cervical humoral mucosal responses in a subset of participants from each group.

In addition, the current study will evaluate the immune responses elicited by an unadjuvanted vaccine regimen as an active control. It is expected that improved immune responses with the adjuvanted regimens in this trial will provide support for the use of adjuvants as components of vaccine regimens planned for testing in later-stage clinical research and development.

While similar vaccine regimens adjuvanted with alum and with MF59® have been conducted and are ongoing in Thailand and in South Africa, these tests remain separated in time and space with samples having been and being tested in different labs with different assays being employed. Therefore, it remains important to compare the two adjuvants directly in a single study in Southern Africa, the geographic location of planned future efficacy studies. In addition, this study will help inform interpretation of results from other planned studies and will give insights into future trial designs to improve vaccine efficacy.

4.2 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).

4.2.1 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

4.2.2 Formulation characteristics

ALVAC-HIV (vCP2438) is formulated as a lyophilized vaccine for injection and is reconstituted with 1.0 mL of sterile sodium chloride solution (NaCl 0.4%) for injection 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)	$\geq 1 \times 10^6$ $CCID_{50}$ and $< 1 \times 10^8$ $CCID_{50}$	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

4.2.3 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.

4.3 Bivalent Subtype C gp120

4.3.1 Constructs

Bivalent Subtype C gp120, manufactured by Novartis Vaccines at Rentschler Biotechnologie (Laupheim, Germany), consists of two 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. The combination of the 2 subtype C gp120 proteins and the MF59® is referred to as Bivalent Subtype C gp120/MF59®. The combination of the 2 subtype C gp120 proteins and Aluminum Hydroxide Suspension is referred to as Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension.

4.3.2 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 briefly below.

Following clone selection, a fed batch cell culture at 500L or 1000L scale is employed for cell propagation. Once the cells reach optimum cell density, the culture is harvested and purified using standard methods. Harvest clarification was performed using a series of depth filters followed by bioburden reduction using a sterilizing-grade filter. The harvest was collected in single use disposable bags and purified including further enrichment for monomer. Additonal processing utilizes multiple filtration steps and a series of chromatography steps that remove process related impurities.

Both TV1.C and 1086.C bulk drug substances are stored frozen at not more than -60°C. The formulations are similar for both drug substances, containing Env antigen, sodium citrate, and sodium chloride, pH 6.5-7.0. Each of the HIV gp120 proteins as final products are tested for pH, appearance, identity, strength (concentration), purity, potency, as well as safety and content uniformity following US Pharmacopoeia methods where applicable.

The 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 MF59® adjuvant

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

Table 4-4 Qualitative composition of MF59®

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

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

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

4.5 Aluminum Hydroxide Suspension

The Aluminum Hydroxide Suspension is provided by the NIH, NIAID, Vaccine Research Center, utilizing Rehydragel HPA from ChemTrade Logistics (Berkeley Heights, New Jersey, USA), diluted with sterile water for injection to a concentration of 5 mg of aluminum per mL and vialed under aseptic conditions consistent with cGMP regulations.

Additional information is provided in the Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension IB Supplement.

4.6 Bivalent Subtype C gp120/MF59® for injection

A final dose of 100 mcg of each recombinant Env protein will be mixed with MF59® adjuvant. The composition of1 dose of the resulting vaccine is shown in Table 4-5.

Table 4-5 Composition of 0.5 mL dose of Bivalent Subtype C gp120/MF59® for injection

Ingredient	Amount in 1 dose	Function
Drug Substances		
TV1.C gp120 protein 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

4.7 Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension for injection

A final dose of 100 mcg of each recombinant Env protein will be admixed with Aluminum Hydroxide Suspension. The composition of 1 dose of the resulting vaccine is shown in Table 4-6.

Table 4-6 Composition of 0.5 mL dose of Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension for injection

Ingredient	Amount in 1 dose	Function
Drug Substances		
TV1.C gp120 protein 1086.C gp120 protein	100 mcg 100 mcg	active active
Adjuvant		
Aluminum hydroxide	625 mcg aluminum	adjuvant
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

4.8 Trial design rationale

The primary objective of the study is to compare the humoral immune responses induced by the MF59®- and alum-adjuvanted vaccine regimens; while the unadjuvanted group is included to provide information about the added value of the adjuvants. Therefore, a smaller sample size for the unadjuvanted controls is sufficient. Other important questions to be answered in this study are to compare the quality, magnitude, kinetics, and duration of the humoral and T-cell responses depending on the adjuvant used or whether ALVAC and proteins are co-adminstered during early vaccinations. The subset of participants who will provide innate immunity and mucosal samples will include 18 participants from each of the vaccine regimens.

Group assignments are randomized to provide unbiased estimates of the safety and immunogenicity parameters of the different study arms. In addition, Groups 1, 2, and 4 are double blinded to study arm assignment. Because Group 3 has a different vaccination schedule and because blood draws for innate immunity assays occur at different timepoints from the other 3 groups in the trial, it would not be possible to include Group 3 in the blind without imposing an excessive number of unnecessary clinic visits, placebo injections, and (possibly) invasive study procedures on all trial participants. For this reason, a decision was taken to not include Group 3 in the blind.

4.8.1 Dose (amount and number)

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 doses of ALVAC-HIV that are within the same range as 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 [39]. 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.

For the Bivalent Subtype C gp120/MF59® vaccine component, the two gp120 subtype C proteins (TV1.C gp120 and 1086.C gp120) will be admixed with the oil-in-water emulsion MF59® by the Pharmacist at each CRS prior to IM administration of a 0.5 mL injection containing 100 mcg of each protein plus MF59® adjuvant.

For the Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension vaccine component, the two gp120 subtype C proteins (TV1.C gp120 and 1086.C gp120) will be admixed with Aluminum Hydroxide Suspension by the Pharmacist at each CRS prior to IM administration of a 0.5 mL injection containing 100 mcg of each protein plus alum adjuvant.

For the Bivalent Subtype C gp120 without adjuvant vaccine component, the two gp120 subtype C proteins (TV1.C gp120 and 1086.C gp120) will be admixed by the Pharmacist at each CRS prior to IM administration of a 0.5 mL injection containing 100 mcg of each protein plus diluent.

Bivalent Subtype C gp120 vaccine has not yet been administered to humans. The 200 mcg total dose was selected based on previous clinical experience. Limited dose range studies performed with Novartis (formerly Chiron) a combination of subtype B SF2 gp120 and subtype E gp120 protein candidates indicated that 50 mcg and 100 mcg doses (ie,150 mcg total) with MF59® adjuvant were immunogenic and well tolerated [40]. Using the same dose of each protein (100 mcg) in all active arms will allow head-to-head comparisons between groups without introducing variability in protein content.

4.8.2 Schedule

This trial will use the same vaccination schedule used in RV144, in HVTN 097 (which is administering the RV144 vaccine regimen in a South African study population), and the primary vaccine regimen in the HVTN 100 trial, which will administer the ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® vaccine regimen in South Africa. Considerations underlying selection of this schedule for RV144 are described in Section 4.11.4.1.

Furthermore, in light of the lower IgA responses seen in HVTN 096 when recombinant NYVAC and AIDSVAX B/E were co-administered at all time points, we will also evaluate the concurrent administration of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® in a separate group. This latter schedule will administer the vaccines at months 0, 1, and 6 to determine if longer interval of the third boost will allow a maturation of antibody responses as seen for influenza vaccines [34]. In addition, data from HVTN 096 shows that antibody responses peaked after the third protein administration in the groups with protein co-administered at every vaccination.

4.8.3 Prime-boost regimen

In Groups 1, 2, and 4, this trial will employ the same strategy as RV144, that is, utilizing the ALVAC vector as a prime and the ALVAC vector/protein dual component boost. Group 3 participants will receive the ALVAC vector/protein dual component concurrently at Months 0, 1, 6, and 12. The HIV Env inserts in the ALVAC vector differ from those in RV144 in that they encode subtype C Env to more closely match the viral strains present in the Southern Africa region [41-44]. As noted above, ALVAC-HIV (vCP2438) encodes the subtype C Env gp120 from HIV-1 strain 96ZM651 in addition to the same Gag and parts of Pol used in RV144 (HIV-1 clade B LAI strain). The Bivalent Subtype C gp120 protein consists of two subtype C recombinant monomeric proteins, TV1.C gp120 and 1086.C gp120.

4.8.4 Choice of control

The vaccine regimen with the unadjuvanted Bivalent subtype C gp120 protein will serve as an active control for the two regimens containing MF59® and alum adjuvants.

4.8.5 Rationale for the innate, early adaptive and mucosal sampling timepoints

Examining the effects that the two adjuvants have on a variety of immune responses relative to each other and to the unadjuvanted protein is an important goal of this study.

Systemic innate immune responses will be measured on Day 1 and Day 3 following the vaccination when participants are first exposed to protein ± adjuvants. For Groups 1, 2, and 4, this represents Day 1 and Day 3 following the third vaccination. For Group 3, this sampling will take place on Day 1 and Day 3 following the first vaccination.

For Groups 1, 2, and 4, early systemic adaptive immune responses will be measured in samples collected at Day 0, Month 0.25, Month 3.25, Month 6.25, and Month 12.25. For Group 3, these samples will be collected at Day 0, Month 0.25, Month 6.25, and Month 12.25. These samples will provide both baseline and postvaccine information.

Systemic and mucosal samples collected at Day 0 provide the baseline values prior to vaccination while those collected at Month 6.5 provide data on peak vaccine-induced mucosal and systemic adaptive immune responses.

Finally, collection at Month 18 will provide the opportunity to assess the durability of mucosal and systemic humoral immune responses 6 months after the last vaccination.

4.9 Plans for future product development and testing

The adjuvants used in this study (MF59® and aluminum hydroxide) have never been compared directly in a clinical trial. It is anticipated that the vaccine regimen given with MF59® will induce a systemic and mucosal Ab response of greater magnitude than the same vaccine regimen given with aluminum hydroxide and that both will surpass the magnitude of immune responses elicited by the unadjuvanted study products. Such a finding would strengthen the rationale for the planned efficacy trials in Southern Africa that will use MF59®. Moreover, the design of future vaccine studies could be adjusted if lower IgA responses are demonstrated in the vaccinees receiving the simulatenous administration of the ALVAC-HIV vector and protein combination at all timepoints compared to individuals receiving initial ALVAC-HIV prime followed by ALVAC-HIV plus protein boost, provided that coadministration at all timepoints is associated with other potentially favorable immune responses, such as high V1V2 binding antibody titers. Future vaccine design could also be impacted if a longer dosing interval allows for improved antibody maturation. If we see that mucosal Ab responses are enhanced in the aluminum hydroxide group (similar to the results observed in the previously cited NHP studies), these data can be used in conjunction with data from other planned studies to better understand immune correlates of HIV risk and to inform improvements in future vaccines and vaccine trial designs.

4.10 Preclinical safety studies

4.10.1 IM local tolerability and systemic toxicity study in New Zealand White Rabbits (Study AB20670)

An objective of the study was to determine the local tolerability and systemic toxicity of ALVAC-HIV (vCP2438)/ALVAC-HIV (vCP2438) with gp120+MF59 vaccines administered by the IM route to New Zealand White Rabbits 7 times at 2-week intervals, followed by a 2-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 1st injection, but returned to normal within 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 hematological 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, 7 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. The study supports the use of this vaccine regimen in human clinical trials.

4.10.2 ALVAC-HIV

Nonclinical safety data from a variety of other ALVAC constructs 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.

4.10.3 Toxicity studies of HIV Env vaccines

The nonclinical safety of 4 doses of the gp120 proteins 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 4.10.1. Overall, the immunizations were clinically and locally well tolerated.

The nonclinical safety of 6 doses of the gp120 proteins, 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 C-reactive protein 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 which 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.

In addition, 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.C 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; and
- Repeat dose toxicity of IM SAAVI DNA-C2 followed by IM SAAVI MVA-C with subtype C gp140/ MF59® in rabbits.

4.10.3.1 Toxicity studies of MF59®

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

Pivotal toxicology studies performed with MF59® include:

- single- and repeat-dose toxicity (including local tolerability),
- genotoxicity,

- sensitization, and
- embryofetal and developmental toxicity.

4.11 Clinical studies

4.11.1 HVTN 100

HVTN 100 protocol version 1.0 enrolled 252 participants between February and May 2015. All Month 12 injections were completed by 2 June 2016.

4.11.1.1 Interim summary of blinded safety and tolerability data

Blinded safety data reported as of November 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 was 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 was 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 (following the 1st injection) and for a right deltoid injection by 2 participants (following the 3rd or 5th injection).

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 cm^2) occurring on Day 2 following the 4th injection, 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 5th vaccination, 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 5th vaccination 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” long, consistent with weight-based guidance for needle length choice provided to sites in the SSPs [45,46].

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 1st injection, 3% after the 2nd, 5% after the 3rd, 2% after the 4th, and 5% after the 5th. Severe systemic reactions have occurred in 2% of participants (4 participants): 2 participants with severe arthralgia occurring after the 1st injection, 1 person with severe malaise and/or fatigue and 1 with severe headache, each after the 5th injection.

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

As of November 2016, 467 adverse events (AEs) have occurred in 181 participants (72% of participants), of which 282 AEs (60%) were mild, 165 were moderate, (35%), 13 (3%) were classified as severe, 3 (0.6%) were classified as potentially life-threatening, and 2 (0.4%) were fatal. AEs were reported by participants most frequently in the Systems Organ Class (SOC) Infections and infestations (132 participants [52% of enrolled participants]), followed by the SOC Investigations (71 participants [28%]). Fourteen AEs occurring in 11 participants have been assessed by the site investigator as being related to study product; 11 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.

Eight SAEs, including 2 deaths, have occurred in 6 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 injury 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, transient ischemic attack, and completed suicide.

4.11.1.3 Discontinued vaccinations and early terminations

As of 21 November 2016, 232 participants have terminated the study: 207 at scheduled study exit (on-time) and 25 participants have terminated the study prematurely. Twenty-two participants have discontinued vaccinations. Clinical events leading to early terminations and discontinuation of vaccinations (DOV) occurred in 6 participants: death from multiple injuries (unrelated to study product); death from completed suicide (unrelated to study product); severe local reactogenicity (DOV); hypertension (DOV for AE unrelated to study product); mild vomiting occurring on the day of 1st vaccination only (participant declined further study participation); psychiatric diagnosis (DOV and early termination for investigator discretion). Reasons for DOV or early termination in other participants included HIV infection, 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. Two pregnancies have been reported to date, with one participant reporting a full term live birth and the other pregnant participant is continuing in follow-up.

4.11.1.4 Summary of Interim Immunogenicity data from HVTN 100

Section [6.1.2] of version 1.0 of HVTN 100 (Part A) describes the immunological criteria guiding the decision whether to advance development of the ALVAC-HIV (vCP2438), Bivalent Subtype C gp120/MF59® regimen. Except for the increased stringency of the

V1V2 response rate criteria (LL of the 95% CI increased from ≥ 45% to ≥ 56%), these same criteria ultimately formed the basis of the decision to proceed forward with HVTN 702, A *pivotal phase 2b/3 multi-site, randomized, double-blind, placebo-controlled clinical trial to evaluate the safety and efficacy of ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59 in preventing HIV-1 infection in adults in South Africa* (Table 4-7). Immunogenicity data from samples collected from HVTN 100 participants at the month 6.5 timepoint (2 weeks post 6 month 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 part A 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 4-1). 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), meeting criteria 1 and 2.

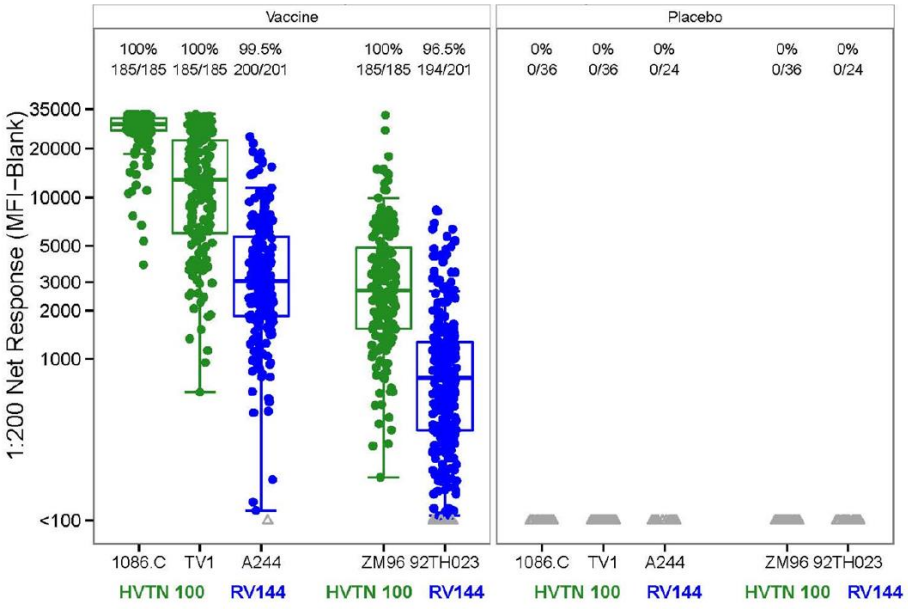

Figure 4-1 Box plots of the binding antibody titers to the vaccine antigens used in RV144 and HVTN 100. The midline of the box plot indicates the median, the ends of the box indicate the 25th and 75th percentiles. Closed dots represent positive responses, open triangles represent negative responses.

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
100	1086	185	26,257.5	8.8	(7.64, 10.2)
RV144	A244	201	2,968.9		
100	TV1	185	10,726.7	3.6	(3.01, 4.34)
RV144	A244	201	2,968.9		
100	ZM96	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 ZM96) 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 (100-RV144) was 16% (95% CI: 6, 16%; *p value* 0.0019). This exceeded the LL response rate difference of -30%, meeting 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 4-9). The cumulative V1V2 response 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.

4.11.2 Other clinical studies with related ALVAC-HIV® vaccines

The proposed ALVAC-HIV vaccine candidate specific for Southern Africa is ALVAC-HIV (vCP2438). The vaccine is most similar to ALVAC-HIV (vCP1521) (the ALVAC-HIV used in the RV144 trial) since it contains the same *gag, pro* and *gp41env TM* components. However, it has been adapted to include ZM96 gp120 *env* insert (subtype C) (rather than the TH023 gp120 *env* insert [subtype E] used for the RV144 study regimen in Thailand).

There is 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)	8202	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
ALVAC-HIV (vCP2438)	2700*	HVTN 702	Ongoing
Total	**13,692****		

* Planned

** Includes planned number of ALVAC recipients in HVTN 702.

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.

4.11.3 Clinical safety experience with related ALVAC-HIV vaccines

4.11.3.1 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 [24,27]. The most relevant data, however, come from the RV144 efficacy study performed in Thailand [4,47], 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 [48] 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:

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

2. 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.

4.11.3.2 Summary of pregnancy occurrence and outcomes

In study RV144 a total of 967 (30.6%) vaccine (vCP1521) and 955 (30.1%) placebo recipients reported a pregnancy during the study while 139 vaccine and 116 placebo recipients reported more than1 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 3165 (5.2%) vaccine female recipients and 139 out of 3169 (4.4%) placebo female recipients (p = 0.13), and in 17.1% and 14.6% (p = 0.13) of vaccine and placebo pregnancies, respectively [47].

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 [49] 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.

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

To date, 2 studies have been conducted and completed in Africa with ALVAC-HIV: HIVNET 007 and HIV Prevention Trials Network (HPTN) 027.

HIVNET 007 [50] 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 [51] 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. 60 infants were enrolled with 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).

In addition, HVTN 097, which is administering the RV144 vaccine regimen but in a “low risk” South African population, is ongoing. As of 20 May 2014, no SAEs or severe AEs related to the study products have been reported in this study.

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 cm^2), 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 adverse eventAE 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.

4.11.3.4 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 all, more than 650 human subjects have received this combination across 9 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®

Study (Country)	ALVAC-HIV®	Protein	Subjects[a]
RV132 [27] (Thailand)	vCP1521	gp120 + MF59® clades B/E made in CHO cells	n = 45
AVEG 022A [52] (USA)	vCP205	gp120 + MF59® clade B made in CHO cells	n = 47
AVEG 029 [53] (USA)	vCP205	gp120 + MF59® clade B made in CHO cells	n = 22
AVEG 202/HIVNET 014 [54] (USA)	vCP205	gp120 + MF59® clade B made in CHO cells	n = 145
AVEG 032 [55] (USA)	vCP205	gp120 +/- p24 + MF59® clade B gp120 made in CHO cells p24 made in *S. cerevisiae*	n = 56
AVEG 026 [56] (USA)	vCP300[b]	gp120 + MF59® clade B made in CHO cells	n = 85
AVEG 012A 012B [57] (USA)	vCP125[c]	gp120 + MF59® clade B made in CHO cells	n = 40
HVTN 100[d] (South Africa)	vCP2438	Bivalent Subtype C gp120 +MF59 Made in CHO cells	n = 210
HVTN 702 (South Africa)	vCP2438	Bivalent Subtype C gp120 +MF59 Made in CHO cells	n = 2700[e]
Total			**N = 650[f]**

[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.
[d] See Section 4.11.1.
[e] Trial currently enrolling. Planned number of vaccinees.
[f] Does not include planned vaccinees in HVTN 702.

4.11.4 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 (see Section 4.1.2 above, [18]).

As the development of assays for measuring immunogenicity has evolved during more than 20 years of testing in humans, 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.

4.11.4.1 ALVAC-protein schedule and immunogenicity

The selection of a vaccination schedule for the large efficacy trial in Thailand (RV144) was based on scientific knowledge at the time [58]. 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 [52,54,59-61]. 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 [60]. 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, two doses of the protein boost were proposed at months 3 and 6 to maximize Ab responses. The RV144 study implemented this vaccination schedule.

This study replicates the RV144 vaccination schedule in Groups 1, 2, and 4. The schedule in Group 3 is based on the observation in HVTN 096 that concurrent administration of subunit protein during priming vaccinations appears to produce a favorable humoral response profile more rapidly.

4.11.4.2 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 4.11.1.4. 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 [27] used ALVAC-HIV (vCP1521) in the same dose and schedule as in study RV144 but 45 subjects in1 of the study arms received a bivalent recombinant

gp120 protein manufactured in CHO cells by Novartis Vaccines and Diagnostics 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 [24] used ALVAC-HIV (vCP1521) in the same dose and schedule as in study RV144, and 97 subjects in two 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 two 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 used 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 [27]	100 mcg CM235* 50 mcg SF2**	MF59®	45	89%	61%	95%	19%	100%
RV135 [24]	100 mcg A244* 100 mcg MN**	alum	50	23%	--	44%	100%	47%
	300 mcg A244* 300 mcg 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 [27]	100 mcg CM235* 50 mcg SF2**	MF59®	45	45	32.66
RV135 [24]	100 mcg A244* 100 mcg MN**	alum	50	12.3	7
	300 mcg A244* 300 mcg MN**	alum	47	14.8	5.4

* clade E Strain
** clade B Strain

Although these 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.

4.11.5 Clinical studies with Novartis HIV-1 subunit protein vaccines

For description of interim safety/tolerability and immunogenicity results from Part A of HVTN 100, see Section 4.11.1.

In addition, recombinant monomeric (gp120) subunit vaccine formulations closely related to Subtype C gp120/MF59® from Novartis Vaccines and Diagnostics (formerly Chiron)

have been tested in clinical trials. In addition, recombinant oligomeric (o-gp140) Env proteins for subtypes B and C from Novartis have been or are currently in clinical trials. Overall, in these studies, recombinant HIV-Env proteins manufactured by Novartis were well tolerated and immunogenic. In most cases, recombinant HIV-Env proteins (either gp120 or gp140) were CHO-based and administered with MF59®, Novartis' proprietary oil-in-water emulsion adjuvant [62]. 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 EU and other countries for use in the elderly. MF59® is also used in a prepandemic H5N1 influenza vaccine (Aflunov®) licensed in EU for use in adults, and in two pandemic H1N1 influenza vaccines (Focetria® and Celtura®), licensed in 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 [27,40,56,63-65]. 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 Republic of South Africa (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 [64]

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
SF2 gp120 (CHO) with MF59®	24	AVEG 024	Completed
SF2 gp120 (CHO)/MF59® and ALVAC	85	AVEG 026	Completed
SF2 gp120 (CHO)/MF59® and ALVAC	22	AVEG 029	Completed
SF2 gp120 (CHO) +/- yeast derived p24/MF59® and ALVAC	56	AVEG 032	Completed
SF2 gp120 (CHO) with MF59®	126	AVEG201	Completed
SF2 gp120 (CHO)/MF59® and ALVAC	140	AVEG 202/HIVNET 014	Completed
SF2 gp120 & CM235 gp120 (CHO)/MF59® and ALVAC	45	RV132	Completed
Subtype B (SF162) gp140 (CHO) /MF59® and Subtype B DNA/PLG	90	HVTN 049	Completed
Subtype B (SF162) gp140 (CHO) /MF59® IN with LTK63	20	C86P1	Completed
Subtype C (TV1) gp140 (CHO)/MF59® and SAAVI DNA-C2 and SAAVI MVA-C	24	HVTN073E	Completed
Subtype C (TV1) gp140 (CHO) & ISS TAT	11	ISS P-002	Terminated*
Subtype C (TV1) gp140 (CHO)/MF59®	36	HVTN 088	Completed
Subtype C (TV1) gp140 (CHO)/MF59® and SAAVI DNA-C2 and SAAVI MVA-C	110	HVTN 086	Completed
Bivalent Subtype C gp120/MF59® and ALVAC-HIV (vCP2438)	210	HVTN 100	Ongoing
Bivalent Subtype C gp120/MF59® and ALVAC-HIV (vCP2438)	2700	HVTN 702	Ongoing

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

In general, these recombinant protein vaccines were immunogenic and well tolerated with no unusual or serious vaccine-associated AEs reported. Most of the reactions were mild to moderate in nature, and of short duration [4,27,40,56,63-67].

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

For description of interim safety/tolerability results from HVTN 100, see Section 4.11.1.1.

In addition, 2 other clinical trials have been conducted recently using Novartis CHO-based subtype B gp140 with MF59®. In addition there have been 4 recent clinical trials using Novartis CHO-based subtype C gp140.

A phase 1 single-center trial (C86P1) was conducted using Novartis CHO-based subtype B gp140 recombinant Env protein in Great Britain by the Mucosal Vaccines for Poverty Related Diseases (MUVAPRED) Consortium to assess safety, tolerability, and immunogenicity of IN administration of subtype B gp140 with and without the mucosal adjuvant LTK63 (detoxified mutant heat labile protein) followed by IM boosting with subtype B gp140/MF59®. This study enrolled 30 healthy volunteers aged 18-45, with 20 to receive gp140. The protocol was amended to halt further IN administration of LTK63 following a report of an AE (ie, facial nerve paralysis) with a possible association with the LTK63 adjuvant in another study [68]. During the study, there was 1 SAE reported of Bell's Palsy (facial nerve paralysis) considered possibly related to the study vaccine LTK63 in a subject who never received any subtype B gp140 protein or any protein with MF59® adjuvant. IN vaccination was reactogenic resulting in upper respiratory tract symptoms including nasal congestion, nasal discomfort, pharyngolaryngeal pain and rhinorrhea. The subtype B gp140 MF59® was well tolerated following IM boost.

Another completed study with Novartis subtype B gp140 MF59® was a multicenter, placebo-controlled trial (HVTN 049) conducted by the HVTN in the United States [69]. Subjects received1 of three doses of a DNA/PLG vaccine (subtype B *gag* DNA/PLG and subtype B *env* DNA/PLG microparticles, at doses of 250/250, 500/500, or 1000/1000 mcg) or placebo (5 to 1 ratio) as a single IM injection at 0, 1 and 2 months, followed by a boost of subtype B gp140 with MF59® (or placebo) at 6 and 9 months. An additional group of subjects received subtype B gp140 with MF59® without DNA prime, administered at 0, 3, and 9 months. Overall 96 healthy, HIV-1-uninfected adult subjects were enrolled and 86 subjects completed all planned vaccinations. There were no SAEs reported as related to study vaccine. There were four events reported as SAEs that were not considered related to the study vaccine. A death attributed to cocaine overdose occurred in1 subject, 10 days after receipt of the second dose of the placebo. One subject had a Grade 3 increase in creatine phosphokinase (CPK) to 2311 U/L 14 days after the first DNA prime vaccination, which resolved within a week. Another subject had a Grade 4 increase in CPK to 4806 U/L 15 days after the first DNA prime vaccination, which resolved within two weeks. Both subjects reported having initiated new exercise programs. One subject experienced severe fatigue 20 days after the fourth immunization (including1 dose of subtype B gp140/MF59®), attributed to working two jobs and long hours. Overall, the regimens were generally well tolerated.

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

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, a phase 1 trial was conducted by the Istituto Superiore di Sanità (ISS) in Italy. One of these trials, HVTN 088, was conducted in the United States in order to evaluate the safety and immunogenicity of a long-interval, cross-clade subtype C gp140/MF59® boost in subjects previously

administered subtype B gp120/MF59® or subtype B gp140/MF59® in previous trials. This includes subjects from the HVTN049 DNA/PLG prime, gp140/MF59® boost study described above. The study enrolled 16 previously vaccinated subjects and 20 naive controls. Individuals were identified who had received a clade B Env protein with MF59® 4-17 years earlier, most in combination with a DNA or ALVAC prime. These individuals were enrolled in 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®. While this study was stopped early due to slow enrollment and subsequent expiration of the study product stability program, no SAEs were reported in this study.

4.11.5.2 Summary of immunogenicity from recent human experience

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

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

homologous SF162 were also detected in all groups following IM boost with subtype B gp140 and MF59® adjuvant.

In addition, in the 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%) 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. 71% of primed participants demonstrated nAb against Tier 1 clade B isolate MN at baseline. After a single booster dose of protein, 100% of the primed participants neutralized MN and 93% showed neutralizing activity against a clade C isolate, MW965.26. Unprimed participants did not demonstrate CD4+ responses or Ab responses to Env until after the second dose, which elicited IgG and IgA responses to vaccine-matched oligomeric TV1 Env in 88% and 50%, respectively. nAb developed to MN in 38% and to MW965.26 in 88% of the unprimed participants.

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

See also Section 4.11.1.4.

4.12 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 aluminum hydroxide.

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, myalgia, arthralgia, nausea/vomiting, lymphadenopathy, asthenia, fever, 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 • Chills, 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, laceration, other transient lesions, itching, 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 or other non-specific injection site reaction • Generalized pruritus • Oral paresthesia • 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 compare the humoral immune responses induced by ALVAC-HIV (vCP2438) prime with 2 ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® boosts or 2 ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension boosts in HIV-uninfected participants

Primary endpoint 1:

Occurrence and level of vaccine-induced systemic IgG Ab binding to the 3 gp120 Env proteins contained in the vaccine regimen (ZM96, TV1.C, and 1086.C) at the Month 6.5 timepoint

Primary objective 2:

To compare the humoral immune responses induced by ALVAC-HIV (vCP2438) prime with 2 ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® boosts to the regimen with 3 ALVAC-HIV (vCP2438) + Bivalent Subtype C /MF59® vaccinations in HIV-uninfected participants

Primary endpoint 2:

Occurrence and level of vaccine-induced serum IgA Ab binding to the 3 gp120 Env proteins contained in the vaccine regimen (ZM96, TV1.C, and 1086.C) at the Month 6.5 timepoint

Primary objective 3:

To evaluate the safety and tolerability of each vaccine regimen through the Month 6.5 timepoint

Primary endpoint 3:

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

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

SAEs, AEs of special interest (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 product(s) administration throughout the study

5.2 Secondary objectives and endpoints

Secondary objective 1:

To further evaluate the humoral and cellular systemic immune responses to the different vaccine regimens

Secondary endpoint 1:

Occurrence and level of vaccine-induced serum IgG Ab binding to the 3 gp120 Env proteins contained in the vaccine regimen (ZM96, TV1.C, and 1086.C) at the Month 12, 12.5, and 18 timepoints

Vaccine-induced serum IgG Ab binding to V2 Env proteins at 6.5, 12, 12.5, and 18 months

Vaccine-induced CD4+ T-cell responses to the HIV proteins included in the vaccine at 6.5, 12, 12.5, and 18 months

Vaccine-induced serum IgG3 Ab binding to Env proteins at 6.5, 12, 12.5, and 18 months

HIV-specific CD4+ T cell polyfunctionality by ICS

Secondary objective 2:

To evaluate the safety and tolerability of each vaccine regimen through Month 18

Secondary endpoint 2:

Endpoints as indicated in primary endpoints 2

5.3 Exploratory objectives

Exploratory objective 1

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

Exploratory endpoint 1

Additional immunogenicity assays may be performed on blood and mucosal samples, including samples at innate and adaptive immunity timepoints, based on the HVTN

Laboratory Assay Algorithm. These assays are outlined in the Laboratory Accessory document.

Exploratory objective 2

To assess whether the gut microbiome correlates with vaccine responses

Exploratory endpoint 2

Gut microbiome characterization via rRNA sequencing using optionally provided stool specimens

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 132 healthy, HIV-uninfected adult participants aged 18 to 40 years.

During the consent process, participants will be given the choice whether or not to participate in the innate/mucosal subset until it is fully accrued. Participants who are not in this subset, will be randomized in a 3:3:3:1 ratio to: (1) ALVAC primes at months 0 and 1 followed by ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59® at Months 3, 6, and 12; (2) ALVAC + ALVAC primes at months 0 and 1 followed by Bivalent Subtype C gp120 adjuvanted with alum at Months 3, 6, and 12; (3) ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59® at all vaccination timepoints (Months 0, 1, 6, and 12); or (4) ALVAC primes at months 0 and 1 followed by ALVAC + Bivalent Subtype C gp120 with no adjuvant at Months 3, 6, and 12. Eligible participants who enroll in the innate/mucosal subset will be randomized to these 4 groups in a 1:1:1:1 ratio. A 3 month accrual period is anticipated. To ensure a relative balance of participants of each sex at birth in the subset and the non-subset portions of the trial, no more than approximately 60% of participants of either sex will be enrolled into the subset or non-subset portions of the trial. Hence, when approximately 43 participants of either sex have been enrolled in the innate/mucosal subset, recruitment for that sex will stop. Similarly, when approximately 36 participants of either sex have been enrolled in the non-subset portion, recruitment for that sex will stop.

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. However, for immunogenicity analyses, it is possible that data may be missing for various reasons, such as participants terminating from the study early, problems in shipping specimens, low cell viability of processed peripheral blood mononuclear cells (PBMCs) or high background. Immunogenicity data from nine phase 1 and1 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of June 2011), indicate that 15% is a reasonable estimate for the rate of missing data at Month 6.5. For this reason, the sample size calculations in Section 6.1.2 account for 15% enrolled participants having missing data for the primary immunogenicity endpoint.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect serious adverse events (SAEs) (see Section 11.2.3) 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 adjuvanted vaccine arm of the study (n = 36), there is a 90% chance of observing at least 1 event if the true rate of such an event is 6.2% or more; and there is a 90% chance of observing no events if the true rate is 0.3% or less. For the unadjuvanted vaccine arm (n = 24), there is a 90% chance of observing at least 1 event if the true rate of such an event is 9.1% or more; and there is a 90% chance of observing no events if the true rate is 0.4% or less. For the vaccine arms combined (n = 132), there is a 90% chance of observing at least 1 event if the true rate of such an event is 1.7% or more; and there is a 90% chance of observing no events if the true rate is

0.08% or less. As a reference, in HVTN vaccine trials from December 2000 through April 2014, about 6.5% of South African participants who received placebos experienced an SAE.

Probabilities of observing 0, 1 or more, and 2 or more events among arms of size 24 and 36 are presented in Table 6-1 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among vaccine arms of size 24 and 36, for different true event rates

True event rate (%)	Pr(0/24)	Pr(1+/24)	Pr(2+/24)	Pr(0/36)	Pr(1+/36)	Pr(2+/36)
0.1	0.976	0.024	<0.001	0.965	0.035	0.001
0.5	0.887	0.113	0.006	0.835	0.165	0.014
1	0.786	0.214	0.024	0.696	0.304	0.050
4	0.375	0.625	0.110	0.230	0.770	0.425
10	0.080	0.920	0.708	0.023	0.977	0.887

An alternative way of describing the statistical properties of the study design is in terms of the 95% 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 [70]. If none of the 132 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total adjuvant-vaccinated population is 2.8. For each individual adjuvanted vaccine arm (n = 36), the 2-sided upper confidence bound for this rate is 9.6%. For the vaccine arm with no adjuvant (n = 24), the 2-sided upper confidence bound for this rate is 13.8%.

Table 6-2 Two-sided 95% CIs based on observing a particular rate of safety endpoints for vaccine arms of size 24, 36, and 132 (pooled)

Observed event rate	CI (%)
0/24	(0.0, 13.8)
1/24	(0.2, 20.2)
2/24	(2.3, 25.8)
0/36	(0.0, 9.6)
1/36	(0.5, 14.2)
2/36	(1.5, 18.1)
0/132	(0.0, 2.8)
1/132	(0.0, 4.2)
2/132	(0.4, 5.4)

6.1.2 Sample size calculations for immunogenicity

The primary immunogenicity objective of this trial is to characterize the vaccine-induced humoral immune responses of the 4 vaccine regimens at Month 6.5. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% CIs for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-3.

Calculations are done using the score test method [70]. The sample size n = 20 and n = 30 assume a 15% missing data rate.

Another primary question of interest regarding immunogenicity is the comparison of the Month 6.5 response rates of insert-directed serum IgG from the binding Ab assay to detect moderate differences in response rates between the 2 arms primed with ALVAC followed by ALVAC + Bivalent Subtype C gp120 adjuvanted either with MF59® or with alum. A separate primary objective is to compare the Month 6.5 response rates of insert-directed serum IgA from the binding Ab assay for the arm primed with ALVAC followed by ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59® to the arm with concurrent administration of ALVAC and Bivalent Subtype C gp120 adjuvanted with MF59® at all vaccination timepoints. Within each of these group comparisons, no adjustment will be made for the use of multiple inserts.

Table 6-3 Two-sided 95% CIs for the true response rate based on observing a particular rate of responses in the vaccinees (n = 20, 30)

n	No. of responses	Observed response rate (%)	CI (%)
20	6	30.0	(14.5, 51.9)
20	10	50.0	(29.9, 70.1)
20	12	60.0	(38.7, 78.1)
20	14	70.0	(48.1, 85.5)
20	16	80.0	(58.4, 91.9)
30	9	30.0	(16.7, 47.9)
30	15	50.0	(33.2, 66.8)
30	18	60.0	(42.3, 75.4)
30	21	70.0	(52.1, 83.3)
30	24	80.0	(62.7, 90.5)

As shown in Table 6-4, there is adequate power for a formal comparison of immunogenicity response rates between adjuvanted vaccine arms of size n = 30 to detect moderate differences in response rates of ~35%. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05.

Table 6-4 Power for comparison of response rates between 2 arms (n1 = 30, n2 = 30)

True response rate Arm 1 (%)	Maximum true response rate in Arm 2 in order to detect a difference	
	80% power	90% power
40	8	5
50	14	10
60	22	18
70	32	27
80	42	37
90	56	51
95	64	59

In addition to comparing insert-directed serum IgG response rates at Month 6.5 between the aluminum hydroxide and MF59® adjuvanted arms, it is also of interest to compare

differences in the magnitudes of the responses. Power calculations use Lachenbruch's 2-part test to compare the response rates and the continuous insert-directed IgG responses at Month 6.5. Given that power is sufficient to compare the response rates, the study will also be well powered for this comparison involving magnitudes. For example, with n = 30 per arm there is 80% power to detect a difference with a true response rate of 92% in 1 arm with GM = 6.31 and variance of 2.06 in responders and a response rate of 80% in the other arm with GM = 5.18 and variance of 2.06 in responders. Response rates of 92% and 94% with GMs of 6.31 and 6.42 (variance 2.06, 1.89 respectively) correspond to the responses observed at Month 6.5 in RV144 with the A244 and 92TH023 inserts. See Table 6-5 for power with other response rates and magnitudes (variance = 2.06 for each arm for true response rate = 92% and variance = 1.89 for true response rate = 94%)

Many other immunogenicity endpoints will also be analyzed to characterize the innate and adaptive immune profiles of the vaccine arms. These include vaccine-induced IgG Ab binding to V2 Env proteins, vaccine-induced CD4+ T-cell responses to the HIV proteins included in the vaccine and vaccine-induced IgG3 Ab binding to Env proteins.

Table 6-5 Power for comparison of response rates and levels between 2 vaccine arms (n1 = 30, n2 = 30)

True response rate (%) and GM Arm 1		Maximum true response rate and GM in Arm 2 in order to detect a difference			
		80% power		90% power	
94	6.42	85	5.25	85	5.05
94	6.42	80	5.37	80	5.15
94	6.42	75	5.51	75	5.18
92	6.31	85	5.15	85	5.00
92	6.31	80	5.18	80	5.07
92	6.31	75	5.22	75	5.14

6.2 Randomization

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

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment group assignments for the 3 heterologous prime boost arms of the study (Groups 1, 2, 4); the ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59 at all vaccination time points will not be blinded (Group 3). 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 (if he or she is in a blinded arm of the study).

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 107 PSRT should be consulted before emergency unblinding occurs.

6.4 Statistical analysis

This section describes the final study analysis, unblinded as to treatment arm assignment. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. The analysis is a modified intent-to-treat analysis in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

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

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

6.4.1 Analysis variables

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

6.4.2 Baseline comparability

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

6.4.3 Safety/tolerability analysis

Since enrollment is concurrent with receiving the first vaccination, all participants will have received at least 1 vaccination and therefore will provide some safety data.

6.4.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition, to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms (malaise and/or fatigue,

myalgia, headache, nausea, vomiting, chills, arthralgia) will be calculated. Kruskal-Wallis tests will be used to test for differences in severity between arms.

6.4.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment arm the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing arms is not planned since interpretation of differences must rely heavily upon clinical judgment.

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

6.4.3.3 Local laboratory values

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

For each local laboratory measure, summary statistics will be presented by treatment arm and time point, as well as changes from baseline for post-enrollment 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 9.10) will be tabulated by treatment arm for each post-vaccination time point. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

6.4.3.4 Reasons for vaccination discontinuation and early study termination

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

6.4.4 Immunogenicity analysis

6.4.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Additional analyses may be performed, limited to participants who received all scheduled injections per protocol. Assay results that are unreliable, from specimens collected outside of the visit window, or from HIV-infected participants postinfection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% CI estimates calculated using the score test method [70]. Fisher's exact tests will be used for the primary analysis comparing the IgG response rates of the arm with ALVAC primes followed by ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59® boosts(Group 1) vs the aluminum hydroxide adjuvanted arm (Group 2) at Month 6.5 with a significant difference declared if the 2-sided p-value is ≤ 0.05. Fisher's exact tests will also be used to compare IgA response rates between the arm with ALVAC primes and Bivalent Subtype C gp120 adjuvanted with MF59® boosts (Group 1) and the arm with ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59® coadministered at all vaccination timepoints (Group 3) at Month 6.5; this is considered a separate primary objective and thus no multiplicity adjustment will be made. Fisher's exact tests will also be used to compare response rates between the unadjuvanted and adjuvanted arms at Month 6.5 and other time points; these analyses are viewed as secondary and therefore no multiplicity adjustment will be made.

For quantitative assay data (eg, level of vaccine-induced IgG Ab binding to the 2 gp120 Env proteins contained in the vaccine regimen), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. The difference between arms at a specific timepoint will be tested with a nonparametric Wilcoxon rank sum test if the data are not normally distributed and with a 2-sample t-test if the data appear to be normally distributed. To test for differences among the 4 vaccine arms, first a Kruskal-Wallis rank test or an F-test (depending on the normality assumption) will be used to test for overall differences. Secondly, if the overall test is significant at the 2-sided 0.05 level, then individual tests comparing the 3 pairs of vaccine arms will be done unless pre-specified. If rank-based tests are used then the tests will be inverted to construct Hodges-Lehmann point-estimates and 2-sided $(1 - 0.05/3) \times 100\%$ CIs about the differences in location centers of the 3 pair-wise comparisons of vaccine arms. An appropriate data transformation (eg, $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance).

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, binding antibody multiplex assay [BAMA]). If treatment arm differences for these assays are best summarized by a mixture model, then Lachenbruch's test statistic [71] will be used to evaluate the composite null hypothesis of equal response rates in the MF59® vs aluminum hydroxide adjuvanted arms and equal response distributions among responders in the 2 arms. This test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

This approach will also be used to compare the arm with ALVAC primes followed by ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59® boosts at Month 3, 6, 12 (Group 1) to the arm with ALVAC + Bivalent Subtype C gp120 adjuvanted with MF59® coadministered at all vaccination timepoints (Group 3).

Based upon previous AIDS Vaccine Evaluation Group (AVEG) and 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 nonparametric tests and generalized linear models fit by generalized estimating equation (GEE) methods, missing data need to be missing completely at random (MCAR). MCAR assumes that the probability of an observation being missing does not depend upon the observed responses or upon any unobserved covariates but may depend upon covariates included in the model (eg, missing more among whites than nonwhites). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then nonparametric tests and GEE methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests. These models will include as covariates all available baseline predictors of the missing outcomes.

If a substantial amount of immunogenicity data are missing (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right- censored, then the linear mixed effects models of Hughes [72] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [73] methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

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

The analysis of the CD4+ T-cell response rate as measured by the ICS assay will be evaluated and compared as described under the general approach. The positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay using the discrete Bonferroni adjustment. The magnitude of response will be analyzed as described for quantitative data in the general approach section. Graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint, with a box plot of data from positive responders superimposed on the individual data values. Statistical testing comparing the magnitudes will be based on positive responders only. The same approach will be used to analyze other T cell subsets of interest.

6.4.5 Analyses prior to end of scheduled follow-up visits

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

6.4.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 107 PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity

A limited unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the Month 6.5 visit and data are available for analysis from at least 80% of these participants. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key HVTN members and investigators. Distribution of reports will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the scheduled follow-up visits.

7 Selection and withdrawal of participants

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

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

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

7.1 Inclusion criteria

General and Demographic Criteria

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

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

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

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

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

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

HIV-Related Criteria:

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

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

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

Laboratory Inclusion Values

Hemogram/Complete blood count (CBC)

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

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

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

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

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

Chemistry

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

Virology

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

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

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

Urine

19. **Normal urine**:

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

Reproductive Status

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

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

- Agree to consistently use effective contraception (Appendix 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 is defined as using:
  - Condoms (male or female), or
  - Diaphragm or cervical cap,

  PLUS 1 of the following methods:
  - Intrauterine device (IUD),
  - Hormonal contraception (in accordance with *Republic of South Africa: National Contraception Clinical Guidelines*),
  - Successful vasectomy in the male partner (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity postvasectomy); or
  - Any other contraceptive method approved by the HVTN 107 PSRT
- Or not be of reproductive potential, such as having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation;
- Or be sexually abstinent.

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

Other

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

7.2 Exclusion criteria

General

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

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

3. **Body mass index (BMI)** ≥ 40; or BMI ≥ 35 with 2 or more of the following: systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, current smoker, known hyperlipidemia

4. **Intent to participate in another study** of an investigational research agent or any other study that requires non-HVTN HIV antibody testing during the planned duration of the HVTN 107 study

5. **Pregnant or breastfeeding**

Vaccines and other Injections

6. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 107 PSRT will determine eligibility on a case-by-case basis.

7. **Non-HIV experimental vaccine(s) received within the last 5 years** in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure in a volunteer's country of residence. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 107 PSRT will determine eligibility on a case-by-case basis. For volunteers who have received an experimental vaccine(s) greater than 5 years ago, eligibility for enrollment will be determined by the HVTN 107 PSRT on a case-by-case basis.

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

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

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

Immune System

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

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

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

14. **Autoimmune disease**

15. **Immunodeficiency**

Clinically significant medical conditions

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

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

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

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

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

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

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

    Exclude a volunteer who:

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

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

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

24. **Hypertension**:

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

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

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

26. **Malignancy** (Not excluded 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)

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

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

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

7.3 Participant departure from vaccination schedule or withdrawal

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

7.3.1 Delaying vaccinations for a participant

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

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

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

- Within 14 days prior to any study injection

  - Receipt of influenza vaccine or any vaccines that are not live attenuated vaccines (eg, pneumococcal)

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

- Pregnancy: for participants who become pregnant, no study vaccinations will be given; except for participants who may have been pregnant during the study but are no longer pregnant as shown by two negative urine pregnancy tests taken from two different urine samples that may be collected on the same day; in this circumstance, the HVTN 107 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 107 Study Specific Procedures.

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

7.3.2 Participant departure from vaccination schedule

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

7.3.3 Discontinuing vaccination for a participant

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

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 107 PSRT).

- Clinically significant condition (ie, a condition that affects the immune system or for which continued vaccinations and/or blood draws may pose additional risk), including but not limited to the following:

  - Pregnancy (Vaccinations will be stopped while a participant is pregnant. If the participant is no longer pregnant and can be vaccinated within an appropriate visit window, vaccinations may resume, see Section 7.3.1);

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

- Any grade 3 lab abnormality or other clinical AE (exception: fever or vomiting and subjective local and systemic symptoms) that is subsequently considered to be related to vaccination; upon review, the PSRT may allow continuation of vaccination if the participant has grade 3 erythema and/or induration;
- SAE that is subsequently considered to be related to vaccination;
- Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 107 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination; or

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

- A study participant who misses a study injection is permitted to continue with subsequent study injections that can still be scheduled within the time interval specified in the *HVTN 107 Study Specified Procedures* (SSP) unless there is a protocol-mandated reason for discontinuation.

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

In addition, vaccinations will be stopped for participants diagnosed with HIV infection. HIV-infected participants will not continue in the trial (see Sections 7.3.4 and 9.7.1).

7.3.4 Participant termination from the study

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

- Participant refuses further participation,

- Participant relocates and remote follow-up or transfer to another HVTN CRS is not possible,

- HVTN CRS determines that the participant is lost to follow-up,

- Participant becomes HIV infected,

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

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

8 Study product preparation and administration

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

8.1 Vaccine regimen

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

Group 1

Treatment 1 (T1): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, 6, and 12

AND

Bivalent Subtype C gp120/MF59® (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and MF59C.1) to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 3, 6, and 12

Group 2

Treatment 2 (T2): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, 6, and 12

AND

Bivalent Subtype C gp120 (an admixture of 100 mcg of TV1.C gp120 and 100 mcg of 1806.C gp120) admixed with Aluminum Hydroxide Suspension to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 3, 6, and 12

Group 3

Treatment 3 (T3): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0, 1, 6, and 12

AND

Bivalent Subtype C gp120/MF59® (an admixture of 100 mcg of TV1.C gp120, 100 mcg of 1086.C gp120, and MF59C.1) to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 0, 1, 6, and 12

Group 4

Treatment 4 (T4): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, 6, and 12

AND

Bivalent Subtype C gp120 (an admixture of 100 mcg of TV1.C gp120 and 100 mcg of 1806.C gp120) admixed with Sodium Chloride for Injection, 0.9% to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 3, 6, and 12

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 of diluent are required to prepare one dose. It must be stored refrigerated (2-8°C). DO NOT FREEZE.

Bivalent Subtype C gp120 composed of two different proteins:

TV1.C gp120 protein [labeled as TV1.C gp120]: The TV1.C gp120 protein will be provided in a 2 mL glass vial containing approximately 0.58 mL (462 mcg protein) of a clear colorless to slightly yellow liquid. The product must be stored frozen at -61°C or colder.

1086.C gp120 protein [labeled as 1086.C gp120]: The 1086.C gp120 protein will be provided in a 2 mL glass vial containing approximately 0.58 mL (462 mcg protein) of a clear colorless to slightly yellow liquid. The product must be stored frozen at -61°C or colder.

MF59® [labeled as MF59C.1] is supplied as an oil-in-water emulsion. The MF59 adjuvant has a milky white opaque appearance and is provided in a glass vial containing a total volume of 0.7 mL. The product must be stored refrigerated at 2 - 8°C. Do not freeze.

The Bivalent Subtype C gp120 and the MF59® are described in further detail in the Bivalent Subtype C gp120/MF59® IB.

Aluminum Hydroxide Suspension appears as an opaque, white gelatinous precipitate in aqueous suspension. It is provided in 3 mL glass vials each containing 0.7 mL of aluminum hydroxide (5 mg/mL as aluminum). The product should be stored refrigerated at 2 - 8° C. Do not freeze.

The study product is described in further detail in the Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension IB supplement.

Sodium Chloride for Injection, 0.9% (normal saline, Sodium Chloride for Injection, 0,9%)

Sodium Chloride for Injection, 0.9% will be used for the Bivalent Subtype C gp120 alone group to maintain blinding between the groups. It 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) (All Groups)

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). 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 syringe should be labeled as “ALVAC-HIV 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 expired syringes are disposed of in accordance with institutional or pharmacy policy for a biological safety level 1 product.

8.3.2 Bivalent Subtype C gp120/MF59® (Groups 1 and 3)

One vial of TV1.C gp120 protein,1 vial of 1086.C gp120 protein, and 1 vial of MF59C.1 will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the TV1.C gp120 and 1086.C gp120 from the freezer and 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 2-8°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 syringe should be labeled as "HVTN 107 study product" and have an overlay applied to the syringe. Although Group 3 is not blinded, this will maintain consistency with the other groups. The syringe must also be labeled for administration in RIGHT deltoid and "Gently roll the syringe prior to administration". 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 vaccine should be stored at 2°C-8°C until administration and if not used within 2 hours, it should be discarded.

8.3.3 Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension (Group 2)

One vial of TV1.C gp120 protein,1 vial of 1086.C gp120 protein, 1 vial of Aluminum Hydroxide Suspension, 1 vial/ampule of Sterile Water for Injection, and 1 empty sterilized vial 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 2-8°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 Aluminum Hydroxide Suspension vial from the refrigerator and the Sterile Water for Injection from storage.

Using aseptic technique, the pharmacist will gently swirl the contents of the vial containing aluminum hydroxide, withdraw 0.35 mL of the aluminum hydroxide and inject it into the empty sterilized vial. The pharmacist will then add 0.35 mL of Sterile Water for Injection to the mixing vial which contains the 0.35 mL of aluminum hydroxide. The mixing vial should be gently swirled and inverted (do not shake). Next, the pharmacist will take the vial containing TV1.C gp120 and withdraw 0.35 mL of TV1.C gp120 from the vial and inject it into the mixing vial (which contains aluminum hydroxide mixed with Sterile Water for Injection). 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 mixing vial (which contains TV1.C gp120 and aluminum hydroxide mixed with Sterile Water for Injection). After gentle swirling and inversion (do not shake vigorously) the pharmacist, using aseptic technique, will withdraw 0.5 mL of the mixed preparation for dosing into a 1 or 2 mL syringe.

The syringe should be labeled as "HVTN 107 study product" and have an overlay applied to the syringe. The syringe must also be labeled for administration in RIGHT deltoid and "Gently roll the syringe prior to administration". 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 vaccine should be stored at 2°C-8°C until administration and if not used within 2 hours, it should be discarded.

8.3.4 Bivalent Subtype C gp120 (Group 4)

One vial of TV1.C gp120 protein,1 vial of 1086.C gp120 protein, Sodium Chloride for Injection, 0.9%, and 1 empty sterilized vial 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 2-8°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 obtain the sodium chloride for injection and 1 empty sterilized vial from storage.

Using aseptic technique, the pharmacist will transfer 0.7 mL of Sodium Chloride for Injection in to the empty sterilized vial. The pharmacist will then gently swirl the contents of the vial containing TV1.C gp120 and withdraw 0.35 mL of TV1.C gp120 from the correct vial and inject it into the mixing vial containing the sodium chloride for injection. 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 mixing vial (which contains TV1.C gp120 and sodium chloride for injection). After gentle swirling and inversion (do not shake vigorously) the pharmacist, using aseptic technique, will withdraw 0.5 mL of the mixed preparation for dosing into a 1 or 2 mL syringe.

The syringe should be labeled as “HVTN 107 study product” and have an overlay applied to the syringe. The syringe must also be labeled for administration in RIGHT deltoid and “Gently roll the syringe prior to administration”. 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 vaccine should be stored at 2°C-8°C until administration and if not used within 2 hours, it should be discarded.

8.4 Administration

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

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

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

For all syringes containing Bivalent Subtype C gp120 with or without adjuvant, the person administering the injection should gently roll the syringe prior to administration of the study product. These syringes will be labeled as "HVTN 107 study product".

If an injection is administered in the contralateral deltoid due to a medical contraindication, the appropriate study staff should document this clearly. Under this circumstance, this is NOT a protocol violation. Two injections administered into the same deltoid should be at least 2.4 cm apart and should be documented in the participant's study record.

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 and the MF59® adjuvant will be supplied by GlaxoSmithKline Biologicals, S.A. and will be available through the CRPMC.

Aluminum Hydroxide Suspension will be provided by the NIH, NIAID, Vaccine Research Center and will be available through the CRPMC.

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

Sterile Water for Injection (used for preparation of Bivalent Subtype C gp120 admixed with Aluminum Hydroxide Suspension) will not be provided through the protocol and must be obtained by the site.

Sodium Chloride for Injection, 0,9% will not be provided through the protocol and must be obtained by the site.

Empty sterilized vials for study product mixing will be provided through the protocol and may be obtained from the CRPMC.

8.6 Pharmacy records

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

8.7 Final disposition of study products

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

9 Clinical procedures

The schedules of clinical procedures are shown in Appendix J, Appendix K, Appendix L, and Appendix M.

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

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

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

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

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

9.1.3 Assessment of Understanding

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

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

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

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

9.2 Pre-enrollment procedures

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

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

- Medical history, documented in the case history record;
- Assessment of Understanding (see Section 9.1.3);
- Assessment of whether the volunteer is at low risk for HIV infection;
- Complete physical examination, including height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots (record the complete generic name for all medications);
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - CBC with differential and platelet count,
  - Chemistry panel (ALT, AST, ALP, and creatinine),
  - Urine dipstick (urinalysis if indicated, see Section 9.9),
  - HBsAg,
  - Anti-HCV Ab,
  - Syphilis test,
  - Urine or serum pregnancy test (volunteers who were born female);
  - Pap smear (Only for volunteers who were born female and who agree to provide cervical samples; not required if volunteer has had Pap smear within previous 3 years with most recent result normal or ASCUS;
- 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 born female and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.

9.2.1 Use of screening results from another HVTN study

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

9.3 Enrollment and vaccination visits

Enrollment is simultaneous with first vaccination. The time interval between randomization and enrollment should not exceed 4 working days. The HVTN CRS registers the participant by scheduling the day 0 visit (enrollment) via the Web-based randomization system, and requests the randomization assignment. Circumstances may require a participant's enrollment visit to be changed. This may exceed the 4-day randomization time limit.

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

- Abbreviated physical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;

- Assessment of baseline reactogenicity parameters;

- Assessment of concomitant medications (as described in Section 9.2);

- Assessment of any new or unresolved AEs/intercurrent illnesses; and

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

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

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

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

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 J, Appendix K, Appendix L, and Appendix M:

- HIV infection assessment including pretest counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate; and
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of behavioral risk assessment questionnaire;
- Specimen collection (blood/mucosal/stool) should be completed prior to vaccination (see Appendix F, Appendix G, Appendix H, and Appendix I);
- For participants who agree to join and who have been randomized to the innate/mucosal subset (see Section 9.5):
    - Urine test for gonorrhea and chlamydia;
    - Vaginal swab for Trichomonas and bacterial vaginosis (for participants providing cervical samples);

- Vaginal swab (if indicated) for hyphae/budding yeast (for participants providing cervical samples);
- Mucosal specimen collection;
- Syphilis serology.

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 J, Appendix K, Appendix L, and Appendix M:

- 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);
- HIV infection assessment including pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Specimen collection (blood/mucosal/stool) should be completed prior to vaccination (see Appendix F, Appendix G, Appendix H, and Appendix I);

- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.9); and
- Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.
- For participants who have been enrolled to the innate/mucosal subset to provide mucosal samples (see Section 9.5):
  - Urine test for gonorrhea and chlamydia;
  - Vaginal swab for Trichomonas and bacterial vaginosis (for participants providing cervical samples);
  - Vaginal swab (if indicated) for hyphae/budding yeast (for participants providing cervical samples);
  - Syphilis serology;
  - Pregnancy test (see Section 9.5);
  - Mucosal specimen collection.

9.5 Innate immunity and mucosal sampling

Innate immunity samples and mucosal samples will be collected at the timepoints indicated in Appendix G, Appendix I, Appendix K, and Appendix M from the subset of 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 born female, a pregnancy test must be performed and be negative prior to any rectal mucosal sampling. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Rectal secretion sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, if still within the visit window. In addition, rectal sampling will not be performed (or may be deferred to a later date if still within the

visit window) if there is a contraindication to rectal secretion sampling, such as an active infection or inflammation of the colorectal area (such as an herpes simplex virus (HSV)-2 outbreak or inflamed hemorrhoids or colitis/diarrhea) or if the participant has any active genital tract infection (GTI).

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

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

Cervical sampling (only for participants who were born female): Participants must report having had a pap smear within the 3 years prior to enrollment, with the latest result reported as normal or ASCUS. A pregnancy test must be performed 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 has an active ulcerative genital lesion or is known to have an active GTI at the scheduled timepoint. Participants providing cervical secretion samples should be advised as follows:

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

Semen sampling (only for participants who were born male): Participants providing semen samples are asked to refrain from ejaculation for at least 48 hours prior to specimen collection. In addition, mucosal sampling will not be performed (or may be deferred to a later date within the visit window) if a participant is known to have an active GTI at the scheduled timepoint.

9.6 Stool sampling

Stool samples will be collected at 2 timepoints from study participants who agree to this procedure: prior to enrollment (before the injection of the vaccine) and at the Month 6.5 timepoint. These samples will be collected using swabs, either via rectal swabs or by taking swabs from stool.

If participants opt to collect stool samples at home, they should be instructed to deliver the sample to the clinic within 24 hours after they are collected.

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. All participants who become HIV infected during the study will be terminated from this study. Potential and enrolled participants identified as HIV infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.7.1 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 J, Appendix K, Appendix L, and Appendix M. 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, Appendix G, Appendix H, and Appendix I). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see Study Specific Procedures [SSP]), which is able to distinguish vaccine-induced 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 (VISP).

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

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

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

9.9 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 (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 [March 2017], except as noted in Section 11.2.2.

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

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

Table 9-1 Schedule of reactogenicity assessments

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

[a] Day of vaccination

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

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

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

9.10.2 Assessment of injection site

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

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

9.10.3 Assessment of lymph nodes

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

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

9.11 Visit windows and missed visits

Visit windows are defined in HVTN 107 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, platelet count, and chemistry panel), pregnancy testing, social impact assessment, and HIV test.

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

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN 107 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, Appendix G, Appendix H, and Appendix I. For tests performed locally, the local lab may assign appropriate tube types.

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

10.2 Total blood volume

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

10.3 Primary immunogenicity timepoint

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

10.4 Endpoint assays: humoral

10.4.1 HIV-1 multiplex Ab assay

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

10.4.2 Neutralizing Ab (nAb) assay

HIV-1–specific nAb assays may be performed on serum samples from study participants taken at the primary immunogenicity timepoint. Specimens from the baseline and other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoint. Tier 1 assays will test neutralization of HIV-1 strains represented in the highly neutralization-sensitive tier 1 viruses. The tier 2 assays will test neutralization of a panel of heterologous primary isolates [74].

10.5 Endpoint assays: cellular

10.5.1 Flow cytometry

Flow cytometry will be used to examine vaccine-specific CD4+ and CD8+ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine construct. ICS parameters will include cytokines such as IFN-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-α, and may include other cytokines (such as cytokines relevant to Th2 and Th17 responses) to identify T cells of specific functionality. Markers of cytotoxic potential (Granzyme B, perforin, and CD57) may also be included. Data will be reported as percentages of CD4+ or CD8+ T cells responding to a specific peptide pool. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

Phenotyping of T cells (eg, T follicular helper [Tfh]), B cells (eg, plasmablasts), dendritic cells, monocytes, natural killer (NK) cells, or other leukocytes may be performed for lineage, maturation, homing, and activation markers using PBMC samples.

10.6 Innate immunity assays

10.6.1 Soluble factors in serum or plasma

Multiplex cytokine array and/or ELISA may be used to measure soluble cytokines, chemokines, and other immunomodulatory factors in the serum or plasma. Analytes may include factors such as IFN-γ, IL-6, TNF-α, IL-10, IP (IFN-γ–induced protein)-10, and/or monocyte chemotactic protein (MCP)-1.

10.6.2 Gene expression

Whole blood will be cryopreserved in a ribonucleic acid (RNA) protection reagent. RNA may be isolated and used for transcriptome analyses. Signatures of gene expression changes will be analyzed over time after vaccination.

10.7 Genotyping

Molecular human leukocyte antigen (HLA) typing may be performed on enrolled participants using cryopreserved PBMC collected at baseline, initially on specimens from participants who demonstrate vaccine-induced T-cell responses at postvaccination timepoints. Other participants (including control recipients) may be HLA-typed to

support future studies of immunological interest at the discretion of the HVTN Laboratory Program. Other markers, such as genes associated with immune responses or HIV-1 disease progression may also be assessed.

10.8 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 time points. Please note that the Lab Assay Algorithm will be updated periodically to include new assays.

10.9 Exploratory studies

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

10.9.1 Microbiome analysis

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

10.10 Other use of stored specimens

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

Other use of specimens is defined as studies not covered by the protocol Appendix F, Appendix G, Appendix H, or Appendix I, or the informed consent form (Appendix A, Appendix E).

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 testing on specimens will occur only after review and approval by the HVTN, the IRB/EC of the researcher requesting the specimens, and the CRS's IRBs/ECs if required.

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

HVTN Regulatory Affairs in writing. In either case, HVTN Regulatory Affairs directs the HVTN Lab Program not to use samples from these participants for such other uses.

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

10.11 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 107 PSRT

The HVTN 107 PSRT is composed of the following members:

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

The clinician members of HVTN 107 PSRT are responsible for decisions related to participant safety.

A medical officer from an organization in Southern Africa designated by the study sponsor will also participate in the PSRT.

The Protocol Team clinic coordinator, project manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 107 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 107 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 107 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 107 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 107 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

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

11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs are graded according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 [March 2017], available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx, except that:

- Unintentional weight loss is required to be reported as an adverse event only if it is considered to be deleterious to the participant's health (see HVTN 107 SSP).
- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:
    - Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area;

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

In general, unsolicited AEs will be collected over 30 days post-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 to pertinent national regulatory authorities (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 AESIs is provided in Appendix N.

The following AEs will be collected and reported throughout the entire study:

- SAEs and AEs requiring expedited reporting to DAIDS (EAEs);
- AESIs;
- New chronic conditions requiring medical intervention for ≥ 30 days;
- New diagnosed or treated STIs; and
- 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 HVTN 107 home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn107/SitePages/Home.aspx). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

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

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

11.2.3 Expedited reporting of AEs to DAIDS

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

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

For questions about DAERS, please contact CRMSsupport@niaid.nih.gov or from within the DAERS application itself. For questions about expedited 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 hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect, or
- is a medically important event or reaction

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

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

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

- ALVAC-HIV (vCP2438)
- Bivalent Subtype C gp120/MF59®
- Bivalent Subtype C gp120 admixed with Aluminum Hydroxide
- Bivalent Subtype C gp120

Clinic staff will report SAEs as indicated in Section 11.3, *Safety pause and prompt PSRT AE review* to HVTN Core or within 3 working days for SAEs not described in Table 11-1, *AE notification and safety pause/AE review rules*, in addition to completing the standard AE form.

11.2.4 Expedited reporting of AEs to 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. SAEs will be reported to the South Africa Medicines Control Council (MCC) within 24 hours of the sponsor becoming aware of each event followed, as needed, with full safety reports deemed reasonably related to study product within the time frame specified in the MCC guidelines.

Site IoRs/designees will submit EAE 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 107 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 107 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

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

Event and relationship to study products	Severity	HVTN CRS action	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and submit forms immediately[a]	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately[a]	Immediate HVTN 107 PSRT notification
SAE, related	Grade 3	Email and submit forms immediately	Prompt HVTN 107 PSRT AE review to consider pause
AE[b], related	Grade 4 or Grade 3	Email and submit forms immediately	Prompt HVTN 107 PSRT AE review to consider pause

[a] Phone numbers and email addresses are found in the Key Contacts under Safety Reporting and Clinical Monitoring on the HVTN 107 home page.

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

For all safety pauses, HVTN Core notifies the HVTN 107 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 107 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 107 PSRT assessment, the trial sponsor or designee(s) notifies pertinent national regulatory authorities as needed.

If an immediate HVTN 107 PSRT notification or prompt HVTN 107 PSRT AE review is triggered, HVTN Core notifies the HVTN 107 PSRT as soon as possible during working hours (US Pacific Time)—or, if the information was received during off hours, by the morning of the next work day. If a prompt HVTN 107 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

The HVTN requires that each CRS submit to its IRB/EC protocol-related safety information (such as safety reports, notification of vaccine holds due to the pause rules, and notification of other unplanned safety pauses). CRSs must also follow all applicable RE reporting requirements.

In addition, all other AEs are reviewed routinely by the HVTN 107 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 107 PSRT AE review criteria.

11.4.2 Weekly review

During the injection phase of the trial, the HVTN 107 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 107 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 107 PSRT, the MCC or other pertinent national regulatory authority, NIH, Office for Human Research Protections (OHRP), or vaccine developer(s). In addition, the conduct of this study at an

individual HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

12 Protocol conduct

This protocol and all actions and activities connected with it will be conducted in compliance with the principles of GCP (ICHe6), and according to DAIDS and HVTN policies and procedures as specified in the *HVTN Manual of Operations,* DAIDS Clinical Research Policies and Standard Procedures Documents including procedures for the following:

- Protocol registration, activation, and implementation;
- Informed consent, screening, and enrollment;
- Study participant reimbursement;
- Clinical and safety assessments;
- Safety monitoring and reporting;
- Data collection, documentation, transfer, and storage;
- Participant confidentiality;
- Study follow-up and close-out;
- Unblinding of staff and participants;
- Quality control;
- Protocol monitoring and compliance;
- Advocacy and assistance to participants regarding negative social impacts associated with the vaccine trial;
- Risk reduction counseling;
- Specimen collection, processing, and analysis;
- Ancillary studies, and
- Destruction of specimens.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 107 *Study Specific Procedures.*

12.1 Social impacts

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

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

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

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

Because this study is evaluating products containing recombinant DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.* Information about the study must be submitted to site Institutional Biosafety Committees (IBC) and must be approved before participants are enrolled at the site. Investigators at each site are responsible for obtaining IBC approval per NIH guidelines *section IV-B07-a-(1).* IBC review and approval must be documented by the investigator and submitted as part of initial protocol registration for this trial. If this protocol is amended, investigators should follow the requirements of their IBC.

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

The Republic of South Africa has laws regarding the use, manufacture, importation, and experimentation of products which are genetically modified. These are contained in the Genetically Modified Organism (GMO) Act 15 of 1997, administered by the South African National Department of Agriculture, Pretoria. The Registrar of GMO shall be consulted on all formal developments relating to this protocol and clinical trial, and as required, a formal application will be made to the Registrar of GMO to review the HVTN 107 clinical trial, to obtain approval for the proposed clinical trial and for the importation of the study products.

Any regulations specific to other countries containing CRSs at which HVTN 107 will be implemented will also be observed.

12.4 Emergency communication with study participants

As in all clinical research, this study may generate a need to reach participants quickly to avoid imminent harm, or to report study findings that may otherwise concern their health or welfare.

When such communication is needed, the CRS will request that its IRB/EC and any applicable RE expedite review of the message. If this review cannot be completed in a timeframe consistent with the urgency of the required communication, the site should contact the participant first, and then notify the IRB/EC and any applicable RE of the matter as soon as possible.

13 Version history

The Protocol Team may modify the original version of the protocol. Modifications are made to HVTN protocols via clarification memos, letters of amendment, or full protocol amendments.

The version history of, and modifications to, Protocol HVTN 107 are described below.

Protocol history and modifications

Date: May 4, 2017

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

Item 1 Added: Optional stool sampling

Item 2 Updated in Section 3, *Overview* and Section 8.5, *Acquisition of study products*: Bivalent Subtype C gp120 and MF59 supplier

Item 3 Updated in Section 3.1, *Protocol Team*

Item 4 Reference updated in Section 4.1.3, *Rationale for the proposed study*

Item 5 Revised in Sections 4.8.1: ALVAC-HIV (vCP2438) dose description

Item 6 Added in Section 4.10, *Preclinical safety studies* and Section 4.10.3, *Toxicity studies of HIV Env vaccines*: Study AB20670

Item 7 Added as new Section 4.11.1, *HVTN 100*: Summary of interim HVTN 100 safety and immunogenicity results

Item 8 Updated in Sections 4.11.2 through 4.11.5: Clinical experience

Item 9 Updated in Section 4.12, *Potential risks of study products and administration*: Table 4-15

Item 10 Revised in Section 7.3.3, *Discontinuing vaccination for a participant*: Criteria for discontinuation

Item 11 Added in Sections 8.3.2 through 8.3.4: Study product storage post-thawing

Item 12 Clarified in Section 8.5, *Acquisition of study products*: Provision of empty sterilized vials and site acquisition of Aluminum Hydroxide Suspension and Sterile Water for Injection

Item 13 Clarified in Section 9.5: Mucosal sampling

Item 14 Clarified in Section 9.13, *Pregnancy*: Pregnancy reporting

Item 15 Updated in Sections 9.7.1, 10.1, and 14: Site lab instructions source

Item 16 Clarified in Section 9.10.2, *Assessment of injection site*: Erythema and induration

Item 17 Corrected in Sections 10.1 through 10.3: Cross-references to Laboratory procedure appendices

Item 18 Updated in Section 10.10, *Other use of stored specimens*: “Other use” definition

Item 19 Updated in Section 9.10, *Assessments of reactogenicity*, 11.2.2, *AE reporting* and Section 14, *Document references (other than literature citations)*: AE grading table version

Item 20 Updated in Section 11.2.2, *AE reporting*: Safety reporting and contact information

Item 21 Updated in Section 11.2.3, *Expedited reporting of AEs to DAIDS*: DAIDS template text

Item 22 Added in Section 11.5, *Study termination*: MCC

Item 23 Updated in Section 14, Document references (other than literature citations): Document URLs

Item 24 Updated: Section 15, *Acronyms and abbreviations*

Item 25 Revised in Appendix A, *Sample informed consent form*: Maximum blood draw volume

Item 26 Updated in Appendix A, *Sample informed consent form*: HVTN protocol template text

Item 27 Updated in Appendix C: Language concerning approved birth control methods

Item 28 Updated in Appendices A and E: Consent language regarding "other uses" of stored samples

Item 29 Updated in Appendix A, Item 26: Study-related injury language

Item 30 Added to "Questions" section in Appendices A and E: Contact titles, IRB/EC name, document references, and MCC contact information

Item 31 Adjusted in Appendices F through I: Blood draw volumes for laboratory assays

Item 32 Revised in Appendices F through I: Endpoint laboratory designations

Item 33 Clarified in footnotes to Appendices J through M: Window for blood and specimen collection prior to vaccination at Visits 2 and 17

Item 34 Added as Appendix O: Protocol signature page

Item 35 Corrected in Clarification Memo 1 to protocol Version 2.0

Item 36 Clarified in Clarification Memo 2 to protocol Version 2.0

Item 37 Minor typographical, formatting, and cross-reference errors have been corrected throughout the protocol document

Date: September 23, 2016

Protocol version: Version 2.0
Protocol modification: Clarification Memo 2

Item 1 Updated in Section 4.2, *ALVAC-HIV (vCP2438)*: Formulation and manufacturing information

Item 2 Updated in Section 4.5, *Aluminum Hydroxide Suspension*: Alum source

Item 3 Updated in Section 8, *Study product preparation and administration*: Formulation information, preparation instructions, and product acquisition

Item 4 Clarified in Section 9.5, *Innate immunity and mucosal sampling*: Pregnancy testing for cervical sampling

Item 5 Clarified in Section 9.9, *Assessments of reactogenicity*: Postvaccination memory tool

Date: September 3, 2015

Protocol version: Version 2.0
Protocol modification: Clarification Memo 1

Item 1 Corrected in Section 9.4, *Follow-up visits*: Outside testing and belief questionnaire removed

Item 2 Corrected in Appendix A, *Sample informed consent form*: Typographical errors

Item 3 Corrected in Appendices F and H: Blood draw for syphilis testing

Item 4 Corrected in Appendices J, K, L, and M: Timepoints for vaccinations (and associated reactogenicity assessments), pregnancy test, CBC, chemistry panel, syphilis testing

Item 5 Corrected in Appendices L and M: Footnoted cross-references to Laboratory Procedures tables

Date: June 23, 2015

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

Item 1 Study design revised: New Group 3 added with concurrent ALVAC-protein administration at Months 0, 1, 6, and 12

Item 2 Updated on cover page, in Sections 3 and 8.5, and in Appendix A: Protein vaccine and MF59 study product provider

Item 3 Updated in Sections 4.3.1, 4.3.2, 4.4, and 4.6: Novartis product descriptions

Item 4 Updated in Section 4.11.4, Clinical studies with Novartis HIV-1 subunit protein vaccines: Safety and immunogenicity information

Item 5 Updated: Table 4-12, Summary of potential risks of study products and administration

Item 6 Added in Section 5.2, Secondary objectives: CD4+ T cell polyfunctionality endpoint

Item 7 Revised: Section 6, Statistical considerations

Item 8 HVTN protocol template updates incorporated into Section 7, Selection and withdrawal of participants

Item 9 HVTN protocol template update incorporated into Section 9.1, Informed consent

Item 10 Section 9.3, Enrollment and vaccination visits: Provision of postvaccination symptom log to study participants made optional

Item 11 HVTN protocol template updates incorporated into Section 11, Safety reporting

Item 12 Document references (other than literature citations) updated

Item 13 Aluminum hydroxide adjuvant information updated

Item 14 Updated: Appendix A, Sample informed consent form

Item 15 Revised: Appendix B, Injection schedule for sample informed consent form

Item 16 Revised: Appendix D, Tables of procedures (for sample informed consent form)

Item 17 Revised for consistency with Appendix A: Appendix E, Sample consent form for use of samples and information in other studies

Item 18 Revised in Appendices F through M: Procedure tables

Item 19 Updated in Section 3.1: Other contributors to the original protocol

Item 20 Updated in Sections 9.9, 11.2.2, and 14: DAIDS AE Grading Table

Item 21 Revised in Section 11.2.2, AE reporting: Exceptions to AE reporting requirements

Item 22 Minor corrections of typographical and formatting errors have been corrected throughout the protocol

Date: August 27, 2014

Protocol version: 1.0
Protocol modification: Original protocol

14 Document references (other than literature citations)

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

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- ABPI. Guidelines for Phase 1 Clinical Trials 2012 Edition. Available at http://www.abpi.org.uk/our-work/library/guidelines/Pages/phase-1-trials-2012.aspx
- Current CDC Guidelines. Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures
- Division of AIDS Protocol Registration Manual. Available at https://www.niaid.nih.gov/sites/default/files/prmanual.pdf
- U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 [March 2017]. Available from: 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
- Reporting Adverse Drug Reactions in South Africa. Available at http://www.mccza.com/documents/ae9635a42.11_ADR_reporting_May03_v1_2.pdf
- Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa. Available at http://www.mccza.com/documents/7c21ba77SA_GCP_guidelines_second_edition_2006.pdf
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- HVTN 107 Special Instructions. Accessible through the HVTN protocol-specific website.

- HVTN 107 Study Specific Procedures. Accessible through the HVTN protocol-specific website.

- HVTN 107 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. Accessible upon request.

- International Conference on Harmonisation (ICH) E6 (R1), Guideline for Good Clinical Practice: Section 4.8, Informed consent of trial subjects. Available at http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html

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

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

- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Available at http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

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

15 Acronyms and abbreviations

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

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

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

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

16 Literature cited

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Appendix A Sample informed consent form

Title: A Phase 1/2a partially double-blinded, randomized clinical trial to characterize the safety and immunogenicity of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120 alone, with MF59® adjuvant, and with alum adjuvant in healthy, HIV uninfected adult participants

HVTN protocol number: HVTN 107

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 study vaccines. HIV is the virus that causes AIDS.

About 132 people will take part in this study at multiple sites. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) and the Bill & Melinda Gates Foundation are paying for the study.

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

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

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

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

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

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

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

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

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

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

4. These study vaccines are experimental.

The study vaccines are called ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120 (gp120 is a protein). From here on, we will call them ALVAC and Protein, or the study vaccines. They are experimental HIV vaccines. That means we do not know whether the study vaccines will be safe to use in people, or whether they will work to prevent HIV infection. These study vaccines are used only in research studies.

The study vaccines were developed by Sanofi Pasteur and Novartis Vaccines and Diagnostics (now GSK Vaccines).

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 vaccine has man-made pieces of a protein found on the outside of HIV. This vaccine will be given in this study either on its own or mixed with one of two adjuvants. An adjuvant is something added to the vaccine to help the immune system respond better. In this study the adjuvants used are called Aluminum hydroxide (alum) or MF59. Alum adjuvants are used in many licensed vaccines that have been given to millions of people. MF59 has been included in flu vaccines licensed in many countries. It has also been included in other vaccines that have been given to over 50,000 people in clinical trials without causing any serious health problems. MF59 is made by Novartis Vaccines and Diagnostics. The alum adjuvant in this study is provided by the US NIH Vaccine Research Center.

These HIV study vaccines have not been given to people before. However, similar ALVAC and Protein vaccines have been given to more than 10,000 people in clinical trials without causing any serious health problems. Most of these people received study vaccines with an alum adjuvant. This includes a study in South Africa, HVTN 097, in which 80 participants received similar ALVAC and protein vaccines with alum adjuvant. Also, more than 300 people have received a similar combination of these vaccines with the MF59 adjuvant in clinical trials without having any serious health problems. The ALVAC and Protein vaccines with MF59 are now being given to 210 participants in another study in South Africa, HVTN 100.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness and feeling tired. Vaccines can also cause pain, redness, swelling, or itching at the injection site. Most people can still do their planned activities after getting a vaccine.

Rarely, people experience side effects that limit their normal activities or make them go to the doctor.

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

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

Risks of the study vaccines:

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

In earlier studies with similar ALVAC and Protein vaccines, some people found it difficult or painful to move their arm for a short while after the injection(s). Some people felt tired or weakened, and some people had swollen lymph nodes. A small number of people had diarrhea, flu-like symptoms, or severe pain or swelling at the place on their arm where they got the injection(s). An even smaller number had trouble sleeping, stomach pain, loss of appetite, an odd taste in their mouths, fainting, acne, eyelid swelling or felt itching at the place on their arm where they got the injection. These problems usually happened within a couple of days of injection(s) and usually went away on their own within a few days. Not everyone had these problems.

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. *South African sites should maintain the following sentence. All other sites should delete:* Only medicines that have been registered in South Africa, or approved for study use, may be used in this study. We will ask you about behaviors that might put you at risk for getting HIV. If you were born female, we will test you for pregnancy. We will also ask if you have ever been allergic to eggs, egg products, or the antibiotic Neomycin.

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

(Sites: 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 unrelated to the study vaccines 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, but we will not pay for it.

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

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

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

Being in the study

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

9. **You will come to the clinic for scheduled visits about 14 times over 18 months.**

   Site: Insert range of visit lengths.

   Visits can last from [#] to [#] hours.

   You may have to come for more visits if you have a lab or health issue.

   If you agree to give extra samples, we may ask you to come in for 3 extra visits (we will describe this more in section 13).

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).

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

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

There are 4 groups in this study. Overall, you will get 8 injections during the study with a needle into your upper arms. The ALVAC vaccine will go into the left arm. The Protein vaccine will go into the right arm.

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

Group	Injection schedule				
	First injection	1 month later	3 months later	6 months later	12 months later
GROUP 1 (about 36 people)	ALVAC	ALVAC	ALVAC + Protein/MF59®	ALVAC + Protein/MF59®	ALVAC + Protein/MF59®
GROUP 2 (about 36 people)	ALVAC	ALVAC	ALVAC + Protein/Alum	ALVAC + Protein/Alum	ALVAC + Protein/Alum
GROUP 3 (about 36 people)	ALVAC + Protein/MF59®	ALVAC + Protein/MF59®		ALVAC + Protein/MF59®	ALVAC + Protein/MF59®
GROUP 4 (about 24 people)	ALVAC	ALVAC	ALVAC + Protein	ALVAC + Protein	ALVAC + Protein

You will have to wait in the clinic for about a half hour after each injection to see if there are any problems. Then for that night and for three more days, you will need to tell the clinic staff how you feel. To help you do this, we can give you tools and show you how to use them. We will ask you the ways we can contact you. If you do not contact us on each of the three days after your injections, we will contact you by the ways you wish to be contacted. If you have a problem, we will continue to check on you until it goes away.

You may have to wait until everyone completes their final study visits to find out which study group you were in. This could be several years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

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

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

- Do regular HIV testing, as well as counseling on your results and on how to avoid getting HIV;
- Perform physical exams;
- Do pregnancy tests if you were born female;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study, and
- Take urine and blood samples.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 225 mL (2 teaspoons to a little less than 1 cup). Your body will make new blood to replace the blood we take out.

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

Site: Insert Appendix 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.

13. We would like to learn more about how your immune system responds to the vaccine by collecting extra samples. We would like to collect blood samples, rectal fluid, cervical fluid, and semen.

Some spots in this study are only open to participants who agree to give these extra samples. Because of this, you may have to agree to give the extra samples in order to join the study. However, if enough people have agreed to give extra samples, we may not need yours, even if you are willing to give them.

Most people are exposed to HIV on their rectum, vagina/cervix, or penis, so it is important to learn if the study vaccines affect those areas. We also want to see if the study vaccines affect your blood the same way as in those areas.

Participants born female who are over 21 years of age need to have had a Pap smear with normal results within the past 3 years in order to give cervical samples. We can tell you where to get a Pap smear if you have not had one within that time.

When we take rectal, cervical, and semen samples, we will also do tests for the following infections: syphilis, gonorrhea, chlamydia, trichomoniasis, and bacterial vaginosis. We may also test you for yeast infection. We will explain what each of these infections is. If

the tests say you have an infection, we will provide counseling and will help you get treatment. This study cannot pay for that treatment. *[Site: Revise preceding sentence if you provide or pay for STI treatment.]*

Blood samples: We will ask you to come in 2 or 3 extra times for blood draws, depending on which group you are in. These extra visits will be 1 day and 3 days after your first vaccination, or 1 day, 3 days, and 7 days after your third vaccination. The amount of blood we would collect at each of these study visits is about 40 to 50 mL (a little less than ¼ cup).

Fluid samples: We will collect fluids from your rectum and from your cervix (if you have one). If you were born male and can provide a sample, we will collect semen at these visits.

We want to collect fluids from these areas 5 times:

- before your first study injection,
- 2 weeks after your second to last study injections,
- at the time of your last study injections,
- 2 weeks after your last study injections, and also
- 6 months after your last study injections.

For all participants who agree to provide extra samples, we will collect rectal fluids by wiping the lining of your rectum with a cotton swab, sponge, or brush, or we may place a special strip of absorbing paper inside the rectum for about 5 minutes. An anoscope, a plastic viewing tube 5-8 centimeters long and 1¼ centimeters wide, may be inserted into the rectum so that the clinician can see better when doing this procedure.

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

- not have receptive anal intercourse (have someone else's penis inside your rectum);
- not put anything into your anus, including cleaning products (creams, gels, lotions, pads, etc.), lubricant, or enemas;
- not douche (even with water); and
- not use any anti-inflammatory creams in or around your anus.

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

For participants who were born female, to collect cervical fluids we will give you a menstrual cup (a small flexible plastic cup, shaped like a bell) to insert into your vagina. The study staff will explain how to insert and remove the cup, or they can do it for you here. We will explain how many cups we will collect and how long you should wear them.

For the 2 days before we collect cervical samples, we ask you:

- not to use any spermicide, lubricants, douche (even with water), or medication in or around your vagina; and

- not to 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.

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

At the end of this consent form, we will ask you if you allow us to collect these samples. If you agree to provide these samples, you can change your mind at any time during the study.

14. We would also like to collect stool samples.

We would like to collect two small samples 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 once at the day of enrollment (month 0) and again at your 6.5 month visit. If you agree, we will give you more information about how we will collect these samples.

You can give stool samples without having any extra clinic visits. You can give stool samples and not give rectal fluid, cervical fluid, or semen.

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

15. We will test your samples for this study.

We will send your samples (without your name) to labs approved by the HVTN to test how your immune system responds to the study products.

Most of these labs will be in South Africa, but some will be in the United States. In rare cases, some of your samples may be sent to labs approved by the HVTN in other countries for research related to this study.

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

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

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

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

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 keep them for use in other studies by HVTN or other researchers. We will call these "extra samples."

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

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* Your samples will be stored in the HVTN repository in South Africa.

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

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

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

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

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

What information is shared with other researchers? The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, 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.

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

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

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

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

People who may see your information are:

- Researchers who use your stored samples and limited information for other research

- Government agencies that fund or monitor the research using your samples or information

- The researcher's Institutional Review Board or Ethics Committee

- The people who work with the researcher

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

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

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 boxed text requires approval from HVTN Regulatory Affairs.

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

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

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

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

- The US National Institutes of Health and its study monitors,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- Sanofi Pasteur and GSK Vaccines and people who work for them,

- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board; and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

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

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

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

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

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

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

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

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

This may happen if:

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

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

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

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

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

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

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

Other Risks

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

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

Risks of routine medical procedures:

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

Personal problems/discrimination/testing HIV antibody positive:

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

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

If you receive a positive test result caused by the study vaccines at any time, we can provide you with free HIV testing for as long as you need it. If this happens, we do not know how long you will test positive due to the study vaccines. If you receive a positive

HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

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

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

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

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

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

Unknown risks:

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

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

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

Benefits

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

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 here. For the care that we cannot provide, we will explain how we will help you get care elsewhere.

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

(Sites: Adjust the language in this paragraph (or remove altogether) so it is applicable to your site.) In this study, our clinic has insurance to cover your medical treatment in case of a study-related injury, following the Association of the British Pharmaceutical Industry guidelines for payment of study-related injury. We can give you a copy of these guidelines. In rare cases, insurance funds may not be enough. In this situation, the vaccine developers have agreed to pay the reasonable cost of medical expenses that arise from injuries caused by the study products.

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

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

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

Questions

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

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

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

This study has been reviewed and approved by a committee called the
[name of local IRB/EC]. If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, contact
[name or title and telephone number of person on IRB or other appropriate organization].

The study has been structured in accordance with the Declaration of Helsinki (last updated October 2013) which deals with the recommendations guiding doctors in biomedical research involving human participants, the Ethics in Health Research: Principles, Structures and Processes Second Edition 2015, and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa. We can provide you with copies of these guidelines if you wish to review them.

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

You can reach a study staff member 24-hours a day at [telephone number].

South African sites: Include the following:

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

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

Fax: (012) 395 9201

e-mail: gouwsj@health.gov.za

Your permissions and signature

28. In section 13 of this form, we told you about optional additional sampling. This includes blood draws at extra visits and also collection of rectal and cervical or semen samples. Please write your initials or make your mark in the boxes next to the options you choose.

☐ I agree to blood draws at extra clinic visits and to provide rectal and cervical or semen samples.

OR

☐ I do not agree to this extra sampling.

29. In section 14 of this form, we told you about collecting stool samples. Please write your initials or make your mark in the box next to the option you choose.

☐ I agree to provide stool samples.

☐ I do not agree to provide stool samples.

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

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

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

OR

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

OR

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

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

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

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

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

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

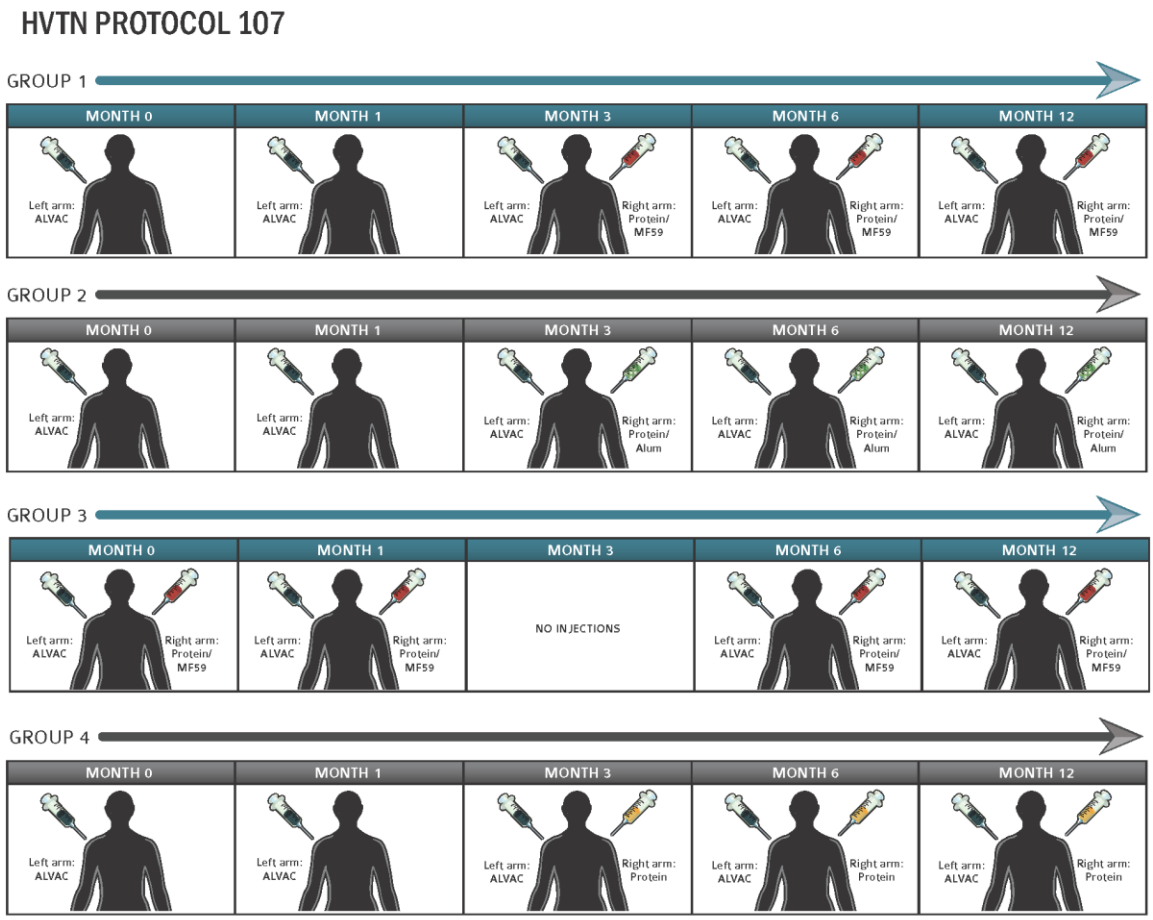
For participants who are unable to read or write, a witness should complete the signature block below:

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

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

Appendix B Injection schedule for sample informed consent form

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



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

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

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

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

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

PLUS 1 of the following methods:

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

You do not have to use birth control if:

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

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

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

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

Main study (Groups 1, 2, and 4)

		Time after 1st injection visit (in months)														
Procedure	Screening visit(s)	First injection visit	¼	1	1½	3	3½	6	6¼	6½	9	12	12¼	12½	15	18
Injection		√		√		√		√				√				
Medical history	√															
Complete physical	√															√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Urine test	√		√											√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female)*	√	√		√		√		√				√			√	
HIV testing & pretest counseling	√					√		√			√	√			√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Stool sample collection (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.

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

Main study (Group 3)

		Time after 1st injection visit (in months)													
Procedure	Screening visit(s)	First injection visit	¼	1	1½	3	6	6¼	6½	9	12	12¼	12½	15	18
Injection		√		√			√				√				
Medical history	√														
Complete physical	√														√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	
Urine test	√		√										√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female)*	√	√		√			√				√			√	
HIV testing & pretest counseling	√					√	√			√	√			√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Stool sample collection (optional)		√							√						

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

Extra visits and sampling (Groups 1, 2, and 4)

Procedure	Screening visit(s)	Time after 1st injection visit (in months)																	
		First injection visit	¼	1	1½	3	3 + 1 day	3 + 3 days	3¼	3½	6	6¼	6½	9	12	12¼	12½	15	18
Injection		√		√		√					√				√				
Medical history	√																		
Complete physical	√																		√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Urine test	√		√														√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female)*	√	√		√		√					√		√		√		√	√	√
HIV testing & pretest counseling	√					√					√			√	√				
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pap smear†	√																		
Rectal/cervical fluids/semen samples		√											√		√		√		√
Stool sample collection (optional)		√											√						
GTI testing (urine and/or swab or blood)		√											√		√		√		√

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

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

† For participants who were born female and who agree to provide cervical fluid samples.

Extra visits and sampling (Group 3)

Procedure	Screening visit(s)	Time after 1st injection visit (in months)															
		First injection visit	+ 1 day	+ 3 days	¼	1	1½	3	6	6¼	6½	9	12	12¼	12½	15	18
Injection		√				√			√				√				
Medical history	√																
Complete physical	√																√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Urine test	√				√										√		
Blood drawn	√	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female)*	√	√				√			√		√		√		√	√	√
HIV testing & pretest counseling	√							√	√			√	√				
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pap smear†	√																
Rectal/cervical fluids/semen samples		√									√		√		√		√
Stool sample collection (optional)		√									√						
GTI testing (urine and/or swab or blood)		√									√		√		√		√

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

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

† For participants who were born female and who agree to provide cervical fluid samples.

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

Title: A Phase 1/2a partially double-blinded, randomized clinical trial to characterize the safety and immunogenicity of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120 alone, with MF59® adjuvant, and with alum adjuvant in healthy, HIV uninfected adult participants

HVTN protocol number: HVTN 107

Site: [Insert site name]

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

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

1. Do I have to agree?

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

2. Where are the samples stored?

Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* Your samples will be stored in the HVTN repository in South Africa.

3. How long will the samples be stored?

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

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

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

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

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

6. Will the HVTN sell my samples and information?

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

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

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

8. What information is shared with other researchers?

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

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

The studies will be related to HIV, vaccines, the immune system and other diseases. Researchers may also do genetic testing on your samples.

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

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 his study.

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

10. What are the risks of genetic testing?

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

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

People who may see your information are:

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

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

Questions

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

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

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

This study has been reviewed and approved by a committee called the
[name of local IRB/EC]. If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, contact
[name or title and telephone number of person on IRB or other appropriate organization].

The study has been structured in accordance with the Declaration of Helsinki (last updated October 2013) which deals with the recommendations guiding doctors in biomedical research involving human participants, the Ethics in Health Research: Principles, Structures and Processes Second Edition 2015, and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa. We can provide you with copies of these guidelines if you wish to review them.

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

You can reach a study staff member 24-hours a day at [telephone number].

South African sites: Include the following:

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

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

Fax: (012) 395 9201

e-mail: mogobm@health.gov.za

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

☐ I allow my extra samples combined with limited 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 combined with limited information to be used in genome wide studies.

OR

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

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

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

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

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

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

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Appendix F Laboratory procedures (Groups 1, 2, and 4)

					Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
					Day	Screening visit[3]	D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
					Weeks		W0			W1	W4	W6	W12			W13	W14	W24	W25	W26	W39	W52	W53	W54	W60	W72	
					Month		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
	Ship to[1]	Assay location[2]	Tube Type[4]	Tube size (vol. capacity)[4]			ALVAC				ALVAC		ALVAC+prot+MF59 OR Alum OR No Adj					ALVAC+prot+MF59 OR Alum OR No Adj				ALVAC+prot+MF59 OR Alum OR No Adj					Total
BLOOD COLLECTION																										
Screening/Diagnostic																										
Screening HIV test	Local lab	Local lab	EDTA	2mL		4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**4**
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**5**
HIV diagnostics[9]	HSML-NICD	HSML-NICD	EDTA	10mL		—	—	—	—	—	—	—	10	—	—	—	—	10	—	—	10	10	—	—	10	20[9]	**70**
STI Serology																										
Syphilis	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**5**
Safety labs																										
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL		5	—	—	—	5	—	5	—	—	—	—	5	—	—	5	—	—	—	5	5	—	**35**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—	—	—	5	—	5	—	—	—	—	5	—	—	5	—	—	—	5	5	—	**35**
Immunogenicity & Virologic assays[6]																										
HLA host genetics[7]	BARC	HVTN Labs	ACD	8.5mL		—	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**17**
Cellular assays																										
ICS	BARC	HVTN Labs	ACD	8.5mL		—	85	—	—	—	—	85	—	—	—	—	85	—	—	***85***	—	85	—	85	—	85	**595**
Phenotyping (pTfh and plasmablasts)	BARC	HVTN Labs	ACD	8.5mL		—	17	—	—	17	—	—	—	—	—	—	—	—	17	—	—	17	17	—	—	—	**85**
Humoral assays																										
Binding Ab Assay	BARC	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	8.5	—	—	—	—	8.5	—	—	***8.5***	8.5	***8.5***	—	8.5	—	8.5	**68**
Neutralizing Ab Assay	BARC	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	8.5	—	—	—	—	8.5	—	—	***8.5***	—	8.5	—	8.5	—	8.5	**60**
Storage																										
PBMC	BARC		ACD	8.5mL		—	42.5	—	—	34	—	59.5	—	—	—	—	59.5	—	59.5	59.5	—	59.5	51	59.5	—	59.5	**544**
Serum	BARC		SST	8.5mL		—	17	—	—	—	—	17	—	—	—	—	17	—	—	17	17	17	—	17	—	17	**136**
Visit total						**24**	**196**	**0**	**0**	**61**	**0**	**189**	**10**	**0**	**0**	**0**	**189**	**10**	**77**	**189**	**36**	**206**	**68**	**189**	**20**	**199**	**1659**
56-Day total						**24**	**220**	**220**	**220**	**281**	**281**	**469**	**199**	**199**	**199**	**199**	**387**	**10**	**87**	**275**	**36**	**206**	**274**	**462**	**20**	**199**	
URINE COLLECTION																										
Urinalysis	Local lab	Local lab				X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	
Pregnancy Test[8]	Local lab	Local lab				X	X	—	—	—	X	—	X	—	—	—	—	X	—	—	—	X	—	—	X	—	
STOOL COLLECTION (OPTIONAL)																										
Stool	BARC	HVTN Labs				—	X[10]	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—	—	—	—	

Shaded visits not required.

[1] BARC = Bio Analytical Research Corporation South Africa (Pty) Ltd. (Johannesburg, South Africa); HSML-NICD = HIV Sero-Molecular Laboratory–National Institute for Communicable Diseases (Johannesburg, South Africa)

[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 Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA)

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (postenrollment).

[6] Immunogenicity assays will be performed at M0 (for binding Ab assay) and M6.5 and 12. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other timepoints.

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

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

[9] At an early termination visit for a withdrawn or terminated participant (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 21 above.

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

Appendix G Laboratory procedures (Groups 1, 2, and 4 – innate and mucosal subset)

					Visit:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
					Day:	Screening visit[3]	D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
					Weeks		W0			W1	W4	W6	W12			W13	W14	W24	W25	W26	W39	W52	W53	W54	W60	W72	
					Month		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
	Ship to[1]	Assay location[2]	Tube Type[4]	Tube size (vol. capacity)[4]			ALVAC				ALVAC		ALVAC+prot+MF59 OR Alum OR No Adj					ALVAC+prot+MF59 OR Alum OR No Adj				ALVAC+prot+MF59 OR Alum OR No Adj					Total
BLOOD COLLECTION																										
Screening/Diagnostic																										
Screening HIV test	Local lab	Local lab	EDTA	2mL	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
HIV diagnostics[9]	HSML-NICD	HSML-NICD	EDTA	10mL	—	—	—	—	—	—	—	—	10	—	—	—	—	10	—	—	10	10	—	—	10	20[9]	70
STI Serology																										
Syphilis	Local lab	Local lab	SST	5mL	5	5	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	5	—	5	—	5	30
Safety labs																										
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	—	—	5	—	5	5	5	5	5	5	5	—	—	5	—	—	—	5	5	—	55
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	—	5	—	5	—	—	—	—	—	5	—	—	5	—	—	—	5	5	—	35
Immunogenicity & Virologic assays[6]																										
HLA host genetics[7]	BARC	HVTN Labs	ACD	8.5mL	—	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17
Cellular assays																										
ICS	BARC	HVTN Labs	ACD	8.5mL	—	42.5	—	—	—	—	42.5	—	—	—	—	—	42.5	—	—	***85***	—	42.5	—	42.5	—	42.5	340
Phenotyping (pTfh and plasmablasts)	BARC	HVTN Labs	ACD	8.5mL	—	17	—	—	17	—	—	—	17	—	—	17	—	—	17	—	—	17	17	—	—	—	119
Humoral assays																										
Binding Ab Assay	BARC	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	8.5	—	—	—	—	—	8.5	—	—	***8.5***	8.5	8.5	—	8.5	—	8.5	68
Neutralizing Ab Assay	BARC	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	8.5	—	—	—	—	—	8.5	—	—	***8.5***	—	8.5	—	8.5	—	8.5	60
Innate Immunity																										
Serum cytokines	BARC	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	—	—	8.5	8.5	8.5	8.5	—	—	—	—	—	—	—	—	—	—	43
RNA Gene Expression	BARC	HVTN Labs	Tempus	3mL	—	3	—	—	—	—	—	—	3	3	3	3	—	—	—	—	—	—	—	—	—	—	15
Phenotyping	BARC	HVTN Labs	ACD	8.5mL	—	8.5	—	—	—	—	—	—	8.5	17	17	8.5	—	—	—	—	—	—	—	—	—	—	60
Storage																										
PBMC	BARC		ACD	8.5mL	—	68	—	—	59.5	—	51	—	—	8.5	8.5	8.5	59.5	—	59.5	59.5	—	59.5	59.5	59.5	—	85	646
Serum	BARC		SST	8.5mL	—	17	—	—	—	—	17	—	—	—	—	—	17	—	—	17	17	17	—	17	—	17	136
Visit total					**24**	**204**	**0**	**0**	**87**	**0**	**138**	**52**	**42**	**42**	**51**	**146**	**10**	**77**	**194**	**36**	**168**	**77**	**151**	**20**	**187**	**1702**	
56-Day total					**24**	**228**	**228**	**228**	**314**	**314**	**452**	**190**	**232**	**274**	**324**	**470**	**10**	**87**	**280**	**36**	**168**	**245**	**396**	**20**	**187**		
URINE COLLECTION																										
Urinalysis	Local lab	Local lab			X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	
Pregnancy Test[8]	Local lab	Local lab			X	X	—	—	—	X	—	—	X	—	—	—	—	X	—	X[11]	—	X	—	X[11]	X	X[11]	
Chlamydia/Gonorrhea	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
CERVICAL/VAGINAL SWAB COLLECTION																										
Trichomonas vaginalis	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Bacterial vaginosis	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Yeast[10]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
MUCOSAL COLLECTION																										
Semen	BARC	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Cervical secretion	BARC	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Rectal secretion	BARC	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
STOOL COLLECTION (OPTIONAL)																										
Stool	BARC	HVTN Labs			—	X[12]	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—	—	—	—	

Shaded visits not required.

[1] BARC = Bio Analytical Research Corporation South Africa (Pty) Ltd (Johannesburg, South Africa); HSML-NICD = HIV Sero-Molecular Laboratory–National Institute for Communicable Diseases (Johannesburg, South Africa)

[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 Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA)

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (postenrollment).

[6] Immunogenicity assays will be performed at M0 (for binding Ab assay) and M6.5 and 12. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other time points

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

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

[9] At an early termination visit for a withdrawn or terminated participant (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 21 above.

[10] Cervical/vaginal swab collection for participants providing cervical mucosal samples–only if clinically indicated.

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

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

Appendix H Laboratory procedures (Group 3)

					Visit:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
					Day:	Screening visit[3]	D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
					Weeks		W0			W1	W4	W6	W12			W13	W14	W24	W25	W26	W39	W52	W53	W54	W60	W72	
					Month		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
	Ship to[1]	**Assay location[2]**	**Tube Type[4]**	**Tube size (vol. capacity)[4]**			**ALVAC+prot+MF59**				**ALVAC+prot+MF59**							**ALVAC+prot+MF59**				**ALVAC+prot+MF59**					**Total**
BLOOD COLLECTION																											
Screening/Diagnostic																											
Screening HIV test	Local lab	Local lab	EDTA	2mL		4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**4**
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**5**
HIV diagnostics[9]	HSML-NICD	HSML-NICD	EDTA	10mL		—	—	—	—	—	—	—	10	—	—	—	—	10	—	—	10	10	—	—	10	20[9]	**70**
STI Serology																											
Syphilis	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**5**
Safety labs																											
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL		5	—	—	—	5	—	5	—	—	—	—	—	—	—	5	—	—	—	5	5	—	**30**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—	—	—	5	—	5	—	—	—	—	—	—	—	5	—	—	—	5	5	—	**30**
Immunogenicity & Virologic assays[6]																											
HLA host genetics[7]	BARC	HVTN Labs	ACD	8.5mL		—	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**17**
Cellular assays																											
ICS	BARC	HVTN Labs	ACD	8.5mL		—	85	—	—	—	—	85	—	—	—	—	—	—	—	***85***	—	85	—	85	—	85	**510**
Phenotyping (pTfh and plasmablasts)	BARC	HVTN Labs	ACD	8.5mL		—	17	—	—	17	—	—	—	—	—	—	—	—	17	—	—	17	17	—	—	—	**85**
Humoral assays																											
Binding Ab Assay	BARC	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	8.5	—	—	—	—	—	—	—	***8.5***	8.5	***8.5***	—	8.5	—	8.5	**60**
Neutralizing Ab Assay	BARC	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	8.5	—	—	—	—	—	—	—	***8.5***	—	8.5	—	8.5	—	8.5	**51**
Storage																											
PBMC	BARC		ACD	8.5mL		—	42.5	—	—	34	—	59.5	—	—	—	—	—	—	59.5	59.5	—	59.5	51	59.5	—	59.5	**485**
Serum	BARC		SST	8.5mL		—	17	—	—	—	—	17	—	—	—	—	—	—	—	17	17	17	—	17	—	17	**119**
Visit total						**24**	**196**	**0**	**0**	**61**	**0**	**189**	**10**	**0**	**0**	**0**	**0**	**10**	**77**	**189**	**36**	**206**	**68**	**189**	**20**	**199**	**1470**
56-Day total						**24**	**220**	**220**	**220**	**281**	**281**	**469**	**199**	**199**	**199**	**199**	**199**	**10**	**87**	**275**	**36**	**206**	**274**	**462**	**20**	**199**	
URINE COLLECTION																											
Urinalysis	Local lab	Local lab				X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	
Pregnancy Test[8]	Local lab	Local lab				X	X	—	—	—	X	—	—	—	—	—	—	X	—	—	—	X	—	—	X	—	
STOOL COLLECTION (OPTIONAL)																											
Stool	BARC	HVTN Labs				—	X[10]	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—	—	—	—	

Shaded visits not required.

[1] BARC = Bio Analytical Research Corporation South Africa (Pty) Ltd (Johannesburg, South Africa); HSML-NICD = HIV Sero-Molecular Laboratory–National Institute for Communicable Diseases (Johannesburg, South Africa)

[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 Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA)

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (postenrollment).

[6] Immunogenicity assays will be performed at M0 (for binding Ab assay) and M6.5 and 12. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other timepoints.

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

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

[9] At an early termination visit for a withdrawn or terminated participant (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 21 above.

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

Appendix I Laboratory procedures (Group 3 – innate and mucosal subset)

					Visit:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
					Day:	Screening visit[3]	D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
					Weeks		W0			W1	W4	W6	W12			W13	W14	W24	W25	W26	W39	W52	W53	W54	W60	W72	
					Month		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
	Ship to[1]	Assay location[2]	Tube Type[4]	Tube size (vol. capacity)[4]			ALVAC+prot+MF59				ALVAC+prot+MF59							ALVAC+prot+MF59				ALVAC+prot+MF59						Total
BLOOD COLLECTION																											
Screening/Diagnostic																											
Screening HIV test	Local lab	Local lab	EDTA	2mL		4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**4**
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**5**
HIV diagnostics[9]	HSML-NICD	HSML-NICD	EDTA	10mL		—	—	—	—	—	—	—	10	—	—	—	—	10	—	—	10	10	—	—	10	20[9]	**70**
STI Serology																											
Syphilis	Local lab	Local lab	SST	5mL		5	5	—	—	—	—	—	—	—	—	—	—	—	—	5	—	5	—	5	—	5	**30**
Safety labs																											
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL		5	5	5	5	5	—	5	—	—	—	—	—	—	—	5	—	—	—	5	5	—	**45**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—	—	—	5	—	5	—	—	—	—	—	—	—	5	—	—	—	5	5	—	**30**
Immunogenicity & Virologic assays[6]																											
HLA host genetics[7]	BARC	HVTN Labs	ACD	8.5mL		—	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**17**
Cellular assays																											
ICS	BARC	HVTN Labs	ACD	8.5mL		—	42.5	—	—	—	—	42.5	—	—	—	—	—	—	—	***85***	—	42.5	—	42.5	—	42.5	**298**
Phenotyping (pTfh and plasmablasts)	BARC	HVTN Labs	ACD	8.5mL		—	17	—	—	17	—	—	—	—	—	—	—	—	17	—	—	17	17	—	—	—	**85**
Humoral assays																											
Binding Ab Assay	BARC	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	8.5	—	—	—	—	—	—	—	***8.5***	8.5	8.5	—	8.5	—	8.5	**60**
Neutralizing Ab Assay	BARC	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	8.5	—	—	—	—	—	—	—	***8.5***	—	8.5	—	8.5	—	8.5	**51**
Innate Immunity																											
Serum cytokines	BARC	HVTN Labs	SST	8.5mL		—	8.5	8.5	8.5	8.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**34**
RNA Gene Expression	BARC	HVTN Labs	Tempus	3mL		—	3	3	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**12**
Phenotyping	BARC	HVTN Labs	ACD	8.5mL		—	8.5	17	17	8.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**51**
Storage																											
PBMC	BARC		ACD	8.5mL		—	34	8.5	8.5	17	—	42.5	—	—	—	—	—	—	59.5	59.5	—	59.5	59.5	59.5	—	85	**493**
Serum	BARC		SST	8.5mL		—	8.5	—	—	—	—	17	—	—	—	—	—	—	—	17	17	17	—	17	—	17	**111**
Visit total						**24**	**166**	**42**	**42**	**64**	**0**	**129**	**10**	**0**	**0**	**0**	**0**	**10**	**77**	**194**	**36**	**168**	**77**	**151**	**20**	**187**	**1395**
56-Day total						**24**	**190**	**232**	**274**	**338**	**338**	**467**	**139**	**139**	**139**	**139**	**139**	**10**	**87**	**280**	**36**	**168**	**245**	**396**	**20**	**187**	
URINE COLLECTION																											
Urinalysis	Local lab	Local lab				X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	
Pregnancy Test[8]	Local lab	Local lab				X	X	—	—	—	X	—	—	—	—	—	—	X	—	X[11]	—	X	—	X[11]	X	X[11]	
Chlamydia/Gonorrhea	Local lab	Local lab				—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
CERVICAL/VAGINAL SWAB COLLECTION																											
Trichomonas vaginalis	Local lab	Local lab				—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Bacterial vaginosis	Local lab	Local lab				—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Yeast[10]	Local lab	Local lab				—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
MUCOSAL COLLECTION																											
Semen	BARC	HVTN Labs				—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Cervical secretion	BARC	HVTN Labs				—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
Rectal secretion	BARC	HVTN Labs				—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	
STOOL COLLECTION (OPTIONAL)																											
Stool	BARC	HVTN Labs				—	X[12]	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—	—	—	—	

Shaded visits not required.

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[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 Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA)

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (postenrollment).

[6] Immunogenicity assays will be performed at M0 (for binding Ab assay) and M6.5 and 12. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other timepoints.

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

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

[9] At an early termination visit for a withdrawn or terminated participant (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 21 above.

[10] Cervical/vaginal swab collection for participants providing cervical mucosal samples–only if clinically indicated.

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

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

Appendix J Procedures at HVTN CRS (Groups 1, 2, and 4)

Visit:	01[a]	02[g]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17[g]	18	19	20	21	Post
Day:		D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
Month:		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
Procedure	Scr.	VAC1				VAC2		VAC3					VAC4				VAC5					
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	X	X	X	X	—	—
Risk reduction counseling	X	X	—	—	X	X	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	X	X	X	X	—
Behavioral risk assessment	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	—	—	X	X	X	X	—	—	—	X	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—	—	X	—	—	—	X	—
Concomitant medications	X	X	—	—	X	X	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	X	X	X	X	—
HIV infection assessment[e]	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	—	—	—	—	X	—	X	—	—	X	X	—	—	X	X
Stool sample collection (optional)[i]	—	(X)[j]	—	—	—	—	—	—	—	—	—	—	—	—	(X)	—	—	—	—	—	—	—
Local lab assessment			—	—																		
Urine dipstick	X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	—	—	X	—	X	—	—	—	—	X	—	—	—	X	—	—	X	—	—
CBC, differential, platelet	X	—	—	—	X	—	X	—	—	—	—	X	—	—	X	—	—	—	X	X	—	—
Chemistry panel (see Section 9.2)	X	—	—	—	X	—	X	—	—	—	—	X	—	—	X	—	—	—	X	X	—	—
Syphilis, Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaccination procedures									—	—	—	—										
Vaccination	—	X	—	—	—	X	—	X	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Reactogenicity assessments[h]	—	X	—	—	—	X	—	X	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Poststudy																						
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

Shaded visits not required.

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

[d] AEs to be collected and reported through 30 days after each vaccination, except as noted in Section 11.2.2.

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

[f] For a participant who was born female, pregnancy test must be performed within 24 hours of vaccination with negative results received prior to vaccination. Pregnancy test to determine initial eligibility may be performed at screening but must also be done on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[g] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration, except for results of a urine or serum pregnancy test, if indicated (see footnote "f"). Lab tests may be drawn within the 14 days prior to vaccination.

[h] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.10).

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

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

Appendix K Procedures at HVTN CRS (Groups 1, 2, and 4 – innate and mucosal subset)

Visit:	01[a]	02[k]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17[k]	18	19	20	21	Post
Day:		D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
Month:		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
Procedure	Scr.	VAC1				VAC2		VAC3					VAC4				VAC5					
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	X	X	X	X	X	X	X	—	—
Risk reduction counseling	X	X	—	—	X	X	X	X	X	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	X	X	X	X	X	X	X	—
Behavioral risk assessment	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	—	—	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—	—	X	—	—	—	X	—
Concomitant medications	X	X	—	—	X	X	X	X	X	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	X	X	X	X	X	X	X	—
HIV infection assessment[e]	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	—	—	—	X	—	—	X	—	—	X	X	—	—	X	X
Local lab assessment																						
Urine dipstick	X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	—	—	X	—	X	—	—	—	—	X	—	X	—	X	—	X	X	X	—
CBC, differential, platelet	X	—	—	—	X	—	X	X	X	X	X	X	—	—	X	—	—	—	X	X	—	—
Chemistry panel (see Section 9.2	X	—	—	—	X	—	X	—	—	—	—	X	—	—	X	—	—	—	X	X	—	—
Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Syphilis	X	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Pap smear[g]	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chlamydia/gonorrhea (urine)[h]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Trichomonas vaginalis[i]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Bacterial vaginosis[i]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Yeast[j]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—

Visit:	01[a]	02[k]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17[k]	18	19	20	21	Post
Day:		D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
Month:		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
Procedure	Scr.	VAC1				VAC2		VAC3					VAC4				VAC5					
Mucosal sample collection (optional)																						
Rectal secretions, cervical secretions, semen	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Stool sample collection (optional)[m]	—	(X)[n]	—	—	—	—	—	—	—	—	—	—	—	—	(X)	—	—	—	—	—	—	—
Vaccination procedures																						
Vaccination	—	X	—	—	—	X	—	X	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Reactogenicity assessments[l]	—	X	—	—	—	X	—	X	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Poststudy																						
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

Shaded visits not required.

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

[b] For specimen collection requirements, see Appendix G

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

[d] AEs to be collected and reported through 30 days after each vaccination, except as noted in Section 11.2.2..

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

[f] For a participant who was born female, pregnancy test must be performed within 24 hours of vaccination with negative results received prior to vaccination. Pregnancy test to determine initial eligibility may be performed at screening but must also be done on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[g] Only for volunteers born female providing cervical samples. Per Section 9.2, women with a most recent normal (or ASCUS) Pap result within 3 years prior to enrollment do not need to have a Pap smear.

[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 and other sample collection required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration, except for results of a urine or serum pregnancy test, if indicated (see footnote "f"). Blood draws and other samples may be collected within the 14 days prior to vaccination.

[l] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.10).

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

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

Appendix L Procedures at HVTN CRS (Group 3)

Visit:	01[a]	02[g]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17[g]	18	19	20	21	Post
Day:		D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
Month:		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
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	X	X	X	—	—
Risk reduction counseling	X	X	—	—	X	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	X	X	X	—
Behavioral risk assessment	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	—	—	X	X	X	X	—	—	—	—	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—	—	X	—	—	—	X	—
Concomitant medications	X	X	—	—	X	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	X	X	X	—
HIV infection assessment[e]	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	—	—	—	—	—	X	X	—	—	X	X	—	—	X	X
Stool sample collection (optional)[i]	—	(X)[j]	—	—	—	—	—	—	—	—	—	—	—	—	(X)	—	—	—	—	—	—	—
Local lab assessment			—	—																		
Urine dipstick	X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	—	—	X	—	—	—	—	—	—	X	—	—	—	X	—	—	X	—	—
CBC, differential, platelet	X	—	—	—	X	—	X	—	—	—	—	—	—	—	X	—	—	—	X	X	—	—
Chemistry panel (see Section 9.2)	X	—	—	—	X	—	X	—	—	—	—	—	—	—	X	—	—	—	X	X	—	—
Syphilis, Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaccination procedures									—	—	—	—										
Vaccination[g]	—	X	—	—	—	X	—	—	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Reactogenicity assessments[h]	—	X	—	—	—	X	—	—	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Poststudy																						
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

Shaded visits not required.

[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 H.

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

[d] AEs to be collected and reported through 30 days after each vaccination, except as noted in Section 11.2.2.

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

[f] For a participant who was born female, pregnancy test must be performed within 24 hours of vaccination with negative results received prior to vaccination. Pregnancy test to determine initial eligibility may be performed at screening but must also be done on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[g] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration, except for results of a serum pregnancy test, if indicated (see footnote "f"). Lab tests may be drawn within the 14 days prior to vaccination.

[h] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.10).

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

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

Appendix M Procedures at HVTN CRS (Group 3 – innate and mucosal subset)

Visit:	01[a]	02[k]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17[k]	18	19	20	21	Post
Day:		D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
Month:		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
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	X	X	X	X	X	—	—
Risk reduction counseling	X	X	X	X	X	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	X	X	X	X	X	—
Behavioral risk assessment	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	—	—	—	—	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—	—	X	—	—	—	X	—
Concomitant medications	X	X	X	X	X	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	X	X	X	X	X	—
HIV infection assessment[e]	X	—	—	—	—	—	—	X	—	—	—	—	X	—	—	X	X	—	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	—	—	—	—	—	X	X	—	—	X	X	—	—	X	X
Local lab assessment																						
Urine dipstick	X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	—	—	X	—	—	—	—	—	—	X	—	X	—	X	—	X	X	X	—
CBC, differential, platelet	X	X	X	X	X	—	X	—	—	—	—	—	—	—	X	—	—	—	X	X	—	—
Chemistry panel (see Section 9.2)	X	—	—	—	X	—	X	—	—	—	—	—	—	—	X	—	—	—	X	X	—	—
Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Syphilis	X	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Pap smear[g]	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chlamydia/gonorrhea (urine)[h]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Trichomonas vaginalis[i]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Bacterial vaginosis[i]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Yeast[j]	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—

Visit:	01[a]	02[k]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17[k]	18	19	20	21	Post
Day:		D0	D1	D3	D7	D28	D42	D84	D85	D87	D91	D98	D168	D175	D182	D273	D364	D371	D378	D455	D546	
Month:		M0			M0.25	M1	M1.5	M3			M3.25	M3.5	M6	M6.25	M6.5	M9	M12	M12.25	M12.5	M15	M18	
Procedure	Scr.	VAC1				VAC2							VAC3				VAC4					
Mucosal sample collection (optional)																						
Rectal secretions, cervical secretions, semen	—	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—	X	—	X	—	X	—
Stool sample collection (optional)[m]		(X)[n]													(X)							
Vaccination procedures																						
Vaccination	—	X	—	—	—	X	—	—	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Reactogenicity assessments[l]	—	X	—	—	—	X	—	—	—	—	—	—	X	—	—	—	X	—	—	—	—	—
Poststudy																						
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

Shaded visits not required.

[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 I.

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

[d] AEs to be collected and reported through 30 days after each vaccination, except as noted in Section 11.2.2..

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

[f] For a participant who was born female, pregnancy test must be performed within 24 hours of vaccination with negative results received prior to vaccination. Pregnancy test to determine initial eligibility may be performed at screening but must also be done on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[g] Only for volunteers born female providing cervical samples. Per Section 9.2, women with a most recent normal (or ASCUS) Pap result within 3 years prior to enrollment do not need to have a Pap smear.

[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 and other sample collection required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration, except for results of a ruine or serum pregnancy test, if indicated (see footnote "f"). Blood draws and other samples may be collected within the 14 days prior to vaccination.

[l] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.10).

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

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

Appendix N Adverse events of special interest

AEs of special interest (AESI) for this protocol include but are not limited to potential immune-mediated diseases; representative examples of AESI are listed below. Updates to AESI will be provided as an appendix to the *HVTN 107 Study Specific Procedures*

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (eg Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: eg non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy). • Narcolepsy	• Systemic lupus erythematosus and associated conditions • Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome • Idiopathic inflammatory myopathies, including dermatomyositis • Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still's disease • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis • Mixed connective tissue disorder	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localized Scleroderma (Morphea)
Vasculitides	**Blood disorders**	**Others**
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis. • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia • Autoimmune aplastic anemia • Autoimmune neutropenia • Autoimmune pancytopenia	• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy) • Autoimmune myocarditis/cardiomyopathy Sarcoidosis • Stevens-Johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon

Appendix O Protocol Signature Page

A Phase 1/2a partially double-blinded, randomized clinical trial to characterize the safety and immunogenicity of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120 alone, with MF59® adjuvant, and with alum adjuvant 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

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

DAIDS Protocol Number: HVTN 107

DAIDS Protocol Version: Version 3.0

Protocol Date: May 4, 2017