PROTOCOL

HVTN 100

A phase 1-2 randomized, double-blind, placebo-controlled clinical trial of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59® in HIV-uninfected adults at low risk of HIV infection

DAIDS DOCUMENT ID 11913

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 PRODUCTS PROVIDED BY

Sanofi-Pasteur
Swiftwater, Pennsylvania, USA
GlaxoSmithKline Biologicals, S.A.
Rixensart, Belgium

May 23, 2017
HVTN 100
Version 3.0

Version 2.0 of HVTN 100 added booster vaccinations and extended follow-up to evaluate the capacity of different boost regimens and schedules to maintain or enhance vaccine-elicited immune responses. Version 3.0 of the protocol implements a single booster vaccination at Month 30 with follow-up to Month 36 and reduces group sizes. A visit 1-week postvaccination has been added to facilitate additional immunogenicity evaluations. Mucosal secretion sampling has been added along with associated STI testing. Additional corrections, clarifications, and updates have been made to consent form language concerning sample testing, to laboratory procedure tables, and to some clinic procedure timepoints. Version 3.0 of HVTN 100 also incorporates changes and corrections implemented through a previous modification to Version 2.0 of the protocol.

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 (CABs) 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. 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

United States (US) Food and Drug Administration (FDA) and other US federal regulations require IRBs or 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). Each HVTN Investigator welcomes IRB/EC questions or concerns regarding these research requirements.

This trial is being conducted exclusively in South Africa, with funding from the US NIH. Due to this, the trial is subject to both US and South African regulations and guidelines on the protection of human research subjects and ethical research conduct. These research regulations and guidelines are based on ethical principles of respect for persons, beneficence and nonmaleficence, and justice. Where there is a conflict in regulations or guidelines, the regulation or guideline providing the maximum protection of human research subjects will be followed.

In compliance with 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 (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 100 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) or a Data and Safety Monitoring Board (DSMB) 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 1-2 randomized, double-blind, placebo-controlled clinical trial of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59® in HIV-uninfected adults at low risk of HIV infection

Primary objective(s) for Part A

- To evaluate the safety and tolerability of 2 doses of ALVAC-HIV (vCP2438) followed by 2 doses of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® in HIV-seronegative low risk South African adults

- To evaluate the immunogenicity of 2 doses of ALVAC-HIV (vCP2438) followed by 2 doses of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® in HIV-seronegative low risk South African adults at the month 6.5 timepoint (2 weeks after completion of the primary immunization series)

Primary objective(s) for Part B

- To evaluate the safety and tolerability of Bivalent Subtype C gp120/MF59 and of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59 when given as boosts in participants previously vaccinated in Part A

- To evaluate the immunogenicity of Bivalent Subtype C gp120/MF59 and of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59 when given as boosts at Month 30 in participants previously vaccinated in Part A at 2 weeks following vaccination

Study products and routes of administration

- ALVAC-HIV (vCP2438) expresses the gene products ZM96 *gp120* (clade C strain) linked to the sequences encoding the HIV-1 transmembrane anchor (TM) sequence of *gp41* (28 amino acids clade B LAI strain) and *gag* and *pro* (clade B LAI strain). It is formulated as a lyophilized vaccine for injection at a viral titer $\geq 1 \times 10^6$ cell culture infectious dose $(CCID)_{50}$ and $< 1 \times 10^8$ $CCID_{50}$ (nominal dose of 10^7 $CCID_{50}$) and is reconstituted with 1mL of sterile sodium chloride solution (NaCl 0.4%) for intramuscular (IM) injection as a single dose

- 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) delivered as a 0.5 mL IM injection

- ALVAC-HIV placebo (Part A): a sterile, lyophilized product that consists of a mixture of virus stabilizer, and freeze drying medium and is reconstituted with 1mL of sterile sodium chloride solution (NaCl 0.4%) for injection as a single dose IM

    - ALVAC-HIV placebo (Part B): sodium chloride for injection, 0,9% delivered as a 1.0 mL IM injection

- Bivalent gp120/MF59® placebo: sodium chloride for injection, 0,9% delivered as a 0.5 mL IM injection

Table 3-1 Schema

Group	N	Primary vaccine regimen				Booster*
		Month 0	Month 1	Month 3	Month 6	Month 12
1	210	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	42	Placebo	Placebo	Placebo + Placebo	Placebo + Placebo	Placebo + Placebo
Total	252					

*For booster dose evaluations, see secondary objectives.

Table 3-2 Schema for Part B

			Booster vaccination
Part A Group	Part B Group†	N	Month 30
1	1a	~30	ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59
	1b	~30	Placebo + Bivalent Subtype C gp120/MF59
2	2	~12	Placebo + Placebo
Total		72	

† Vaccine recipients in Part A (Group 1) are randomized to Group 1a or 1b. Part A placebo recipients (Group 2) continue to receive placebo injections in Part B.

Numbers shown for each Part B group are approximate and will depend on how many Part A vaccine recipients and placebo recipients are enrolled in Part B.

Participants

Part A: 252 healthy, HIV-1–uninfected volunteers aged 18 to 40 years; 210 vaccinees, 42 placebo recipients

Part B: 72 HVTN 100 study participants who have not met discontinuation of vaccination criteria described in Section 7.4.3 and termination from study criteria as described in 7.4.4 and who agree to provide mucosal secretion samples, as described in Section 9.5.

Design

Multicenter, randomized, placebo controlled, double-blind trial

Duration per participant

36 months

Estimated total study duration

40 months

Study sponsor

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

Study product providers

- ALVAC-HIV (vCP2438): Sanofi-Pasteur (Swiftwater, Pennsylvania, USA)
- Bivalent subtype C gp120/MF59®: GlaxoSmithKline Biologicals, S.A. (Rixensart, Belgium) [formerly Novartis Vaccines and Diagnostics (Cambridge, Massachusetts, 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 Molecular and Serology Laboratory, National Institute for Communicable Diseases (Johannesburg, South Africa)

Endpoint assay laboratories

- HIV Sero-Molecular Laboratory–National Institute for Communicable Diseases (HSML-NICD) (Johannesburg, South Africa)
- Cape Town HVTN Immunology Laboratory (CHIL) (Cape Town, South Africa)
- Duke University Medical Center (Durham, North Carolina, USA)
- FHCRC/University of Washington (Seattle, Washington, USA)

Study sites

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

Safety monitoring

- HVTN 100 PSRT; NIAID Data and Safety Monitoring Board (DSMB)

3.1 Protocol Team

Protocol leadership

Chair	Linda-Gail Bekker The Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa 011-27-21-650-6959 linda-gail.bekker@hiv-research.org.za	*Statistician*	Zoe Moodie SCHARP, FHCRC 206-667-7077 zoe@scharp.org
Cochair	Fatima Laher Perinatal HIV Research Unit, Chris Hani Baragwanath Hospital, Soweto, South Africa 011-27-11-989-9720 laherf@phru.co.za	*Medical officer*	Mary Allen DAIDS, NIAID 240-627-3036 mallen@niaid.nih.gov
Protocol Team leader	Nicole Grunenberg HVTN Core, FHCRC 206-667-2043 ngrunenb@fhcrc.org	*Laboratory lead*	Georgia Tomaras HVTN Laboratory Program 919-681-5598 gdt@duke.edu

Other contributors to the original protocol

Core medical monitor	Nicole Grunenberg HVTN Core, FHCRC	*DAIDS protocol pharmacist*	Katherine Shin DAIDS, NIAID 240-627-3047
Regional medical liaison	Simba Takuva HCRISA	*Clinical safety specialist*	Jill Zeller HVTN Core, FHCRC
Vaccine developer representatives	Carlos DiazGranados Sanofi Pasteur	*Clinical trials manager*	Carissa Karg HVTN Core, FHCRC
	Sanjay Phogat Sanofi Pasteur	*SCHARP Clinical data manager*	Huguette Redinger SCHARP, FHCRC
	Niranjan Kanesa-thasan Novartis Vaccines and Diagnostics		Jennifer Schille SCHARP, FHCRC
	Susan Barnett Novartis Vaccines and Diagnostics	*SCHARP Senior clinical data manager*	Gina Escamilla SCHARP, FHCRC
	Marguerite Koutsoukos GSK	*SDMC Associate director of lab science*	April Randhawa SCHARP, FHCRC
	Olivier van der Meeren GSK	*Statistical research associate*	Shannon Grant SCHARP, FHCRC
	Francois Roman GSK	*DAIDS Project Officer*	Michael Pensiero DAIDS, NIAID

Laboratory Program representatives	On Ho HVTN Laboratory Program, FHCRC	*Protocol development manager*	Carter Bentley HVTN Core, FHCRC
	Eva Chung HVTN Laboratory Program, FHCRC	*Community engagement unit representative*	Genevieve Meyer HVTN Core, FHCRC
	John Hural HVTN Laboratory Program, FHCRC	*Community educator/recruiter*	Mluleki Nompondwana Emavundleni CRS, UCT CTU
	Erica Anderson-Nissen HVTN Cape Town HIV Immunology Laboratory	*Technical editor*	Erik Schwab HVTN Core, FHCRC
Regulatory affairs	Elizabeth Briesemeister HVTN Core, FHCRC	*Community Advisory Board (CAB) members*	Jerry Phore Klerksdorp CRS
Clinic coordinator	Dorothie Van der Vendt Cape Town CRS		Goduka Mfana Soweto CRS
			Mzwandile Phanziso Emavundleni CRS, UCT CTU

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. This is the first phase 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. As a 'first-in-human' test of this particular vaccine regimen, the trial will be conducted in South Africa among individuals at low risk of HIV infection.

With approximately 6.1 million people living with HIV as of 2012 [5], South Africa's epidemic remains the largest in the world and the Sub-Saharan region bears the preponderant burden of the HIV epidemic with almost 70% of all infections worldwide [6]. The vast majority of newly acquired infections in this region occur during unprotected heterosexual intercourse. Clearly, effective methods for preventing the acquisition and transmission of HIV-1 are urgently needed for this region.

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]. In sum, 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 (see, eg, [16,17]). This protocol and overall plan takes into consideration that the predominant clade circulating in the Southern African region is HIV-1 subtype C [18-28].

Although the results of the RV144 trial are modest, these provide the first indications that a prophylactic vaccine can reduce HIV acquisition risk [4]. This protocol describes a "next step" phase 1-2 study and is the first in a set of clinical trials which test vaccine products relevant to this region, and in a regimen that will build on and potentially enhance the level of the protection seen in the RV144 trial.

4.1.1 The RV144 trial

The RV144 trial was conducted by the US Military HIV Research Program and the Thailand Ministry of Health in a community-based sample of more than 16 000 HIV-1 uninfected participants in Thailand and results were published in 2009 [4]. This community-based study enrolled individuals aged 18 to 30 years with varying degrees of HIV risk. The clinical trial evaluated the heterologous prime-boost combination of canarypox prime ALVAC-HIV (vCP1521), expressing clade E env and clade B gag and pro, followed by the AIDSVAX® clades B/E gp120 protein boost. These products were based on viruses commonly circulating in Thailand at the time. This vaccine regimen demonstrated 31.2% efficacy when compared with placebo (n = 51 vs. n = 74, respectively; p = 0.04) at 3.5 years [4]. Although evaluation of vaccine efficacy at 12 months post vaccination was not included in the pre specified analysis, substantially greater reduction in acquisition was observed one year post vaccination (estimated 60.5%, p = 0.02) with the vaccine effect waning over time to 31% cumulative through 3.5 years [29].

4.1.2 Correlates of risk (CoR) in RV144

To better understand how the RV144 vaccine regimen reduced the risk of HIV infection, a large consortium of independent laboratories worked together systematically to ensure maximal information could be derived from samples obtained from participants who were vaccinated but became infected compared with those vaccinated but uninfected at the end of the trial. A case control study was performed on 41 infected vaccine recipients, 205 uninfected vaccine recipients (5:1) and 40 placebo recipients (20 infected and 20 uninfected) within the RV 144 clinical trial to identify CoR [30]. Among the 6 primary immunological variables selected for the correlates analysis (5 different antibody [Ab] responses and CD4+ T cell cytokine production) that were measured at the 2 weeks after the final vaccination visit (ie, at or near peak immunogenicity), 2 immune CoR of HIV acquisition were identified among vaccine recipients in the RV144 case control study. The first was the presence of immunoglobulin G (IgG) Ab that bound to a scaffolded gp70 V1V2 recombinant protein; this variable correlated inversely with infection rate (ie, higher V1V2 Ab→lower infection rate). The second was plasma Env-specific binding IgA, which correlated directly with infection rate (ie, higher immunoglobulin A [IgA] Ab to Env→higher infection rate). The other 4 primary variables correlated inversely with infection rate only when the level of IgA binding was low. Notably, neither low levels of V1V2 Ab nor high levels of Env-specific IgA were associated with higher rates of infection than those found in the placebo group [30].

4.1.3 Rationale for the proposed vaccine regimen for South Africa

This clinical trial will be conducted in South Africa, where clade C is the predominant circulating subtype [31] and will evaluate the safety and immunogenicity of the regionally adapted next-generation ALVAC/protein vaccine regimen. The regimen will comprise 2 administrations of ALVAC-HIV (vCP2438) containing clade C ZM96 gp120 env along with clade B (LAI) gag, pro and gp41 env TM at Months 0 and 1 followed by 3 administrations of the same ALVAC-HIV along with Bivalent Subtype C gp120/MF59®, comprising recombinant TV1.C and 1086.C gp120 Env proteins mixed with MF59® adjuvant, at Months 3, 6, and 12.

The vaccine regimen in this trial strikes a balance between maintaining a close link to the RV144 vaccine regimen and the need to target the HIV strains circulating in the region of

the world where deployment of the vaccine is envisioned. In addition, the vaccine design and vaccination schedule have been altered in ways that aim at improving the magnitude and duration of vaccine-elicited immune responses beyond those observed in RV144. These changes have been made under the scientific assumption that improvements or extensions of immune responses will translate into improvements in vaccine efficacy.

The gag, pro, and gp41 env components of ALVAC-HIV (vCP2438) are the same as in ALVAC-HIV (vCP1521), the ALVAC-HIV used in the RV144 trial. Adaptations of the RV144 vaccine regimen to South Africa include use of the 96ZM651 gp120 env insert (subtype C) rather than the TH023 gp120 env insert (clade E) used in RV144, and inclusion of 2 subtype C gp120 Env proteins (TV1.C and 1086.C) in boost vaccinations (rather than the clade B and E proteins used in RV144).

The TV1.C and 1086.C gp120 Env proteins comprising Bivalent Subtype C gp120 were selected from among a long list of candidate clade C gp120 proteins according to a predefined algorithm incorporating, among other factors: genetic relatedness to regional HIV strains, CCR5 binding, capacity in animal studies to elicit key epitope-specific Ab, percent monomer expression (associated with receptor and co-receptor binding and induction of nAb), expression/secretion levels in stable cell lines, stability, and immunogenicity in animal models (including evidence of recognition of key epitopes identified in the RV144 correlates studies) [32,33]. Use of gp120 monomers rather than trimers has been prioritized to support comparisons with the RV144 results [34].

MF59® was selected as the protein adjuvant for multiple reasons, including its established safety record and previous experience with this adjuvant in similar prime-boost HIV vaccine regimens [35,36]. In addition, this adjuvant is proprietary of Novartis Vaccines and Diagnostics, the developer of the gp120 protein vaccine components. Based on related human experience, it is expected that MF59® will provide significant dose sparing compared to proteins adjuvanted with alum, while potentially improving the magnitude and subsequent durability of the immune responses elicited by the RV144 regimen. In addition, it has capacity to provide a more balanced Th1/Th2 response than alum [37]. MF59® was originally approved in Europe to enhance the immunogenicity of seasonal influenza vaccine among the elderly [38]. MF59®-adjuvanted influenza vaccine is now licensed in more than 20 countries with more than 100 million doses having been distributed. Recently influenza vaccine formulated with MF59® has been shown to be safe in young children and to significantly enhance immune responses and efficacy in this group [39]. MF59® is also used in a prepandemic H5N1 influenza vaccine (Aflunov®) licensed in the European Union (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 [40].

4.1.4 Rationale for Part B

Based on interim safety and immunogenicity data for the primary vaccination series (ie, Months 0, 1, 3, and 6), a decision has been made to advance the HVTN 100 vaccine regimen to a pivotal efficacy trial in South Africa (see Section 4.5) This trial is designated HVTN 702. In this context, understanding the durability of vaccine-elicited immune responses and the extent to which those responses can be maintained or enhanced by subsequent booster vaccinations has assumed great importance. Part B of HVTN 100 seeks to provide this understanding by characterizing the immune responses elicited by different booster vaccinations at Month 30.

Participants in Part B will receive vaccinations at Month 30 with ALVAC-HIV (vCP2438) plus Bivalent Subtype C gp120/MF59, with Bivalent Subtype C gp120/MF59 alone, or placebo. Vaccinees in Part A of HVTN 100 (ie, those assigned to Group 1) will be randomized to Group 1a or 1b (see Table 3-2) in 1:1 proportions. In order to maintain blinding throughout the trial, placebo recipients in Part A (Group 2) will also receive placebo vaccinations at Month 30.

Part B of HVTN 100 thus provides an opportunity to characterize the immune responses elicited by booster vaccinations at Month 30 and, importantly, in the event that vaccine-induced immune CoR or correlates of vaccine protection are identified in the planned HVTN 702 trial, samples from Part B of HVTN 100 can be evaluated to determine whether booster vaccinations can maintain or even enhance those correlates. This information may help inform investigation of boost strategies should the vaccine regimen be advanced toward an application for marketing authorization.

Questions concerning boost intervals and vaccine product selection have been explored previously with respect to the RV144 ALVAC-gp120 protein vaccine regimen in follow-on protocols RV305 and RV306. Since the HVTN 100 vaccine regimen was designed to mimic the efficacious vaccine in RV144 but with adaptations to the clade C epidemic in sub-Saharan Africa (see Section 4.1.3) and HVTN 097 demonstrated that the RV144 vaccine regimen elicited responses in South African vaccinees similar to those seen among Thai vaccinees in RV144, information from the RV305 and RV306 studies may suggest what to expect with late boosts to the HVTN 100 vaccine regimen. Notwithstanding differences in boosting schedule and study products between these studies and HVTN 100, these results have been useful in informing the design of Part B in HVTN 100.

In RV305, RV144 participants who had completed the RV144 vaccination series per protocol received ALVAC+AIDSVAX, AIDSVAX alone, or ALVAC alone 6-8 years after completing the primary RV144 vaccination sequence. Vaccinations took place at Weeks 0 and 24. The trial was planned to enroll 162 participants (45 vaccinees and 9 placebo recipients in each of the 3 groups); 97% of those enrolled completed the planned vaccinations. Among the key findings in this study are the following:

- Boosts with AIDSVAX alone and with ALVAC/AIDSVAX boosted antibody responses to levels significantly higher than those attained by the primary vaccine regimen. Specifically,

    - AIDSVAX and ALVAC/AIDSVAX boosts generated peak IgG responses to gp120 A244gD and to vaccine-matched 92TH023 gp70 V1V2 that were significantly superior (following the first boost) and at least as robust (following the second boost) to the peak responses observed in RV144 following the 4th vaccination; boosting with ALVAC alone did not boost these IgG responses; and, while IgG responses declined at a rate similar to that observed in RV144, responses remained substantially higher 6 and 12 months post final vaccination than in RV144 (Karasavvas and Akapirat, unpublished data).

    - With respect to IgG subclass responses to gp120 A244gD and to vaccine-matched 93TH023, late boosts in RV305 elicited IgG1 and IgG4 responses that were higher than those observed in RV144; IgG3 responses were not boosted to the levels observed in RV144 (Karasavvas and Akapirat, unpublished data).

- Although it has been demonstrated previously that antibodies of the IgG3 and IgG1 subclasses that target V1V2 gp70 antigens were associated with antibody-mediated effector functions (eg, ADCC) that were linked to a reduced risk of infection in RV144 [41-43] and, conversely, that IgG4 can interfere with such Fc-mediated effector functions, ADCC responses were boosted after each injection of either AIDSVAX or ALVAC/AIDSVAX, but not with ALVAC alone. TH023-specific ADCC responses were stronger with ALVAC/AIDSVAX combination boosts compared to AIDSVAX alone while MN-specific ADCC responses were similar between the 2 boosting regimens. (Ferrari, unpublished data).

- Mucosal cytokine profiles differed between the AIDSVAX and ALVAC/AIDSVAX regimen, although the implications for the differences are unclear.

The RV306 trial enrolled 360 new Thai volunteers (327 vaccinees; 33 placebo recipients) in a study to evaluate the impact of the timing of an additional vaccine boosts to the RV144 regimen and to gather new immunogenicity data. Vaccinees in all groups were primed with the RV144 regimen (4 vaccinations), which elicited IgG responses to gp120 and to scaffolded gp70 V1V2 similar to the peak levels observed in RV144. Three groups were boosted once with ALVAC+AIDSVAX at either 6, 9, or 12 months after completion of the primary regimen; a fourth group was boosted with AIDSVAX alone 6 months after completion of the primary regimen; the 5th group did not receive a late boost. Note that the boosts in RV306 occur far sooner than the first boost in RV305. Preliminary (blinded) findings from this study included:

- AIDSVAX and ALVAC/AIDSVAX boosts 6 months after completion of the primary (RV144) vaccine regimen elicited IgG responses to vaccine-matched antigens and to scaffolded V1V2 antigens comparable to peak levels following the primary vaccine regimen.

- Booster vaccinations elicited IgA response levels substantially lower than those elicited by the primary (RV144) regimen. IgG geometric mean titers (GMT) following boosting were approximately 100-fold higher than IgA. Note that the ratio of IgG to IgA responses was observed to correlate with efficacy in the RV144 trial [44].

- Booster vaccinations increased neutralizing antibody response rates and titers to levels above those observed following the primary (ie, RV144) vaccine regimen; to one vaccine-matched antigen, late boosts at 9 and 12 months after the primary regimen elicited statistically higher neutralizing antibody titers than a boost 6 months after the primary regimen (Polonis and Wieczorek, unpublished data).

- Late boosts increased the number of gp120-specific B cell responders with a significant increase in magnitude and in frequency of plasmablasts (Schuetz, unpublished data).

- There was no evidence of induction of activated CD4+/CCR5+ T cells following the late boost vaccinations.

Based on the failure of boosting with ALVAC-HIV alone to boost antibody responses in RV305, Part B does not include a late boost with ALVAC-HIV alone. However, aside from notation that activated T cells were not induced by serial boosting in RV306,

information concerning the impact of boost vaccinations on the rate, magnitude, and quality of cellular immune responses is lacking at this point in time. Hence a critical factor that may distinguish boosts that include the vector from protein-only boosts is currently unknown.

The results of late boosts in RV305 and RV306 suggest the hypothesis that a Month 30 boost of the Part A regimen with Bivalent Subtype C gp120/MF59 with and without ALVAC-HIV (vCP2438) will elicit immune response levels 2 weeks following the boost that are similar to or better than the immune responses observed 2 weeks following the Month 6 vaccination. These include immune responses previously shown to be inversely correlated with risk of HIV infection in the RV144 efficacy trial.

4.1.5 Rationale for mucosal secretion sampling in Part B

While it has long been understood that immune responses at mucosal surfaces are critical in prevention of viral infections (see, eg, [45-47]), until recently the immunogenicity of investigational HIV-1 vaccines has been evaluated primarily in the periphery. 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 [48,49]. In order to determine if key peripheral immune responses (especially V1V2 IgG responses) shown to be associated with a lowered risk of immune HIV infection [30] are also present in the genital mucosa and to assess whether these differences between systemic and mucosal immune responses observed in macaques are recapitulated in humans, mucosal secretion sampling has been added in Part B of the study.

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 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 of 1.0 mL as a single dose. The composition of one dose of ALVAC-HIV (vCP2438) is provided in Table 4-2.

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

Ingredient	Amount in one dose	Function
ALVAC-HIV (vCP2438)	$> 1 \times 10^6$ $CCID_{50}$ 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

For Part A of the study, ALVAC-HIV (vCP2438) Bulk Drug Substance and Drug Product are manufactured by IDT Biologika GmbH, Am Pharmapark, Dessau-Rosslau, Germany, under contract to Sanofi Pasteur.

For Part B of the study, 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 viral seed lot 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/MF59®

4.3.1 Constructs

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

4.3.2 Manufacturing and formulation

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

Following clone selection, a fed batch cell culture at 250L scale is employed for cell propagation. Once the cells reach optimum cell density, the culture is harvested and purified using standard methods. The conditioned media is concentrated by ultrafiltration. The manufacturing process utilizes a weak cation exchange chromatography step, CM-Fractogel, which provides purification as well as viral reduction. Concentration (by ultrafiltration) is then used, followed by exchange into the formulation buffer.

After these process steps, both subtype C gp120 protein processes include viral reduction filtration (nanofiltration) followed by 0.22 µm filtration and bulk fill. Both TV1.C and 1086.C bulk drug substances are stored frozen at not more than -60°C. For both drug substances, the formulation is the same, containing 0.4 mg/mL Env antigen, sodium citrate, and sodium chloride, pH 6.0.

The 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	diluting agent

Additional information is provided in the IB.

4.3.3 MF59® adjuvant

The Novartis MF59® adjuvant is an oil-in-water emulsion with a squalene internal oil phase and a citrate buffer external aqueous phase. Two non-ionic surfactants, sorbitan trioleate and polysorbate 80, serve to stabilize the emulsion. The bulk formula is shown in Table 4-4.

Table 4-4 Composition of MF59® per liter

Name of Ingredients	Quantity per Litre*	Function	Reference to Standards
Squalene	39.0 g	oil phase	In-house specification
Polysorbate 80	4.7 g	surfactant	USP/NF
Sorbitan Trioleate	4.7 g	surfactant	USP/NF
Sodium Citrate, dihydrate	2.65 g	buffer	USP/NF
Citric Acid, monohydrate	0.17 g	buffer	USP/NF
Water for Injection	q.s. 1 L	aqueous phase	Ph.Eur. and USP/NF
Nitrogen	overlay	inert gas	USP/NF

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

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

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

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

Additional information is provided in the IB.

4.3.4 Bivalent Subtype C gp120/MF59® for injection

A final dose of 100mcg of each recombinant Env protein will be mixed with MF59® adjuvant. The composition of one 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 one dose	Function
Drug Substances		
TV1.C gp120 protein 1086.C gp120 protein	100 mcg 100 mcg	active active
Adjuvant (MF59®)		
Squalene	9.75 mg	oil phase
Polysorbate 80	1.175 mg	surfactant
Sorbitan Trioleate	1.175 mg	surfactant
Excipients		
Sodium Citrate, Dihydrate Citric Acid, Monohydrate Sodium Chloride Water for injections	1.39 mg 0.051 mg 4.38 mg qs to 0.5 mL	buffer buffer tonicity modifying agent solvent

4.4 Trial design rationale

No preventive efficacy was demonstrated when AIDSVAX® rgp120 subunit vaccines (B/B' in North America and the Netherlands, in high risk men who have sex with men; B/E in Thailand, in injection drug users) were administered alone [50,51]. However, the modified intention-to-treat results of the community-based RV144 trial in Thailand in individuals at low and high risk demonstrated safety and modest efficacy of an ALVAC-HIV (vCP1521) plus AIDSVAX® B/E protein regimen. The promising findings of RV144 provide a compelling rationale for studies aimed at replicating or enhancing these results in other regions of the world.

This trial is part of a program that tests a vaccine regimen similar to that used in RV144. It aims not only to compare data with RV144 results, but also to build upon that trial design in three important ways: adaptation to the clade C strains of HIV-1 predominant in South Africa, an additional booster dose at month 12 aimed at prolonging the durability of peak immunogenicity, and use of different adjuvant having the potential to enhance the magnitude and durability of the immune responses elicited by the vaccine (see Section 4.1.3).

Safety and immunogenicity data from this trial will inform a decision whether to advance the vaccine regimen to a pivotal phase 3 trial. Hence the primary immunogenicity endpoints have been selected to support evaluation of the hypothesis that 2 doses of ALVAC-HIV (vCP2438) followed by 2 doses of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® will result in immune responses at least comparable to those induced by the RV144 vaccine regimen.

For Part B see Section 4.1.4.

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

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

HVTN 039 is the only study that has compared the safety and immunogenicity of ALVAC-HIV (vCP1452) given at the standard dose ($10^{7.25}$ $CCID_{50}$) to a dose 5.6 times higher (10^8 $CCID_{50}$), and placebo. The high-dose ALVAC-HIV (vCP1452) resulted in unacceptable levels of reactogenicity, without evidence of immunogenicity improvements. 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.

The desired improvements in overall and protective immune responses will rely on the use of a more potent protein adjuvant and adjustment of the vaccination schedule.

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

Bivalent Subtype C gp120/MF59® vaccine has not yet been administered to humans. The 200 mcg total dose was selected based on previous clinical experience. Limited dose range studies performed with Novartis (formerly Chiron) subtype B SF2 gp120 and subtype E gp120 protein candidates indicated that 50 mcg and 100 mcg, totalling 150 mcg, doses with MF59® adjuvant were immunogenic and well tolerated [52].

4.4.2 Schedule

Safety and immunogenicity will be assessed for ALVAC-HIV (vCP2438) (months 0, 1, 3, 6, 12) and for Bivalent Subtype C gp120/MF59® (months 3, 6, 12).

The vaccine administration schedule encompasses the schedule used in RV144 but with an additional boost at month 12 in response to the apparent wane in efficacy observed in RV144 from 12 to 36 months.

Addition of a booster vaccination at month 12 is supported by immunogenicity data from nonhuman primate (NHP) studies performed with another pox-protein regimen. Both neutralizing and binding Ab levels dropped after completion of the primary immunization, while the re-boost at week 49 was able to bring back the Ab responses to the higher level [53]. In addition, administration of a late ALVAC-HIV/AIDSVAX® boost (in RV 305) approximately 7-9 years after the final vaccination in RV144 demonstrated that immune responses were restored or improved compared to the responses observed at the primary timepoint in RV144 [54].

The selected dose schedule is therefore a five-dose schedule of ALVAC-HIV (vCP2438), a four-dose primary vaccination schedule with ALVAC-HIV administered at 0, 1, 3 and 6 months plus protein boost administered at 3 and 6 months (replicating the RV144 schedule); and a single booster vaccination with both ALVAC-HIV (vCP2438) and protein boost administered at 12 months.

For Part B see Section 4.1.4.

4.4.3 Choice of placebo

Part A

The placebo for ALVAC-HIV (vCP2438) is a sterile, lyophilized product that consists of a mixture of virus stabilizer, and freeze drying medium and is reconstituted with sterile sodium chloride (0.4% NaCl) for a single 1 mL dose, to administer IM.

Placebo for Bivalent Subtype C gp120/MF59® is sodium chloride for injection, 0,9%.

Part B

In Part B, the placebo for both ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59® is sodium chloride for injection, 0,9%.

4.4.4 Prime-boost regimen

The prime-boost strategy consists of ALVAC-HIV (vCP2438) prime with Bivalent Subtype C gp120/MF59® boost.

The concept of priming with one HIV vaccine followed by boosting with another heterologous HIV vaccine emerged in the 1990s as a strategy to enhance vaccine immunogenicity and, potentially, vaccine efficacy. While Env glycoproteins elicit strong humoral responses, they have not been associated with potent cellular immunity, particularly CD8-specific cytotoxic T lymphocyte (CTL) responses. On the other hand, although viral vector constructs, including ALVAC, often elicit both Ab and cell-mediated responses, it was observed that the level of Ab could be increased significantly by subsequent administration of Env glycoproteins. In addition, prime-boost vaccine regimens were explored as means of increasing the breadth of HIV strains to which vaccination might induce responses and also as a means of generating both potent CTL and potent Ab responses, a combination believed to be important in conferring protective immunity against HIV infection [55,56]. These desirable immune response characteristics were often observed in early phase clinical trials of a variety of prime-boost vaccine regimens [56-66].

Among the many prime-boost vaccine regimens tested during this period were canarypox-derived (ALVAC) vector primes containing differing arrays of recombinant HIV genes and HIV Env glycoprotein boosts. By 1998, early phase clinical trials of ALVAC-protein regimens had shown:

- These regimens with glycoproteins adjuvanted with alum or MF59® were well tolerated in several hundred HIV-uninfected, healthy volunteers;

- HIV-specific immune responses were not dampened in vaccinia-experienced individuals;

- The prime-boost regimens induced large repertoires of HIV-specific Ab, neutralizing activity against some laboratory HIV isolates, Ab capable of inducing antibody-dependent cellular cytotoxicity (ADCC), and HIV-specific CTL activity against a broad range of targets [56,67].

In NHP studies, heterologous prime-boost regimens afforded better protection against viral challenge than did sequential injections of ALVAC-HIV vaccines alone, suggesting a potential connection between the characteristic responses elicited by the heterologous ALVAC-protein prime-boost regimens and protection from HIV infection [68].

On the basis of results from nonclinical studies and early-phase clinical trials, ALVAC-HIV (vCP1521) and HIV gp120 Env became the first heterologous prime-boost vaccine regimen to enter efficacy testing in humans in the RV144 trial in Thailand. Comparison of the immunogenicity observed in the RV144 trial to that from the VAX003 efficacy study previously conducted in Thailand, showed that the nAb response after two doses of gp120 protein following priming with ALVAC-HIV (vCP1521) [4] exceeded the nAb

response seen after two inoculations with the same bivalent gp120 protein without preceding ALVAC-HIV (vCP1521) administration [52], pointing to a priming effect by ALVAC-HIV (vCP1521) that benefited the immune response in the RV144 study [69]. Hence, one of the primary advantages to prime-boost regimens posited by nonclinical studies and supported in early phase clinical trials, was borne out in comparison of results from 2 large later-phase clinical trials conducted in the same country.

Furthermore, recent data suggest that priming by ALVAC-HIV (vCP1521) may favourably influence the quality of Ab responses. The IgG3 HIV-1 Env responses induced by the ALVAC-protein prime-boost regimen were significantly higher than those induced by the homologous gp120 prime-boost regimen, which indicates that ALVAC priming before a protein boost modifies the IgG subclass profile in a manner that may substantially change the HIV-1 inhibitory functions of the vaccine-elicited IgG responses [41,42]. IgG1 and IgG3 are the most functional of the IgG subclasses in that they have been associated with HIV-1 neutralization, complement fixation, phagocytosis, ADCC, and antibody dependent cellular viral inhibition (ADCVI) [70].

4.5 Plans for future product development and testing

This phase 1-2 trial is designed to establish a preliminary safety record in the low risk South African adult population for priming vaccinations of ALVAC vector with HIV clade C (ZM96) gp120 *env* and clade B (LAI) *gag, pro,* and *gp41 env* inserts followed by boost vaccinations of the ALVAC vector in combination with gp120 Env proteins (TV1.C and 1086.C) with MF59® adjuvant. Safety and immunogenicity data from this trial will inform a decision whether to advance the vaccine to a pivotal phase 3 trial and, should the phase 3 trial demonstrate sufficient preventive efficacy, an application for marketing authorization in the Republic of South Africa (RSA) will follow. Such additional studies as are required by governing regulatory authorities to support such an application will also be performed.

Should the HVTN 702 study demonstrate preventive efficacy sufficient to support an application for a marketing authorization, data generated by Part B of HVTN 100 may help to inform subsequent evaluation of boosting strategies to prolong the protective effects of the vaccine.

4.6 Nonclinical studies

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

The objective of the study was to determine the local tolerability and systemic toxicity of ALVAC-HIV (vCP2438)/ALVAC-HIV (vCP2438) with gp120+MF59 vaccines and DNA-HIV-PT123 with gp120+MF59 vaccines administered by the IM route to New Zealand White Rabbits 7 or 6 times, respectively, 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 which 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.

4.6.2 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
- Hypersensitivity study with ALVAC-HIV (vCP125) in guinea pigs

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

4.6.3 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 4.6.1.1. 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 one of the components (TV1 gp120) in the proposed Bivalent Subtype C gp120/MF59® vaccine, and hence is very similar in sequence. Overall, toxicology studies revealed that both the subtype B gp140 and subtype C gp140 vaccines with MF59® were well tolerated and testing revealed no adverse local or systemic effects.

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

- Subchronic IM toxicity study of Biocine® HIV Thai E gp120/SF 2 gp120 vaccine in rabbits

- Repeat dose toxicity of IM HIV DNA/PLG prime followed by IM subtype B gp140/MF59® in rabbits

- Repeat dose toxicity of intranasal (IN) subtype B gp140 with an LTK63 adjuvant followed by IM subtype B gp140 with MF59® in rabbits

- Repeat dose toxicity of IM SAAVI DNA-C2 followed by IM SAAVI MVA-C with subtype C gp140/ MF59® in rabbits

4.6.3.1 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 immunological adjuvant. These findings are readily reversible within days to 1 to 2 weeks. In repeat-dose toxicology studies in dogs, there were no effects on cardiovascular or central nervous system (safety pharmacology) parameters. MF59® is not genotoxic (Ames test) or clastogenic (mouse micronucleus), is not a dermal sensitizer (Guinea pig), and was not teratogenic (rat and rabbit) or a developmental toxicant (rat).

Pivotal toxicology studies performed with MF59® include:

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

4.7 Clinical studies

4.7.1 HVTN 100 experience to date

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

4.7.1.1 Interim summary of blinded safety and tolerability data

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

The vaccine regimen is very well tolerated thus far with the vast majority experiencing no or mild local reactogenicity symptoms. Mild pain and/or tenderness 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 (at injection timepoint #1) and for a right deltoid injection by 2 participants (1 at injection timepoint #3 and 1 at injection timepoint #5).

For the left deltoid, grade 1 (mild) erythema and/or induration injection site reactions were reported by 6% of participants and grade 2 (moderate) reactions by 5% of participants. For the right deltoid, grade 1 erythema and/or induration injection site

reactions were reported by 4% of participants and grade 2 reactions by 2% of participants. Three participants have reported erythema and/or induration reactions meeting grade 3 criteria (severe) based on size (≥ 10cm diameter or ≥ 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 of the 4th injection timepoint, which resolved within 4 days. This participant also self-reported severe induration on Day 3, which resolved within 1 day. The participant returned to clinic on Day 4 for examination, and clinic staff observed severe erythema alone without induration or swelling. Antibiotics, analgesics, and antihistamines were prescribed and the participant was discontinued from further vaccinations but continued in follow-up. Another person reported grade 3 induration and erythema in the right deltoid on Day 3 after the 5th vaccination timepoint, resolving by Day 5. Antibiotics, anti-inflammatory and analgesic medications were prescribed and were taken for 3 days. Another person reported severe erythema and induration reactions in the left deltoid occurring on Day 0 after the 5th vaccination timepoint and resolving by Day 3. Antihistamine, oral steroid and analgesic/anti-inflammatory medications were taken. In all 3 participants, the needle used for injection was < 1.5" long, consistent with weight-based guidance for needle length choice provided to sites in the SSPs [71,72].

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.

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

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

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

4.7.1.3 Discontinued vaccinations and early terminations

As of July 14, 2016, Seventeen participants have terminated the study prematurely and 16 participants have discontinued vaccinations. Clinical events leading to early terminations and discontinuation of vaccinations (DOV) occurred in 5 participants: death from multiple injuries (unrelated to study product); severe local reactogenicity (DOV); hypertension (DOV for AE unrelated to study product); mild vomiting occurring on the day of 1st vaccination only (participant declined further study participation); psychiatric diagnosis (DOV and early termination for investigator discretion). Six other participants have terminated the study due to HIV infection. Reasons for DOV or early termination in other participants included participant refusal, unable to contact, unable to schedule within visit windows, unable to adhere to study schedule, relocation, desiring to fall pregnant, desiring to donate eggs, unwilling to use contraception, and pregnancy. One pregnancy has been reported to date and this participant is continuing in follow-up.

4.7.1.4 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 4-6). 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 4-6 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 4-7). Hence criteria 1 and 2 were met.

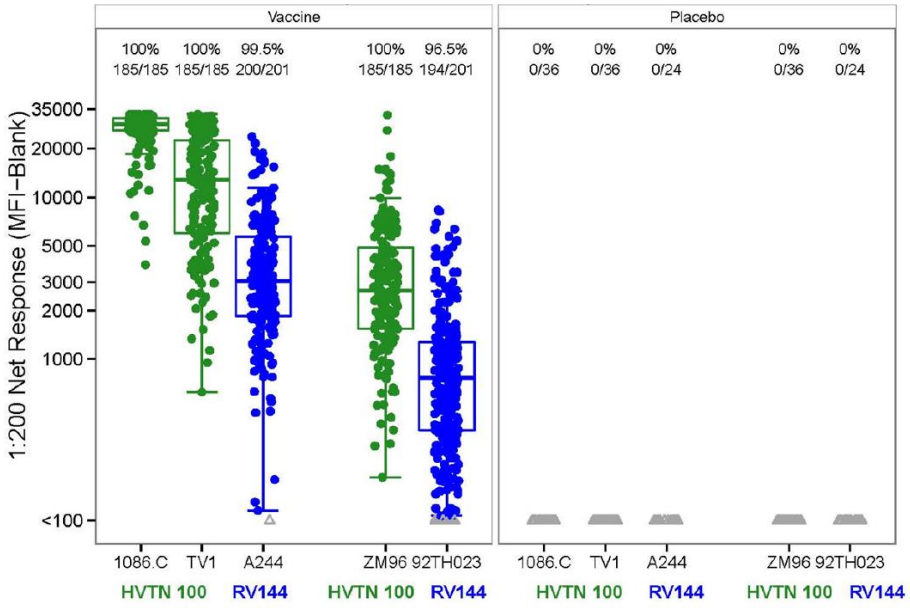


Figure 4-1 Box plots of the binding antibody titers to the vaccine antigens used in RV144 and HVTN 100. The midline of the box plot indicates the median; the ends of the box indicate the 25th and 75th percentiles. Closed dots represent positive responses, open triangles represent negative responses.

Table 4-7 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% and 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 4-8). 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 4-8 HVTN 100 Binding Antibody Env V1V2 Response Rates, Per-Protocol Cohort (Go/No-Go Criterion 4)

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

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

4.7.2 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 closest 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 that inform the expected safety, tolerability, and immunogenicity profile of the new vaccine (see Table 4-9). In all, more than 10,000 human subjects have received ALVAC-HIV vaccines in clinical trials. The majority of these trials have been performed with ALVAC-HIV

(vCP205), (vCP1452), or (vCP1521). In these vaccines, different HIV gene inserts are introduced into the ALVAC vector.

Table 4-9 Recombinant ALVAC-HIV vaccine in human adult prevention trials

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

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

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

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

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

4.7.3 Clinical safety experience with related ALVAC-HIV vaccines

4.7.3.1 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 [73,74]. The most relevant data, however, come from the large efficacy study performed in Thailand [4,75], 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.

De Bruyn et al [76] characterized the tolerability and safety profile of ALVAC-HIV (vCP205) and ALVAC-HIV (vCP1452) (along with other ALVAC vectors) 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.

4.7.3.2 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 than one 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 [75].

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

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

To date, 3 studies have been conducted and completed in Africa with ALVAC-HIV: HIVNET 007, HIV Prevention Trials Network (HPTN) 027 (see also Section 4.7.1), and HVTN 097.

HIVNET 007 [78] 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 [79] 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).

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 AE compared to 85% of placebo recipients. There were 2 SAEs and both were unrelated to treatment: thermal burn in a vaccinee and substance-induced psychotic disorder in a placebo recipient. There were no Grade 4 (life threatening) or 5 (death) AEs. There were 5 Grade 3 AEs in vaccine recipients (6.25%) and 2 Grade 3 events in 1 placebo recipient (5.3%), all deemed unrelated to study treatment. These included alanine transaminase (ALT) increase, headache, hypertension, abnormal loss of weight, and thermal burn in vaccinees and substance-induced psychotic disorder and abnormal loss of weight in 1 placebo recipient. Moderate AEs were experienced by 61.25% of vaccinees and 50% of placebo recipients. AEs considered related to the vaccine included itching at the injection site, lymph node swelling, faster heartbeat, abdominal pain, flu-like illness, diarrhea, injection site skin lump, and muscle spasms. Each of these reactions were mild or moderate, only occurred in 1 person (except for the skin lump which occurred in 3 participants), and did not last long. All participants recovered without sequelae. A few participants had changes in their laboratory, blood, and urine test results that were considered related to the vaccinations and all returned to normal. Overall, the study indicated that the vaccine regimen used in RV144 appears safe and well-tolerated in South Africans.

4.7.3.4 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, 440 human subjects have received this combination across 7 clinical trials (Table 4-10). No safety signal of concern was identified in these studies (see also Section 4.7.1).

Table 4-10 Clinical studies performed with ALVAC-HIV and gp120+MF59®

Study (Country)	ALVAC-HIV	Protein	Subjects[a]
RV132 [74] (Thailand)	vCP1521	gp120 + MF59® clades B/E made in Chinese hamster ovary (CHO) cells	n = 45
AVEG 022A [80] (USA)	vCP205	gp120 + MF59® clade B made in CHO cells	n = 47
AVEG 029 [81] (USA)	vCP205	gp120 + MF59® clade B made in CHO cells	n = 22
AVEG 202/HIVNET 014 [82] (USA)	vCP205	gp120 + MF59® clade B made in CHO cells	n = 145
AVEG 032 [83] (USA)	vCP205	gp120 +/- p24 + MF59® clade B gp120 made in CHO cells p24 made in *S. cerevisiae*	n = 56
AVEG 026 [84] (USA)	vCP300[b]	gp120 + MF59® clade B made in CHO cells	n = 85
AVEG 012A 012B [85] (USA)	vCP125[c]	gp120 + MF59® clade B made in CHO cells	n = 40
Total			**N = 440**

[a] Number of subjects who received both the ALVAC-HIV prime and the gp120/MF59® boost.
[b] ALVAC-HIV (vCP300) is similar to vCP205 and contains additional sequences encoding Pol and Nef epitopes.
[c] ALVAC-HIV (vCP125) contains the gene for gp160 from clade B.

4.7.4 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 [30]).

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.

4.7.4.1 ALVAC-protein schedule and immunogenicity

The selection of a vaccination schedule for the large efficacy trial in Thailand (RV144) was based on existing scientific knowledge at the time [86]. 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. Section 4.4.2 explains the rationale for schedule modifications to address the goal of improving upon the RV144 results.

Prior to RV144, several schedules of administration with ALVAC and protein boost were examined in multiple clinical trials [67,80,82,87,88]. 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 [88]. 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.

4.7.4.2 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 200mcg, 100mcg of each protein) adjuvanted with MF59®. Both vaccine components have been adapted to target the predominant HIV clade circulating in South Africa (clade C). Interim immunogenicity data from HVTN 100 are summarized in Section 4.7.1.4. 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 [74] used ALVAC-HIV (vCP1521) in the same dose and schedule as in study RV144 but 45 subjects in one 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 [73] 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 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 4-11 summarizes the nAb response rates for the pertinent study arms from these studies.

Table 4-11 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 [74]	100mcg CM235* 50 mcg SF2**	MF59®	45	89%	61%	95%	19%	100%
RV135 [73]	100mcg A244* 100mcg MN**	alum	50	23%	--	44%	100%	47%
	300mcg A244* 300mcg MN**	alum	47	31%	--	64%	98%	71%

* clade E Strain
** clade B Strain

The geometric mean (GM) nAb titers were also reported in these studies for 2 of the clade E strains. Data are summarized in Table 4-12.

Table 4-12 nAb GM titers to clade E strains

Study	gp120 dose	Adjuvant	N	NPO3 Strain	CM244 Strain
RV132 [74]	100mcg CM235* 50 mcg SF2**	MF59®	45	45	32.66
RV135 [73]	100mcg A244* 100mcg MN**	alum	50	12.3	7
	300mcg A244* 300mcg MN**	alum	47	14.8	5.4

* clade E Strain
** clade B Strain

Although the data should be interpreted with caution, they suggest that 100mcg of gp120 protein adjuvanted with MF59® can induce Ab responses after ALVAC prime at least comparable to and possibly greater than 300mcg of gp120 protein adjuvanted with alum after ALVAC prime. These data suggest that MF59® allows for protein dose sparing compared with the less potent alum adjuvant.

4.7.5 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 4.7.1.

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 many clinical trials. In addition, recombinant oligomeric (o-gp140) Env proteins for subtypes B and C from Novartis have been or are currently in clinical trials. Overall, in these studies, recombinant HIV-Env proteins manufactured by Novartis were well tolerated and immunogenic. In most cases, recombinant HIV-Env proteins (either gp120 or gp140) were CHO-based and administered with MF59®, Novartis' proprietary oil-in-water emulsion adjuvant [38]. 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 [52,74,84,89-91]. Two clinical trials were conducted using Novartis CHO-based subtype B gp140 recombinant Env protein with MF59®. There are three ongoing phase 1 studies with Novartis CHO-based subtype C gp140/MF59® being conducted by the NIH-sponsored HVTN in the US and the RSA. Table 4-13 summarizes clinical trial experience with Novartis gp120 and gp140 recombinant vaccine candidates.

Table 4-13 Novartis recombinant gp120 and gp140 vaccines in human clinical trials [90]

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	145	AVEG 202	Completed
SF2 gp120 & CM235 gp120 (CHO)/MF59® and ALVAC	45	RV132	Completed
Subtype B (SF162) gp140 (CHO) /MF59® and Subtype B DNA/PLG	90	HVTN 049	Completed
Subtype B (SF162) gp140 (CHO) /MF59® IN with LTK63	20	C86P1	Completed
Subtype C (TV1) gp140 (CHO) & ISS TAT	30	ISS P-002	Completed
Subtype C (TV1) gp140 (CHO)/MF59®	20	HVTN 088	Completed
Subtype C (TV1) gp140 (CHO)/MF59® and SAAVI DNA-C2 and SAAVI MVA-C	24	HVTN073E	Completed
Subtype C (TV1) gp140 (CHO)/MF59® and SAAVI DNA-C2 and SAAVI MVA-C	114	HVTN 086	Completed

In general, these recombinant protein vaccines were immunogenic and well tolerated with no unusual or serious vaccine-associated AEs reported. Most of the reactions were mild to moderate in nature, and of short duration [4,51,52,74,84,89-92].

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

For description of interim safety/tolerability results from Part A of HVTN 100, see Section 4.7.1.

In addition, two other clinical trials have been conducted recently using Novartis CHO-based subtype B gp140 with MF59®. In addition there are four ongoing clinical trials using Novartis CHO-based subtype C gp140.

A phase 1 single-center trial (C86P1) was conducted using Novartis CHO-based subtype B gp140 recombinant Env protein in Great Britain by the Mucosal Vaccines for Poverty Related Diseases (MUVAPRED) Consortium to assess safety, tolerability, and immunogenicity of IN administration of subtype B gp140 with and without the mucosal adjuvant LTK63 (detoxified mutant heat labile protein) followed by IM boosting with subtype B gp140/MF59®. This study enrolled 30 healthy volunteers aged 18-45, with 20 to receive gp140. The protocol was amended to halt further IN administration of LTK63 following a report of an AE (ie, facial nerve paralysis) with a possible association with the LTK63 adjuvant in another study [93]. During the study, there was one 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.

The other completed study with Novartis subtype B gp140 MF59® was a multicenter, placebo-controlled trial (HVTN 049) conducted by the HVTN in the United States [94]. Subjects received one 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 1000/1000mcg) or placebo (5 to 1 ratio) as a single IM injection at 0, 1 and 2 months, followed by a boost of subtype B gp140 with MF59® (or placebo) at 6 and 9 months. An additional group of subjects received subtype B gp140 with MF59® without DNA prime, administered at 0, 3, and 9 months. Overall 96 healthy, HIV-1-uninfected adult subjects were enrolled and 86 subjects completed all planned vaccinations. There were no SAEs reported as related to study vaccine. There were four events reported as SAEs that were not considered related to the study vaccine. A death attributed to cocaine overdose occurred in one subject, 10 days after receipt of the second dose of the placebo. One subject had a Grade 3 increase in creatine phosphokinase (CPK) to 2311 U/L, 14 days after the first DNA prime vaccination, which resolved within a week. Another subject had a Grade 4 increase in CPK to 4806 U/L 15 days after the first DNA prime vaccination, which resolved within two weeks. Both subjects reported to have initiated new exercise programs. One subject experienced severe fatigue 20 days after the fourth immunization (including one dose of subtype B gp140/MF59®), attributed to working two jobs and long hours. Overall, the regimens were generally well tolerated.

There are three ongoing phase 1 studies with Novartis subtype C gp140/MF59® being conducted by the HVTN in the US and the RSA as well as one phase 1 trial being conducted by the Istituto Superiore di Sanità (ISS) in Italy. One of these trials, HVTN 088, is being conducted in the United States in order to evaluate the safety and immunogenicity of a long-interval, cross-clade subtype C gp140/MF59® boost in subjects previously administered subtype B gp120/MF59® or subtype B gp140/MF59® in previous trials. This included subjects from the HVTN049 DNA/PLG prime, gp140/MF59® boost study described above. The study is fully enrolled with 16 previously vaccinated subjects

and 20 naive controls. Individuals were identified who had received a clade B Env protein with MF59® 4-17 years earlier, most in combination with a DNA or ALVAC prime. These individuals were enrolled in HVTN088 to receive a clade C protein boost in an open label phase 1 trial. There have been 3 SAEs reported in this trial, one involving traumatic injury, one instance of gastroenteritis, and one of appendicitis. All of these were assessed as unrelated to study agents.

Another ongoing study, HVTN 073E, is being conducted in the US and RSA as an extension to the previous HVTN 073/SAAVI03 study. This extension study examines 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 is fully enrolled, with 27 subjects. There was one report of endometrial intra-epithelial neoplasia resulting in hospitalization for hysterectomy, which was assessed as unrelated to study agents.

The third ongoing HVTN study, HVTN 086, is being conducted in the RSA. It is evaluating the safety and immunogenicity of various combinations of SAAVI DNA-C2, SAAVI MVA-C, and Novartis subtype C gp140/MF59®. This study enrolled 184 subjects. To date, 6 SAEs have been reported in this study, one case of acute tonsillitis that required hospitalization, one of schizophrenia requiring hospitalization (later determined to be a pre-existing condition), one of pelvic inflammatory disease, one soft-tissue injury, one instance of anemia, and one instance of alcohol-related cardiomyopathy. All were assessed as not related to the study products.

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

4.7.5.2 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 one subject following the second protein boost. Similarly in the group of subjects who received subtype B gp140/MF59® without a DNA/PLG prime, a nearly complete response to the SF162 isolate was observed at the second vaccination (all but one subject) which lasted through the third vaccination. Binding Ab titers against Env, measured by enzyme-linked immunosorbent assay (ELISA), were detected following the first subtype B gp140/MF59® boost and were very high following the second boost administration.

The C86P2 MUVAPRED IN study, demonstrated immunogenicity with considerable IgG and IgA Ab responses to subtype B gp140 in serum, cervical, and vaginal secretions of

subjects following IN administration of subtype B gp140 with the adjuvant LTK63 and an IM boost with subtype B gp140 and M59 adjuvant. nAb responses against the homologous SF162 were also detected in all groups following IM boost with subtype B gp140 and MF59® adjuvant.

In addition, although the subtype C gp140/MF59® HVTN 088 long interval boost study is currently ongoing in the long term safety follow up stage, all vaccine administrations have been completed and preliminary results are available. Sixteen previously primed volunteers and 20 naïve volunteers each received 2 doses of the subtype C gp140/MF59® given 6 months apart. HIV-1 specific CD4+ and CD8+ T-cell responses were measured by an ICS assay. Ab responses were measured with a Luminex binding Ab assay and a nAb assay in TZM-bl Cells. Despite the long interval (4-17 years from prior protein/MF59® administration), 31% of primed participants demonstrated CD4+ T-cell responses to Env at baseline, which increased to 75% after a single protein boost. IgG and IgA responses to subtype C gp140/MF59® were present in 64% (IgG) and 7% (IgA) of primed participants at baseline, and rose to 93% and 85%, respectively, after one dose of protein. 71% of primed participants demonstrated nAb against Tier 1 clade B isolate MN at baseline. After a single booster dose of protein, 100% of the primed participants neutralized MN and 93% showed neutralizing activity against a clade C isolate, MW965.26. Unprimed participants did not demonstrate CD4+ responses or Ab responses to Env until after the second dose, which elicited IgG and IgA responses to TV1 trimeric Env in 88% and 50%, respectively. nAbs developed to MN in 38% and to MW965.26 in 88% of the unprimed participants.

See also Section 4.7.1.

4.8 Potential risks of study products and administration

Table 4-14 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®.

Table 4-14 Summary of potential risks of study products and administration

Common (> 10%)	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 antibody test result
Less common (1% to 10%)	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, or bleeding related to the injection procedure
Uncommon (< 1%) or rare (< 0.1%)	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
Unknown frequency or theoretical risks	Muscle damage at the injection site Autoimmune disease Effects on a participant's response to an approved HIV vaccine administered in the future Effects on susceptibility to HIV, if the participant is exposed to HIV Effects on the course of HIV infection/disease, if the participant is infected with HIV Effects on the fetus and on pregnancy

5 Objectives and endpoints

5.1 Primary objectives and endpoints

Part A

Primary objective 1:

To evaluate the safety and tolerability of 2 doses of ALVAC-HIV (vCP2438) followed by 2 doses of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® in HIV-seronegative low risk South African adults

Primary endpoints 1:

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

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

SAEs 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

Primary objective 2:

To evaluate the immunogenicity of 2 doses of ALVAC-HIV (vCP2438) followed by 2 doses of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® in HIV-seronegative low risk South African adults at the month 6.5 timepoint (2 weeks after completion of the primary immunization series)

Primary endpoints 2:

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

Vaccine-induced IgG Ab binding to 3 V1V2-scaffolded Env proteins

Vaccine-induced CD4+ T cell responses to the HIV proteins included in the vaccine

Part B

Primary objective 3:

To evaluate the safety and tolerability of Bivalent Subtype C gp120/MF59 and of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59 when given as boosts at Month 30 in participants previously vaccinated in Part A

Primary endpoints 3:

Number and frequency of severe local and systemic reactogenicity signs and symptoms (pain, tenderness, maximum severity of pain and/or tenderness, erythema, induration, fever, malaise/fatigue, myalgia, headache, nausea, vomiting, chills, arthralgia) occurring within 3 days of each vaccine/placebo dose

AEs by body system, Medical Dictionary for Regulatory Activities (MedDRA) Preferred Term, severity, and assessed relationship to study products occurring within 30 days of each vaccine/placebo dose

SAEs throughout the study

AEs leading to early participant withdrawal or early discontinuation of study product(s) administration 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

Primary objective 4:

To evaluate the immunogenicity of Bivalent Subtype C gp120/MF59 and of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59 when given as boosts at Month 30 in participants previously vaccinated in Part A at 2 weeks following vaccination

Primary endpoints 4:

Occurrence and level of vaccine-induced IgG Ab binding to Env proteins contained in the vaccine

Vaccine-induced IgG Ab binding to V1V2-scaffolded Env proteins

Vaccine-induced CD4+ T cell responses to the HIV proteins included in the vaccine

5.2 Secondary objectives and endpoints

Part A

Secondary objective 1:

To evaluate the safety and tolerability of 2 doses of ALVAC-HIV (vCP2438) followed by 3 doses of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® in HIV-seronegative low risk South African adults

Secondary endpoint 1:

Endpoints as indicated in primary endpoints 1

Secondary objective 2:

To further characterize the HIV-1 specific immunogenicity of ALVAC-HIV (vCP2438) followed by ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59®

Secondary endpoints 2:

Contingent upon results from the primary immunogenicity objective described above (primary objective 2), additional immunogenicity assays may be conducted at the Month 6.5 timepoint, such as occurrence and level of various vaccine-induced immunoglobulin isotypes and HIV-1 nAb

Secondary objective 3:

To compare the immunogenicity of ALVAC-HIV (vCP2438) and ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® in HIV-seronegative low risk South African adults at the Month 12.5 timepoint (2 weeks after booster immunization) to the immunogenicity of the primary regimen at the Month 6.5 timepoint

Secondary endpoint 3:

Endpoints as in primary endpoints 2, and secondary endpoints 2 as applicable, at the Month 6.5 and Month 12.5 timepoints

Part B

Secondary objective 4:

To evaluate the durability of immune responses at Months 30 and 36

Secondary endpoint 4:

Endpoints as in primary endpoints 4 and secondary endpoints 5, as applicable

Secondary objective 5:

To further characterize the HIV-1 specific immunogenicity of ALVAC-HIV (vCP2438) followed by ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® at 2 weeks following vaccination at Month 30

Secondary endpoints 5:

Additional immunogenicity assays based on the HVTN Laboratory Assay Algorithm and as informed by correlates analyses in HVTN 702 (as applicable)

5.3 Exploratory objectives

Exploratory objective 1:

To describe self-reported risk behavior of male and female participants and perceived treatment assignment

Exploratory objective 2:

Additional immunogenicity assays may be performed on blood samples and mucosal samples, including samples from other timepoints, based on the HVTN Laboratory Assay Algorithm

Exploratory objective 3:

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

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target 252 healthy, HIV-uninfected participants aged 18 to 40 years at low risk of HIV infection. 210 participants will receive the ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59® regimen and 42 will receive placebos, randomized in a 5:1 ratio in Part A. To ensure a relative balance in participants of each sex at birth, no more than approximately 60% of trial participants of either sex will be enrolled into the trial in Part A and Part B. Hence, when approximately 150 participants of either sex have been enrolled in Part A (approximately 43 in Part B), recruitment for that sex will stop. In Part B, eligible participants who were randomized to the vaccine (Group 1) in Part A will be randomized to Group 1a or 1b while eligible participants who were randomized to placebo (Group 2) in Part A will continue to receive placebo injections in Part B. The sample sizes for Part B are estimated to be ~30 for each of Groups 1a and 1b and ~12 for Group 2. The availability of immunogenicity data at the later study visits in Part B may be somewhat lower than the projected sample sizes due to dropout over time. Differential dropout across the groups in Part B is not expected due to randomization and blinding.

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 9 phase 1 and 1 phase 2a HVTN trials, which began enrolling after June 2005 (data as of June 2011) indicate that 14% is a reasonable estimate for the rate of immunogenicity missing data at Month 6.5. For this reason, the sample size calculations in Section 6.1.2 account for 14% of enrolled participants having missing data for the primary immunogenicity endpoints as well as for immunogenicity endpoints for Part B.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect SAEs (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 the vaccine arm of the study (n = 210), there is a 90% chance of observing at least 1 event if the true rate of such an event is 1.1% or more; and there is a 90% chance of observing no events if the true rate is 0.05% or less. For the combined vaccine Groups 1a and 1b in Part B of the study with n = 60 in total, there is a 90% chance of observing at least 1 event if the true rate of such an event is 3.8% or more; and there is a 90% chance of observing no events if the true rate is 0.17% or less. As a reference, in HVTN vaccine trials from December 2000 through September 2010, about 4% of participants who received placebos experienced an SAE.

Probabilities of observing 0, 1 or more, and 2 or more events for arms of size 42 and 210 are presented in Table 6-1 for a range of possible true AE 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, for vaccine groups of size 60, (Groups 1a and 1b in Part B) and 210 (Group 1 in Part A), for different true event rates

True event rate (%)	Pr(0/60)	Pr(1+/60)	Pr(2+/60)	Pr(0/210)	Pr(1+/210)	Pr(2+/210)
0.1	0.942	0.058	0.002	0.810	0.190	0.019
0.5	0.740	0.260	0.037	0.349	0.651	0.283
1	0.547	0.453	0.121	0.121	0.879	0.622
4	0.086	0.914	0.698	< 0.001	> 0.999	0.998
10	0.002	0.998	0.986	< 0.001	> 0.999	> 0.999

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval (CI) for the true rate of an AE 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 [95]. If none of the 210 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 1.8%. If none of the 60 participants from Part B Groups 1a and 1b combined experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 6.02%.

Table 6-2 Two-sided 95% CIs based on observing a particular rate of safety endpoints for vaccine groups of size 60 (Groups 1a and 1b combined in Part B) and 210 (Group 1 in Part A)

Observed event rate	CI (%)
0/60	(0.00, 6.02)
1/60	(0.29, 8.86)
2/60	(0.92, 11.36)
0/210	(0.00, 1.80)
1/210	(0.02, 2.65)
2/210	(0.26, 3.41)

6.1.2 Sample size calculations for immunogenicity

The primary immunogenicity objective of this trial is to provide data to guide the decision of whether to advance development of the ALVAC-HIV (vCP2438), Bivalent Subtype C gp120/MF59® regimen. Immunogenicity data from the study regimen will be 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 this phase 1-2 trial will be analyzed contemporaneously using qualified assays in the same laboratories (along with placebo samples for blinding).

Computer simulations (details provided below) were run to determine the sample size of the vaccine regimen for this trial and the RV144 comparator arm based on estimates of the power to conclude the vaccine regimen has potential for clinical development and the probability of incorrectly drawing this conclusion.

With n = 180 evaluable samples for the vaccine regimen under study in this trial and n = 180 for the comparator RV144 arm, there is 91% power to conclude the vaccine regimen has potential for clinical development and there is a 3% probability of erroneously concluding the regimen has potential for clinical development (Figure 6-1). The regimen in this study is assumed to have potential for clinical development if the following conditions hold for the LL of the 95% CI:

Env Ab response rate ≥ 75% for at least 2 of the 3 *env* inserts

GM titer ratio of ≥ 0.5 relative to RV144 samples for at least 2 of the 3 *env* inserts (and above response rate threshold is met for these)

Difference in ALVAC Env-specific CD4 response rate in HVTN 100 and RV144 samples ≥ -30%

Env V1V2 response rate ≥ 45% for at least 1 of the 3 env inserts

In the simulation study, seven binary responses, corresponding to 3 Env inserts, 1 CD4+ T cell and 3 V1V2 variables, were simulated with underlying multivariate normal variables with correlations equal to Spearman correlations estimated from RV144 data. Other correlations were considered but had minimal effect on the results. The RV144 comparator arm results were simulated with underlying multivariate normal variables with parameters estimated in the RV144 correlates analysis [30] with response rates of 92%, 94%, 98%, 71.5%, 63.8% for the 3 Env Ab, 1 CD4+ T cell, and 1 V1V2 variables respectively. The GM titers for the Env insert responses in the RV144 comparator arm were set equal to those estimated in RV144 with GMs=6.31, 6.42, and 8.05 with variance-covariance matrix with rows = (2.02 1.92 1.30), (1.92 1.89 1.32), (1.30 1.32 2.06). For scenarios in which the regimen has the potential for clinical development, the V1V2 and Env insert response rates were set equal to those observed in RV144; the Env insert GMs were set equal to 90% of the responses observed in RV144, and the CD4+ T cell response was set equal to 5% less than the RV144 response rate. For scenarios in which the regimen does not have potential, the response rates for the Env inserts were set equal to 74% (with GMs set equal to 49% of those seen in RV144), the CD4+ T cell response rate was set equal to 31% less than that seen in RV144, and the V1V2 response rates were set equal to 44%, just failing to meet the above conditions. The error rate is then computed as the maximum probability of concluding potential for further development under scenarios where in truth not all 3 of the above conditions are met. The power is computed as the minimum probability of concluding potential for further development under scenarios where in truth all of the above conditions are met.

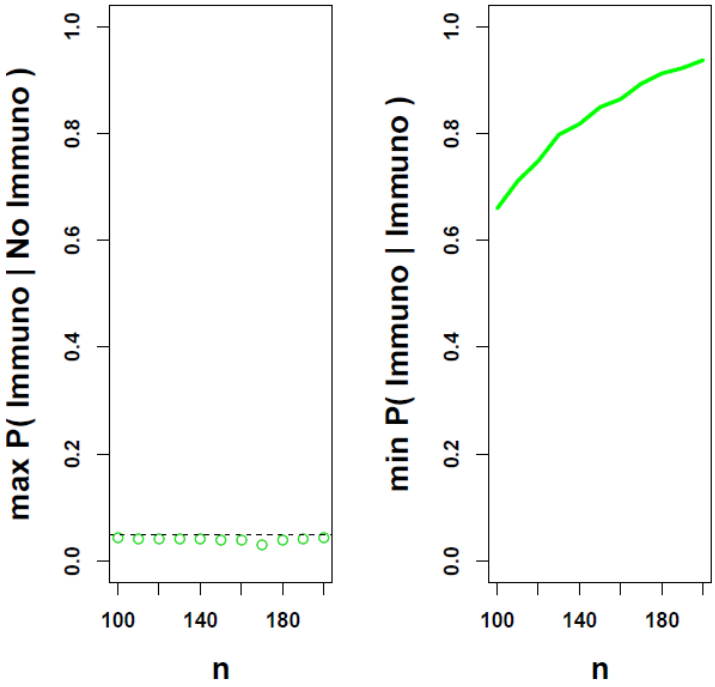


Figure 6-1 Plots display the maximum probability of erroneously concluding the vaccine regimen has potential for clinical development (left panel) and the minimum probability of correctly concluding the vaccine regimen has potential for clinical development (right panel) over a range of sample sizes (n = 100-200).

To provide a sense of the level of precision with which the true immune responses can be estimated with this sample size, CIs for various hypothetical observed response rates are provided in Table 6-3. No adjustment for multiple comparisons will be made for the use of multiple assays. 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 [95]. Note, n = 180 assumes a 14% rate of missing data.

Table 6-3 Two-sided 95% CIs for the true response rate based on observing a particular rate of responses in the vaccinees (n = 180)

No. of responses	Observed response rate (%)	CI
90	50	(42.8, 57.2)
108	60	(52.7, 66.9)
126	70	(62.9, 76.2)
144	80	(73.6, 85.2)
162	90	(84.7, 93.6)

CIs for various hypothetical observed response rates are provided in Table 6-4 for the sample sizes of Groups 1a and 1b in Part B. Table 6-5 contains CIs for the pooled Part B vaccine Groups 1a and 1b. No adjustment for multiple comparisons will be made for the use of multiple assays. Two-sided 95% CIs for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-4 and Table 6-5; calculations are done using the score test method [95]. Note, n = 26 (52) assumes a 14% rate of missing data.

Table 6-4 Two-sided 95% CIs for the true response rate based on observing a particular rate of responses in the Part B Groups 1a and 1b vaccinees (n = 26 each group)

No. of responses	Observed response rate (%)	CI

13	50	(32.1, 67.9)
16	60	(42.5, 77.6)
18	70	(50.0, 83.5)
21	80	(62.1, 91.5)
23	90	(71.0, 96.0)

Table 6-5 Two-sided 95% CIs for the true response rate based on observing a particular rate of responses in the pooled vaccinees in Part B (Groups 1a and 1b combined, n = 52 in total)

No. of responses	Observed response rate (%)	CI
26	50	(36.9, 63.1)
31	60	(46.1, 71.8)
36	70	(55.7, 80.1)
42	80	(68.1, 89.2)
47	90	(79.4, 95.8)

6.2 Randomization

The randomization sequence for Parts A and B will be obtained by computer-generated random numbers and provided to each HVTN CRS through the SDMC's Web-based randomization system. The randomization will be done in blocks to ensure balance across arms. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining blinding of the treatment assignments (except in emergency situations as specified in the HVTN Manual of Operations [MOP]).

6.3 Blinding

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

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

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 100 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. 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 characteristic, safety, and immunogenicity for primary- and secondary-objective analyses.

6.4.2 Baseline comparability

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

6.4.3 Safety/tolerability analysis

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

6.4.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms will be calculated. Wilcoxon rank sum tests will be used to test for differences in severity between arms.

6.4.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment arm the number and percentage of participants experiencing an AE within a System Organ Class or within Preferred Term 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 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 timepoint, as well as changes from baseline for postenrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the DAIDS AE Grading Table (see Section 9.9) will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

6.4.3.4 Reasons for vaccination discontinuation and early study termination

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

6.4.4 Immunogenicity analysis

6.4.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Additional analyses will also 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 [95]. No adjustment will be made to the vaccine arm estimates for the false positive rates in the placebo arms.

For quantitative assay data (eg, GM Ab titers from the binding Ab multiplex assay [BAMA]), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. An appropriate data transformation (eg, $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance).

The 95% CIs for the primary immunogenicity endpoints will be examined to determine whether the conditions are met to indicate that the vaccine regimen has the potential for clinical development.

For the secondary objective regarding the comparison of immunogenicity at Month 6.5 and Month 12.5, 95% CIs for the difference in response rates and in the quantitative responses will be provided.

More sophisticated analyses employing repeated measures methodology (for example, repeated measures analysis of variance [ANOVA] or generalized estimating equations) may be utilized to incorporate immune responses over several timepoints and to test for differences over time. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05$.

Based upon previous early phase HVTN trials in the RSA, missing up to 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 [96] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [97] methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

6.4.5 Analyses prior to end of scheduled follow-up visits

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

6.4.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared for review by the DSMB, which will evaluate the safety and integrity of the ongoing trial periodically.

6.4.5.2 Immunogenicity

An unblinded statistical analysis by treatment assignment for the primary immunogenicity endpoints will be performed when all participants have completed the primary immunogenicity visit (Month 6.5) and the data are available for analysis from all of these participants. An unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after all of the primary immunogenicity analyses are complete. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key stakeholders. 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 for Part A

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** (per Low Risk Guidelines for South African sites, available on the HVTN 100 protocol web page)

and committed to maintaining behavior consistent with low risk of HIV exposure through the last required protocol clinic visit

Laboratory Inclusion Values

Hemogram/CBC

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

11. **WBC** = 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 urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination or negative serum beta human chorionic gonadotropin (β-HCG)

pregnancy test within 24 hours prior to initial vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

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

    - Agree to consistently use effective contraception (see Appendix C) for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment through the last required protocol clinic visit. Effective contraception for participants in South Africa is defined as using 2 methods, including the following:
        - 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 100 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

7.2 Exclusion criteria for Part A

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 study that includes HIV testing during the planned duration of the HVTN 100 study

5. **Pregnant, breastfeeding, or lactating**

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 100 PSRT will determine eligibility on a case-by-case basis.

7. **Non-HIV experimental vaccine(s) received within the last 5 yea**rs in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure by the South Africa Medicines Control Council (MCC). For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 100 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 100 PSRT on a case-by-case basis.

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

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

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

Immune System

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

12. **Serious adverse reactions to vaccines or to vaccine components such as eggs, egg products, or neomycin,** including history of anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain. (Not excluded 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 or moderate, 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 high dose inhaled corticosteroids, or
    - In the past year has either of the following:
        - Greater than 1 exacerbation of symptoms treated with oral/parenteral corticosteroids;
        - Needed emergency care, urgent care, hospitalization, or intubation for asthma.

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

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

24. **Hypertension**:

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

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

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

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

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

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

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

7.3 Eligibility criterion for Part B

HVTN 100 study participants who have met neither discontinuation of vaccination criteria described in Section 7.4.3 nor criteria for termination from the study as described in Section 7.4.4. For those participants who experienced a prior SAE, consultation with the HVTN 100 PSRT is required prior to subsequent vaccinations in Part B.

Participants who enter Part B under protocol Version 3.0 must agree to provide mucosal samples, as described in Section 9.5.

7.4 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.

7.4.1 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)
- Prevaccination 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; in this circumstance, the HVTN 100 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 100 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.

7.4.2 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 7.4.1 and 7.4.3).

7.4.3 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 100 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:
  - 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; or
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 100 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).

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 7.4.4 and 9.6).

7.4.4 Participant termination from the study

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

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

8 Study product preparation and administration

CRS pharmacists should consult the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for standard pharmacy operations. The protocol schema is shown in Table 3-1 and Table 3-2 (Part B). See the IBs 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.

8.1.1 Part A

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

Placebo 2 (P2): Placebo for ALVAC-HIV to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, 6 and 12

AND

Placebo for Bivalent Subtype C gp120/MF59® administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Months 3, 6 and 12.

8.1.2 Part B

Group 1a

Treatment 1a (T1a): ALVAC-HIV (vCP2438) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at Month 30

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 Month 30

Group 1b

Treatment 1b (T1b): Placebo for ALVAC-HIV (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at Month 30

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 Month 30

Group 2

Placebo 2 (P2): Placebo for ALVAC-HIV (Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM in LEFT deltoid (unless medically contraindicated) at Month 30

AND

Placebo for Bivalent Subtype C gp120/MF59® (Sodium Chloride for Injection, 0.9%) to be administered as 0.5mL IM in RIGHT deltoid (unless medically contraindicated) at Month 30

8.2 Study product formulation

8.2.1 Part A

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

ALVAC-HIV (vCP2438) is provided as a lyophilized, white to beige product. It must be stored refrigerated (2-8°C). DO NOT FREEZE. Once reconstituted with 1mL of Diluent 0.4% NaCl, it appears as a clear to slightly opalescent solution, colorless with possible presence of particles or filaments.

Placebo for ALVAC-HIV [Labeled as ALVAC-HIV Placebo]

Placebo for ALVAC-HIV is a sterile, lyophilized product that consists of a mixture of virus stabilizer, and freeze drying medium. It is provided as a lyophilized, white to beige product. It must be stored refrigerated (2-8°C). DO NOT FREEZE. Once reconstituted with 1mL of Diluent 0.4% NaCl, it appears as a clear to slightly opalescent solution, colorless with possible presence of particles or filaments.

Diluent for ALVAC-HIV (vCP2438) and Diluent for Placebo for ALVAC-HIV [Labeled as Diluent 0.4% NaCl]

The diluent is provided in a vial filled with 1.2 -1.3 mL of sterile sodium chloride solution (NaCl 0.4%). It must be stored refrigerated (2-8°C). DO NOT FREEZE.

Bivalent gp120 composed of two different proteins:

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

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

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

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

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

Placebo for Bivalent Subtype C gp120/MF59® (Sodium Chloride for Injection, 0,9%)

Sodium Chloride for Injection, 0,9% will be used for the Bivalent Subtype C gp120/MF59®. Product must be stored as directed by the manufacturer.

8.2.2 Part B

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 1mL of Diluent 0.4% NaCl, it appears as a clear to slightly opalescent solution, colorless with possible presence of particles or filaments.

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

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

The diluent is provided in a vial filled with a volume to deliver 0.5 mL of sterile sodium chloride solution (NaCl 0.4%). Two vials will be needed to reconstitute each vial of ALVAC-HIV (vCP2438). It must be stored refrigerated (2-8°C).

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

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

Bivalent gp120 composed of two different proteins:

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

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

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

MF59® [labeled as MF59C.1] is supplied as an oil-in-water emulsion. The MF59 adjuvant 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. Protect from light. Do not freeze.

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

Placebo for Bivalent Subtype C gp120/MF59® (Sodium Chloride for Injection, 0,9%)

Sodium Chloride for Injection, 0,9% will be used for the Bivalent Subtype C gp120/MF59®. Product must be stored as directed by the manufacturer.

8.3 Preparation of study products

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

8.3.1 ALVAC-HIV (vCP2438)

8.3.1.1 Part A

One vial of ALVAC-HIV (vCP2438) and 1 vial 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 1 mL from the vial containing diluent (NaCl 0.4%) and slowly inject (the 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. (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 pharmacist will apply an overlay to the syringe.

The syringe should be labeled as “ALVAC-HIV or placebo 1 mL” as well as “Administer in Left Deltoid”. The study product should be administered within 2 hours of the time the vaccine is reconstituted.

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

8.3.1.2 Part B

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 pharmacist will apply an overlay to the syringe.

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

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

8.3.2 Placebo for ALVAC-HIV

8.3.2.1 Part A

One vial of Placebo for ALVAC-HIV and 1 vial of diluent (NaCl 0.4%) is 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 1 mL from the vial containing diluent (NaCl 0.4%) and slowly inject the diluent into the vial containing the lyophilized placebo. The pharmacist will then set the vial aside and allow the vial to sit for up to 3 minutes to allow for dissolution. The pharmacist will gently swirl the vial to assure the contents are well dissolved. DO NOT SHAKE. (Note: Presence of particles or filaments in the dissolved solution is possible). Using aseptic technique, the pharmacist will then withdraw the entire contents for the Placebo for ALVAC-HIV vial into a 2, 3, or 5 mL syringe. The pharmacist will apply an overlay to the syringe.

The syringe should be labeled as "ALVAC-HIV or placebo 1 mL" as well as "Administer in Left Deltoid". The study product should be administered within 2 hours of the time the vaccine is reconstituted. Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in accordance with institutional or pharmacy policy for a biological safety level 1 product.

8.3.2.2 Part B

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

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

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

8.3.3 Bivalent Subtype C gp120/MF59 vaccine

8.3.3.1 Part A

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

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

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

8.3.3.2 Part B

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

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

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

The syringe should be labeled as "Bivalent Subtype C gp120/MF59 or Placebo" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid. The syringe containing study product should be bagged for transport to the clinic where it will be administered. This study product should be administered immediately, defined as within 30 minutes as per Immunization Action Coalition (IAC) recommendations. If this is not possible, the site pharmacist may be permitted to label the syringe with a DO NOT USE AFTER 2 hour date and time from preparation. In this case, the vaccine should be stored at 2°C-8°C until administration and if not used within 2 hours, it should be discarded.

8.3.4 Placebo for Bivalent Subtype C gp120/MF59 vaccine

8.3.4.1 Part A

Using aseptic technique, the pharmacist will withdraw 0.5 mL of Sodium Chloride for Injection, 0,9% into a 1 or 2 mL syringe.

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

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

8.3.4.2 Part B

Using aseptic technique, the pharmacist will withdraw 0.5 mL of Sodium Chloride for Injection, 0,9% into a 1 or 2 mL syringe. The needle should be removed and the syringe capped.

The syringe should be labeled as "Bivalent Subtype C gp120/MF59 or Placebo" and have an overlay to maintain blinding. The syringe must also be labeled for administration in RIGHT deltoid. The syringe containing study product should be bagged for transport to the clinic where it will be administered. In order to maintain blinding and be consistent with the active product, this study product should be administered immediately, defined as within 30 minutes as per IAC recommendations. If this is not possible, the site pharmacist may be permitted to label the syringe with a DO NOT USE AFTER 2 hour date and time from preparation. In this case, the study product should be stored at 2°C-8°C until administration and if not used within 2 hours, it should be discarded.

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

8.3.5 Procedures to preserve blinding

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

8.4 Administration

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

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

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

For all syringes containing Bivalent gp120/MF59 or placebo, the person administering the injection should gently roll the syringe prior to administration of the 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

Part A

The ALVAC-HIV (vCP2438) or Placebo was manufactured by IDT Biologika for Sanofi Pasteur. It will be provided (along with the Diluent) by Sanofi Pasteur.

Bivalent Subtype C gp120 and MF59® will be provided by Novartis Vaccines and Diagnostics, Inc.

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0,9%) will not be provided through the protocol and must be obtained by the site.

Part B

The ALVAC-HIV (vCP2438) and Diluent (0.4% NaCl) will be provided by Sanofi Pasteur.

Bivalent Subtype C gp120 and MF59® will be provided GlaxoSmithKline Biologicals, S.A.

Placebo for Bivalent Subtype C gp120/MF59 (Sodium Chloride for Injection, 0,9%) and Placebo for ALVAC-HIV (Sodium Chloride for Injection, 0,9%) will not be provided through the protocol and must be obtained by the site.

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

8.6 Pharmacy records

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

8.7 Final disposition of study products

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

9 Clinical procedures

The schedules of clinical procedures are shown in Appendix H and Appendix I.

9.1 Informed consent

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

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

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

Note: As defined in the DAIDS Protocol Registration Manual, an RE is "Any group other than the local IRB/EC responsible for reviewing and/or approving a clinical research protocol and site-specific 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 Protocol Registration Office's Regulatory Support Center (RSC).

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 consent addendum for participants who will enter Part B of the protocol is located in Appendix B.

An optional separate consent form for other uses of samples is located in Appendix E. Note that participants need only sign the consent addendum in Appendix B in order to enter Part B of the study.

Each HVTN CRS is responsible for developing protocol-specific consent forms for local use, based on the sample protocol-specific consent forms in Appendix A, Appendix B, and Appendix E. The consent forms 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, Code of Federal Regulations (CFR) Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonisation (ICH) E6, *Good Clinical Practice: Consolidated Guidance* 4.8.

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

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

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

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

9.1.3 Assessment of Understanding

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

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

IRB/EC and any applicable RE may require that a participant has signed either a screening or protocol-specific consent document prior to administering the Assessment of Understanding. The consent process (including the use of the Assessment of Understanding) should be explained thoroughly to the IRB/EC and any applicable RE, whose recommendations should be followed.

9.2 Pre-enrollment procedures

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

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

- Medical history, documented in the case history record;
- Assessment of Understanding (see Section 9.1.3);
- Assessment of whether the volunteer is at low risk for HIV infection;
- Complete physical examination, including height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots;
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - CBC with differential and platelet count,
  - Chemistry panel (ALT, AST, ALP, and creatinine),
  - Urine dipstick (urinalysis if indicated, see Section 9.8),
  - HBsAg,
  - Anti-HCV Ab,
  - Syphilis test,
  - Urine or serum pregnancy test (volunteers who were born female);
- Administration of behavioral risk assessment questionnaire;
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html);
- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.6; and

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

9.2.1 Use of screening results from another HVTN study

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

9.2.2 Part B eligibility visit

Prior to administration of study injections at Month 30, the eligibility of Part A participants to continue into Part B of the study is assessed. This assessment can take place over the course of several contacts/visits, up to and including before vaccination at Month 30.

After the appropriate informed consent has been obtained (see Appendix B) and before study product administration in Part B, the following procedures are performed:

- Assessment of Understanding (see Section 9.1.3);
- 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 concomitant medications (as described in Section 9.2);
- Assessment of new or unresolved AEs/intercurrent illnesses;
- 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);
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the placebo;
- HIV infection assessment including pretest counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Laboratory tests, including:
    - HIV diagnostic test,

  - CBC with differential and platelet count,
  - Chemistry panel (ALT, AST, ALP, and creatinine),
  - Urine dipstick (urinalysis if indicated, see Section 9.8),
  - Urine or serum pregnancy test (volunteers who were born female);
- Risk reduction counseling (as described in Section 9.6); and
- Pregnancy prevention assessment (as described in Section 9.2 and 9.7).

After eligibility has been confirmed, the participant may be randomized for Part B.

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

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

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

Immediately following vaccination, the participant remains in the clinic for observation. An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes. Before leaving the clinic, the participant is given the postvaccination symptom log and is instructed on how to complete it. The site will make arrangements to obtain daily reports of reactogenicity events from the participant during the reactogenicity period (as described in Section 9.9).

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

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

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

- 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
- Specimen collection, blood and/or mucosal (should be completed prior to vaccination)
- For participants entering Part B (see Appendix I):
    - Urine test or swab for gonorrhea and chlamydia;
    - Vaginal swab for Trichomonas and bacterial vaginosis (for participants providing cervical samples);
    - Vaginal swab (if indicated) for hyphae/budding yeast (for participants providing cervical samples);
    - Syphilis serology; and
    - Mucosal specimen collection.

9.4 Follow-up visits

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

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

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

- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the placebo;
- Administration of behavioral risk assessment questionnaire;
- 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;
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Specimen collection (blood and/or mucosal);
- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.8).
- For participants entering Part B (see Appendix I):
  - Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.
  - Urine test or swab for gonorrhea and chlamydia;
  - Vaginal swab for Trichomonas and bacterial vaginosis (for participants providing cervical samples);
  - Vaginal swab (if indicated) for hyphae/budding yeast (for participants providing cervical samples);
  - Syphilis serology; and
  - Mucosal specimen collection.

9.5 Mucosal sampling

Mucosal samples will be collected at the timepoints indicated in Appendix G and Appendix I from study participants who enter Part B under protocol Version 3.0.

Participants providing cervical, rectal, or semen samples will be tested for the following infections at visits specified in Appendix G and Appendix I: gonorrhea, chlamydia, and syphilis. Participants providing 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 a herpes simplex virus (HSV)-2 outbreak or inflamed hemorrhoids or colitis/diarrhea] or if the participant has any active genital tract infection (GTI).

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

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

Cervical fluid sampling (only for participants who were born female): Participants who are 21 years of age and older must report having had a Pap smear within:

- the 3 years prior to sample collection with the latest result reported as normal or ASCUS (atypical squamous cells of undetermined significance), OR
- the 5 years prior to sample collection, with the latest result reported as normal, or ASCUS with no evidence of high risk HPV.

If no Pap smear was done within the last 3 years prior to sample collection (or within the last 5 years, if high risk HPV testing was performed), the volunteer must have a Pap smear with the result reported as normal or ASCUS prior to sample collection.

A pregnancy test must be performed on the day of cervical sampling. The pregnancy test can be performed after collection has taken place. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Cervical sampling should be deferred if a participant is menstruating, but should be performed as soon as possible, if still within the visit window. In addition, cervical sampling will not be

performed (or may be deferred to a later date if still within the visit window) if a participant is known to have an active ulcerative genital lesion or an active GTI at the scheduled timepoint. Participants providing cervical secretion samples should be advised as follows:

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

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

9.6 HIV counseling and testing

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

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

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

Potential participants identified as being HIV infected during screening are not enrolled. All participants who become HIV infected during the study will be terminated from this study. Potential and enrolled participants identified as HIV infected will be referred for

medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.6.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

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

- Participants will have physical examinations at visits specified in Appendix H and Appendix I. 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 and Appendix G). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the HVTN 100 Study Specific Procedures), which is able to distinguish vaccine-induced Ab responses from actual HIV infections.
- All participants can receive HIV-1 diagnostic testing from the site following their last scheduled visit until they are told that they did not receive an HIV vaccine or that they do not have vaccine-induced seropositivity.
- All participants who received vaccine product and who have vaccine-induced positive or indeterminate HIV-1 serology (as measured by the standard anti-HIV Ab screening tests) at or after the study is unblinded will be offered poststudy HIV-1 diagnostic testing (per the HVTN poststudy HIV-1 testing algorithm) periodically and free of charge as medically/socially indicated (approximately every 6 months).

9.6.2 VISP registry

Experimental HIV vaccines may induce Ab production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP) (see Section 9.6.1). In order to provide poststudy HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of poststudy testing and counseling services to HIV vaccine recipients. The registry contains the names of all study participants, unless they request that their names be removed.

9.7 Contraception status

Contraception status is assessed and documented at every scheduled clinic visit for a participant who was born female and who is sexually active in a way that could cause that participant to become pregnant. Prior to enrollment and throughout the study, staff will ask participants to verbally confirm their use of adequate contraceptive methods. A participant who was born female and is sexually active in a way that could cause that

participant to become pregnant should be reminded at all scheduled clinic visits of the importance of using contraception and should be referred to specific counseling, information, and advice as needed. (Specific contraception requirements are listed in Section 7.1). This reminder should be documented in the participant's study record.

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

9.8 Urinalysis

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

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

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

9.9 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), 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 are instructed to record symptoms using a postvaccination symptom log and to contact the site daily during the assessment period. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 3 to resolution. Participants are instructed to contact the clinic for events that arise during the period between vaccination and the next scheduled visit. In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved.

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

Table 9-1 Schedule of reactogenicity assessments

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

[a] Day of vaccination

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

9.9.1 Assessment of systemic and local symptoms

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

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

9.9.2 Assessment of injection site

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

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

9.9.3 Assessment of lymph nodes

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

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

9.10 Visit windows and missed visits

Visit windows are defined in HVTN 100 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 7.4.2 and Section 7.4.3 for resolution.

9.11 Early termination visit

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

9.12 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given during the pregnancy, but remaining visits and study procedures should be completed unless medically contraindicated. For participants who are no longer pregnant, see Section 7.4.1. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome.

10 Laboratory

10.1 HVTN CRS laboratory procedures

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

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

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

10.2 Total blood volume

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

10.3 Primary immunogenicity timepoint

The primary immunogenicity timepoint for Part A is at visit 10 (day 182) (ie, 2 weeks after the fourth vaccination visit) and for Part B at visit 19 (ie, 2 weeks after the sixth 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 in the primary and secondary endpoints, assays for humoral and cellular responses may be performed on participants at other timepoints; the schedule is shown in Appendix F and Appendix G.

10.4 Endpoint assays: humoral

10.4.1 HIV-1 multiplex Ab assay

Total binding IgG and IgA Ab to HIV Env proteins contained in the vaccine regimen will be assessed on plasma/serum samples from study participants taken at the primary immunogenicity timepoint and baseline. In addition, binding to cross-clade Env proteins and IgG isotypes will be assessed. Specimens from other timepoints as well as other HIV antigens may also be assayed based on the results of the initial assay. Mucosal samples collected at the M30 and M30.5 visits (Visits 17 and 19) may also be evaluated using this assay.

10.4.2 HIV-1 nAb assays

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.

10.5 Endpoint assays: cellular

10.5.1 Flow cytometry

Flow cytometry will be used to examine vaccine-specific CD4+ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins in the vaccine. Data for CD8+ T-cell responses will also be collected in the same assay. ICS parameters will include cytokines such as IFN-γ, interleukin 2 (IL-2), and tumor necrosis factor alpha (TNF-α), and may include other cytokines to identify T cells of specific functionality (such as Th2 and Th17). Data will be reported as percentages of CD4+ T cells responding to a specific peptide pool. Additional cell surface markers, cytokines, or functional markers may also be analyzed to assess additional T and B cell phenotypes.

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

10.7 Lab assay algorithm

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

10.8 Exploratory studies

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

10.9 Other use of stored specimens

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

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

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

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

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

10.10 Biohazard containment

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

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

11 Safety monitoring and safety review

11.1 Safety monitoring and oversight

11.1.1 HVTN 100 PSRT

The HVTN 100 PSRT is composed of the following members:

- DAIDS medical officer representative,
- Protocol chair and cochair,
- Protocol Team leader,
- Core medical monitor,
- Regional medical liaison, and
- Clinical safety specialist.

The clinician members of HVTN 100 PSRT listed above are responsible for decisions related to participant safety.

A medical officer from an in-country organization 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 100 PSRT meetings.

11.1.2 DSMB review of cumulative safety data

The DSMB assesses the effects of the study vaccine during the trial and may give advice to the HVTN 100 Protocol Team.

The DSMB will periodically review accumulating unblinded safety data by group. Prior to each meeting, the SDMC will provide the DSMB with data as described in Section 6.4.5.1. Reports will be cumulative, generated from an up-to-date data file.

Based upon the reports, the DSMB will determine whether to recommend that the study should be continued, modified, or stopped for safety reasons.

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 100 PSRT and DSMB (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 100 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 100 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 refaxed before the end of the next business day after receiving the new information.

11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.1 [March 2017], available on the RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables, 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 100 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);

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 MCC (Section 11.2.3) and (2) if the AE meets the criteria for a safety pause/prompt AE review (Section 11.3).

AEs leading to early participant withdrawal or early discontinuation of study product(s) administration will be collected and reported throughout the study.

Sites are expected to notify the CSS of any serious safety concern requiring their attention (see Table 11-1). Telephone numbers and email addresses are listed in the Key Resource Guide of the HVTN 100 Study Specific Procedures. 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 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/MCC

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

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

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

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

An AE is considered to be an SAE by the most recent version of the MCC guidelines as described in *Reporting Adverse Drug Reactions in South Africa* (http://www.mccza.com/documents/ae9635a42.11_ADR_reporting_May03_v1_2.pdf), if it:

- results in death,
- is life-threatening,
- 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.

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.

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 include blood dyscrasias or convulsions not resulting in hospitalization, or development of drug dependency or drug abuse.

The sponsor or designee(s) prepares and files expedited reports to appropriate regulatory authorities and ECs within the timelines required by the South African MCC guidelines, which are detailed in *Reporting Adverse Drug Reactions in South Africa*, Version 1.2, December 2012.

Any SAE that is considered unexpected and for which the contribution of the study products cannot be ruled out will qualify for expedited reporting to the South African Medicines Control Council (MCC). 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 MCC are:

- ALVAC-HIV (vCP2438)
- Placebo for ALVAC-HIV (vCP2438)
- Bivalent Subtype C gp120/MF59®
- Placebo for Bivalent Subtype C gp120/MF59®

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.

11.3 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 100 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 100 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

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

Event and relationship to study products	Severity	HVTN CRS action	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and fax forms immediately[a]	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and fax forms immediately[a]	Immediate HVTN 100 PSRT notification
SAE, related	Grade 3	Email and fax forms immediately	Prompt HVTN 100 PSRT AE review to consider pause
AE[b], related	Grade 4 or Grade 3	Email and fax forms immediately	Prompt HVTN 100 PSRT AE review to consider pause

[a] Phone numbers and email addresses are listed in HVTN 100 Study Specific Procedures, Key Resource Guide.

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

For all safety pauses, HVTN Core notifies the HVTN 100 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 DSMB.

Once a trial is paused, the HVTN 100 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the DSMB 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 100 PSRT assessment, the trial sponsor or designee(s) notifies the South Africa MCC as needed.

If an immediate HVTN 100 PSRT notification or prompt HVTN 100 PSRT AE review is triggered, HVTN Core notifies the HVTN 100 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 100 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 IND safety reports, notification of vaccine holds due to the pause rules, and notification of other unplanned safety pauses). CRSs must also follow all applicable RE reporting requirements.

In addition, all other AEs are reviewed routinely by the HVTN 100 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 the HVTN Core for events requiring expedited reporting, and events that meet safety pause criteria or prompt HVTN 100 PSRT AE review criteria.

11.4.2 Weekly review

During the injection phase of the trial, the HVTN 100 PSRT review clinical safety reports on a weekly basis and conduct 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 100 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 100 PSRT, MCC, 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 100 *Study Specific Procedures.*

12.1 Social impacts

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

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

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

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

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

12.3 Specific regulatory considerations for South Africa

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 100 clinical trial, to obtain approval for the proposed clinical trial and for the importation of the study products.

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 100 are described below.

Protocol history and modifications

Date: May 23, 2017

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

Item 1 Revised: Vaccination and visit schedules

Item 2 Added: Mucosal secretion sampling

Item 3 Added to Part B visit schedule: One week post-vaccination visit

Item 4 Revised: Appendix B, *Addendum to Sample informed consent form (for Part B)*

Item 5 Revised in Appendix D, *Tables of procedures (for sample informed consent forms)*: Part B procedure tables

Item 6 Revised in Appendix G, *Laboratory procedures for Part B*: Mucosal sampling, STI, testing, and follow-up duration

Item 7 Revised in Appendices F and G: Endpoint laboratory designations

Item 8 Corrected in Appendices F and G: Neutralizing antibody assay

Item 9 Revised in Appendix I, *Procedures at HVTN CRS for Part B*: Mucosal sampling, STI, testing, and follow-up duration

Item 10 Removed in Section 8.2.2, *Part B*: Do not freeze instruction

Item 11 Revised in Sections 8.3.1.2 and 8.3.2.2: Time limit within syringe for administration of ALVAC-HIV study product or placebo

Item 12 Clarified in Section 9.1.2, *Protocol-specific consent forms*: Participants entering Part B

Item 13 Removed in Section 9.2, *Pre-enrollment procedures*: Requirement to record generic medication names

Item 14 Updated in Section 9.6.1: Reference for HVTN HIV testing algorithm

Item 15 Updated in Section 9.9, *Assessments of reactogenicity*, Section 11.2.2, *AE reporting* and Section 14, *Document references (other than literature citations)*: AE grading table version and exceptions

Item 16 Updated in Section 10.1: Source for site lab instructions

Item 17 Clarified in footnotes to Appendices G and I: Window for specimen collection prior to Part B vaccination

Item 18 Removed in Appendix I: Confirmation HIV results provided to participant at Month 36 study visit

Item 19 Clarified in Appendix I footnote "e": Pregnancy test results required prior to vaccination

Item 20 Added as Appendix J: Protocol signature page

Item 21 Updated in Sections 3.1, 11.1.1, and 15: Protocol team

Item 22 Updated throughout protocol: Literature reference numbering, cross-references, and document URLs

Item 23 Updated per Clarification Memo 1 to Version 2.0: Part B visit numbering

Item 24 Corrected per Clarification Memo 1 to Version 2.0: Behavioral risk assessment timepoints

Item 25 Corrected per Clarification Memo 1 to Version 2.0: UW-VSL removed from Appendix G footnote

Item 26 Updated in boxed text: Summary of revisions

Item 27 Updated in Section 13: Version history

Item 28 Minor typographical and formatting errors have been corrected throughout the protocol document

Date: December 20, 2016

Protocol version: Version 2.0
Protocol modification: Clarification Memo 1

Item 1 Updated in Appendices G and I : Visit numbering

Item 2 Corrected in footnote 2 to Appendix G, *Laboratory procedures for Part B*: UW-VSL removed

Item 3 Corrected in Appendix I, *Procedures at HVTN CRS for Part B*: Behavioral Risk Assessment timepoints

Date: September 9, 2016

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

Item 1 Added as HVTN 100, Part B: Evaluation of booster vaccinations at Months 24 and 36

Item 2 Added following Title page: Brief amendment description

Item 3 Version 1.0 content marked Part A

Item 4 Updated in Section 3, *Overview*: Part B primary objectives, schema, participants description, study duration, and endpoint assay laboratories

Item 5 Updated in Section 3, *Overview*, Section 4.4.3, *Choice of placebo*, and Section 8.2, *Study product formulation*: ALVAC-HIV placebo in Part B

Item 6 Updated in Section 3.1, *Protocol team*: Team members and team roles

Item 7 Updated on Title page and in Section 3, *Overview*: Study product provider

Item 8 Added: Section 4.1.4, *Rationale for Part B*

Item 9 Updated in Section 4.2, *ALVAC-HIV (vCP2438)*: Formulation and manufacture

Item 10 Updated in Section 4.3.4, *Bivalent Subtype C gp120/MF59® for injection*: Buffer quantities

Item 11 Added in Section 4.5, *Plans for future product development and testing*: Potential impact of Part B results

Item 12 Added in Section 4.6, *Nonclinical studies* and Section 4.6.3, *Toxicity studies of HIV Env vaccines*: Study AB20670

Item 13 Added as new Section 4.7.1, *HVTN 100 experience to date*: Summary of interim HVTN 100 safety and immunogenicity results

Item 14 Updated in Sections 4.7.2 through 4.7.5: Clinical experience

Item 15 Updated in Section 4.8, *Potential risks of study products and administration*: Table 4-14

Item 16 Added in Section 5, *Objectives and endpoints*: Part B primary and secondary objectives and endpoints

Item 17 Added in Section 6.1, *Accrual and sample size calculations* and Section 6.2, *Randomization* for Part B

Item 18 Added as Section 7.3: *Eligibility criterion for Part B*

Item 19 Added in Section 7.4.3, *Discontinuing vaccination for a participant*:

Item 20 Updated in Section 8, *Study product preparation and administration*:

Item 21 Added in Section 9, *Clinical procedures*: Part B clinical procedures

Item 22 Updated in Section 10, *Laboratory*: Primary timepoints and cross-references to Laboratory procedures appendices

Item 23 Update in Section 13: *Version history*

Item 24 Updated in Section 14: *Document references*

Item 25 Updated in Section 16: *Literature cited*

Item 26 Added as Appendix B: *Addendum to Sample informed consent form*

Item 27 Added to Appendix D, *Tables of procedures (for sample informed consent forms)*: Part B table of procedures

Item 28 Added as Appendix G: *Laboratory procedures for Part B*

Item 29 Clarified in Appendix H: Unblinding

Item 30 Added as Appendix I: *Procedures at HVTN CRS for Part B*

Item 31 Corrected per Clarification Memo 1: Study day for visit 13 Part A Laboratory and CRS procedures Appendices

Item 32 Clarified per Clarification Memo 2: Serum pregnancy tests prior to vaccination, screening HIV infection assessment, and provision of test results to participants

Item 33 Updated per Clarification Memo 3: DAIDS AE Grading Table version

Item 34 Clarified per Clarification Memo 4: Exceptions to AE reporting

Item 35 Minor typographical and formatting errors have been corrected throughout the protocol document

Date: April 2, 2015

Protocol version: Version 1.0
Protocol modification: Clarification Memo 4

Item 1 Clarified: Exceptions to Version 2.0 of the DAIDS AE Grading Table

Date: February 26, 2015

Protocol version: Version 1.0
Protocol modification: Clarification Memo 3

Item 1 Updated: References to DAIDS AE Grading Table

Date: February 5, 2015

Protocol version: Version 1.0
Protocol modification: Clarification Memo 2

Item 1 Clarified: Time limit for pregnancy tests prior to vaccination

Item 2 Corrected in Appendix F, *Procedures at HVTN CRS*: Confirm HIV test results provided to participant at Visit 15

Date: November 13, 2014

Protocol version: Version 1.0
Protocol modification: Clarification Memo 1

Item 1 Corrected in Appendix E, *Laboratory Procedures* and Appendix F, *Procedures at HVTN CRS*: Study day for Visit 13

Date: May 5, 2014

Protocol version: 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 Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 [March 2017] Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual
- *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
- *Republic of South Africa: National Contraception Clinical Guidelines.* December 2012.
- *Ethics in Health Research: Principles, Processes and Structures (Second edition, 2015)*, Department of Health, Republic of South Africa. Available at http://www.nhrec.org.za/docs/Documents/EthicsHealthResearchFinalAused.pdf
- *Declaration of Helsinki (last updated October 2013)*, World Medical Association. Available at http://www.wma.net/en/30publications/10policies/b3/
- *Guidelines on Ethics for Medical Research: HIV Preventive Vaccine Research.* Available at http://www.mrc.ac.za//ethics/ethicsbook5.pdf
- *South Africa Genetically Modified Organism (GMO) Act 15 of 1997.* Available at http://www.saflii.org/za/legis/num_act/gmoa1997286/

- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 100 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 100 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN 100 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
- International Conference on Harmonisation (ICH) E6 (R1), Guideline for Good Clinical Practice: Section 4.8, Informed consent of trial subjects. Available at http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html
- Participants' Bill of Rights and Responsibilities. Accessible through the HVTN website.
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 2016. Available at http://osp.od.nih.gov/sites/default/files/resources/NIH_Guidelines.pdf
- NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research. Available at http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html
- Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks, July 2008.
- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://www.niaid.nih.gov/sites/default/files/documents/daids-sourcedocpolicy.pdf
- Title 21, Code of Federal Regulations, Part 50. Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50
- Title 45, Code of Federal Regulations, Part 46. Available at http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html
- Immunization Action Coalition, *Vaccines with Diluents: How to Use Them*. Available at http://www.immunize.org/catg.d/p3040.pdf
- US Centers for Disease Control and Prevention. Vaccine Storage & Handling Toolkit, June 2016. Available at http://www.cdc.gov/vaccines/hcp/admin/storage/toolkit/storage-handling-toolkit.pdf

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

15 Acronyms and abbreviations

Ab	antibody
ADCC	antibody-dependent cellular cytotoxicity
ADCVI	antibody dependent cellular viral inhibition
AE	adverse event
ALT	alanine aminotransferase
ANOVA	analysis of variance
ART	antiretroviral therapy
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
CBC	complete blood count
CCID	cell culture 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 intervals
CoR	correlate(s) of risk
CPK	creatine phosphokinase
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
CSS	Clinical safety specialist
CTL	cytotoxic T lymphocyte
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
DHVI	Duke Human Vaccine Institute
DOV	discontinuation of vaccination
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
EU	European Union
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
HBsAg	hepatitis B surface antigen
HCRISA	Hutchinson Centre Research Institute of South Africa
HCV	hepatitis C virus
HLA	human leukocyte antigen
HPTN	HIV Prevention Trials Network
HSML-NICD	HIV Sero-Molecular Laboratory, National Institute for Communicable Diseases
HVTN	HIV Vaccine Trials Network
IAC	Immunization Action Coalition
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IFN-γ	interferon gamma
IgA	immunoglobulin A
IgG	immunoglobulin G
IL-2	Interleukin 2
IM	intramuscular
IN	intranasal
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
mcg	microgram
MedDRA	Medical Dictionary for Regulatory Activities
MMR	measles, mumps, and rubella
MOP	Manual of Operations
MUVAPRED	Mucosal Vaccines for Poverty Related Diseases
nAb	neutralizing antibody
NAEPP	US National Asthma Education and Prevention Program
NHP	nonhuman primate
NIAID	(US) National Institute of Allergy and Infectious Diseases

NIH	US National Institutes of Health
OHRP	US Office for Human Research Protections
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PI	Principal Investigator
PSRT	Protocol Safety Review Team
RAB	(DAIDS) Regulatory Affairs Branch
RE	regulatory entity
RSA	Republic of South Africa
RSC	(DAIDS) Regulatory Support Center
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SMB	Safety Monitoring Board
SPF	specific pathogen free
SPT	(DAIDS) Safety and Pharmacovigilance Team
TB	tuberculosis
TM	transmembrane
TNF-α	tumor necrosis factor alpha
VISP	Vaccine induced seropositivity
WBC	white blood cells

* 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

1. UNAIDS. Ethical considerations in biomedical HIV prevention trials. **2007**.

2. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research. **1979**.

3. Council for International Organizations of Medical Sciences (CIOMS). International ethical guidelines for biomedical research involving human subjects. Bull Med Ethics **2002**;17-23.

4. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med **2009**;361:2209-20.

5. UNAIDS. Global Report: Report on the global AIDS epidemic 2013. **2013**;UNAIDS / JC2502/1/E.

6. UNAIDS. Report on the Global AIDS Epidemic. **2012**;UNAIDS / JC2417E.

7. WHO in partnership with UNICEF and UNAIDS. Global Report on HIV Treatment 2013: Results, Impact and Opportunities. **2014**.

8. AVERT. HIV Treatment as Prevention. http://www.avert.org/hiv-treatment-as-prevention.htm. 2012. Last accessed: 12-13-2012

9. Schouten EJ, Jahn A, Ben-Smith A, Makombe SD, Harries AD, Aboagye-Nyame F, Chimbwandira F. Antiretroviral drug supply challenges in the era of scaling up ART in Malawi. J Int AIDS Soc **2011**;14 Suppl 1:S4.

10. Eholie SP, Aoussi FE, Ouattara IS, Bissagnene E, Anglaret X. HIV treatment and care in resource-constrained environments: challenges for the next decade. J Int AIDS Soc **2012**;15:17334.

11. Powers KA, Ghani AC, Miller WC, Hoffman IF, Pettifor AE, Kamanga G, Martinson FE, Cohen MS. The role of acute and early HIV infection in the spread of HIV and implications for transmission prevention strategies in Lilongwe, Malawi: a modelling study. Lancet **2011**;378:256-68.

12. Dodd PJ, Garnett GP, Hallett TB. Examining the promise of HIV elimination by 'test and treat' in hyperendemic settings. AIDS **2010**;24:729-35.

13. Celum C, Baeten JM. Tenofovir-based pre-exposure prophylaxis for HIV prevention: evolving evidence. Curr Opin Infect Dis **2012**;25:51-7.

14. van der Straten A, Van Damme L, Haberer JE, Bangsberg DR. Unraveling the divergent results of pre-exposure prophylaxis trials for HIV prevention. AIDS **2012**;26:F13-F19.

15. Van DL, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, Malahleha M, Owino F, Manongi R, Onyango J, Temu L, Monedi MC, Mak'Oketch P, Makanda M, Reblin I, Makatu SE, Saylor L, Kiernan H, Kirkendale S, Wong C, Grant R, Kashuba A, Nanda K, Mandala J, Fransen K, Deese J, Crucitti T, Mastro TD, Taylor D. Preexposure prophylaxis for HIV infection among African women. N Engl J Med **2012**;367:411-22.

16. Fauci AS, Folkers GK. Toward an AIDS-free generation. JAMA **2012**;308:343-4.

17. World Health Organization. HIV/AIDS, HIV Vaccines. http://www.who.int/hiv/topics/vaccines/Vaccines/en/. 2013. Last accessed: 7-30-2013

18. Bredell H, Williamson C, Sonnenberg P, Martin DJ, Morris L. Genetic characterization of HIV type 1 from migrant workers in three South African gold mines. AIDS Res Hum Retroviruses **1998**;14:677-84.

19. Van Harmelen JH, Van der RE, Loubser AS, York D, Madurai S, Lyons S, Wood R, Williamson C. A predominantly HIV type 1 subtype C-restricted epidemic in South African urban populations. AIDS Res Hum Retroviruses **1999**;15:395-8.

20. Novitsky V, Smith UR, Gilbert P, McLane MF, Chigwedere P, Williamson C, Ndung'u T, Klein I, Chang SY, Peter T, Thior I, Foley BT, Gaolekwe S, Rybak N, Gaseitsiwe S, Vannberg F, Marlink R, Lee TH, Essex M. Human immunodeficiency virus type 1 subtype C molecular phylogeny: consensus sequence for an AIDS vaccine design? J Virol **2002**;76:5435-51.

21. Guevara H, Johnston E, Zijenah L, Tobaiwa O, Mason P, Contag C, Mahomed K, Hendry M, Katzenstein D. Prenatal transmission of subtype C HIV-1 in Zimbabwe: HIV-1 RNA and DNA in maternal and cord blood. J Acquir Immune Defic Syndr **2000**;25:390-7.

22. Sher R. HIV infection in South Africa, 1982-1988--a review. S Afr Med J **1989**;76:314-8.

23. Van Harmelen JH, Wood R, Lambrick M, Rybicki EP, Williamson AL, Williamson C. An association between HIV-1 subtypes and mode of transmission in Cape Town, South Africa. AIDS **1997**;11:81-7.

24. Peeters M, Toure-Kane C, Nkengasong JN. Genetic diversity of HIV in Africa: impact on diagnosis, treatment, vaccine development and trials. AIDS **2003**;17:2547-60.

25. McCormack GP, Glynn JR, Crampin AC, Sibande F, Mulawa D, Bliss L, Broadbent P, Abarca K, Ponnighaus JM, Fine PE, Clewley JP. Early evolution of the human immunodeficiency virus type 1 subtype C epidemic in rural Malawi. J Virol **2002**;76:12890-9.

26. Jacobs GB, Loxton AG, Laten A, Robson B, van Rensburg EJ, Engelbrecht S. Emergence and diversity of different HIV-1 subtypes in South Africa, 2000-2001. J Med Virol **2009**;81:1852-9.

27. Romani B, Glashoff R, Engelbrecht S. Molecular and phylogenetic analysis of HIV type 1 vpr sequences of South African strains. AIDS Res Hum Retroviruses **2009**;25:357-62.

28. Jacobs GB, Laten A, van Rensburg EJ, Bodem J, Weissbrich B, Rethwilm A, Preiser W, Engelbrecht S. Phylogenetic diversity and low level antiretroviral resistance mutations

in HIV type 1 treatment-naive patients from Cape Town, South Africa. AIDS Res Hum Retroviruses **2008**;24:1009-12.

29. Robb ML, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Kunasol P, Khamboonruang C, Thongcharoen P, Morgan P, Benenson M, Paris RM, Chiu J, Adams E, Francis D, Gurunathan S, Tartaglia J, Gilbert P, Stablein D, Michael NL, Kim JH. Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post-hoc analysis of the Thai phase 3 efficacy trial RV 144. Lancet Infect Dis **2012**;12:531-7.

30. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med **2012**;366:1275-86.

31. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. AIDS **2006**;20:W13-W23.

32. Barnett S. Selection of 2 subtype C gp120 proteins for Phase 2b proof-of-concept trials in RSA . HVTN Full Group Meeting, Washington, DC, June 2, 2011, **2011**

33. Dey A. Progress on selection and development of subtype C gp120s for post-RV144 proof-of-concept clinical trials . HVTN Full Goup Meeting, Washington, D.C.May 31, **2012**

34. Ruffin N, Borggren M, Euler Z, Fiorino F, Grupping K, Hallengard D, Javed A, Mendonca K, Pollard C, Reinhart D, Saba E, Sheik-Khalil E, Skold A, Ziglio S, Scarlatti G, Gotch F, Wahren B, Shattock RJ. Rational design of HIV vaccines and microbicides: report of the EUROPRISE annual conference 2011. J Transl Med **2012**;10:144.

35. Schultze V, D'Agosto V, Wack A, Novicki D, Zorn J, Hennig R. Safety of MF59 adjuvant. Vaccine **2008**;26:3209-22.

36. Pellegrini M, Nicolay U, Lindert K, Groth N, Della CG. MF59-adjuvanted versus non-adjuvanted influenza vaccines: integrated analysis from a large safety database. Vaccine **2009**;27:6959-65.

37. Coffman RL, Sher A, Seder RA. Vaccine adjuvants: putting innate immunity to work. Immunity **2010**;33:492-503.

38. O'Hagan DT, Ott GS, De GE, Seubert A. The mechanism of action of MF59-An innately attractive adjuvant formulation. Vaccine **2012**;30:4341-8.

39. Vesikari T, Knuf M, Wutzler P, Karvonen A, Kieninger-Baum D, Schmitt HJ, Baehner F, Borkowski A, Tsai TF, Clemens R. Oil-in-water emulsion adjuvant with influenza vaccine in young children. N Engl J Med **2011**;365:1406-16.

40. Gasparini R, Amicizia D, Lai PL, Panatto D. Aflunov((R)): a prepandemic influenza vaccine. Expert Rev Vaccines **2012**;11:145-57.

41. Yates NL, Liao HX, Fong Y, DeCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang ZY, Seaton KE, Berman PW, Alpert MD, Evans DT, O'Connell RJ, Francis D, Sinangil F, Lee C, Nitayaphan S, Rerks-Ngarm S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Pinter A, Zolla-Pazner S, Gilbert PB, Nabel GJ, Michael NL, Kim JH, Montefiori DC, Haynes BF, Tomaras GD. Vaccine-Induced Env V1-V2 IgG3 Correlates with Lower HIV-1 Infection Risk and Declines Soon After Vaccination. Sci Transl Med **2014**;6:228ra39.

42. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, Dugast AS, Schoen MK, Rolland M, Suscovich TJ, Mahan AE, Liao L, Streeck H, Andrews C, Rerks-Ngarm S, Nitayaphan S, de Souza MS, Kaewkungwal J, Pitisuttithum P, Francis D, Michael NL, Kim JH, Bailey-Kellogg C, Ackerman ME, Alter G. Polyfunctional Fc-Effector Profiles Mediated by IgG Subclass Selection Distinguish RV144 and VAX003 Vaccines. Sci Transl Med **2014**;6:228ra38.

43. Chung AW, Kumar MP, Arnold KB, Yu WH, Schoen MK, Dunphy LJ, Suscovich TJ, Frahm N, Linde C, Mahan AE, Hoffner M, Streeck H, Ackerman ME, McElrath MJ, Schuitemaker H, Pau MG, Baden LR, Kim JH, Michael NL, Barouch DH, Lauffenburger DA, Alter G. Dissecting Polyclonal Vaccine-Induced Humoral Immunity against HIV Using Systems Serology. Cell **2015**;163:988-98.

44. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, Bonsignori M, Moody MA, Fong Y, Chen X, Poling B, Nicholson CO, Zhang R, Lu X, Parks R, Kaewkungwal J, Nitayaphan S, Pitisuttithum P, Rerks-Ngarm S, Gilbert PB, Kim JH, Michael NL, Montefiori DC, Haynes BF. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. Proc Natl Acad Sci U S A **2013**;110:9019-24.

45. Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. Nature **2010**;464:217-23.

46. Iwasaki A. Exploiting Mucosal Immunity for Antiviral Vaccines. Annu Rev Immunol **2016**;34:575-608.

47. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. Annu Rev Med **2011**;62:127-39.

48. Bomsel M, Tudor D, Drillet AS, Alfsen A, Ganor Y, Roger MG, Mouz N, Amacker M, Chalifour A, Diomede L, Devillier G, Cong Z, Wei Q, Gao H, Qin C, Yang GB, Zurbriggen R, Lopalco L, Fleury S. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. Immunity **2011**;34:269-80.

49. Vaccari M, Gordon SN, Fourati S, Schifanella L, Liyanage NP, Cameron M, Keele BF, Shen X, Tomaras GD, Billings E, Rao M, Chung AW, Dowell KG, Bailey-Kellogg C, Brown EP, Ackerman ME, Vargas-Inc, Whitney S, Doster MN, Binello N, Pegu P, Montefiori DC, Foulds K, Quinn DS, Donaldson M, Liang F, Lore K, Roederer M, Koup RA, McDermott A, Ma ZM, Miller CJ, Phan TB, Forthal DN, Blackburn M, Caccuri F, Bissa M, Ferrari G, Kalyanaraman V, Ferrari MG, Thompson D, Robert-Guroff M, Ratto-Kim S, Kim JH, Michael NL, Phogat S, Barnett SW, Tartaglia J, Venzon D, Stablein DM, Alter G, Sekaly RP, Franchini G. Adjuvant-dependent innate and adaptive immune signatures of risk of SIVmac251 acquisition. Nat Med **2016**;22:762-70.

50. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, Heyward WL, Jobes DV, Popovic V, Self SG, Sinangil F, Burke D, Berman PW. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis **2005**;191:666-77.

51. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis **2005**;191:654-65.

52. Pitisuttithum P, Nitayaphan S, Thongcharoen P, Khamboonruang C, Kim J, de Souza M, Chuenchitra T, Garner RP, Thapinta D, Polonis V, Ratto-Kim S, Chanbancherd P, Chiu J, Birx DL, Duliege AM, McNeil JG, Brown AE. Safety and immunogenicity of combinations of recombinant subtype E and B human immunodeficiency virus type 1 envelope glycoprotein 120 vaccines in healthy Thai adults. J Infect Dis **2003**;188:219-27.

53. Pantaleo G. Second Generation Poxvirus Vector Platforms: NYVAC +/- DNA . AIDS Vaccine 2012, Boston, MA, 9-12 September, **2012**

54. Robb ML. Looking back to move forward: Understanding ALVAC/AIDSVAX immune responses. AIDS Vaccine 2013, Barcelona, Spain. 2013.

55. Barnett SW, Klinger JM, Doe B, Walker CM, Hansen L, Duliege AM, Sinangil FM. Prime-boost immunization strategies against HIV. AIDS Res Hum Retroviruses **1998**;14 Suppl 3:S299-S309.

56. Excler JL, Plotkin S. The prime-boost concept applied to HIV preventive vaccines. AIDS **1997**;11 Suppl A:127-37.

57. Callan MF. The evolution of antigen-specific CD8+ T cell responses after natural primary infection of humans with Epstein-Barr virus. Viral Immunol **2003**;16:3-16.

58. Callan MF, Tan L, Annels N, Ogg GS, Wilson JD, O'Callaghan CA, Steven N, McMichael AJ, Rickinson AB. Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus In vivo. J Exp Med **1998**;187:1395-402.

59. Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, Riddell SR. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med **1995**;333:1038-44.

60. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, Farthing C, Ho DD. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol **1994**;68:4650-5.

61. Appay V, Papagno L, Spina CA, Hansasuta P, King A, Jones L, Ogg GS, Little S, McMichael AJ, Richman DD, Rowland-Jones SL. Dynamics of T cell responses in HIV infection. J Immunol **2002**;168:3660-6.

62. Paroli M, Propato A, Accapezzato D, Francavilla V, Schiaffella E, Barnaba V. The immunology of HIV-infected long-term non-progressors--a current view. Immunol Lett **2001**;79:127-9.

63. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, Montefiori DC, Xu Y, Herndon JG, Wyatt LS, Candido MA, Kozyr NL, Earl PL, Smith JM, Ma HL, Grimm BD, Hulsey ML, McClure HM, McNicholl JM, Moss B, Robinson HL. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Vaccine **2002**;20:1949-55.

64. Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. Nature **2000**;408:605-9.

65. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol **2008**;8:247-58.

66. Mulligan MJ, Weber J. Human trials of HIV-1 vaccines. AIDS **1999**;13:105-12.

67. Tartaglia J, Excler JL, El Habib R, Limbach K, Meignier B, Plotkin S, Klein M. Canarypox virus-based vaccines: prime-boost strategies to induce cell-mediated and humoral immunity against HIV. AIDS Res Hum Retroviruses **1998**;14 Suppl 3:S291-S298.

68. Andersson S, Makitalo B, Thorstensson R, Franchini G, Tartaglia J, Limbach K, Paoletti E, Putkonen P, Biberfeld G. Immunogenicity and protective efficacy of a human immunodeficiency virus type 2 recombinant canarypox (ALVAC) vaccine candidate in cynomolgus monkeys. J Infect Dis **1996**;174:977-85.

69. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Gilbert P, de Souza MS, McLinden R, Tovanabutra S, Laurence-Chenine A, Sanders-Buell E, Moody MA, Bonsignori M, Ochsenbauer C, Kappes J, Tang H, Greene K, Gao H, LaBranche CC, Andrews C, Polonis VR, Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Self SG, Berman PW, Francis D, Sinangil F, Lee C, Tartaglia J, Robb ML, Haynes BF, Michael NL, Kim JH. Magnitude and Breadth of the Neutralizing Antibody Response in the RV144 and Vax003 HIV-1 Vaccine Efficacy Trials. J Infect Dis **2012**;206:431-41.

70. Tomaras GD, Haynes BF. HIV-1-specific antibody responses during acute and chronic HIV-1 infection. Curr Opin HIV AIDS **2009**;4:373-9.

71. Centers for Disease Control and Prevention. Vaccine Administration Recommendations and Guidelines. http://www.cdc.gov/vaccines/hcp/admin/recs-guidelines.html. 12-16-2015. Last accessed: 7-18-2016

72. Poland GA, Borrud A, Jacobson RM, McDermott K, Wollan PC, Brakke D, Charboneau JW. Determination of deltoid fat pad thickness. Implications for needle length in adult immunization. JAMA **1997**;277:1709-11.

73. Nitayaphan S, Pitisuttithum P, Karnasuta C, Eamsila C, de Souza M, Morgan P, Polonis V, Benenson M, VanCott T, Ratto-Kim S, Kim J, Thapinta D, Garner R, Bussaratid V, Singharaj P, El Habib R, Gurunathan S, Heyward W, Birx D, McNeil J, Brown AE. Safety and immunogenicity of an HIV subtype B and E prime-boost vaccine combination in HIV-negative Thai adults. J Infect Dis **2004**;190:702-6.

74. Thongcharoen P, Suriyanon V, Paris RM, Khamboonruang C, de Souza MS, Ratto-Kim S, Karnasuta C, Polonis VR, Baglyos L, Habib RE, Gurunathan S, Barnett S, Brown AE, Birx DL, McNeil JG, Kim JH. A phase 1/2 comparative vaccine trial of the safety and immunogenicity of a CRF01_AE (subtype E) candidate vaccine: ALVAC-HIV (vCP1521) prime with oligomeric gp160 (92TH023/LAI-DID) or bivalent gp120 (CM235/SF2) boost. J Acquir Immune Defic Syndr **2007**;46:48-55.

75. Pitisuttithum P, Rerks-Ngarm S, Bussaratid V, Dhitavat J, Maekanantawat W, Pungpak S, Suntharasamai P, Vanijanonta S, Nitayapan S, Kaewkungwal J, Benenson M, Morgan P, O'Connell RJ, Berenberg J, Gurunathan S, Francis DP, Paris R, Chiu J, Stablein D, Michael NL, Excler JL, Robb ML, Kim JH. Safety and reactogenicity of canarypox ALVAC-HIV (vCP1521) and HIV-1 gp120 AIDSVAX B/E vaccination in an efficacy trial in Thailand. PLoS One **2011**;6:e27837.

76. de Bruyn G, Rossini AJ, Chiu YL, Holman D, Elizaga ML, Frey SE, Burke D, Evans TG, Corey L, Keefer MC. Safety profile of recombinant canarypox HIV vaccines. Vaccine **2004**;22:704-13.

77. Russell ND, Graham BS, Keefer MC, McElrath MJ, Self SG, Weinhold KJ, Montefiori DC, Ferrari G, Horton H, Tomaras GD, Gurunathan S, Baglyos L, Frey SE, Mulligan MJ, Harro CD, Buchbinder SP, Baden LR, Blattner WA, Koblin BA, Corey L. Phase 2 study of an HIV-1 canarypox vaccine (vCP1452) alone and in combination with rgp120: negative results fail to trigger a phase 3 correlates trial. J Acquir Immune Defic Syndr **2007**;44:203-12.

78. Cao H, Kaleebu P, Hom D, Flores J, Agrawal D, Jones N, Serwanga J, Okello M, Walker C, Sheppard H, El Habib R, Klein M, Mbidde E, Mugyenyi P, Walker B, Ellner J, Mugerwa R. Immunogenicity of a recombinant human immunodeficiency virus (HIV)-canarypox vaccine in HIV-seronegative Ugandan volunteers: results of the HIV Network for Prevention Trials 007 Vaccine Study. J Infect Dis **2003**;187:887-95.

79. Kintu K, Andrew P, Musoke P, Richardson P, Asiimwe-Kateera B, Nakyanzi T, Wang L, Fowler MG, Emel L, Ou SS, Baglyos L, Gurunathan S, Zwerski S, Jackson JB, Guay L. Feasibility and safety of ALVAC-HIV vCP1521 vaccine in HIV-exposed infants in Uganda: results from the first HIV vaccine trial in infants in Africa. J Acquir Immune Defic Syndr **2013**;63:1-8.

80. Gupta K, Hudgens M, Corey L, McElrath MJ, Weinhold K, Montefiori DC, Gorse GJ, Frey SE, Keefer MC, Evans TG, Dolin R, Schwartz DH, Harro C, Graham B, Spearman PW, Mulligan M, Goepfert P. Safety and immunogenicity of a high-titered canarypox vaccine in combination with rgp120 in a diverse population of HIV-1-uninfected adults: AIDS Vaccine Evaluation Group Protocol 022A. J Acquir Immune Defic Syndr **2002**;29:254-61.

81. Sabbaj S, Mulligan MJ, Hsieh RH, Belshe RB, McGhee JR. Cytokine profiles in seronegative volunteers immunized with a recombinant canarypox and gp120 prime-boost HIV-1 vaccine. NIAID AIDS Vaccine Evaluation Group. AIDS **2000**;14:1365-74.

82. Gorse GJ, Patel GB, Mandava MD, Arbuckle JA, Doyle TM, Belshe RB. Cytokine responses to human immunodeficiency virus type 1 (HIV-1) induced by immunization with live recombinant canarypox virus vaccine expressing HIV-1 genes boosted by HIV-1(SF-2) recombinant GP120. Vaccine **2001**;19:1806-19.

83. A study to test the safety of three experimental HIV vaccines. http://www.clinicaltrials.gov/ct2/show/NCT00000946?term=NCT00000946&rank=1. 5-16-2012. Last accessed: 8-10-2012

84. Evans TG, Keefer MC, Weinhold KJ, Wolff M, Montefiori D, Gorse GJ, Graham BS, McElrath MJ, Clements-Mann ML, Mulligan MJ, Fast P, Walker MC, Excler JL, Duliege AM, Tartaglia J. A canarypox vaccine expressing multiple human immunodeficiency virus type 1 genes given alone or with rgp120 elicits broad and durable CD8+ cytotoxic T lymphocyte responses in seronegative volunteers. J Infect Dis **1999**;180:290-8.

85. Clements-Mann ML, Weinhold K, Matthews TJ, Graham BS, Gorse GJ, Keefer MC, McElrath MJ, Hsieh RH, Mestecky J, Zolla-Pazner S, Mascola J, Schwartz D, Siliciano R, Corey L, Wright PF, Belshe R, Dolin R, Jackson S, Xu S, Fast P, Walker MC, Stablein D, Excler JL, Tartaglia J, Paoletti E, et al. Immune responses to human immunodeficiency virus (HIV) type 1 induced by canarypox expressing HIV-1MN gp120, HIV-1SF2 recombinant gp120, or both vaccines in seronegative adults. NIAID AIDS Vaccine Evaluation Group. J Infect Dis **1998**;177:1230-46.

86. Russell ND, Hudgens MG, Ha R, Havenar-Daughton C, McElrath MJ. Moving to human immunodeficiency virus type 1 vaccine efficacy trials: defining T cell responses as potential correlates of immunity. J Infect Dis **2003**;187:226-42.

87. Belshe RB, Gorse GJ, Mulligan MJ, Evans TG, Keefer MC, Excler JL, Duliege AM, Tartaglia J, Cox WI, McNamara J, Hwang KL, Bradney A, Montefiori D, Weinhold KJ. Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. NIAID AIDS Vaccine Evaluation Group. AIDS **1998**;12:2407-15.

88. Franchini G, Gurunathan S, Baglyos L, Plotkin S, Tartaglia J. Poxvirus-based vaccine candidates for HIV: two decades of experience with special emphasis on canarypox vectors. Expert Rev Vaccines **2004**;3:S75-S88.

89. Graham BS, Keefer MC, McElrath MJ, Gorse GJ, Schwartz DH, Weinhold K, Matthews TJ, Esterlitz JR, Sinangil F, Fast PE. Safety and immunogenicity of a candidate HIV-1 vaccine in healthy adults: recombinant glycoprotein (rgp) 120. A randomized, double-blind trial. NIAID AIDS Vaccine Evaluation Group. Ann Intern Med **1996**;125:270-9.

90. Gilbert PB, Chiu YL, Allen M, Lawrence DN, Chapdu C, Israel H, Holman D, Keefer MC, Wolff M, Frey SE. Long-term safety analysis of preventive HIV-1 vaccines evaluated in AIDS vaccine evaluation group NIAID-sponsored Phase I and II clinical trials. Vaccine **2003**;21:2933-47.

91. Kim JH, Pitisuttithum P, Kamboonruang C, Chuenchitra T, Mascola J, Frankel SS, DeSouza MS, Polonis V, McLinden R, Sambor A, Brown AE, Phonrat B, Rungruengthanakit K, Duliege AM, Robb ML, McNeil J, Birx DL. Specific antibody responses to vaccination with bivalent CM235/SF2 gp120: detection of homologous and heterologous neutralizing antibody to subtype E (CRF01.AE) HIV type 1. AIDS Res Hum Retroviruses **2003**;19:807-16.

92. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, Hu D, Tappero JW, Choopanya K. Randomized, Double-Blind, Placebo-Controlled Efficacy

Trial of a Bivalent Recombinant Glycoprotein 120 HIV-1 Vaccine among Injection Drug Users in Bangkok, Thailand. J Infect Dis **2006**;194:1661-71.

93. Lewis DJ, Huo Z, Barnett S, Kromann I, Giemza R, Galiza E, Woodrow M, Thierry-Carstensen B, Andersen P, Novicki D, Del Giudice G, Rappuoli R. Transient facial nerve paralysis (Bell's palsy) following intranasal delivery of a genetically detoxified mutant of Escherichia coli heat labile toxin. PLoS One **2009**;4:e6999.

94. Spearman P, Lally MA, Elizaga M, Montefiori D, Tomaras GD, McElrath MJ, Hural J, De Rosa SC, Sato A, Huang Y, Frey SE, Sato P, Donnelly J, Barnett S, Corey LJ. A trimeric, V2-deleted HIV-1 envelope glycoprotein vaccine elicits potent neutralizing antibodies but limited breadth of neutralization in human volunteers. J Infect Dis **2011**;203:1165-73.

95. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat **1998**;52:119-26.

96. Hughes JP. Mixed effects models with censored data with application to HIV RNA levels. Biometrics **1999**;55:625-9.

97. Rotnitzky A, Robins J. Analysis of semi-parametric regression models with non-ignorable non-response. Stat Med **1997**;16:81-102.

Appendix A Sample informed consent form

Title: A phase 1-2 randomized, double-blind, placebo-controlled clinical trial of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59® in HIV-uninfected adults at low risk of HIV infection

HVTN protocol number: HVTN 100

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 a new HIV vaccine combination. HIV is the virus that causes AIDS.

About 252 people will take part in this study at multiple sites. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

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 chance of becoming infected with HIV if you are exposed to the virus.**

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

The study uses 2 different vaccines. These are ALVAC-HIV (vCP2438) and bivalent gp120/MF59. From here on, we will call them ALVAC and Protein. These are experimental HIV vaccines. That means we do not know whether the vaccines will be safe to use in people, or whether they will work to prevent HIV infection. These vaccines are used only in research studies. They were developed by Sanofi Pasteur and Novartis Vaccines and Diagnostics.

The ALVAC vaccine is made out of canarypox virus. Canarypox virus infects birds but cannot grow in human cells. 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.

A study in South Africa, HVTN 097, is giving a similar ALVAC vaccine to about 80 participants. So far, no one has had serious health problems.

The Protein vaccine has man-made pieces of a protein found on the outside of HIV. These protein pieces are mixed with an adjuvant called MF59. An adjuvant is something added to the vaccine to help the immune system respond better. 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.

This combination of study vaccines has 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. Also, over 300 people have received a similar combination of ALVAC and protein vaccines with the MF59 adjuvant in clinical trials without having any serious health problems.

General risks of vaccines:

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.

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Most people can still do their planned activities after getting a vaccine. Rarely, people experience side effects that limit their normal activities or make them go to the doctor.

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:

In earlier studies with similar ALVAC and Protein vaccines, some people found it hard to move their arm for a short while after the injection(s). Also, some people felt tired or weakened, and some people had swollen lymph nodes. A small number of people had diarrhea after receiving the injections. 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 in 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 has 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 or HIV testing. Also during the study, you should not donate blood or tissue.

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

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

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

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

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

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

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

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

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

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

Being in the study

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

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

   Site: Insert number of visits and range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)

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

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

   We may contact you after the study ends (for example, to tell you about the study results).

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

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

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

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

10. **We will give you either the study vaccines or placebos.**

    Not everyone in this study will get the study vaccines. Some people will get placebos (non-vaccine). The ALVAC placebo has all the same ingredients as the vaccine but no HIV-related pieces. The Protein placebo is sterile salt water. We will compare the results from people who got the placebos with results from people who got the study vaccines.

You have a 5-in-6 chance of receiving the study vaccines.

Site: Modify the randomization metaphor in the paragraph below as appropriate to your local culture.

Whether you get the study vaccines or the placebos is completely random, like flipping a coin.

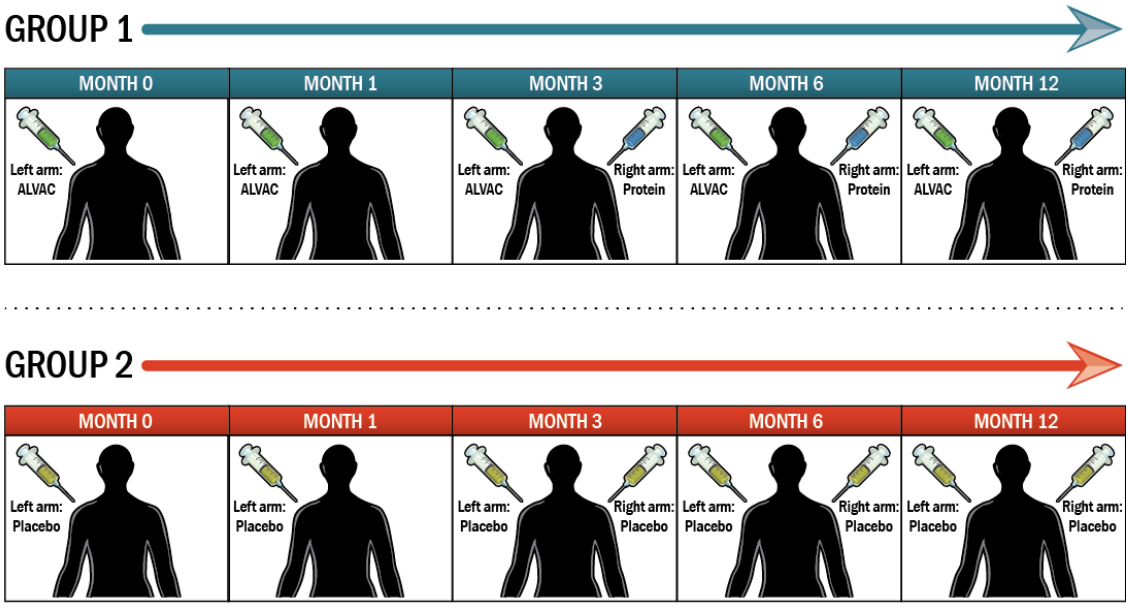
The clinic staff have no say in whether you get the study vaccines or the placebos. They will not know which one you are getting, and neither will you. Only the pharmacist at your site will have this information while the study is going on.

You will have to wait until everyone completes their final study visits to find out whether you got the study vaccines or the placebos. 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.

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

You will be in one of 2 groups. You will get 8 injections into the upper arms during the study. At some visits you will get one injection. At other visits, you will get two injections. The ALVAC vaccine or its placebo will go into the left arm. The Protein vaccine or its placebo will go into the right arm.

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



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 write down how you are feeling and if you have any symptoms. Contact the 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;
- Take blood and urine samples;
- Do pregnancy tests if you were born female (If you have had a complete hysterectomy [removal of the uterus and ovaries, verified by medical records], you are not required to have pregnancy tests.);
- 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.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 200 mL (a little less than 3 teaspoons to a little less than ¾ cup). Your body will make new blood to replace the blood we take out.

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

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

We will review the results of these procedures and tests with you at your next visit, or sooner if necessary. We will also offer you counseling and referral for needed care.

13. We will counsel you on avoiding HIV infection.

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

- What you think may cause risky behavior for you.
- Methods to avoid getting HIV.

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

14. We will test your samples for this study.

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

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

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

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

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

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

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

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

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* The central repositories for the HVTN are located in the United States and South Africa. While most of your samples will be stored in South Africa, some may be shipped to the US for some tests, including genetic tests.

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

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

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

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

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

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

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

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

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

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

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

People who may see your information are:

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

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

- The researcher's Institutional Review Board or Ethics Committee

- The people who work with the researcher

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

16. 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.

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

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

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

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

- The US NIH, its study monitors, and its chosen South African representatives,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- The South African Medicines Control Council,
- Sanofi Pasteur and Novartis Vaccines and Diagnostics and people who work for them,
- The HVTN and people who work for them,
- The Data and 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.

17. 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.

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

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

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

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

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

care, and about other studies you may want to join. We will not provide or pay for any of your HIV care directly.

Other risks

20. 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, muscle damage, and (rarely) infection where the needle was inserted. Taking blood can cause a low blood cell count (anemia), making you feel tired.

Personal problems/discrimination/testing HIV antibody positive:

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

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

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

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

If someone believes you are infected with HIV even if you are not, you could face discrimination and other problems. For example, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military in some countries (outside of South Africa). 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.

Site: Modify the preceding paragraph if applicable.

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

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

Embarrassment/anxiety:

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

Risks of disclosure of your personal information:

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

Risks of genetic testing:

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

Unknown risks:

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

We do not know if getting these study vaccines will affect how you respond to any future approved HIV vaccine. It could be that a future HIV vaccine may not work as well for

you because you got the study vaccines. Currently, no HIV vaccine has been approved for use.

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

Benefits

21. 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

22. 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

23. 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

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

Your health is important to us. We will help you get the medical care you need.

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

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

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

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

Questions

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

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

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

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

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

Your permissions and signature

26. In Section 15 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please write your initials or make your mark in the box next to the option you choose.

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

OR

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

OR

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

27. 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 Addendum to Sample informed consent form (for Part B)

Title: A phase 1-2 randomized, double-blind, placebo-controlled clinical trial of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59® in HIV-uninfected adults at low risk of HIV infection

HVTN protocol number: HVTN 100

Site: [Insert site name]

Thank you for your participation in the HVTN 100 study. Please read this consent form or ask someone to read it to you. The study staff will talk with you about the information in it. You are free to ask questions at any time.

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are continuing HVTN 100 by adding a second part to the study with more clinic visits and injections. We will now call the first part of the study that you were in Part A, and we will call the second part of the study Part B. We are inviting you to join Part B because you have participated in the HVTN 100 study already.

The body's immune response to vaccines gets weaker as time passes. For this reason, most vaccines need to be given again to build up the immune responses again. This is called "boosting." We want to learn about how the body responds to boosting with the study vaccines.

If you decide to continue in 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.

Review of study information

The study is testing 2 experimental vaccines. One is ALVAC-HIV (vCP2438) and we call it the ALVAC vaccine. The other is Bivalent Subtype C gp120/MF59 and we call it the Protein vaccine. There is also a placebo of sterile salt water in this study.

The study was designed to look at 3 main 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.)

The study opened on 2 February 2015. On 26 May 2015 the study was fully enrolled with 252 participants.

New information about the study vaccines

All study injections were complete by 2 June 2016. As of July 2016, about 225 participants were still in the study.

In the Part A consent form, we told you that the Protein vaccine was developed by Novartis Vaccines and Diagnostics and that this company may review your study records. The Protein vaccine is now owned by GlaxoSmithKline (GSK). This means that GSK may review your study records, not Novartis.

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

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

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

Most of the symptoms participants had are common side effects of vaccines or of getting injections. We do not know yet which participants got the study vaccines and which got the placebo because the study is still “blinded”. So we do not know if the study vaccines caused these side effects. We will not know this until part B is complete.

So far the study vaccines have produced the immune responses we hoped to see. We don’t know yet if the study vaccines will protect people from HIV. A larger study is being started to answer that question.

Part B of the study

We are continuing the study to learn how well boosting the study vaccines improves immune responses. About 72 participants will be enrolled in Part B of the study.

It is completely up to you whether or not to continue in the study. If you decide not to continue, or if you leave it after you have agreed to continue, your other care at this clinic and the benefits or rights you would normally have will not be affected.

If you decide to continue in Part B of the study, we will check to make sure you are eligible. This involves:

- A brief physical exam
- An HIV test
- Blood and urine tests
- Questions about medications you are taking
- Questions about any problems you may have experienced from being in the study
- Questions about any HIV testing you have had outside the study
- A pregnancy test if you were born female.

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

Also, to be in Part B of the study, you must be willing to give samples of your rectal fluids and also cervical fluid or semen. The collection procedures are described below.

Being in Part B of the study

If you are eligible and want to join Part B, here is what will happen:

You will come to the clinic for scheduled visits about [#] times over 6 months.

Site: Insert number of visits and range of visit lengths. (There is site-specific variation in the number of possible follow-up visits between protocol-mandated visits.)

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

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

At these study visits, we will do the same things we did at the study visits in Part A. We will review with you the consent form for Part A. The information in it about what we will do at study visits remains correct.

Site: Paste table of procedures for Part B (Appendix D) in this section or distribute it as a separate sheet if it is helpful to your study participants.

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 Part B.

If you were born female and could become pregnant, you will need to continue using birth control in Part B of the study.

In Part A, the clinic staff did not know whether you got the study vaccines or the placebo, and neither did you. It will be the same in Part B. Only the pharmacist at your site will have this information while the study is going on.

In Part A, we told you that you would have to wait until everyone completed their final study visits to find out which study products you got. Because we are continuing this study, we will not tell you which study products you got in Part A until after everyone in Part B completes their final study visits. This could be several more years. If you have a serious medical problem and need to know what you got before the end of Part B, we can tell you.

In Part B you will get 2 injections into the upper arms. The ALVAC vaccine or its placebo will go into the left arm. The Protein vaccine or its placebo will go into the right arm.

People who got the study vaccines in Part A will get one or both of the study vaccines in Part B.

- Some will get the ALVAC vaccine and the Protein vaccine.
- Some will get the Protein vaccine and placebo.
- Some will get placebos.

People who got placebos in Part A will continue to get placebos in Part B.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 20 mL and 220 mL (about 1½ tablespoons to a little less than 1 cup). Your body will make new blood to replace the blood we take out.

Sampling of rectal fluid and cervical fluid or semen

We want to see how the study vaccines affect the parts of the body where people may be exposed to HIV: their rectum, vagina, and penis. We especially want to see whether the immune system makes antibodies in these parts of the body.

Collecting rectal fluid along with cervical fluid (if you were born female) or semen (if you were born male) is required in Part B of this study. If you do not want to give these samples, you cannot join Part B of the study.

We will collect these samples 2 times: at your injection visit and at the visit 2 weeks after the injection visit.

When we collect these samples, we will test you for gonorrhea, chlamydia, and syphilis. And if you were born female, we will also test you for pregnancy, trichomoniasis, bacterial vaginosis, and if needed, for a yeast infection. We will explain what these tests are for and we will give you the results. If you need care, we will tell you about the care we can give you at this clinic. We will also tell you about care we can help you get elsewhere. We will ask you to avoid some activities for 2 days before we collect these samples. This will help make sure your samples give accurate lab readings.

Rectal fluid

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

For the 2 days before we collect your rectal fluid:

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

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

Cervical fluid

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

To collect cervical fluid, we will give you a menstrual cup (a small flexible plastic cup, shaped like a bell) to insert into your vagina. We will explain how to insert and remove the cup, or we 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 your cervical fluid,

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

Do not insert the menstrual cup:

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

Semen

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

For at least 2 days before providing the semen:

- Do not have sex, including oral sex;
- Do not ejaculate;
- Do not use anything with lubricants;
- Do not put saliva on your 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.

Risks of collecting rectal and cervical fluids:

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

New information about testing your samples

In Part A, we told you that we will send your samples (without your name) to a lab to see how your immune system responds to the study products. We said that the researchers may do limited genetic testing on your samples. Limited genetic testing involves only some of your genes, not all of your genes (your genome).

We are now adding the following information about the use of your samples in this study:

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

Use of your samples in other studies

When you joined Part A, you signed a separate consent form about using your extra samples and information in other studies. The information in that consent form still applies to your samples and information from Part B. In that consent form you told us whether or not you allowed use of your samples and information in other studies. We will continue to follow your decision in that consent form unless you tell us you have changed your mind. We can review it with you if you would like.

Other information

The rest of the information in the consent forms you signed when you joined Part A has not changed. This includes:

- the information about study procedures,
- birth control,
- stopping your injections,
- the risks and benefits of being in the study,
- your rights and responsibilities,
- how your privacy is protected,
- how study-related injuries would be handled and whom to contact if you have questions or problems during the study.

Questions

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

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

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

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

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].

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

If you agree to continue in Part B of 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 continue. 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 provide rectal fluid samples.
- If you were born female, you agree to provide cervical fluid samples.
- If you were born male, you agree to provide semen samples.
- You agree to continue in this study.

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

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

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

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

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

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

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

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

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

Effective birth control means using 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.

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

Part A

			Time after 1st injection visit (in months)												
Procedure	Screening visit(s)	First injection visit	½	1	1.5	2	3	3.5	6	6.5	9	12	12.5	15	18
Injection		√		√			√		√			√			
Medical history	√														
Complete physical	√														√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	
Urine test	√		√							√			√		
Blood drawn	√	√	√		√		√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female)*	√	√		√			√		√			√		√	
HIV testing and pretest counseling	√						√		√		√	√		√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

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

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.

Part B

		Time after enrollment in Part A (in months)			
Procedure	Eligibility visit(s)	30	30.25	30.5	36
Injection		√			
Complete physical					√
Brief physical	√	√	√	√	
Urine test	√			√	
Blood drawn	√	√	√	√	√
Pregnancy test (participants born female)*	√	√		√	√
HIV testing and pretest counseling	√	√			√
Risk reduction counseling	√	√	√	√	√
Questions/questionnaire	√	√	√	√	√
Mucosal sampling		√		√	
Sexually transmitted infection (STI) testing		√		√[§]	

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

[§] STI testing only if the participant has new STI symptoms since the month 30 visit.

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.

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

Title: A phase 1-2 randomized, double-blind, placebo-controlled clinical trial of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59® in HIV-uninfected adults at low risk of HIV infection

HVTN protocol number: HVTN 100

Site: [Insert site name]

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

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

1. Do I have to agree?

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

2. Where are the samples stored?

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

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

3. How long will the samples be stored?

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

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

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

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

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

6. Will the HVTN sell my samples and information?

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

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

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

8. What information is shared with other researchers?

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

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

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

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

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

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

10. What are the risks of limited 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 IRB or EC
- The people who work with the researcher

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

Questions

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

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

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

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

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

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

OR

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

OR

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

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

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

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

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

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

Appendix F Laboratory procedures for Part A

				Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
				Day:	Screening visit[3]	D0	D14	D28	D42	D56	D84	D98	D168	D182	D273	D364	D378	D455	D546	
				Weeks		W0	W2	W4	W6	W8	W12	W14	W24	W26	W39	W52	W54	W65	W78	
				Month		M0	M0.5	M1	M1.5	M2	M3	M3.5	M6	M6.5	M9	M12	M12.5	M15	M18	
						ALVAC		**ALVAC**			**ALVAC+prot**		**ALVAC+prot**			**ALVAC+prot**				
Procedure	**Ship to[1]**	**Assay location[2]**	**Tube Type[4]**	**Tube size (vol. capacity)[4]**		**placebo**		**placebo**			**placebo**		**placebo**			**placebo**				**Total**
BLOOD COLLECTION																				
Screening/Diagnostic																				
Screening HIV test	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**5**
HBsAg/anti-HCV/Syphilis	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**5**
HIV in-study diagnostic test[9]	HMSL-NICD	HSML-NICD	EDTA	10mL	—	—	—	—	—	—	10	—	10	—	10	10	—	10	20	**70**
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**
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	—	y	—	—	y	—	—	y	—	***y***	—	y	y	y	y	**0**
Storage																				
PBMC	BARC		ACD	8.5mL	—	59.5	—	—	59.5	—	—	59.5	—	59.5	—	59.5	59.5	—	59.5	**417**
Serum	BARC		SST	8.5mL	—	17	—	—	17	—	—	17	—	17	—	17	17	17	17	**136**
Visit total					**20**	**187**	**10**	**0**	**180**	**0**	**10**	**180**	**10**	**180**	**10**	**180**	**180**	**46**	**190**	**1383**
56-Day total					**20**	**207**	**217**	**217**	**397**	**377**	**190**	**370**	**10**	**190**	**10**	**180**	**360**	**46**	**190**	
URINE COLLECTION																				
Urinalysis	Local lab	Local lab			X	—	X	—	—	—	—	—	—	X	—	—	X	—	—	
Pregnancy Test[8]	Local lab	Local lab			X	X	—	X	—	—	X	—	X	—	—	X	—	X	—	

[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. 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 9.11), blood should be drawn for HIV diagnostic testing, as shown for visit 15 above.

y = SST blood collected for binding Ab assays and serum storage will also cover specimen needs for neutralizing Ab assays; no separate blood draw is needed.

Appendix G Laboratory procedures for Part B

Procedure	Ship to[1]	Assay location[2]	Tube Type[4]	Tube size (vol. capacity)[4]	Visit: 16	17[14]	18	19	20	Total
					Day: Part B eligibility visit[3]	D910	D917	D924	D1092	
					Weeks:	W130	W131	W132	W156	
					Month:	M30	M30.25	M30.5	M36	
						Vacc #6				
BLOOD COLLECTION										
Diagnostic										
HIV diagnostics[8]	HSML-NICD	HSML-NICD	EDTA	10mL	10	10	—	—	20[8]	**40**
Safety labs										
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	—	5	—	**10**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	5	—	**10**
STI Serology										
Syphilis[10]	Local lab	Local lab	SST	5mL	—	5	—	5[13]	—	**10**
Immunogenicity & Virologic assays[6]										
Cellular assays										
ICS	BARC	HVTN Labs	ACD	8.5mL	—	59.5	—	59.5	85	**204**
pTfh and plasmablasts	BARC	HVTN Labs	ACD	8.5mL	—	42.5	42.5	—	—	**85**
Humoral assays										
Binding Ab Assay	BARC	HVTN Labs	SST	8.5mL	—	8.5	—	8.5	8.5	**26**
Neutralizing Ab Assay	BARC	HVTN Labs	SST	8.5mL	—	y	—	y	y	**0**
Storage										
PBMC	BARC		ACD	8.5mL	—	59.5	—	59.5	59.5	**179**
Plasma	BARC		ACD	8.5mL	—	—	z	—	—	**0**
Serum	BARC		SST	8.5mL	—	17	—	17	17	**51**
Visit total					**20**	**202**	**43**	**160**	**190**	**614**
56-Day total					**20**	**222**	**265**	**424**	**190**	
URINE COLLECTION										
Urinalysis	Local lab	Local lab			X	—	—	X	—	
Pregnancy Test[7]	Local lab	Local lab			X	X	—	X[9]	X	
Chlamydia/Gonorrhea[10]	Local lab	Local lab			—	X	—	X[13]	—	
CERVICAL/VAGINAL SWAB COLLECTION[11]										
Trichomonas vaginalis	Local lab	Local lab			—	X	—	X[13]	—	
Bacterial vaginosis	Local lab	Local lab			—	X	—	X[13]	—	
Yeast	Local lab	Local lab			—	X	—	X[13]	—	
MUCOSAL COLLECTION										
Semen	BARC	HVTN Labs			—	X[12]	—	X	—	
Cervical Secretions	BARC	HVTN Labs			—	X[12]	—	X	—	
Rectal Secretions	BARC	HVTN Labs			—	X[12]	—	X	—	

[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 (SAID-NICD, Johannesburg, South Africa); Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

[3] Eligibility visit applies to participants who terminated from the study. It is not needed for participants who are currently in study. The eligibility visit may occur over the course of several contacts/visits up to and including the visit day for visit 17.

[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 from Part A (for binding Ab assay) and M30.5. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other timepoints.

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

[8] At an early termination visit (see Section 9.11), blood should be drawn for HIV diagnostic testing, as shown for visit 20 above.

[9] 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.

[10] Syphilis testing by serology and Chlamydia and gonorrhea testing by urine will only be performed for participants providing mucosal samples.

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

[12] Mucosal specimens must be collected prior to vaccination once the participant is confirmed to have met mucosal specimen collection criteria specified in Section 9.5.

[13] STI tests at M30.5 will only be performed if the participant is symptomatic following their STI tests at M30.

[14] Specimens collected at the vaccination visit may be obtained within the 14 days prior to vaccination, except for a pregnancy test (see footnote "7" above).

y = SST blood collected for binding Ab assays and serum storage will also cover specimen needs for neutralizing Ab assays; no separate blood draw is needed.

z = Up to 10 × 1mL aliquots of ACD plasma will be harvested for storage during PBMC processing; no separate blood draw is needed.

Appendix H Procedures at HVTN CRS for Part A

Visit:	01[a]	02	03	04	05	06	07	08	09	10	11	12	13	14	15	Post
Day:		D0	D14	D28	D42	D56	D84	D98	D168	D182	D273	D364	D378	D455	D546	
Week		W0	W2	W4	W6	W8	W12	W14	W24	W26	W39	W52	W54	W65	W78	
Month:		M0	M0.5	M1	M1.5	M2	M3	M3.5	M6	M6.5	M9	M12	M12.5	M15	M18	
Procedure	Scr.	VAC1		VAC2			VAC3		VAC4			VAC5				
Study procedures[b]																
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Signed protocol consent	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—
Screening for low risk of HIV infection	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	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 questionnaire	X	—	—	—	—	—	X	—	X	—	X	—	—	X	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	—
Outside testing and belief questionnaire	—	—	—	—	—	—	—	—	—	X	—	—	—	X	X	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[d]	X	—	—	—	—	—	X	—	X	—	X	X	—	X	X	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	X	—	X	—	X	X	—	X	X
Local lab assessment																
Screening HIV test	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Urine dipstick	X	—	X	—	—	—	—	—	—	X	—	—	X	—	—	—
Pregnancy (urine or serum HCG)[e]	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[f]	—	X	—	X	—	—	X	—	X	—	—	X	—	—	—	—
Reactogenicity assessments[g]	—	X	—	X	—	—	X	—	X	—	—	X	—	—	—	—
Poststudy																
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X[h]

[a] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.
[b] For specimen collection requirements, see Appendix F.
[c] Pregnancy prevention compliance occurs only with participants who were born female and are capable of becoming pregnant.
[d] Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant. See Section 9.6.
[e] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination (serum pregnancy tests may be performed within 24 hours preceding vaccination). Pregnancy test to determine eligibility may be performed at screening or on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with 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.
[f] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration. Lab tests may be drawn within the 3 days prior to vaccination.
[g] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.9).
[h] Unblinding of all participants will occur after Part B has been completed.

Appendix I Procedures at HVTN CRS for Part B

Visit:	16	17[k]	18	19	20	Post
Day:	Part B eligibility visit[a]	D910	D917	D924	D1092	
Week		W130	W131	W132	W156	
Month:		M30	M30.25	M30.5	M36	
Procedure		VAC6				
Study procedures[b]						
Signed screening consent (if used)	—	—	—	—	—	—
Signed Part B protocol consent	X	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—
Complete physical exam	—	—	—	—	X	—
Confirm eligibility, randomize	X	—	—	—	—	—
Abbreviated physical exam	X	X	X	X	—	—
Risk reduction counseling	X	X	X	X	X	—
Pregnancy prevention assessment[c]	X	X	X	X	X	—
Behavioral risk assessment questionnaire	—	X	—	—	X	—
Social impact assessment	X	X	X	X	X	—
Social impact assessment questionnaire	X	—	—	—	X	—
Outside testing and belief questionnaire	X	—	—	—	X	—
Concomitant medications	X	X	X	X	X	—
Intercurrent illness/adverse experience	X	X	X	X	X	—
HIV infection assessment[d]	X	X	—	—	X	—
Confirm HIV test results provided to participant	—	X	—	X	—	X
Local lab assessment						
Urine dipstick	X	—	—	X	—	—
Pregnancy (urine or serum HCG)[e]	X	X	—	X[f]	X	—
CBC, differential, platelet	X	—	—	X	—	—
Chemistry panel (see Section 9.2)	X	—	—	X	—	—
Pap smear[g]	X	—	—	—	—	—
Syphilis[h]	—	X	—	X[i]	—	—
Chlamydia/gonorrhea[h]	—	X	—	X[i]	—	—
Trichomonas vaginalis[j]	—	X	—	X[i]	—	—
Bacterial vaginosis[j]	—	X	—	X[i]	—	—

[a] May occur over the course of several contacts/visits up to and including day 910 prior to vaccination

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

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

[d] Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant. See Section 9.6.

[e] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination with negative results received prior to vaccination (serum pregnancy tests may be performed within 24 hours preceding 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.

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

[g] Only for participants who were born female and who enter Part B under protocol Version 3.0. Pap smear is not required if volunteer has had a Pap smear within the previous 3-5 years with most recent result normal or ASCUS, or if participant is less than 21 years old. See Section 9.5.

[h] Syphilis testing by serology and urine testing for Chlamydia and gonorrhea will be done only for participants who enter Part B under protocol Version 3.0 . Specimen collection for this testing will take place at the time of mucosal sampling, prior to vaccination (if scheduled).

[i] Test at M30.5 will only be performed if the participant is symptomatic following their STI tests at M30.

[j] Cervical/vaginal swabs will only be collected from participants who enter Part B under protocol Version 3.0 and who provide a cervical secretion sample. Swabs for bacterial vaginosis and yeast will only be collected if clinically indicated. Specimen collection for this testing will take place at the time of mucosal sampling prior to vaccination.

Visit:	**16**	**17[k]**	**18**	**19**	**20**	**Post**
Day:	Part B eligibility visit[a]	D910	D917	D924	D1092	
Week		W130	W131	W132	W156	
Month:		M30	M30.25	M30.5	M36	
Procedure		VAC6				
Yeast[j]	—	X	—	X[i]	—	—
Mucosal sample collection						
Rectal secretions, cervical secretions, semen	—	X	—	X	—	—
Vaccination procedures						
Vaccination	—	X	—	—	—	—
Reactogenicity assessments[l]	—	X	—	—	—	—
Poststudy						
Unblind participant	—	—	—	—	—	X

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

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

Appendix J Protocol Signature Page

A phase 1-2 randomized, double-blind, placebo-controlled clinical trial of clade C ALVAC-HIV (vCP2438) and Bivalent Subtype C gp120/MF59® in HIV-uninfected adults at low risk of HIV infection

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 100

DAIDS Protocol Version: Version 3.0

Protocol Date: May 23, 2017