HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 137

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV-uninfected adults

DAIDS DOCUMENT ID 38559

IND 019275 HELD BY DAIDS

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

International AIDS Vaccine Initiative (IAVI)
New York, New York, USA

Access to Advanced Health Institute (AAHI)
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Division of AIDS (DAIDS), National Institutes of Health
Rockville, Maryland, USA

The Dale and Betty Bumpers Vaccine Research Center (VRC)
Bethesda, Maryland, USA

September 26, 2022
Final
HVTN 137
Version 5.0

Contents

1 Ethical considerations

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

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

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

- HVTN scientists and operational staff are committed to substantive community input—into the planning, conduct, and follow-up of its research—to help ensure that locally appropriate cultural and linguistic needs of study populations are met. Community Advisory Boards (CAB) are required by DAIDS and supported at all HVTN research sites to ensure community input.

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

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

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

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

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

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

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

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

2 IRB/EC review considerations

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

2.1 Minimized risks to participants

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

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

2.2 Reasonable risk/benefit balance

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

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

2.3 Equitable participant selection

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

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

2.4 Appropriate informed consent

45 CFR 46.111 (a) 4 and 5 and 21 CFR 56.111 (a) 4 and 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 137 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

2.6 Protect privacy/confidentiality

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

Privacy refers to an individual's right to be free from unauthorized or unreasonable intrusion into his/her private life and the right to control access to individually identifiable information about him/her. The term "privacy" concerns research participants or potential research participants as individuals whereas the

term “confidentiality” is used to refer to the treatment of information about those individuals. This protocol respects the privacy of participants by informing them about who will have access to their personal information and study data (see Appendix A, Appendix B and Appendix C). The privacy of participants is protected by assigning unique identifiers in place of the participant’s name on study data and specimens. In the United States, research participants in HVTN protocols are protected by a Certificate of Confidentiality from the US NIH, which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form. In addition, each staff member at each study site in this protocol signs an Agreement on Confidentiality and Use of Data and Specimens with the HVTN. In some cases, a comparable confidentiality agreement process may be acceptable. Each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV-uninfected adults

Primary objective(s)

- To evaluate the safety and tolerability of intramuscular (IM) administration of BG505 SOSIP.664 gp140 with the following adjuvants: cytidine phosphoguanosine (CpG) 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum alone

- To evaluate and compare the neutralizing antibody (nAb) response induced by BG505 SOSIP.664 gp140 formulated in different adjuvants (CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum alone)

- To evaluate the safety and tolerability of Trimer 4571 formulated in 3M-052-AF + Alum

- To evaluate the neutralizing antibody response induced by Trimer 4571 formulated in 3M-052-AF + Alum

Study products and routes of administration

- BG505 SOSIP.664 gp140: A stable, soluble cleaved trimeric HIV envelope (Env) gp140 glycoprotein based on the HIV-1 subtype A strain BG505 envelope sequence. To be administered as 100 mcg dose admixed with different adjuvants as 0.5 mL IM injection in the deltoid muscle.

- Trimer 4571 (VRC-HIVRG096-00-VP) (labeled as HIV-1 Trimer 4571 Vaccine): Consists of an HIV-1 envelope (Env) trimer variant derived from the clade A HIV-1 strain BG505. To be administered IM as 100-mcg dose admixed with 5 mcg of 3M-052-AF and 500 mcg of Alum.

- CpG 1018 adjuvant (labeled as CpG 1018 Drug Product): A cytosine phosphoguanosine oligodeoxynucleotide (CpG-ODN) designated 1018 containing a short unmethylated immunostimulatory DNA sequence (CpG), a toll-like receptor (TLR) 9 agonist. To be administered IM as 300 mcg dose admixed with BG505 SOSIP.664 gp140 and 500 mcg of Alum.

- 3M-052-AF adjuvant (labeled as AP 60-702): An aqueous formulation (AF) of the small molecule imidazoquinoline immune response modifier (IRM) 3M-052; TLR7/8 agonist. To be administered IM as 1 mcg or 5 mcg admixed with BG505 SOSIP.664 gp140 and 500 mcg of Alum in Part A and at the

highest tolerated dose from Part A admixed with BG505 SOSIP.664 gp140 and 500 mcg of Alum in Part B. To be administered IM as 3 mcg with BG505 SOSIP.664 gp140 and 500 mcg of Alum (Group 7) or 5 mcg with Trimer 4571 and 500 mcg of Alum (Group 8) in Part C

- GLA-LSQ adjuvant (labeled as AP 10-602): A liposomal formulation of the synthetic TLR4 ligand glucopyranosyl lipid A (GLA) with the saponin *Quillaja saponaria* fraction 21 (QS-21). To be administered IM as 5 mcg GLA and 2 mcg QS-21 admixed with BG505 SOSIP.664 gp140.

- Alum adjuvant (labeled as Aluminum Hydroxide Suspension): Aluminum hydroxide to be administered IM as 500 mcg (Aluminum content) admixed with the BG505 SOSIP.664 gp140 and Trimer 4571 (and adjuvants) as shown.

- Placebo: Tris-NaCl

Table 3-1 Schema

	N (V:P)	Protein Dose	Adjuvant dose	Injection schedule in months (days)		
Part A 3M-052-AF + Alum dose escalation						
				M0 (D0)	**M2 (D56)**	**M6 (168) Optional 2nd Boost***
Group 1	5:1	100 mcg	1 mcg 3M-052-AF + 500 mcg Alum	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)
Group 2	10:1	100 mcg	5 mcg 3M-052-AF + 500 mcg Alum	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)
Part B Adjuvant comparison						
				M0 (D0)	**M2 (D56)**	**M6 (D168)**
Group 3	20:2	100 mcg	300 mcg CpG 1018 + 500 mcg Alum	BG505 SOSIP.664 gp140 + (CpG 1018 + Alum)	BG505 SOSIP.664 gp140 + (CpG 1018 + Alum)	BG505 SOSIP.664 gp140 + (CpG 1018 + Alum)
Group 4	20:2	100 mcg	5 mcg 3M-052-AF + 500 mcg Alum	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)
Group 5	20:2	100 mcg	GLA-LSQ (5 mcg GLA + 2 mcg QS-21)	BG505 SOSIP.664 gp140 + GLA-LSQ	BG505 SOSIP.664 gp140 + GLA-LSQ	BG505 SOSIP.664 gp140 + GLA-LSQ
Group 6	20:2	100 mcg	500 mcg Alum	BG505 SOSIP.664 gp140 + Alum	BG505 SOSIP.664 gp140 + Alum	BG505 SOSIP.664 gp140 + Alum
Part C 3M052-AF + Alum Optimization for Elicitation of Neutralizing Activity with Trimers						
Group 7**	10:1	100 mcg	3 mcg 3M-052-AF + 500 mcg Alum	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)
Group 8***	10:1	100 mcg	5 mcg 3M-052-AF +500 mcg Alum	Trimer 4571 + (3M-052-AF + Alum)	Trimer 4571 + (3M-052-AF + Alum)	Trimer 4571 + (3M-052-AF + Alum)
Total	**127: 115 vaccine recipients / 12 placebo recipients**					

* Participants consenting to an optional second boost vaccination will undergo procedures as shown in the Part A with Optional Second Boost - specific clinical and laboratory procedure tables which are included in Appendix K and Appendix R.
** Enrollment in Part C will be stepwise by Group (Group 7 will fully enroll before Group 8 begins enrollment)
*** Enrollment into Group 8 will be restricted to 1 participant per day for the first 5 participants, and enrollment will then pause after the first 5 participants are enrolled in Group 8. Details are described in Section 11.3.3.

Participants

127 healthy, HIV-1–uninfected volunteers aged 18 through 50 years (inclusive); 115 vaccine recipients, 12 placebo recipients.

Design

Multicenter, randomized, controlled, double-blind trial

Duration per participant

Part A: 8 months of scheduled clinic visits (main study) plus an adverse event of special interest (AESI) health contact at month 14

Part A Optional Second Boost: additional 12 months of scheduled clinic visits (main study) after the third vaccination.

Part B: 18 months of scheduled clinic visits (main study)

Part C: 18 months of scheduled clinic visits (main study)

Estimated total study duration

41 months (includes enrollment, planned safety holds, and follow-up).

Investigational New Drug (IND) sponsor

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

Study product providers

- BG505 SOSIP.664 gp140: International AIDS Vaccine Initiative (IAVI) (New York, New York, USA)
- Trimer 4571 (BG505 DS-SOSIP.664 gp140 Env): The Dale and Betty Bumpers Vaccine Research Center (VRC) (Bethesda, Maryland, USA)
- CpG 1018 (CpG 1018 Drug Product): Division of AIDS (DAIDS), NIAID, NIH (Rockville, Maryland, USA)
- 3M-052-AF (AP 60-702): Access to Advanced Health Institute (AAHI) (Seattle, Washington, USA)
- GLA-LSQ (AP 10-602): DAIDS, NIAID, NIH (Rockville, Maryland, USA)
- Alum (Aluminum Hydroxide Suspension): DAIDS, NIAID, NIH (Rockville, Maryland, USA)
- Tris-NaCl Diluent: IAVI (New York, New York, USA)

Core operations

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

Statistical and data management center (SDMC)

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

HVTN Laboratory Center (LC)

HIV diagnostic laboratory

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

Endpoint laboratories

- Duke University Medical Center (Durham, North Carolina, USA)
- Fred Hutchinson Cancer Research Center/University of Washington (Seattle, Washington, USA)
- Cape Town HVTN Immunology Laboratory (CHIL) (Cape Town, South Africa)

Study sites

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

Safety monitoring

HVTN 137 PSRT; HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

Protocol leadership

Chair	M. Juliana McElrath Seattle Vaccine Trials Unit 206-667-6704 jmcelrat@fredhutch.org	*Statistician*	Holly Janes SCHARP, Fred Hutch 206-667-6353 hjanes@fredhutch.org
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		Clinical trials manager	Julie Hunt HVTN Core, Fred Hutch
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	David Berger Seattle CRS	*Protocol development managers*	Rufi Dalvi Kajari Mondal HVTN Core, Fred Hutch
Community Advisory Board (CAB) members	Ward Rushton Vanderbilt University	*Community engagement unit representatives*	Rafael Gonzalez HVTN Core, Fred Hutch
	Andrew Braun Seattle Vaccine Trials Unit	*Community educator/recruiter*	Keith Richardson Vanderbilt University

4 Background

4.1 Rationale for trial concept

An estimated 1.8 million people with new HIV-1 infections were reported worldwide in 2017 (4). Based on models predicting the future rate of decline in new infections, the HIV epidemic is unlikely to end by sustaining current treatment and prevention approaches alone (5). The development of a safe, highly effective HIV vaccine remains the utmost priority to induce long-lived immunity against HIV-1 infection. Such a vaccine implemented globally, alone or in combination with novel prevention strategies such as monoclonal antibodies (mAbs) for immunoprophylaxis or pre-exposure prophylaxis with antiretrovirals, holds the greatest promise to halt the HIV epidemic.

To confer protection, an efficacious HIV vaccine, like most licensed viral vaccines, is thought to require the induction of antibodies (Ab) that can recognize circulating strains of HIV upon encounter and prevent infection. One of the major goals in new HIV immunogen development is to activate B cells whose receptors can recognize and bind to antigens resembling functional components on the envelope glycoprotein spike on the virus particle, and thereby produce neutralizing antibodies (nAbs) that prevent HIV entry and infection of a susceptible CD4-expressing host target cell.

This phase 1 HVTN protocol is a landmark study. First, it will test a new vaccine design, BG505 SOSIP.664 gp140, the first clinically produced, stabilized soluble Env trimer exposing multiple HIV-1 broadly neutralizing antibody (bnAb) epitopes (6, 7). The soluble native-like trimer design is a recent advance in the HIV vaccine field, and it is envisioned that the trimer platform will have wide applicability in tailoring immunogens as components of a neutralizing antibody-based HIV vaccine regimen.

Second, this protocol will evaluate BG505 SOSIP.664 gp140 separately combined with four different adjuvant formulations that activate different innate immune pathways; a separate placebo group will receive Tris-buffered saline. Three adjuvants are newly developed in this decade (GLA-LSQ, 3M-052-AF, and CpG 1018) and the fourth is the licensed Alum adjuvant (Alhydrogel) as an active control. The study aims to identify at least one novel trimer-adjuvant formulation that induces more potent, durable neutralizing antibodies to the tier 2 BG505 subtype A viral strain than the trimer-Alum formulation. This study provides a unique opportunity to compare a single common antigen, the first-in-class stable Env SOSIP trimer, with four clinical-grade adjuvants available in humans. The in-depth analyses of safety and immunological effects may lead to identification of a more suitable adjuvant than Alum for Env immunogens in future studies, as well as a platform for novel adjuvant discovery in first in human studies. For example, building on information obtained in Part A of this protocol, we will evaluate a stabilized Env SOSIP trimer (Trimer 4571) in combination with 3M-052-AF to see if neutralization activity in human sera can be improved by combining a

stabilized trimer with a promising adjuvant. Trimer 4571 has two amino acid differences with BG505 SOSIP.664, designed to mimic the vulnerable prefusion state. By fixing the trimeric protein into this configuration, in theory this should allow for more visibility to the immune system and greater degree of neutralization. This theory will be directly tested in Part C. In addition, Part C will also test whether an intermediate dose (3 mcg) of the 3M-052 AF plus Alum leads to equivalent neutralization to the high dose (5 mcg, evaluated in Parts A and B) when combined with the original BG505 SOSIP.664 and establish the tolerability of this dose The intended purpose of this arm is to test whether an intermediate dose of this promising adjuvant can drive similar immune responses and therefore will be important for clinical development of this adjuvant.

4.1.1 BG505 SOSIP.664 gp140: a native-like soluble Env trimer

Because of the remarkable sequence and antigenic diversity of HIV-1 Env worldwide, vaccine-induced antibodies will likely need to neutralize a high percentage of circulating strains and with high potency to provide protection. Such broadly neutralizing antibodies (bnAbs) emerge in > 15% of persons within two years of HIV-1 infection, indicating that humans are capable of making these functional Abs (8-13). Nevertheless, no preventive vaccine candidate and regimen has elicited human bnAbs after three decades of evaluation. The failures can be explained in large part by two reasons, discussed below.

First, the Env immunogens tested thus far in clinical trials, while often highly immunogenic in the induction of Abs to immunodominant epitopes, fail to adequately display the neutralizing epitopes in the relevant conformation of the functional Env spike and therefore, with rare exceptions, only induce neutralizing antibodies to easily neutralized tier 1 HIV-1 strains, a panel primarily composed of lab-adapted strains (14, 15). Thus, one of the greatest challenges in HIV vaccine development has been to design Env immunogens that resemble the native Env trimeric structure of the virion-associated spike, affording B cells access to conformationally correct neutralizing epitopes, and to feasibly manufacture the trimer in sufficient amounts with long-term stability for clinical testing. Over the past decade, John Moore and colleagues described and improved a cleaved SOSIP trimer design platform to successfully produce a stable recombinant Env gp140 trimer that structurally mimics the native HIV-1 Env spike (16-20). The prototype, BG505 SOSIP.664 gp140, is the first of this class now manufactured for clinical use and will be evaluated in this phase 1 HVTN protocol. The gp140 sequence is modified from a tier 2 HIV-1 subtype A, CCR5-dependent, transmitted founder virus isolated from a 6-week-old Kenyan infant infected at birth (21, 22).

Second, analysis of the molecular and structural properties of bnAbs reveal unusual features and complex maturation pathways, including high levels of somatic mutation in the variable domains, long heavy chain complementarity determining regions, and autoreactivity with host antigens. The bnAbs induced in natural infection only occur in a fraction of people after the initial autologous nAb response, and this is driven by progressive exposure to HIV-1 variation within the epitopic regions. Thus, it is unlikely that a single Env immunogen, including the

BG505 SOSIP.664 gp140 trimer alone with near native Env virion structure, will be able to induce bnAbs in uninfected persons and within the time frame of an immunization schedule typical for licensed protein-based viral vaccines. Instead, a sequential vaccine approach will likely be needed to activate the relevant naïve precursor B cells and steer the appropriate affinity maturation of memory B cells to produce bnAbs. Nevertheless, we expect that a stable trimer such as BG505 SOSIP.664 gp140 may induce nAbs in some proportion (up to 50%) of vaccine recipients to the autologous HIV-1 BG505 subtype A tier 2 strain, based on reports from animal studies summarized below. The study will determine if nAbs to other tier 2 viruses emerge in HVTN 137 vaccine recipients, but this will likely be a variable effect and we expect a low response rate and heterologous tier 2 nAb titers. Nevertheless, this will be an important benchmark for the vaccine field and can guide strategies for future Env immunogen designs.

The interactions of the BG505 SOSIP.664 gp140 trimer with bnAbs have been extensively characterized and have contributed a wealth of insight into improved designs of Env immunogens as well as the identification of potent bnAbs as mAbs for HIV immune prophylaxis and possibly treatment (23). Since its construction, several other trimers have been successfully engineered and are being evaluated in animal models now and as clinical germline-targeting immunogens and boosts for lineage and epitope structure-based designs in the near future. While the soluble cleaved SOSIP.664 design platform is the current lead strategy for stabilized cleaved trimer immunogens, others include the native flexibly-linked (NFL) and uncleaved prefusion optimized (UFO) platforms. It is anticipated that the native-like soluble Env trimers will be important designs in the prime-boost sequential bnAb pathway regimens, and they will offer the advantage of presenting more than one bnAb epitope region.

The BG505 SOSIP.664 gp140 trimer has now been evaluated as an immunogen in a variety of animal models, including wild-type and bnAb unmutated common ancestor (UCA) knock-in mice, guinea pigs, rats, rabbits, rhesus macaques, and cows. One key rationale for this study will be to define the immunogenicity of this trimer in humans in comparison to the various animal species. The preclinical studies supporting this protocol's IND (see Section 4.4) were designed to use appropriate trimer-adjuvant formulations, dose levels, and schedule as planned in this study, while other reported studies have used primarily research grade BG505 SOSIP.664 gp140 combined with adjuvants that were frequently not available for clinical use. Despite potential differences in experimental design of these animal studies from the planned clinical trial, these comparative data will have utility in defining the suitability of each animal model in guiding antibody-inducing vaccine development in humans. Importantly, additional stabilizing approaches to ensure a closed, prefusion form and minimize presentation of the fully open, CD4 receptor-bound form have been undertaken since the manufacturing of the BG505 SOSIP.664 product that will be tested here. These approaches are aimed at reducing the induction of tier 1 nAbs directed at the Env V3 immunodominant and non-neutralizing CD4 binding site (CD4bs) epitopes. Despite the reduction of responses to these potentially less desirable antibodies, no increases in Ab breadth or autologous neutralization were observed in mice or rabbits (24) following immunization with these modified trimers.

The recent review by Sanders and Moore (23) nicely summarizes many of the key animal studies using BG505 SOSIP.664 as an immunogen, and a few other recent studies in rhesus macaques and cows are presented in Table 4-1.

Table 4-1 Assessment of antibody responses in rabbits, nonhuman primates, and cows following BG505 SOSIP gp140 immunization

• **Mapping neutralization epitopes recognized by BG505 autologous nAbs:** defined by mapping autologous neutralizing Ab specificities in rabbits and rhesus macaques previously immunized with the BG505 SOSIP.664 gp140. Those observed were epitopes in 1) a glycan hole at either residue 241 or 289 in gp120, 2) an area at residue 465 near the junction of the gp120 V5 loop and beta 24 strand and influenced by amino acid changes in the structurally nearby C3 region (the C3/465 epitope, and 3) in the V1 region of gp120 (25).
• **Induction of autologous tier 2 nAbs:** nine of twelve rhesus macaques developed autologous tier 2 nAbs after BG505 SOSIP.v5.2 with adjuvant (ISCOMATRIX or R848 (a TLR7/8 agonist) and monophosphoryl lipid A (MPL) (a TLR4 agonist) encapsulated in Poly(lactic-co-glycolic acid) (PLGA) immunization. The best correlate of tier 2 nAb induction was lymph node germinal center B cell magnitude and T follicular helper (Tfh) properties of Env-specific CD4+ T cells (26).
• **Protection against SHIVBG505 challenge**: 78 rhesus macaques were immunized three times with BG505 SOSIP.664 gp140. 12 animals with either low or high titers received a fourth immunization and subsequently were repeatedly challenged with homologous SHIVBG505 intrarectally. Animals with high-titers of autologous nAbs were protected (no infection or lowered peak viremia) until the titers waned. An autologous serum ID50 nAb titer of ~1:500 was consistent with >90% protection from the median challenge dose (27).
• **Immune response kinetics**: Rhesus macaques receiving bilateral, adjuvanted, subcutaneous (SC) immunization with BG505 SOSIP.664 gp140 generated tier 2 nAbs after two immunizations 8 weeks apart, and these were further enhanced by a third immunization with BG505 SOSIP.664 gp140. Neutralizing Abs were strongly associated with germinal center reactions, as assessed by lymph node B and T cells obtained by fine needle aspiration (28).
• **bnAb induction by BG505 SOSIP.664 vaccination in cows**: Four cows immunized with a single injection of BG505 SOSIP.664 rapidly generated broad, potent antibodies. One cow more extensively analyzed for neutralization against a 117 cross-clade HIV-1 panel developed 20% breadth within 42 days, and this increased to 96% breadth at 381 days. A mAb isolated from this cow neutralized 72% of isolates and was found to have an ultra-long heavy chain complementarity-determining region 3 (HCDR3) (60 amino acids) (29).

4.1.2 Trimer 4571 (BG505 DS-SOSIP.664 gp140): A Stabilized Soluble Trimer

The prototype, BG505 SOSIP.664 gp140, is the first of the stable trimer class manufactured for clinical use. The HIV envelope is dynamic, however, and can have multiple conformations. Since HIV can assume multiple conformations, it is not known which conformation is optimal to target for inducing tier 2 neutralizing antibodies. The rationale for engineering the two cysteine residue changes into Trimer 4571 was to fix the antigen into the mature, pre-fusion closed form (a "vulnerable" state) (Figure 4-1). This "DS" form of the BG505 SOSIP.664 demonstrates substantial binding to canonical broadly neutralizing antibodies and virtually no binding to poorly neutralizing epitopes in preclinical models (30).

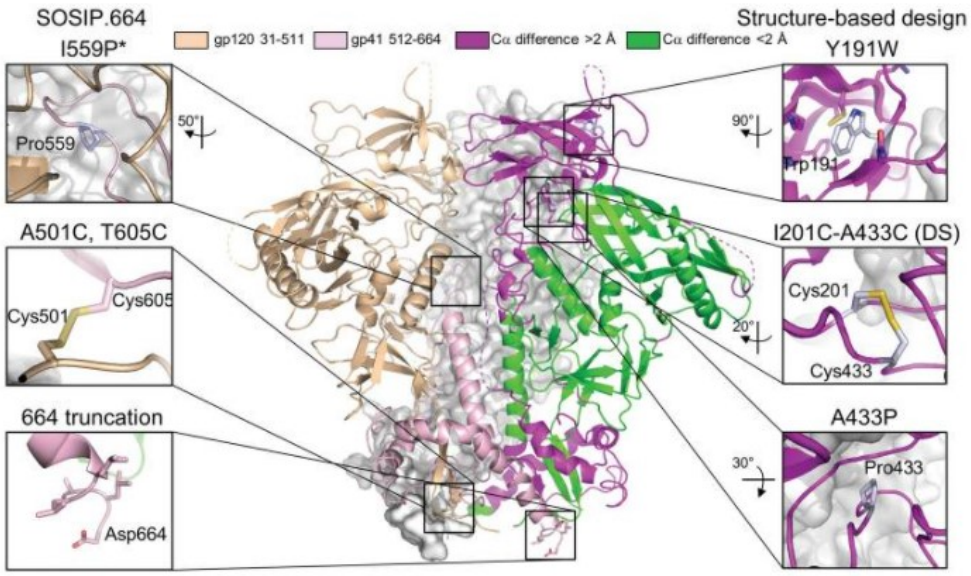

Figure 4-1 Structure of Trimer 4571 Trimer 4571 has two specific mutations introducing two cysteine residues at positions 201 and 433 A number of candidates were screened and this trimer was determined to have two desirable properties: 1) it was conformationally stable and 2) it was not "triggered" by binding to CD4 which can lead to exposure of non-neutralizing epitopes.

Rationale for Trimer 4571

In proof-of-concept preclinical studies in guinea pigs, Trimer 4571 adjuvanted with Alum-induced autologous neutralizing antibodies to BG505.W6M.C2 after the second administration, which increased after the third administration. Rhesus macaques immunized via IM injection with research-grade Trimer 4571 produced neutralizing antibodies to BG505.W6M.C2.T332N (see the Investigator's Brochure [IB] for more detail on these BG505 variants).

4.1.3 Adjuvants

Since HIV preventive vaccine clinical trials began in 1987, more than a dozen unique adjuvants have been evaluated with HIV-1 Env protein or peptide immunogens (31-35). Alum and MF59 have been the most frequently used in formulation with subunit Env immunogens, but others have been evaluated in phase 1 trials, including emulsions, saponins, surfactants, particles, and TLR4 agonists. Among these are QS-21, monophosphoryl lipid A (MPL), muramyl dipeptide (MDP), muramyl tripeptide phosphatidylethanolamine (MTP-PE), liposome-encapsulated lipid A, Montanide ISA-51, AS02A, AS01B, AS01E, and more recently glucopyranosyl lipid A (GLA). One adjuvant, Montanide ISA-51, was highly reactogenic at doses delivered (32, 36). Newer formulations elicited mild-to-moderate increased antibody and T cell responses (eg, QS-21, AS01B, and liposome-encapsulated lipid A) (37), and these components are now in use in a number of new vaccine formulations for malaria, HIV, and other viral pathogens. Unfortunately, specimens needed for retrospective analysis were not archived from these trials and, until recently, the immunologic tools to explore at

the molecular level patterns of innate and adaptive vaccine/adjuvant–induced immune responses were unavailable.

The development of new generation adjuvants beyond Aluminum and emulsion formulations, together with a deeper understanding of the mechanistic role of adjuvant formulations in stimulating innate immunity, offer opportunities to improve candidate vaccine designs intended to prevent HIV infection. In addition, renewed interest in novel adjuvants for use in clinical HIV subunit vaccines has emerged with the findings from the RV144 trial (38-42) that more potent and durable antibodies with distinct effector functions may be important to elicit. In addition, the potential utility of improved adjuvants is well recognized for use with Env immunogens designed to induce immune responses along the pathway to bnAbs. Thus, we seek to combine a novel new class of HIV Env immunogen with three novel adjuvant formulations signaling through specific TLRs, either TLR4 (GLA-LSQ), TLR7/8 (3M-052-AF), or TLR9 (CpG 1018), all of which show promise in altering the potency and quality of antibody responses in preclinical studies.

Several recent reviews highlight the role of adjuvants in modern vaccines, present their mechanisms of action when known, and attest to the need to develop more clinically effective adjuvant/immunogen combinations for HIV and other disease targets (35, 43-47). Novel adjuvants have been shown to increase the magnitude and breadth of vaccine-induced antibodies in animal models (48-52) and, less frequently, in humans (53). Nevertheless, while the past several years have seen much progress in understanding immunologic effects of adjuvants and their underlying signaling mechanisms in in vitro and animal models, only a few studies have been performed in humans, particularly head-to-head adjuvant comparisons, in HIV vaccine clinical trials (54).

Molecular immunostimulants and carrier systems comprise the two main adjuvant categories (55). Most immunostimulants act as ligands for pattern recognition receptors. These include toll-like receptors, nucleotide-binding oligomerization domain (NOD)-like receptors, helicases and C-type lectin receptors. Current novel adjuvants primarily act as agonists targeting TLR3, 4, 7/8 and 9. These adjuvants can stimulate, increase or regulate immune responses to the antigen, which could be critical for poorly immunogenic antigens such as HIV-1 envelope bnAb epitopes. Desirable adjuvant properties may include increasing the breadth of immunity, enhancing responses in persons with advanced age or immune impairments, extending the durability of vaccines in newborns or the elderly, reducing the need for repeated booster immunizations, sparing antigen dose, and targeting specific types of immune responses.

Development of new adjuvants for clinical use has been challenging, and because of safety concerns a limited number have advanced to licensure. In some cases, enhanced adjuvant potency has been accompanied by increased reactogenicity and systemic toxicity. This may be due to irritant effects at the site of injection or release of proinflammatory mediators and vasoactive substances (56, 57), and infrequently to the breakdown of self-tolerance, possibly leading to autoimmune disorders (58). Rappouli et al described vaccine adjuvant development as “one of

the slowest processes in the history of medicine" (59). This highlights the need to develop standardized predictive models to assess safety, including adverse events (AE), pre-existing genetic variations in innate immunity and the application of systems approaches to define early biomarkers of adjuvant activity and safety signals, such as genomics, transcriptomics, proteomics and metabolomics. This study offers a unique opportunity to systematically assess the safety of multiple adjuvants with one vaccine antigen and contribute a potential expanded safety marker panel. While discordant adjuvant effects may be seen when formulated with different antigens, a safety marker panel that can be used in future studies of novel HIV and non-HIV vaccine adjuvants could be beneficial in preventing infections of major public health importance.

4.1.4 Recent preclinical investigations with HIV vaccine-adjuvant and delivery formulations

One challenge in understanding adjuvant effects in humans relates to species differences in innate cell receptor expression and tissue distribution, and the regulation of innate signaling responses, which may limit translation of preclinical experimental findings to clinical applications. TLR expression on antigen presenting cells (APCs) varies between mouse and human. In inbred mice, functional TLR8 is not expressed on APCs, whereas in humans, myeloid dendritic cells (mDCs) express both TLR4 and TLR8. Human plasmacytoid DCs (pDCs) and B cells express intracellular TLR7 and TLR9, which recognize viral and bacterial nucleic acids and mediate the production of type I interferons (IFNs). However, in mice, TLR7 is primarily expressed by CD8- mDCs (43) and TLR9 in CD8- mDCs and in B cells (60). Less is known about how responses to TLR ligand stimulation compare between rhesus macaques and humans. In vitro stimulation studies (61) indicate that the response pattern of human and rhesus macaque pDCs and B cells to TRL7/8 and TLR9 is comparable, despite some differences in surface phenotype of responding cells. A comprehensive evaluation of rhesus macaque innate responses in blood and draining lymph node (LN) following TLR agonist intradermal injection highlights the distinct signatures of early innate responses both locally and systemically, and provides a major step toward addressing species differences in recognizing TLR agonists now being used in non-human primate vaccine models and advanced to clinical studies (62). These insights into species differences in innate responses can influence the selection of suitable animal models for preclinical immunogenicity and toxicity studies in developing candidates for clinical testing.

Francica and Seder performed a longitudinal analysis of adjuvant effects and HIV Env-specific B cell receptor (BCR) responses in rhesus macaques following Env protein vaccination with eight different adjuvants (63). The immunogens were HIV subtype C Env gp140 (Novartis) alone or formulated with Alum or MF59 with or without agonists of TLR4 or 7, or with poly IC:LC or immune stimulating complexes (ISCOMs). Increased anti-Env binding titers and neutralization titers to tier 1A viruses were observed among the adjuvanted groups in contrast to the un-adjuvanted group after four immunizations; notably the Alum/TLR7, MF59 and ANE/TLR4 recipients produced the highest mean IgG endpoint titers. Moreover, the MF59, poly IC:LC and ISCOM formulations elicited marked

increases in Env-specific memory B cells after two or four immunizations, although these contracted by 12 weeks after the fourth immunization. Various adjuvant formulations did not modulate changes in overall somatic hypermutation levels and HCDR3 length, properties consistent with but not sufficient for development of bnAbs. This investigation provides an important framework for assessing B cell and antibody responses with HIV vaccines that can be applied in preclinical and clinical models.

An additional study by Francica and colleagues (64) assessed eight adjuvants combined with an Env gp140 immunogen to examine and compare innate transcriptional effects with the potency and quality of Env-specific immune responses in rhesus macaques. Among the adjuvant groups assessed, the Alum/TLR7 and poly IC:LC adjuvants were the most potent in assays characterizing both Ab and cellular responses. This hierarchy of potency was sustained throughout the longitudinal follow up. Of note, antiviral interferon gene signatures during innate timepoints post vaccination correlated with Fc-receptor binding across all adjuvant groups. The study also described the impact of adding a TLR7 agonist to Alum, finding that the TLR7 agonist suppressed expression of inflammatory genes commonly seen with Alum alone, and the combination enhanced upregulation of antiviral and interferon genes; the overall effect of the Alum/TLR combination was to boost Env binding Ab titers 3-10-fold compared to Alum alone.

Franchini and collaborators (65-67) conducted extensive investigations in a rhesus macaque simian immunodeficiency virus (SIV) vaccine challenge model to elucidate and validate correlates of protection associated with the mildly efficacious RV144 Thai trial regimen that used a recombinant canarypox vector (ALVAC)-SIV and gp120 formulated either in Alum or MF59. Vaccine efficacy against SIVmac251 intrarectal challenge was associated with Alum-induced, but not with MF59-induced, Env-dependent mucosal innate lymphoid cells producing interleukin (IL)-17, gp120 V2-specific mucosal IgG, and activation of the Ras pathway (65). Follow-up studies have pointed to the potential importance of mucosal anti-V2 antibodies at the site of virus challenge (67) and the ability of vaccine-induced antibodies to inhibit infectivity of virus that it binds and captures (66). An additional study from the Franchini and Sekaly laboratories (68) evaluated various priming vectors (ALVAC, DNA, Ad26) with SIV inserts followed by pathogenic SIV challenge. They used a systems biology approach to determine innate and adaptive responses associated with protection. The authors found that the activation of hypoxia and the inflammasome in CD14+16- monocytes, CD4+ Th2 cells with gut-homing markers and anti-V2 Abs correlated with reduced risk of SIV acquisition. Larger confirmatory studies are in progress to understand the impact of these adjuvants and vectors on SIV protection, particularly at mucosal sites, which may inform future human trials with similar regimens. In addition, it will be important to assess some of these effects with the adjuvant responses in this protocol.

Examination of early events in vaccine antigen-adjuvant priming in animal models are lending insight into new methods to induce desired immunity (26, 69-71). Irvine and colleagues reported heightened antibody responses by sustained

delivery of HIV antigens through either repeated injections or osmotic pumps over 1-2 weeks (71). Exponentially increasing dosing was superior to a single full dose priming; findings were consistent with antigen retention in draining lymph nodes and expanded germinal center B and T follicular helper (Tfh) cell responses. Thus, regulating the kinetics of antigen and adjuvant delivery may stimulate increased vaccine potency. Monitoring germinal center reactivities in this protocol potentially would be useful in understanding the qualitative effects of various adjuvants and the potential for inducing tier 2 HIV nAbs.

Havenar-Daughton and colleagues have employed repeated direct probing of germinal center responses by fine needle lymph node aspirates in nonhuman primate (NHP) studies and found that induction of neutralizing antibodies against tier 2 autologous HIV strains best correlated with germinal center B cell magnitude and Env-specific CD4+ Tfh cells (26). Furthermore, based on murine, NHP and human data, Havenar-Daughton and Crotty (70) found that significantly higher levels of plasma CXCL13 were associated with heightened germinal center reactivity, attesting to the potential use of CXCL13 as a biomarker in blood to follow germinal center activities promoting functional antibody responses and persistence. Similar sampling strategies are under evaluation in licensed vaccine and early phase human HIV vaccine studies and are planned in this protocol (72).

Taken together, these animal studies support the further study of novel adjuvanted vaccines and their potential for advanced evaluation in clinical trials. Clearly new HIV vaccine-adjuvant formulations are needed to induce Abs with greater potency and persistence. Probing the dynamic and molecular effects of complex adjuvant formulations with novel HIV Env immunogens is critical for advancing candidate adjuvanted vaccine regimens that induce desired immune response profiles.

4.1.5 Clinical studies

We anticipate that the native-like soluble Env trimers will be important designs in the sequential bnAb pathway regimens, particularly as more native-like Envs may be required as booster immunogens, and they will offer the advantage of presenting more than one bnAb epitope region. Additional designs will include stabilized Env trimers expressed as DNA and in viral vectors (73). Two clinical trials with stabilized soluble trimers will be underway when this protocol begins, IAVI W001 evaluating the same BG505 SOSIP.664 gp140 vaccine as in this protocol but formulated with $AS01_B$, and a NIH Vaccine Research Center (VRC) phase 1 study with HIVRGP096-00-VP with Alum, a DS-SOSIP trimer.

IAVI W001 (ClinicalTrials.gov Identifier: NCT03699241) is a randomized double-blinded, placebo-controlled, dose-escalation phase 1 clinical trial evaluating safety and immunogenicity of recombinant HIV BG505 SOSIP.664 gp140 vaccine formulated with $AS01_B$ (GlaxoSmithKline [GSK]) administered IM in HIV-uninfected study participants. This is the first in human study of BG505 SOSIP.664. The protocol was activated in December 2018 and the first vaccination was administered in March 2019. The study will enroll 36 U.S. participants (30 vaccine recipients/6 placebo recipients) in Seattle and Boston,

evaluating three dose levels (30, 100, and 300 mcg) of BG505 SOSIP.664 gp140. Later in 2019, 24 study participants (20 vaccine recipients/4 placebo recipients) are scheduled to be enrolled in Nairobi, Kenya, and to receive either 30 or 100 mcg of BG505 SOSIP.664 gp140. As of August 9, 2019, 6 participants have been enrolled in Group 1 (30 mcg dose). To date there have been no immune mediated diseases, no deaths, no pregnancies, no incident HIV infections, and no Grade 3 or higher hematology, chemistry, or urine laboratory values. No volunteers have been discontinued from the trial. This trial is completely enrolled and near completion.

The NIH VRC has manufactured Trimer 4571, a CHO-cell line derived DS-SOSIP.664 trimer design, and its ligand-free crystal structure reveals a soluble stabilized prefusion-closed Env trimer (30, 74). This new HIV vaccine, designated HIVRGP096-00-VP with Alum, is being evaluated for safety and immunogenicity in 25 healthy adults in a first in human phase 1 trial (ClinicalTrials.gov Identifier: NCT03783130) that began in March 2019. The clinical trial evaluated two doses of the trimer, 100 and 500 mcg, each delivered either IM or subcutaneous (SC).

For HIV vaccines, MF59, a squalene-based oil-in-water emulsion, has been formulated with several HIV-1 envelope proteins for extensive clinical testing and is presently in phase 2b efficacy evaluation (HVTN 702) in South Africa as part of an ALVAC/HIV vector prime and adjuvanted bivalent gp120 boost regimen. In addition to AS03 and AS04, the Adjuvant System family (GSK) (75, 76) includes two adjuvants containing monophosphoryl lipid A (MPL) and *Quillaja saponaria* fraction 21 (QS-21), AS01 and AS02, both of which have been evaluated in phase 1 HIV vaccine studies (33, 77, 78). AS01 has been formulated with various new Env immunogens for clinical testing, including the eOD-GT8 Env immunogen (IAVI G001) and BG505 SOSIP.664 gp140 in the IAVI W001 trial described above. Table 4-2 summarizes novel adjuvant formulations in HIV vaccine clinical trials.

Table 4-2 Adjuvant formulations in HIV vaccine clinical trials

Adjuvant	Vaccine	Study
MF-59 (Novartis/Seqirus)	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59	Pivotal phase 2b/3 trial (HVTN 702), prime-boost regimen in South Africa
	HIV-DNA and bivalent gp120 (all subtype C)	Phase 1/2a trial (HVTN 107 and HVTN 108), 2 adjuvant arms
	ALVAC-HIV (vCP2438 and bivalent subtype C gp120/MF59)	2-adjuvant–arm study (in planning)
$AS01_B$ (GSK): Liposome-based QS-21 + monophosphoryl lipid A	HIV-DNA and bivalent up120/AS01B (all subtype C)	Phase 1/2a trial (HVTN 108), 2 adjuvant arms
	eOD GT8 60-mer nanoparticle	Germline targeting Env immunogen (IAVI G001)
	BG505 SOSIP.664 gp140	Well-ordered native-like soluble Env trimer (IAVI W001)
	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/AS01B	2-adjuvant–arm study (HVTN 120)
GLA-SE AAHI): glucopyranosyl lipid A in stable emulsion	Polyvalent gp120	Phase 1 trial (HVTN 124)
	CH505 gp120	Phase 1 trial, B-cell lineage (HVTN 115)

4.2 Trial design rationale

4.2.1 Dose (amount and number)

Part A: BG505 SOSIP.664 gp140 admixed with adjuvants will be administered two times.

- BG505 SOSIP.664 gp140: 100 mcg
- 3M-052-AF adjuvant: 1 mcg and 5 mcg
- Alum adjuvant: 500 mcg (Aluminum content)

Part A optional second boost: a third administration of BG505 SOSIP.664 gp140 admixed with 3M-052-AF and Alum (see below for rationale).

- BG505 SOSIP.664 gp140: 100 mcg
- 3M-052-AF adjuvant: 1 mcg and 5 mcg
- Alum adjuvant: 500 mcg (Aluminum content)

HVTN studies have tested HIV Env antigen doses ranging from 20 to 600 mcg. The effects of adjuvants may be less pronounced at high antigen doses, based on data from HVTN 041 (79) and animal studies. Therefore, 100 mcg is chosen as an

intermediate dose that may be sufficient to generate immune responses and induce potential detectable distinguishing features among different adjuvant groups.

3M-052 has been tested in patients with cancer. This will be the first-in-human study in which 3M-052 is used as a vaccine adjuvant. Based on the preclinical and clinical data, we will test 1 and 5 mcg of 3M-052 in aqueous formulation (3M-052-AF) to ensure that the adjuvant is safe.

Alum has been used safely as an adjuvant in licensed vaccines up to 850 mcg per dose. The dose of 500 mcg is sufficient for adsorption of 3M-052-AF and BG505 SOSIP.664.

Part B: BG505 SOSIP.664 gp140 admixed with adjuvants will be administered three times.

- BG505 SOSIP.664 gp140: 100 mcg dose
- 3M-052-AF adjuvant: 5 mcg dose
- CpG 1018 adjuvant: 300 mcg dose
- GLA-LSQ adjuvant: 5 mcg GLA and 2 mcg QS-21
- Alum adjuvant: 500 mcg (Aluminum content)

In the licensed HEPLISAV-B hepatitis B vaccine, 3 mg dose of CpG 1018 is used. The dose of 300 mcg will be used in this study, based on formulation data with Alum and BG505 SOSIP.664.

The same dose of GLA-LSQ was used in a TB vaccine study and the safety data were acceptable (ClinicalTrials.gov Identifier: NCT02508376).

Part C: BG505 SOSIP.664 gp140 admixed with adjuvant (Group 7) and Trimer 4571 admixed with adjuvant (Group 8) will be administered 3 times on the same schedule as Part B.

The 100-mcg dose of Trimer 4571 was found to elicit trimer-specific binding antibodies in participants in VRC018 and was well tolerated. We selected the 100 mcg dose of Trimer 4571 to permit direct comparisons with the participants who were given the 100 mcg dose of the BG505 SOSIP.664 gp140 trimer in Parts A and B.

- BG505 SOSIP.664 gp140: 100-mcg dose
- Trimer 4571: 100 mcg-dose
- 3M-052-AF adjuvant: 3- and 5-mcg dose
- Alum adjuvant: 500 mcg (Aluminum content)

4.2.2 Schedule

The schedule of vaccinations in Part A is 0 and 2 months. An optional third vaccination – Part A optional second boost – will be administered to participants who received two vaccinations at 0 and 2 months in Part A and consent to one additional (third) immunization with the same dose of protein and adjuvant.

The schedule of vaccinations in Part B and Part C is 0, 2, and 6 months. This second schedule and a similar schedule 0, 1, 6 months are commonly used for subunit recombinant vaccines. This is the same schedule as licensed HPV vaccines Gardasil and Gardasil 9. Hepatitis B vaccines Engerix-B and Recombivax HB use 0, 1, 6 month schedule. In addition, recent studies from the Crotty laboratory showed that immune responses in lymph nodes escalate over 3 weeks after immunization in rhesus macaques (26), suggesting that the second dose may better activate desirable immune responses if it is given at a later timepoint such as month 2 rather than month 1.

Rationale for Optional Third Dose to Participants in Part A:

There are two major justifications for offering all participants who received two doses in Part A the opportunity to receive a third dose of the same products they have already received, which is also one of the same three dose regimens included in Part B.

First, disruptions from the ongoing COVID-19 pandemic allowed for an unanticipated opportunity to take a preliminary look at the immunologic response in participants in Part A. These data are now available and look extremely promising. Serum from 1 out of 5 participants in the low dose adjuvant group and 3 out of 10 participants in the high dose adjuvant group neutralized BG505/T332N, albeit with low titers (see Figure 4-2 below). **This is the first example of vaccine elicited autologous tier 2 neutralization in humans**. The internal controls strongly suggest that the neutralizing effect is specific to HIV and not an artifact of the pseudovirus assay because no neutralizing activity against the MLV control virus was detected in the assays with MLV. A major question for HIV vaccinology is whether these neutralization titers can be boosted with a third dose. To test whether this low-tier neutralization could be improved, the protocol was modified to allow participants in Part A an optional third boost. Indeed, 3/5 participants in the 5-mcg dose group had high autologous tier-2 neutralization. The 1-mcg dose group had 1 participant (out of 3) with low-level neutralization, suggesting that the dose of adjuvant may matter.

Second, delays associated with the ongoing COVID-19 pandemic have altered the timelines of anticipated studies such that it is important to understand the role of additional vaccinations with products containing 3M-052 AF. Waiting until Part B is fully enrolled and data is available will take substantially more time (~18 months) as compared to offering participants an additional boost in Part A. The additional boost dose was added to Part A due to a need to evaluate the 3M-052-AF for planned future studies.

Rationale for Adding Part C: Optimization of 3M-052-AF

A third dose of BG505 SOSIP.664 and 3M-052-AF + Alum was offered to participants in Part A (see above). Data from participants who provided additional informed consent are now available and are presented in Figure 4-2.

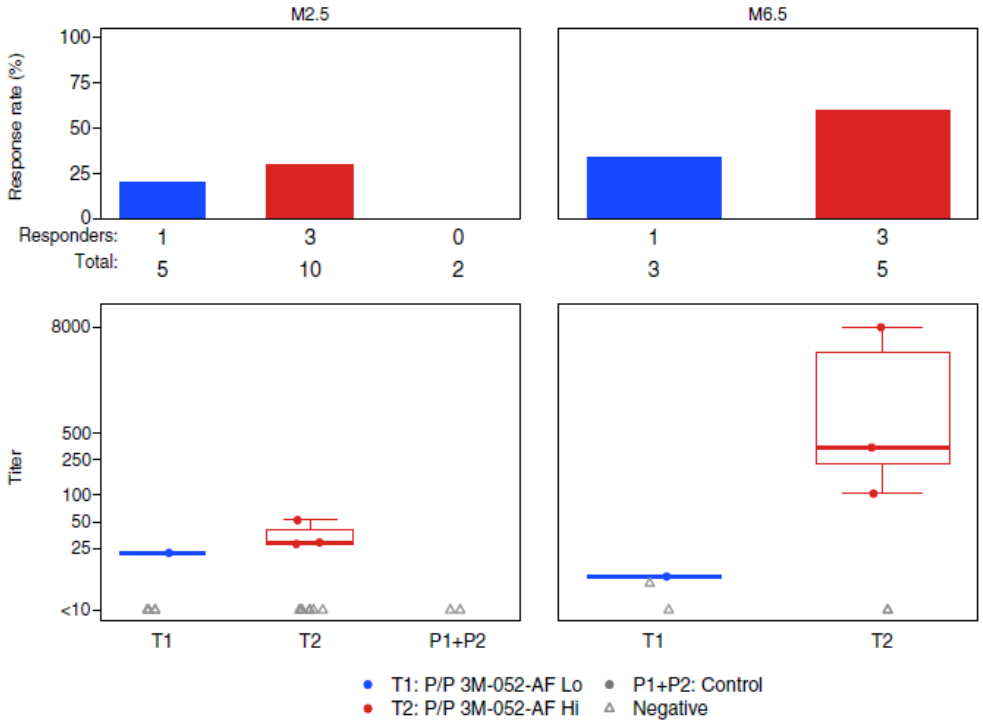

Figure 4-2 Response rate and ID50 neutralizing antibody titers against the autologous tier 2 virus BG505/T332N 2 weeks post second and third vaccination in HVTN 137 Part A.

The high neutralization (with maximum ID50 titers of ~8,000) seen in some of the participants in Part A is the first time such high titers have been observed following vaccination in humans (to our knowledge).

The protocol team therefore desires to build on these exciting results with a goal of optimizing the 3M-052 AF + Alum for advancement of this product.

Using the same schedule, we propose to determine whether a 3-mcg dose of 3M-052 AF + Alum is sufficient to drive tier 2 autologous neutralization with less systemic reactogenicity than observed with the 5-mcg dose.

Additionally, we propose to test whether the VRC trimer 4571 adjuvanted with 3M-052 AF leads to the development of tier 2 (both autologous and heterologous) neutralizing antibodies. The VRC 4571 trimer is a stabilized BG505 trimer designed to better elicit nAbs. The VRC trimer 4571 was formulated in Alum in a recent VRC phase 1 trial, but no autologous nAbs were elicited. This trial used Alum as an adjuvant. To test whether there is a conceptual issue with trimer design or, alternatively, whether the adjuvant is a critical mediator for neutralization, we proposed to add an arm testing whether VRC 4571 leads to

neutralization of the autologous and other tier 2 viruses. This is a critical question for the field because it will determine whether the conceptual framework (that alterations in the stabilization of a trimer will lead to improved neutralization) is accurate.

4.2.3 Choice of control

Tris-NaCl Diluent will be administered as a placebo control.

4.2.4 Rationale for mucosal specimens and sample collection by leukapheresis, lymph node aspiration, bone marrow aspiration

4.2.4.1 Mucosal specimens

Env antibody IgG and IgA titers will be examined in rectal and vaginal tissues and secretions. These studies will determine the amount of antibodies available post 3rd immunization at mucosal sites of potential HIV exposure and the antibody levels that can be achieved with use of different adjuvants.

4.2.4.2 Leukapheresis

Blood mononuclear cells will be collected by leukapheresis to increase cell yield after the 2nd immunization in consenting participants. This will provide sufficient cell numbers to analyze the B cell receptor sequence repertoire, including detection of signatures associated with broad neutralizing antibodies, as well as B cell function and specificities. Similarly, the additional cell yields will permit comprehensive T cell analyses, including T cell receptor repertoire, function, and epitope specificities.

4.2.4.3 Lymph node fine needle aspiration (FNA)

Draining axillary lymph node cells will be examined for germinal center reactivities after vaccination and the influence of various adjuvants on germinal center B cell, CD4+ T follicular cell, and myeloid cell functions. We will also direct efforts to find germinal cell reactivity biomarkers in peripheral blood for future studies that can be used to potentially predict Env-specific broad neutralizing activities.

4.2.4.4 Bone marrow aspiration

Long-lived plasma cells resident in bone marrow will be examined to determine adjuvant effects on durability of antibody responses. Additional immune cells in the marrow will be characterized that potentially support these effects in the bone marrow microenvironment.

4.3 Preclinical safety studies

Several preclinical studies have been performed to assess the potential toxicity of the vaccine-adjuvant formulations that will be evaluated in this clinical trial. The design of these studies is outlined in Table 4-3.

Table 4-3 Summary of preclinical safety studies

Study number	Product	Dose (mcg*)	Type of study	Animal	N**	Dose groups	Route	Schedule
Covance 8394847	BG505 SOSIP.664 g140 3M-052-AF + Alum CpG 1018 + Alum GLA-LSQ (GLA+QS-21)	50 0.5+250 and 2.5+250 150+250 2.5+1.0	Toxicity	Rat	15m, 15f per dose group	5	IM	0, 3, 6, 9 weeks
12-728	HEPLISAV-B (HBsAg+CpG 1018)	20+3000 and 4+600	Toxicity	Rat	15m, 15f per dose group	2	IM	0, 2, 4, 6 weeks
2416-001	ID93 GLA-LSQ (GLA+QS-21)	0 or 20 0 or 20+40	Toxicity	Rabbit	10m, 10f per dose group	4	IM	0, 2, 4, 6 weeks
11943	H5 VLP 3M-052-SE	20 0, 2, 10	Toxicity	Rat	15m, 15f per dose group	4	IM	0, 3, 6, 9 weeks
NA	3M-052	0, 1.25, 3.75, or 6.25 mg/kg	Toxicity	Mouse	10-16m, 10-16f per dose group	4	SC	0, 1, 2, 3 weeks
NA	3M-052	0, 1.25, 2.5, or 5.0 mg/kg	Toxicity	Cynomolgus monkeys	5m, 5f per dose group	4	SC	0, 1, 2, 3 weeks

* Except where noted.

** m = male; f = female

4.3.1 IND-enabling, GLP repeat dose toxicity study of BG505 SOSIP.664 gp140 with adjuvants GLA-LSQ, CpG 1018+Alum, 3M-052-AF+Alum in rats (Covance study 8394847)

The objective of this IND-enabling study was to evaluate the safety and tolerability of BG505 SOSIP.664 gp140 + adjuvants in rats. The study was conducted in rats because two adjuvants (3M-052-AF and CpG 1018) were deemed unlikely to enhance the immune response to the antigen in rabbits. We have demonstrated an active immune response in rats in a pilot study following immunization with the various adjuvanted investigational vaccines that would be evaluated in the current study. Likewise, rats historically have been used in safety evaluation studies and are recommended by appropriate regulatory agencies.

The animals were dosed by IM injection in week 0, 3, 6, and 9. Ten animals per sex per group were assigned to the main study. Five animals per sex per group were in the 4-week dose-free recovery period. To make a direct comparison of the toxicity of each investigational vaccine, the dosing interval and recovery periods were identical across all adjuvanted vaccines evaluated. This also facilitated management of study logistics and interpretation of the study data. Repetition of dosing once every three weeks ensured that the animals had sufficient time in between doses to recover from any potential toxic effects.

The mixing proportion of the various experimental vaccines were identical to that proposed in the clinical trial in terms of concentration of BG505 SOSIP.664 gp140 and adjuvant levels used. However, the dose volume in the nonclinical study was limited to the maximum tolerated volume for IM injections in rats of 0.25 mL, whereas the clinical dose volume is 0.5 mL.

In a pre-IND technical consultation, the FDA concurred with the study design and proposed in-life activities (see Table 4-4).

Table 4-4 Covance 8394847 group designations and dose levels in rats

Group	Test article(s)	No. of Animals		Dose Level (mcg)				
		Male	Female	BG505 SOSIP.664 gp140	Alum	3M-052	CpG	GLA/ QS-21
1	Control	15	15	0	0	0	0	0
2	BG505 SOSIP.664 gp140 + Alum + Low 3M-052	15	15	50	250	0.5	0	0
3	BG505 SOSIP.664 gp140 + Alum + High 3M-052	15	15	50	250	2.5	0	0
4	BG505 SOSIP.664 gp140 + Alum + CpG 1018	15	15	50	250	0	150	0
5	BG505 SOSIP.664 gp140 + GLA-LSQ	15	15	50	0	0	0	2.5 GLA/ 1.0 QS-21

Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, dermal irritation scoring, body temperature, ophthalmic observations, and clinical and anatomic pathology. Blood samples were collected for antibody, α-acid glycoprotein, and α-2-macroglobulin (acute phase markers) analyses.

No test article or adjuvant-related mortality; clinical observations (other than dermal irritation findings at the dose site); or effects on body weight, food consumption, body temperature, ophthalmic observations, or organ weight parameters were observed during the dosing or recovery phase.

Dermal irritation, included erythema (barely perceptible to well-defined) and edema (barely perceptible to slight), were typically observed only after the second dose and were observed between 1 and 72 hours postdose. Erythema and edema were considered study product related since controls were largely unaffected. In general, no adjuvant combination caused a markedly higher incidence or severity of dermal irritation. The pattern of dermal irritation was generally consistent with injection site irritation findings noted microscopically, with the clinical pathology changes, and with the acute phase markers response identified in the study. No abnormal dermal observations were noted during the recovery phase.

During the dosing phase, all dose groups administered BG505 SOSIP.664 gp140 had minimal test article-related hematology, coagulation, and/or clinical chemistry changes at 72 hours post dose after the first and last dose that were consistent with injection site inflammation and/or the immune response elicited by the test articles. These changes included minimally higher fibrinogen and/or globulin concentrations, neutrophil count, and/or red blood cell distribution width; minimally lower albumin concentration, reticulocyte count, and/or albumin:globulin ratio; and/or minimally prolonged prothrombin time.

Acute phase marker proteins were evaluated 24 and 72 hours after the first and last dose and at terminal sacrifice. Minimal to moderately higher levels of both acute phase proteins were identified in all groups administered the adjuvanted vaccine.

Collectively, these clinical pathology changes were generally most pronounced 72 hours after the last vaccination during the dosing phase and in animals administered adjuvants containing Alum (Groups 2, 3, and 4). A dose response was generally evident in animals administered ≥ 0.5 mcg 3M-052-AF (Groups 2 and 3).

During the recovery phase, these test article-related clinical pathology changes fully reversed, except for minimally lower reticulocyte count and albumin concentration in males administered the adjuvants containing 2.5 mcg 3M-052-AF or 150 mcg CpG 1018, which partially reversed.

Very few gross necropsy findings were identified. One female rat from the GLA-LSQ group had discoloration of the last injection site that correlated

microscopically with mononuclear cell inflammation. Macroscopic finding of thickening observed at the last injection site in two female rats from the high dose 3M-052-AF group lacked confirmatory microscopic correlates; its etiology remains unresolved. No vaccine induced macroscopic findings were identified at recovery necropsy.

At terminal sacrifice, test article-related microscopic findings were observed in both vaccination sites (right and left thigh musculature) and the left quadriceps muscle, stifle joint and sciatic nerve. Histologically it was characterized by mixed cell inflammation, mononuclear cell inflammation, and/or mononuclear cell infiltrates.

Mixed cell inflammation, an acute physiological response was limited to tissues in the left hindlimb, the site of the last vaccination and often extended into the left quadriceps muscle and stifle joint. The mixed inflammation was characterized by infiltrates of neutrophils, macrophages, and lymphocytes and foci of necrosis containing cellular debris, edema, and/or hemorrhage. This finding was observed in all vaccinated group; however, with higher incidence and severity in Groups 2, 3, and 4, compared with Groups 5; therefore, mixed cell inflammation appeared to be exacerbated by Alum administration.

Mononuclear cell inflammation, characterized by infiltrates of lymphocytes and histiocytes (often vacuolated and forming clusters) with occasional fibroplasia, was noted at both injection sites, which often extended into the quadriceps muscle, stifle joint, and/or sciatic nerve (epineurium). Mononuclear cell inflammation was considered a more chronic reaction to the test article. This finding was noted in all BG505 SOSIP.664 gp140 treated groups without a clear relationship with any tested adjuvant and was considered related to BG505 SOSIP.664 gp140. No GLA-LSQ–related mononuclear cell inflammation was noted in the right thigh of Group 5 animals, which was consistent with a more rapid resolution of inflammation than groups administered test articles containing Alum.

At the recovery sacrifice, test article-related microscopic findings were limited to mononuclear cell inflammation and infiltrates and generally occurred at a lower incidence and severity (compared with the terminal sacrifice), consistent with partial recovery. Group 5 animals had the lowest incidence of test article-related microscopic findings during the recovery phase.

In conclusion, administration of BG505 SOSIP.664 gp140 with various combinations of adjuvants was well tolerated when administered by intramuscular injection to rats once every 3 weeks for 10 weeks (four total doses). Seroconversion was observed in all groups administered the adjuvanted BG505 SOSIP.664 gp140. Local dermal reactivity to the administered vaccine was characterized by reversible dose site edema and erythema of minimal severity. Clinical pathology changes were of minimal severity, consistent with injection site inflammation and/or the immune response elicited by the test articles, and

generally most pronounced 72 hours after the last vaccination and in animals administered adjuvants containing Alum. Microscopically, vaccination site inflammation (mixed and mononuclear cell) was observed in all test article-treated groups; however, mixed cell inflammation appeared to be most severe in animals administered test articles adjuvanted with Alum, while mononuclear cell inflammation (and infiltrates) was without a clear relationship with any tested adjuvant and was considered related to BG505 SOSIP.664 gp140. Partial recovery of test article-related microscopic findings was noted at the recovery sacrifice.

As part of the nonclinical repeat dose toxicity study, an immunogenicity assessment of serum samples was done by a binding antibody multiplex assay (BAMA). The antibody assay was conducted in compliance with Good Clinical Laboratory Practice (GCLP). Rat samples were tested for IgG antibodies to BG505 SOSIP.664 gp140 prevaccination, 72 hours after the last vaccination and towards the end of the four-week dose free recovery phase. Prevaccination samples were used for baseline assessment.

At both the post vaccination collection interval, a 100% seroconversion response rate was evident in Groups 2 through 5 vaccinated with adjuvanted BG505 SOSIP.664 gp140. None of the control animals administered saline showed positive results post vaccination. On both days, the magnitude of the immune response to BG505 SOSIP.664 gp140 was significantly higher in Groups 2, 3, and 4 in comparison to Group 5 as shown in Table 4-5.

Table 4-5 Geometric mean titer of MFI-background-blank dilution at 1:5000 and 95% confidence intervals

		Geometric Mean Titer (MFI)			
Timepoint	**Parameter**	**BG505 SOSIP.664 + 250 mcg Alum + 0.5 mcg 3M052 (Group 2)**	**BG505 SOSIP.664 + 250 mcg Alum + 2.5 mcg 3M052 (Group 3)**	**BG505 SOSIP.664 + 250 mcg Alum + 150 mcg CpG (Group 4)**	**BG505 SOSIP.664 + 2.5 mcg GLA/ 1 mcg QS-21 (Group 5)**
72 hours after last vaccination	Mean	13,003	14,490	15,324	1955
	Range	12,057-16,519	13,437-18,392	14,227-18,846	1869-3404
End of dose free recovery phase	Mean	16,238	18,486	17,348	3140
	Range	14,055-19,847	16,706-20,971	15,281-20,532	2259-7479

4.3.2 GLP repeat dose toxicity study of hepatitis B vaccine HEPLISAV-B in rats (study 12-728)

Dynavax's licensed hepatitis B vaccine HEPLISAV-B consists of a hepatitis B antigen and CpG 1018 adjuvant. Dynavax has completed a series of toxicity studies, including CpG 1018 single-dose toxicity studies in rabbits and baboons, CpG 1018 repeat-dose toxicity studies in mice, rats, and cynomolgus monkeys,

CpG 1018 genotoxicity studies, CpG 1018 + hepatitis B surface antigen (HBsAg) reproductive and developmental toxicity study, and repeat-dose toxicity studies of CpG 1018 + HBsAg in mice and rats. The objective of rat repeat dose study #12-728 was to evaluate the toxicity of 2 dose levels: 3000 mcg CpG 1018 + 20 mcg HBsAg/dose and 600 mcg CpG 1018 + 4 mcg HBsAg/dose when administered by IM injection to Sprague-Dawley rats once every 2 weeks over a 6-week period (total of 4 doses). The studies all demonstrated acceptable safety profile of CpG 1018 and CpG 1018 + HBsAg.

4.3.3 GLP repeat dose toxicity study of TB vaccine ID93 with adjuvant GLA-LSQ in rabbits (study 2416-001)

The objective of this study by AAHI was to evaluate the potential toxicity of the TB vaccine ID93 ± GLA-LSQ adjuvant in male and female New Zealand White rabbits following repeated IM administrations, and to evaluate the reversibility of effects after a four-week recovery period.

Eighty rabbits were administered control article or test article for four bi-weekly doses, as described in Table 4-6. Half of the animals of each sex from each dose group were euthanized for terminal postmortem examinations approximately 48 hours after the final dose. The remaining animals from each dose group were retained and observed for a postdosing recovery period of four weeks following the final dose, followed by euthanasia and postmortem examination.

Table 4-6 Treatment Groups for Rabbit Toxicity Study #2416-001

Group	Treatment	ID93 Dose (mcg)	GLA Dose (mcg)	QS-21 Dose (mcg)	Injection Volume (mL)	Injection Route	Terminal Animal #s		Recovery Animal #s	
							M	F	M	F
1	Control (0.9% saline)	-	-	-	0.75	IM	5	5	5	5
2	GLA-LSQ	-	20	40	0.75	IM	5	5	5	5
3	ID93	20	-	-	0.75	IM	5	5	5	5
4	ID93 + GLA-LSQ	20	20	40	0.75	IM	5	5	5	5

All study rabbits survived to the scheduled necropsies. No treatment-related clinical findings or injection site reactogenicity were observed. No treatment-related or toxicologically significant effects were observed for body weights, body weight changes, food consumption, body temperatures, ophthalmology, serum protein electrophoresis, and organ weights.

Alterations in various clinical pathology parameters were noted throughout the study and were likely attributed to treatment with the GLA-LSQ adjuvant component of the vaccine (with or without ID93); however, these findings were reversible and no longer observed near the end of the recovery period suggesting that these adjuvant-related findings may be of minimal toxicological significance, and none of these findings resulted in any limiting toxicity.

No test article-related gross findings were noted at the terminal and recovery necropsies. There were test article-related microscopic findings in the left sciatic nerve perineurium (mixed cell infiltrates), left administration site (serocellular crust; mixed cell infiltrates in the subcutaneous tissue and skeletal muscle), right administration site (mixed cell infiltrates in the subcutaneous tissue and skeletal muscle), and testes (multinucleated giant cells) on Study Day 45 in groups receiving GLA-LSQ, ID93, and/or ID93 + GLA-LSQ. At the end of the 28-day recovery period (on Study Day 71), most of these microscopic findings were no longer present or were generally decreased. Additional findings (previously not seen on Study Day 45) were noted in the lungs (mixed cell infiltrates), mandibular lymph nodes (erythrophagocytosis), stomach (mononuclear cell infiltrates), and testes (multinucleated giant cells) in groups receiving GLA-LSQ, ID93, and/or ID93 + GLA-LSQ; these additional findings were limited to a single gender and were generally seen in only one treatment group.

Repeated administration of the ID93 + GLA-LSQ vaccine, or its components, by IM injection was well-tolerated as there were no treatment-related and/or toxicologically-significant findings noted throughout the study for most of the endpoints evaluated except for clinical pathology and microscopic pathology. The increases in globulin, fibrinogen, blood cells (WBCs, heterophils, and monocytes), and microscopic findings of mixed or mononuclear cell infiltrates are consistent with the administration of immunogenic substances (ie, the ID93 antigen and the GLA-LSQ adjuvant). As most of these findings were reversible and no longer seen near or at the end of the 28-day recovery period (on Study Day 71) and/or were limited to a single gender, these findings are considered to be of less toxicological significance than if these findings persisted until the end of the recovery period and/or were seen in both sexes.

Administration of the ID93 + GLA-LSQ vaccine or the ID93 antigen component alone produced appropriate IgG titers following repeated administration. Immunogenicity analyses also demonstrated that total IgG titers were approximately 10 times higher in rabbits receiving ID93 + GLA-LSQ compared to total IgG titers in rabbits receiving ID93 alone.

4.3.4 GLP repeat dose toxicity study of flu vaccine H5-VLP + 3M-052-SE adjuvant in rats (study 11943)

This study in rats was based on the intent to use 3M-052-SE adjuvant with flu antigen as a flu vaccine by AAHI. The repeat dose toxicity study in Sprague Dawley rats was sponsored by AAHI where the toxicity of 3M-052-SE (SE – Stable Emulsion) was evaluated in combination with an influenza hemagglutinin antigen. The highest dose of 3M-052 in the study is four times as high as the high dose in our toxicity study (Covance study 8394847). AAHI's IND 17789 for the indication of pre-exposure prophylaxis against influenza received a safe to proceed notification on November 29, 2017.

The objective of the study was to evaluate the toxicity of influenza H5 virus like particle (H5-VLP) antigen adjuvanted with 3M-052-SE, an oil-in-water emulsion formulation. A single dose of the H5-VLP antigen at 20 mcg was evaluated in combination with 0, 2, or 10 mcg of 3M-052-SE. Rats were dosed three times 21 days apart. Ten rats/sex/group were allocated to the main study group and were necropsied on Day 45. Five rats/sex/group that were dosed identically were assigned to a 4-week dose free recovery period and were necropsied on Day 71. The majority of rats exhibited slight erythema at the injection site starting on Day 1-2 post dose that persisted up to 6 days. Decreased body weight was noted in the adjuvanted vaccine groups after 24 hours after all three doses with a partial recovery after each occurrence. Decreased food consumption was observed and closely mirrored the body weight changes. Body weight and food consumption changes were not apparent in the recovery phase. A minimal increase of body temperature was observed at 24 hours post dose in the adjuvanted vaccine groups. In a majority of animals, the average increase in body temperature was < 1°C. The absolute WBC and neutrophil counts were increased while the platelets count was decreased in the two adjuvanted vaccine groups consequent to the inflammatory response at the injection site. These changes resolved by the end of the recovery period. Coagulation parameter changes attributed to the adjuvanted vaccine included increased activated partial thromboplastin time (APTT) and fibrinogen levels that resolved by the end of the recovery period in male rats. Clinical chemistry changes included increases in serum globulin and total protein levels and decreases in albumin/globulin (A/G) ratio which are consistent with an immune response to an antigen. Increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels correlated with vacuolation in the liver and was considered to be of minimal toxicological significance. C-reactive protein (CRP) was increased in the two adjuvanted vaccine groups and was consistent with an acute phase response following vaccination.

At Day 45, the adjuvanted vaccine groups had antigen-specific IgG titers that were 10 times higher that the group receiving the antigen alone. On a log10 scale the mean total IgG titer in the antigen alone and the antigen adjuvanted groups were 5 and 6 respectively. An increase in spleen weight was identified in both sexes and correlated with increased myelopoiesis. Microscopic changes were noted in the adjuvanted vaccine groups. The injection site showed inflammatory cell infiltration, degeneration/necrosis and hemorrhage. An increased myeloid to erythroid ratio was noted in the bone marrow and was interpreted as a secondary response to the inflammation at the injection site. The incidence and severity of liver vacuolation was increased in the 10 mcg adjuvanted vaccine group female animals. Single cell necrosis was identified in the pancreas of animals administered high doses of the adjuvant in combination with the vaccine. Degeneration of the white matter of the spinal cord was seen in one female administered 10 mcg of 3M-052-SE. With the exception of the mononuclear inflammatory cell infiltration at the injection site, all other microscopic changes resolved by the end of the recovery phase. None of the toxicological findings were considered significant with the exception of injection site findings.

4.3.5 GLP repeat dose toxicity study of 3M-052 in sesame oil formulation for cancer treatment in mice

This study was preparatory to testing 3M-052 for use in intratumoral therapy by 3M. The mouse was chosen as the rodent species because the antitumor efficacy of 3M-052 was demonstrated in mouse models. 3M-052 in a sesame oil/ethanol formulation was administered to mice SC once weekly over a 22-day period (4 total doses) at doses of 0, 1.25, 3.75, and 6.25 mg/kg body weight. (The dose tested was significantly higher than the dose planned in the current BG505 SOSIP.664 gp140 + Adjuvants study.) In comparison, the 3M-052 dose in the IND-enabling rat study (Covance 8394847) is in the range of 0.008 mg/kg body weight, more than 100-fold lower than the low dose in the mice study.

Dose sites were rotated weekly among left and right dorsoscapular and dorsolumbar sites. Necropsies were performed the day following the last dose. Additional animals were included in the 0 and 6.25 mg/kg dose groups to assess reversibility of treatment effects; these animals were necropsied 15 days after the last dose.

Effects at the injection site were observed in mice following SC administration. Scabbing occurred at a similar incidence in all groups, as did the microscopic finding of granulomatous inflammation. Microscopic findings of epithelial hyperplasia, necrosis, exudate, and ulceration occurred exclusively or at much greater incidence in the 3M-052–treated mouse groups versus the control group.

One female in the 6.25 mg/kg group was found dead 15 days after the last dose. For 2 to 3 days prior to death, this animal exhibited a swollen abdomen and yellow ventral staining. Autolysis precluded histologic examination and the cause of death was not determined. This death was considered to be potentially test article-related, with test article-related effects possibly persisting or evolving in the recovery period.

Statistically significant test article-related changes occurred in serum protein, RBC, and WBC parameters in all 3M-052–treated male groups. Changes in these parameters were consistent with 3M-052 pharmacologic activity, were within or very close to historic control ranges, and were not considered adverse.

Test article-related effects on organ weights at the end of treatment included higher liver and spleen weights or weight ratios in all 3M-052–treated male and female groups. The increased spleen weights correlated to microscopic findings of extramedullary hematopoiesis (EMH) and mild lymphoid hyperplasia. Both EMH and lymphoid hyperplasia were consistent with the pharmacologic effects of 3M-052. The degree of EMH and lymphoid hyperplasia were not considered adverse. There were no histologic correlates to the liver weight changes.

At necropsy at the end of treatment, grossly enlarged lymph nodes were noted in all 3M-052–treated male and female groups. The lymphoid hypercellularity was consistent with 3M-052 pharmacologic activity and was not considered adverse.

There were no 3M-052–related changes in body weights, food consumption, ophthalmology, or coagulation parameters either at the end of treatment or at the end of the 14-day recovery period.

Many of the toxicities reported in this repeat dose study might not be relevant from a clinical perspective since the 3M-052 in this study will be given much lower dose levels (1 or 5 mcg total dose; maximum approximately 0.000025 to 0.000125mg/kg for a 40 kg participant or ~1/10,000 the lowest dose administered in this mouse study) and at greater intervals.

4.3.6 GLP repeat dose toxicity study of 3M-052 in sesame oil formulation for cancer treatment in cynomolgus monkeys

This study was preparatory to testing 3M-052 for use in intratumoral therapy by 3M. 3M-052 in a sesame oil/ethanol formulation was administered to cynomolgus monkeys (5/sex/group) by SC route once weekly over a 22-day period (4 total doses) at doses of 0, 1.25, 2.5, and 5.0 mg/kg body weight. The doses tested were significantly higher than the dose planned in the current BG505 SOSIP.664 gp140 + Adjuvants study. In comparison, the 3M-052 dose in the IND-enabling rat study (Covance 8394847) is in the range of 0.008 mg/kg body weight, more than 100-fold lower than the low dose (1.25 mg/kg) in the cynomolgus monkey study.

There were no deaths during the study. Compared with the control group, inappetence was noted more frequently in the 3M-052 groups and was noted in the recovery phase as well as the treatment phase. During the recovery period, all 3M-052–treated groups gained weight. Body temperatures measured 1 day post dose were statistically significantly increased in all 3M-052 male groups (but not in females), and in most individual animals the temperatures exceeded laboratory historical control values. Body temperatures were within normal range when measured during the recovery period.

In cynomolgus monkeys, clinical signs of swelling and sores, and microscopic findings of ulceration occurred at greater incidence and/or severity at the injection sites of the 3M-052–treated groups versus the control group. The control and 3M-052 groups showed similar incidence of adipose tissue necrosis, ulceration, and (at recovery) dermal scar tissue formation. Clinical signs of discharge, and microscopic findings of inflammatory cells at the injection site, occurred exclusively in the 3M-052–treated cynomolgus monkey groups. The discharge was usually described as purulent and persisted into the recovery phase. The inflammatory cell population changed over time, being primarily neutrophils at the end of dosing and predominantly lymphocytes at the end of the recovery period. These findings were considered adverse.

Test article-related hematology changes occurred in all 3M-052 groups. These changes were frequently statistically significant and/or outside of laboratory historic control values. Generally, the severity of effects was similar in the mid

and high doses, which in turn were slightly more severe than the low dose effects. None of the hematology changes were considered adverse.

Serum protein changes occurred in all 3M-052 groups. These changes were frequently statistically significant and/or outside of laboratory historic control values. Generally, the severity of effects was similar in the mid and high doses, which in turn were slightly more severe than the low dose effects. The changes in serum proteins were not considered adverse.

Changes in serum chemistry parameters were observed only in the 3M-052 male groups. At the end of the recovery period, all of these parameters were comparable to the vehicle control. Based on reversibility of the findings, these changes were not considered adverse.

At necropsy at the end of treatment, there were multiple organ changes in all 3M-052 treated male and female groups, manifested as changes in organ weights and/or histology. None of the inflammatory/mononuclear infiltrates were considered to cause injury or functional impairment. Thymus changes were considered to be related to an indirect, stress-related effect of 3M-052. At the recovery necropsy, the histologic changes appeared to be resolving.

4.3.7 Preclinical Toxicology Studies of Trimer 4571

Preclinical toxicology studies of Trimer 4571 formulated with Alum were conducted in New Zealand White Rabbits (VRC-HIVRGP096-00-VP). No treatment-related or toxicologically significant clinical findings were determined. For full details, please consult the investigator's brochure. These data have been reinforced by clinical data from clinical studies (please see Section 4.5.5 below).

4.4 Preclinical immunogenicity studies

Various preclinical immunogenicity studies have been performed evaluating the BG505 SOSIP.664 with various adjuvant formulations, as summarized in Table 4-7.

Table 4-7 Summary of preclinical immunogenicity studies

Study number	Product	Animal	N	Dose groups	Route	Schedule	Assay
CAVD 708 (Covance 0066-18)	BG505 SOSIP.664 gp140, 3M-052-Alum, CpG 1018, GLA-LSQ, VRCplex, Alum	Guinea pig	6 females per group	9	IM	0, 8, 20 weeks	Neutralizing Ab, binding Ab
CAVD 724 (Covance 0102-18)	BG505 SOSIP.664 gp140, 3M-052-AF, CpG 1018, GLA-LSQ, Alum	Rat	2 females, 2 males per group	4	IM	0, 2, 4 weeks	Binding Ab, neutralizing Ab
CAVD 749 (Covance 0147-18)	BG505 SOSIP.664 gp140, 3M-052-AF, CpG 1018, GLA-LSQ, GLA-SE, LMQ, Alum	Rabbit	5 females per group	7	IM	0, 8, 20 weeks	Neutralizing Ab, binding Ab
CAVD 641 (by Bali Pulendran Lab)	BG505 SOSIP.664 gp140, 3M-052-Alum, GLA-LSQ,	Rhesus macaques	14 males per group	3	SC, IM	0, 8, 24 weeks	Neutralizing Ab, binding Ab

4.4.1 Immunogenicity of BG505 SOSIP.664 gp140 + adjuvants in guinea pigs (CAVD 708)

The objective of the guinea pig study, CAVD 708, was to assess the immunogenicity of BG505 SOSIP.664 gp140 + adjuvants, measured by neutralizing antibody and binding antibody responses. The adjuvants used in the study were based on the proposed clinical trial plan. Guinea pigs have been shown to develop neutralizing antibody responses against the tier 2 autologous virus after immunization with BG505 SOSIP.664 gp140 + Poly IC adjuvant (80), and after immunization with glycan modified BG505 SOSIP.664 gp140 with Adjuplex adjuvant (81). Guinea pigs are also known to respond to TLR4, 7/8, and 9 agonists. Adjuvant doses were selected based on recommendations of the adjuvant providers. Some variations of formulation and/or doses were tested to identify the optimal conditions. The groups, adjuvants, and doses are shown in Table 4-8. Note that 3M-052-Alum was prepared by mixing 3M-052-AF with Alum and vialed. VRCplex is the same adjuvant as Adjuplex.

Table 4-8 CAVD 708 guinea pig groups, antigen + adjuvants, and doses

Group (N=6)	Antigen / Adjuvant Assignment	Antigen Dose	Adjuvant dose
1	BG505 SOSIP.664 gp140 / **Alum**	30 mcg	500 mcg
2	BG505 SOSIP.664 gp140 / **GLA-LSQ**	30 mcg	5 mcg GLA, 2 mcg QS-21
3	BG505 SOSIP.664 gp140 / **3M-052-Alum,** low dose	30 mcg	2 mcg 3M-052, 100 mcg Alum
4	BG505 SOSIP.664 gp140 / **3M-052-Alum,** high dose	30 mcg	5 mcg 3M-052, 500 mcg Alum
5	BG505 SOSIP.664 gp140 / **CpG 1018**	30 mcg	100 mcg
6	BG505 SOSIP.664 gp140 / **CpG 1018 / Alum**, mixing 1	30 mcg	CpG 100 mcg, Alum 500 mcg (CpG mix with Alum first)
7	BG505 SOSIP.664 gp140 / **CpG 1018 / Alum**, mixing 2	30 mcg	CpG 100 mcg, Alum 500 mcg (Antigen will mix with Alum first)
8	BG505 SOSIP.664 gp140 / **VRCplex**	30 mcg	80 mcL
9	BG505 SOSIP.664 gp140 only	30 mcg	None

Guinea pigs were immunized at weeks 0, 8, and 20. Injection volume was 500 mcl total, with 250 mcl at each injection site (in the left and right rear quadriceps muscle). Antibody responses were measured at weeks 10, 22, 28, 36, and 40. Neutralization antibody titers to autologous BG505.T332N and selected tier 2 HIV-1 strains were measured by the Comprehensive Antibody Vaccine Immune Monitoring Consortium (CAVIMC)/Montefiori lab. Binding antibody binding titers to the homologous antigen were measured by the CAVIMC/Tomaras Lab. The results showed that BG505 SOSIP.664 gp140 + adjuvants were immunogenic in guinea pigs.

Autologous and heterologous serum neutralizing antibodies to tier 2 HIV-1 strains were detected in guinea pig serum at various titers, and in general the magnitude of responses depended on the adjuvant groups. High nAb responses against the tier 1A virus MW965.26 (a neutralization sensitive virus) was elicited in all of the adjuvant groups, while the response was remarkably lower in the no-adjuvant group 9. The responses reached peak at week 22, 2 weeks after the 3rd dose, and decreased somewhat over the next 18 weeks until study termination at week 40 (see Figure 4-3).

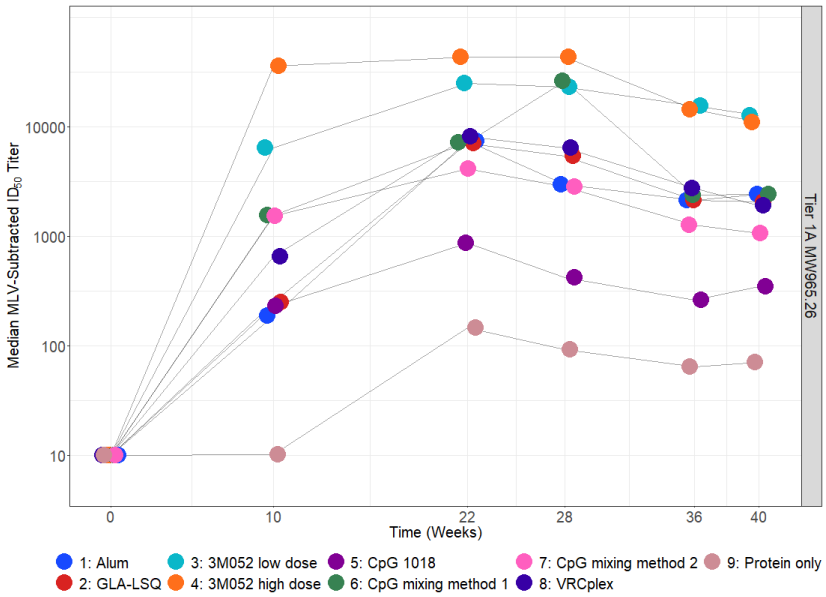

Figure 4-3 ID50 neutralizing antibody titers against the tier 1A virus MW965.26 after administration of BG505 SOSIP.664 gp140 with various adjuvants

The detection of nAbs recognizing the autologous tier 2 virus BG505 T332N is the main endpoint for this study. At the peak timepoint (week 22), the nAb titers were the highest in 3M052-Alum and VRCplex groups (ID_{50} 500-1000), lower in the CpG 1018/Alum and Alum groups (ID_{50} 100-600), and lowest in the GLA-LSQ, CpG 1018, and no-adjuvant groups (ID_{50} below 100). The titers in general decreased over time after week 22 or 28; however, the pattern of kinetics varied across different adjuvant groups (see Figure 4-4). Group 1 and 4 titers fell at week 28 and rose again at week 36. Peak response was at week 28 for Group 7 and 8.

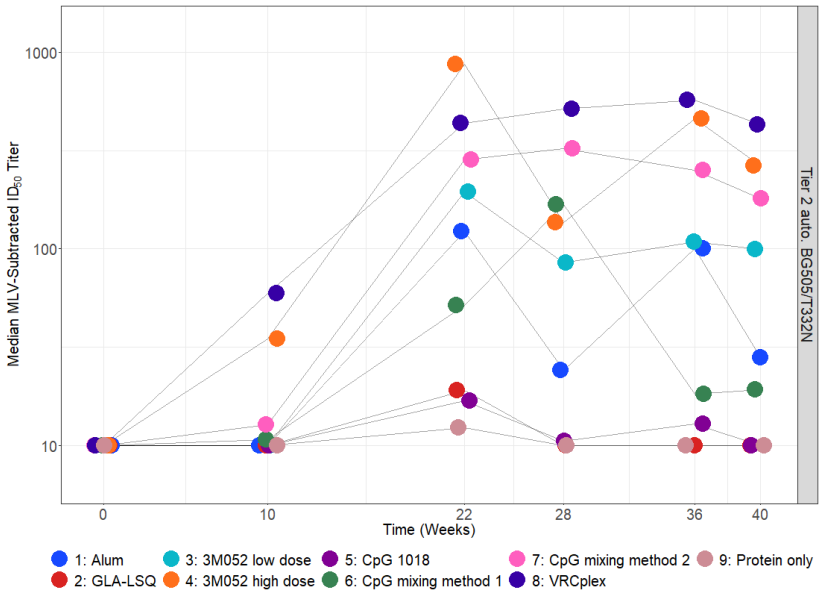

Figure 4-4 Neutralizing antibody responses against the autologous tier 2 virus BG505.T332N after administration of BG505 SOSIP.664 gp140 with various adjuvants

Neutralizing antibody responses to heterologous subtype C viruses 25710 and CE1176 were also elicited in selected adjuvant groups, with ID_{50} near or below 100 (lower than that to BG505 T332N) (see Figure 4-5 for responses to tier 2 HIV-1 CE1176).

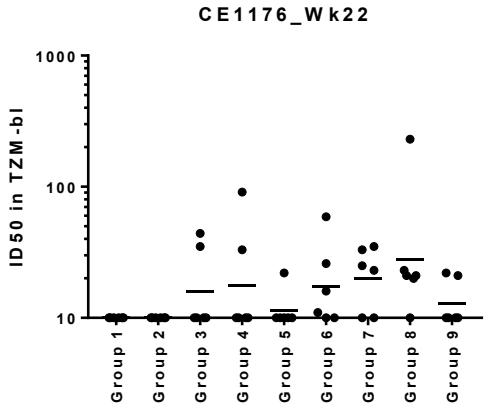

Figure 4-5 Neutralizing antibody responses against the heterologous tier 2 virus CE1176 after administration of BG505 SOSIP.664 gp140 with various adjuvants. For group definitions, see Table 4-8.

Binding antibody responses to the autologous antigen varied across adjuvant groups. It was the highest in 3M-052-Alum, lower in CpG 1018/Alum and Alum groups, and lowest in GLA-LSQ and no-adjuvant groups (Figure 4-6). The responses were pronounced after 2 doses at week 10, reached peak after 3 doses at week 22, and declined slowly over time. Assays for binding antibody responses from week 28, 36, and 40 timepoints are in progress.

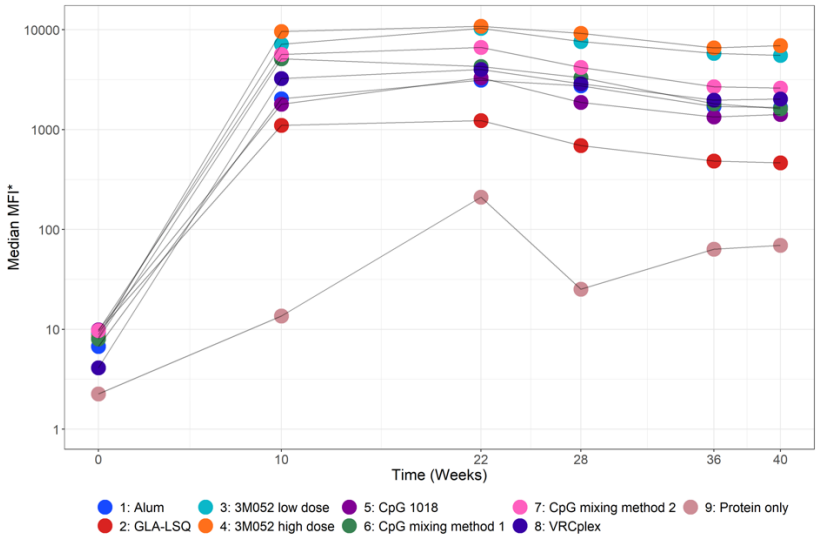

Figure 4-6 Binding antibody responses to BG505 SOSIP.664 gp140 administered with various adjuvants

4.4.2 Immunogenicity of BG505 SOSIP.664 gp140 + Adjuvants in rats

The objective of this study, CAVD 724, was to evaluate the immunogenicity (antibody responses) of BG505 SOSIP.664 gp140 + adjuvants in rats. The purpose of the study is to ensure that rats are a suitable model for the toxicity study. The study groups are shown in Table 4-9.

Table 4-9 CAVD 724 groups, antigen + adjuvants, and doses

Group	N	Antigen + Adjuvant	Antigen Dose	Adjuvant dose
1	4	BG505 SOSIP.664 gp140 only	50 mcg	None
2	4	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	50 mcg	5 mcg 3M-052-AF, 250 mcg Alum
3	4	BG505 SOSIP.664 gp140 + (CpG 1018 + Alum)	50 mcg	1500 mcg CpG 1018, 250 mcg Alum
4	4	BG505 SOSIP.664 gp140 + GLA-LSQ	50 mcg	2.5 mcg GLA, 1 mcg QS-21

Animals were immunized in week 0, 2, and 4. Injection volume was 250 mcl total, with 250 mcl in the left or right rear quadriceps muscle alternatively. BG505 SOSOP.664 gp140-specific binding antibody titers were measured on day 43. The results indicated that BG505 SOSOP.664 gp140 were immunogenic in rats, and the magnitude of the binding antibody responses varied depending on the adjuvants (Figure 4-7). Little neutralizing antibody response to the autologous tier 2 virus BG505 T332N was observed but the binding antibody results assured us that rat is the appropriate model for toxicity study.

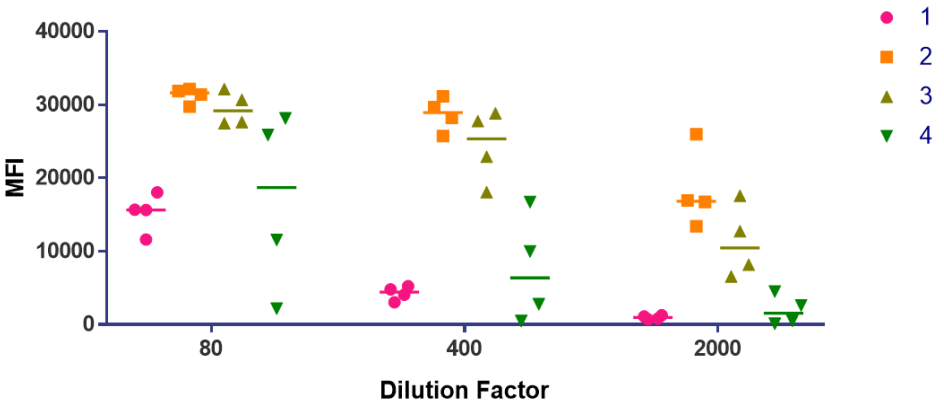

Figure 4-7 Binding antibody responses to BG505 SOSIP.664 gp140 adjuvanted with Alum (Group 1), 3M-052+Alum (Group 2), CpG 1018+Alum (Group 3), or GLA-LSQ (Group 4) in rats. For group definitions, see Table 4-9 above.

4.4.3 Immunogenicity of BG505 SOSIP.664 gp140 + Adjuvants in rabbits

The objective of this study, CAVD 749, is to assess the immunogenicity of BG505 SOSIP.664 gp140 + adjuvants in rabbits measured by neutralizing antibody and binding antibody responses. Immune responses to BG505

SOSIP.664 gp140 with ISCOMATRIX adjuvant in rabbits were robust and have been reported extensively in the literature. However, it was considered that rabbits may not respond to adjuvants that are TLR7/8 or TLR9 agonists (3M-052 and CpG 1018). This study will bridge past studies and will also compare several additional TLR4 agonists (Table 4-10).

Table 4-10 CAVD 749 groups, antigen + adjuvants, doses, and dosing schedule

Group	N	Antigen + Adjuvant	Antigen Dose	Adjuvant dose	Dosing schedule in weeks
1	5	BG505 SOSIP.664 gp140 only	30 mcg	None	0, 8, and 20
2	5	BG505 SOSIP.664 gp140 + Alum	30 mcg	500 mcg	0, 8, and 20
3	5	BG505 SOSIP.664 gp140 + GLA-LSQ	30 mcg	25 mcg GLA, 10 mcg QS-21	0, 8, and 20
4	5	BG505 SOSIP.664 gp140 + (3M-052-AF + Alum)	30 mcg	5 mcg 3M-052-AF, Alum 500 mcg	0, 8, and 20
5	5	BG505 SOSIP.664 gp140 + (CpG 1018 + Alum)	30 mcg	CpG 1018 300 mcg, Alum 500 mcg	0, 8, and 20
6	5	BG505 SOSIP.664 gp140 + LMQ	30 mcg	10 mcg 3D-MPL, 25 mcg QS-21	0, 8, and 20
7	5	BG505 SOSIP.664 gp140 + GLA-SE	30 mcg	25 mcg GLA, 2% squalene	0, 8, and 20

Animals were immunized in week 0, 8, and 20. Injection route was IM. Injection volume was 500 mcl total, with 250 mcl at each injection site (in the left and right rear quadriceps muscle). At week 10, some of the animals across all groups developed neutralizing antibody responses against the tier 1A virus MW965.26 and the autologous tier 2 virus BG505 T332N (Figure 4-8).

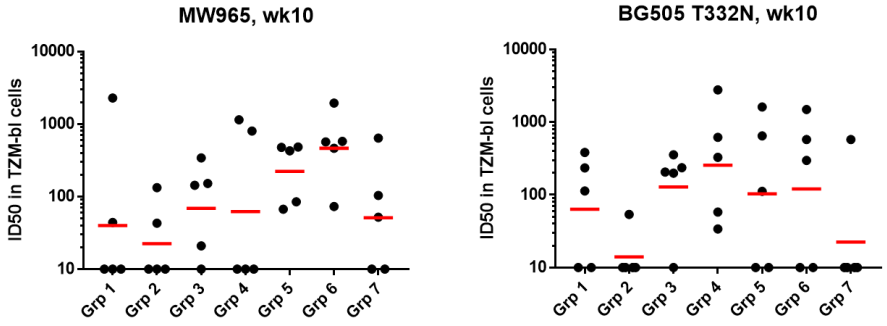

Figure 4-8 Neutralizing antibody responses to BG505 SOSIP.664 gp140 with or without adjuvants in rabbits at week 10

At week 22, most of animals in all groups developed neutralizing antibody responses against the tier 1A virus MW965.26 and the autologous tier 2 virus BG505 T332N (Figure 4-9).

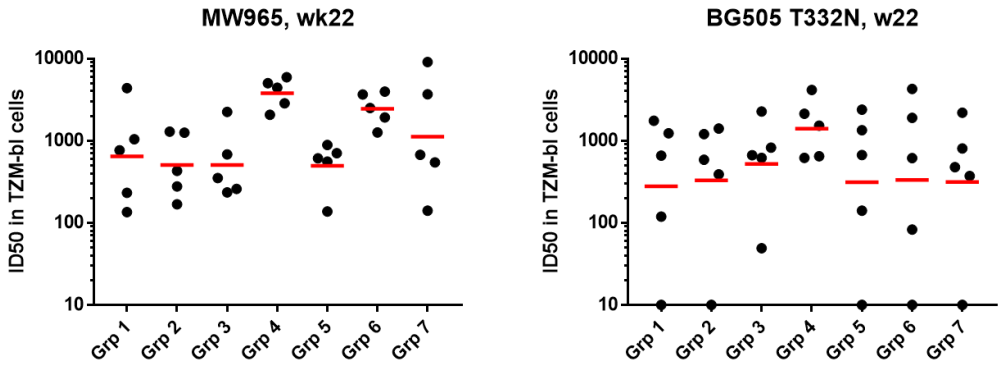

Figure 4-9 Neutralizing antibody responses to BG505 SOSIP.664 gp140 with or without adjuvants in rabbits at week 22

Binding antibody responses at week 22 were robust across all groups (Figure 4-10).

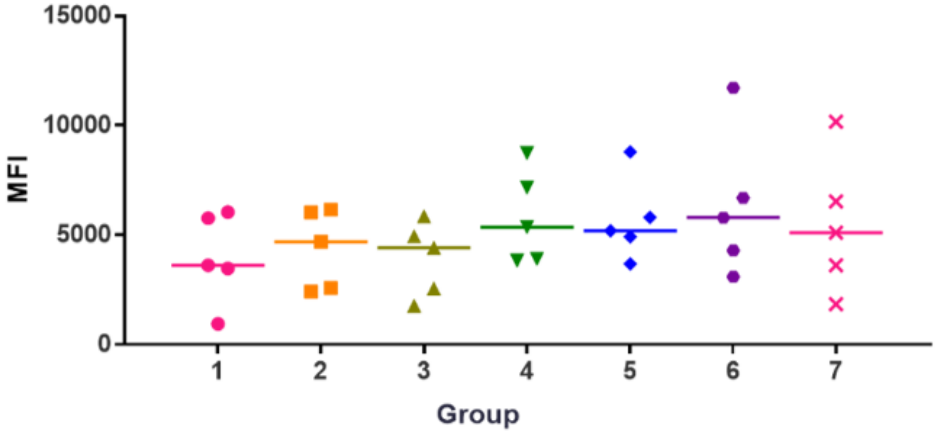

Figure 4-10 Binding antibody responses to BG505 SOSIP.664 gp140 with or without adjuvants in rabbits at week 22. The samples were tested at 1:800 dilution.

4.4.4 Immunogenicity of BG505 SOSIP.664 gp140 + 3M-052-Alum in rhesus macaques

The objective of this study, CAVD 641, was to assess the immunogenicity of BG505 SOSIP.664 gp140 with 3M-052-Alum adjuvant by SC or IM routes, in comparison to GLA-LSQ adjuvant, in rhesus macaques. The study was conducted by Bali Pulendran's lab at Emory University. 3M-052-Alum was prepared by mixing 3M-052-AF with Alum and vialed.

The groups are specified in Table 4-11. At each immunization, two sites (left and right leg quadriceps) were injected, 30 mcg of 3M-052-Alum at each site for a total dose of 60 mcg. Animals were immunized at weeks 0, 8, and 24.

Table 4-11 CAVD 641 antigen + adjuvant, routes, doses, and dosing schedule

Group	N	Antigen + Adjuvant	Antigen Dose	Adjuvant dose	Dosing schedule in weeks
1	3	BG505 SOSIP.664 gp140 + 3M-052-Alum, SC	100 mcg	60 mcg 3M-052	0, 8, 24
2	3	BG505 SOSIP.664 gp140 + 3M-052-Alum, IM	100 mcg	60 mcg 3M-052	0, 8, 24
3	3	BG505 SOSIP.664 gp140 + GLA-LSQ, IM	100 mcg	12.5 mcg GLA 5 mcg QS-21	0, 8, 24

Robust autologous tier 2 neutralizing antibody responses were observed. The responses persist for at least 4 months post vaccination (Figure 4-11). There was limited breadth of tier 2 HIV neutralizing antibodies.

After the second immunization, SC immunization appears to induce a slightly higher autologous tier 2 response than IM immunization. This trend disappeared following the third immunization.

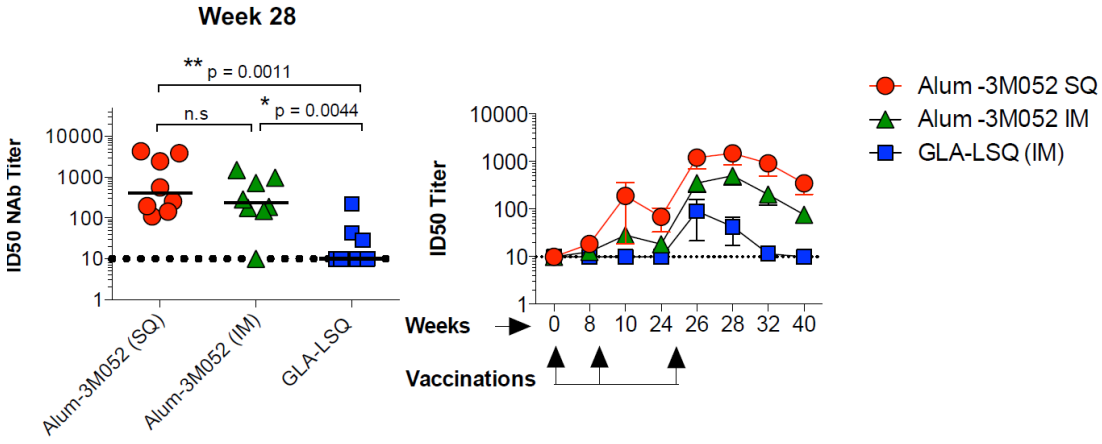

Figure 4-11 Neutralizing antibody responses to BG505 SOSIP.664 gp140 adjuvanted with 3M-052-Alum or GLA-LSQ

Robust and persistent GC and Tfh responses were observed in the draining LN. Long lived bone marrow plasma cell responses were also observed.

4.4.5 Immunogenicity of Trimer 4571

In the VRC018 trial, Trimer-4571–specific antibody titers in serum samples were measured by Electrochemiluminescence (ECLIA) using a Meso Scale Discovery (MSD) platform at baseline and at 2 weeks after the third product administration. Both the 100-mcg (n = 3) and 500-mcg (n = 5) IM and SC doses elicited Trimer-4571–specific antibodies, with geometric mean AUCs 8- fold and 40-fold over background, respectively. No tier-2 neutralizing activity was observed.

In pre-clinical studies, Trimer 4571 adjuvanted with Alum elicited autologous tier-2 neutralizing antibodies in guinea pigs (50 mcg of Trimer 4571) and rhesus

macaques (100 mcg of Trimer 4571). For full details, please see the Investigator's Brochure.

4.5 Clinical studies

4.5.1 Clinical studies of HEPLISAV-B

HEPLISAV is a hepatitis B virus vaccine comprised of recombinant, yeast cell-derived hepatitis B surface antigen (HBsAg) and CpG 1018. HEPLISAV is a sterile liquid dosage form that is administered as an IM injection. The CpG 1018 in HEPLISAV is a synthetic 22-mer phosphorothioate oligodeoxynucleotide (PS ODN) containing an immunostimulatory sequence that is an agonist for Toll-like receptor 9. The desired biological activity of CpG 1018 is to enhance the generation of antibodies to HBsAg by mimicking the immunostimulatory activity of single-stranded viral and bacterial DNA. HEPLISAV is administered as a single dose of 0.5 mL, containing 20 mcg HBsAg and 3000 mcg CpG 1018.

Cumulatively, 10,049 subjects (10,038 subjects 18 years of age and older and 11 subjects below 18 years old) and 386 adult subjects with chronic kidney disease 18 years of age and older have received HEPLISAV in clinical trials. Before it was approved by the FDA, that vaccine was tested in more than 9500 people.

HEPLISAV appeared to be well tolerated in clinical trials in healthy adults. The reactogenicity profile of HEPLISAV is similar to Engerix-B (a non-adjuvanted HBV vaccine) in time course and severity. Compared with Engerix-B, HEPLISAV caused more local reactions and fewer systemic reactions. Common adverse events (AEs) determined by investigators to be related to HEPLISAV included injection site erythema, headache, nasopharyngitis, myalgia, fatigue, diarrhea, nausea, injection site swelling, back pain, injection site hematoma, injection site pain, pain in extremity, dizziness, oropharyngeal pain, postprocedural hematoma, arthralgia, injection site pruritus, and increased white blood cell count. SAEs, autoimmune adverse events, and deaths have been infrequent in the HEPLISAV clinical program. One SAE of Wegener's granulomatosis (also called granulomatosis with polyangiitis [GPA]) has been reported in a recipient of HEPLISAV; this event was considered by the investigator as possibly related to trial vaccine (82). In one randomized, observer-blind, active-controlled, multicenter study in the United States in which 5587 participants received at least 1 dose of HEPLISAV-B and 2781 participants received at least 1 dose of Engerix-B, acute myocardial infarction (AMI) was reported in 0.25% (n = 14) of HEPLISAV-B recipients and 0.04% (n = 1) of Engerix-B recipients. Additional evidence, including information on temporal relationship and baseline risk factors, does not support a causal relationship between HEPLISAV-B administration and AMI. No deaths have been considered by the investigator to be related to HEPLISAV.

For more information, see the Investigator's Brochure (IB).

4.5.2 Clinical studies of ID93 + GLA-LSQ

A phase 1, randomized, double blind clinical trial evaluated the safety, tolerability, and immunogenicity of the vaccine candidates ID93 + AP10-602 (GLA-LSQ) and ID93 + GLA-SE administered IM in in 70 healthy adults 18-49 years of age (ClinicalTrials.gov Identifier: NCT02508376).

The four treatment groups are outlined in Table 4-12. General safety was evaluated for 28 days following each injection at days 1, 29, and 57. Subjects were monitored for approximately 422 days (one year following the third study injection), including safety laboratory analyses done just prior to and 7 days following each study injection. Blood samples were obtained for immunological assays (Luminex, intracellular cytokine staining at Days 1 and 71, and antibody analysis at Days 1 and 85).

Table 4-12 Protocol DMID 12-0109 treatment groups

Cohort	N	Treatment Assignment	Route	Volume	Injection Schedule
1	20	10 mcg ID93 + 5 mcg AP10-602*	IM	0.5 mL	Days 1, 29, and 57
2	20	10 mcg ID93 + 10 mcg AP10-602		1 mL	
3	20	10 mcg ID93 + 5 mcg GLA-SE		0.5 mL	
4	10	10 mcg ID93		0.5 mL	

* AP10-602 is GLA-LSQ.

All participants have completed the study. All treatment regimens were safe and well tolerated. There were no Grade 4 adverse events and no related SAEs, AESIs, or deaths. No participants withdrew or discontinued vaccination due to adverse reactions. The most common local reactions were mild to moderate injection site pain, tenderness, warmth, and pruritus. The most common systemic reactions were mild to moderate headache, fatigue, and myalgia. No significant safety issues have been noted.

4.5.3 Clinical studies of 3M-052

3M-052 (MEDI9197) has been evaluated in a clinical study using a sesame oil formulation to evaluate its effects following intratumoral injection (ClinicalTrials.gov Identifier NCT02556463). In this study, 52 cancer patients with metastatic or locally advanced solid tumors received 3M-052 dissolved in sesame oil intratumorally at doses of 55, 37, 12, and 5 mcg alone, in combination with the anti-PD-L1 antibody durvAlumab delivered IV, or with durvAlumab plus radiation therapy. Two dose-limiting-toxicities were observed in this study: Grade 3 cytokine release syndrome at 37 mcg and Grade 4 cytokine release syndrome at 55 mcg. The most common (≥ 15%) drug related adverse events (AEs) were pyrexia, fatigue, decreased lymphocyte count, chills, nausea, arthralgia, and injection site pain (83). Based on this safety record, the 37 mcg dose of 3M-052 was defined as the maximum tolerated dose for this study.

One patient with Stage IV recurrent anal squamous cell carcinoma died following a second round of intrahepatic tumoral injection of 12mcg 3M-052 plus IV durvAlumab. Two weeks prior, per-protocol liver biopsy performed demonstrated necrotic metastatic lesions. This death could not be attributed to a specific cause (autopsy and post-mortem CT scan were not performed). However, the study investigator considered the event to be related to 3M-052 and the study procedure. Medimmune, the trial sponsor, informed the FDA that this event was potentially related to hemorrhagic shock due to a ruptured liver metastasis. The FDA required protocol alterations prohibiting injection of deep-seated lesions before allowing continuation of the clinical trial.

HVTN 300 (NCT04915768)**:** This study is a first-in-human, unblinded trial testing a 300 mcg dose of a CH505 TF chTrimer (a stabilized, chimeric SOSIP Env trimer) in combination with a 5 mcg 3M-052-AF + 500 mcg Alum delivered via split injection into both the right and left deltoids. The planned schedule is 5 total vaccinations at months zero, two, four, eight, and twelve.

As of September 7, 2022, the study is ongoing and all 13 participants have received the first vaccination, 10 participants have received the second vaccination, 9 participants have received the third vaccination, 9 participants have received the fourth vaccination, and 6 participants have received the fifth vaccination. Five participants have discontinued further vaccinations, 1 due to a panic attack after the first injection (this participant had a history of panic attacks before being part of the study), 3 due to reactogenicity events and 1 was lost to follow-up. Out of these 5, 2 terminated from the study early (panic attack and lost to follow-up) and 3 have remained in the study for follow-up.

All participants experienced at least some local reactogenicity during the trial, mostly mild to moderate. One (1) participant experienced severe pain/tenderness in both the right and left injection sites 3 days following the fourth vaccination, though it lasted only one day.

All participants reported some systemic reactogenicity during the course of the trial to date, mostly mild to moderate. For example, 11 of 13 participants experienced systemic reactogenicity after the first dose. Five (5) participants reported Grade 3 (severe) systemic reactogenicity during the trial through September 7, 2022. One (1) of these 5 participants received a subsequent vaccination without any Grade 3 reactogenicity, one (1) experienced an additional repeat Grade 3 systemic reactogenicity symptom, one (1) has not yet received a subsequent vaccination, and two (2) declined to receive additional vaccinations after their first Grade 3 systemic reactogenicity event. All Grade 3 events resolved within the 7-day reactogenicity period; the longest duration for severe reactogenicity was 2 days.

There have been no related SAEs, AESIs, or deaths as of September 7, 2022.

4.5.4 Clinical studies of the proposed product combination

No previous clinical studies of BG505 SOSIP.664 gp140 with any of the proposed adjuvants (GLA-LSQ, CpG 1018 + Alum, 3M-052-AF + Alum, or Alum) have been performed.

HVTN 137 (NCT04177355): HVTN 137 Part A is a first-in-human, double-blinded, dose-escalation study testing the combination of 100-mcg BG505 SOSIP.664 gp140 with 3M-052-AF at 2 doses, including 1 mcg (Group 1) and 5 mcg (Group 2), both combined with 500-mcg Alum. The study is ongoing and remains blinded to within-group treatment assignment. As of October 18, 2021, both the 1-mcg 3M-052-AF group (6 participants total; 5 receiving protein/adjuvant, 1 receiving placebo) and the 5-mcg 3M-052-AF group (11 participants total; 10 receiving protein/adjuvant, 1 receiving placebo) completed enrollment. Part A of the initial protocol specified that participants in Groups 1 and 2 would receive 2 total doses, 1 at month zero and the other at month two.

Five out of 6 participants received 2 doses of the 1-mcg dose (1 participant discontinued for reasons unrelated to vaccination) and 10 out of 11 participants received 2 doses of the 5-mcg dose. One participant in the 5-mcg dose group (Group 2) decided to discontinue vaccination due to Grade-3 induration/erythema first noted on Day 8 postvaccination. The maximum size of both the induration and erythema was measured at 17 x 17 cm on Day 8 (Grade 3). The erythema resolved over 3 days and the induration subsided to less than 5 cm on Day 11 (Grade 1). The induration was measured at 2 x 2 cm on Day 14 but did not completely resolve until 41 days post injection. At no point was the pain/tenderness greater than mild, and the participant continued to work. It is unknown at present whether this participant received placebo or study product. Another participant in Group 2 experienced 2 days of Grade-3 induration and erythema from Days 6-7 postvaccination that resolved completely by Day 8. In consultation with the Protocol Safety Review Team, the participant did receive the second dose, which was uneventful. In addition, 4 participants reported Grade-3 systemic reactogenicity events.

The protocol was amended and participants in Groups 1 and 2 were given the option to receive a third dose for 3 total doses. Nine (out of 17) participants elected to receive a third dose. There were no unsolicited Grade-3 or -4 AEs and no related SAEs, AESIs, or deaths in either group after this third dose. Both local and systemic reactogenicity were similar between all 3 doses in participants who received 3 doses in Part A.

HVTN 137 Part B is evaluating the safety and immunogenicity of 100-mcg BG505 SOSIP.664 gp140 in combination with 3 TLR agonists, including 5-mcg 3M-052 AF + 500-mcg Alum, and Alum alone. Enrollment is now complete and 88 participants have been enrolled. Of these 88 participants, 20 have been randomized to the 3M-052-AF + Alum group. All 88 participants have received at least two doses and, as of June 7th, 81 have received the third dose. Given that the

trial remains blinded across the 4 treatment arms, interpretation of the blinded safety data from HVTN 137 Part B is not possible, but there have been no related SAEs, AESIs, deaths, or unplanned study pauses. No additional grade 3 local reactogenicity events have been observed.

Overall, BG505 SOSIP.664 with 3M-052-AF + Alum was generally well tolerated, with no unsolicited Grade-3 or -4 AEs; no related SAEs, AESIs, or deaths; and no unplanned study pauses. Other than the persistent erythema in one of the HVTN 137 Part A Group 2 participants described above, all reactogenicity symptoms resolved within 14 days and generally within 7 days. For more detailed information, please see the IB.

HVTN 300 (NCT04915768)**:** Refer to Section 4.5.3 for more information.

4.5.5 Clinical studies of Trimer 4571

A summary of the clinical studies for Trimer 4571 with Alum is shown in Table 4-13.

VRC-018: Data on the safety and immunogenicity of Trimer 4571 + Alum from VRC 018 are available. As of August 2021, final data from this study are available. No related SAEs, related grade 4 AEs, or related MAAEs were reported or other unexpected reactions, and no study pause criteria were met at any time. The immunologic assays and analysis for this study are ongoing.

NIAID- 19-I-0069: As of January 5, 2022, enrollment in the study is ongoing, with 12 participants receiving Trimer 4571 with Alum adjuvant. The dose was administered as a boost and was generally well tolerated. No SAEs were reported, and no adverse events resulted in study product discontinuation, including no SUSARs.

For additional information, see the Investigator's Brochure (IB).

Table 4-13 Clinical studies for Trimer 4571 with Alum

Study	ClinicalTrials.gov NCT # / Study Status	Participant HIV status	Number receiving Trimer 4571 + Alum	Route / Dose	Schedule	Safety data (ClinicalTrials.gov)
VRC 018	NCT03783130 / Completed	Negative	16	IM or SC at 100- or 500-mcg Trimer and 500-mcg Alum	0, 2, 5 months	No serious adverse events
NIAID 19-I-0069	NCT03878121 / Enrolling	Negative	Max 100	IM at 500-mcg Trimer with 500-mcg Alum	Boost post adenovirus vector prime	As of Jan 5, 2022, 12 participants have received study product. No SAEs, no SUSARs
PI: M Choudhary, Univ of Pittsburg	NCT04985760 / Enrolling	Positive	24	IM at 100- or 500-mcg Trimer and 500-mcg Alum	0, 2, 5 months	As of Jan 1, 2022, 5 participants have received Trimer 4571. No related SAES, no SUSARs

4.6 Potential risks of study products and administration

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

Common	• Mild to moderate injection site pain, tenderness, erythema, or swelling/induration/edema • Malaise/fatigue, myalgia, or headache in the first few days following injection • A vaccine-induced positive HIV antibody test result
Less common	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, nausea, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, other transient lesions, itching, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis • Muscle damage at the injection site
Theoretical risks	• Autoimmune disease • Effects on a participant's response to an approved HIV vaccine administered in the future • Effects on susceptibility to HIV, if the participant is exposed to HIV • Effects on the course of HIV infection/disease, if the participant is infected with HIV • Effects on the fetus and on pregnancy

5 Objectives and endpoints

5.1 Objectives and endpoints for Part A

5.1.1 Primary objectives and endpoints for Part A

Primary objective 1:

To evaluate the safety and tolerability of intramuscular (IM) administration of BG505 SOSIP.664 gp140 with 3M-052-AF + Alum

Primary endpoint 1:

Signs and symptoms of local and systemic reactogenicity, laboratory measures of safety, adverse events (AEs), expedited AEs (EAEs), and AEs of special interest (AESIs)

5.1.2 Secondary objectives and endpoints for Part A

Secondary objective 1:

To evaluate and compare immune responses elicited by BG505 SOSIP.664 gp140 with each 3M-052-AF +Alum dose regimen

Secondary endpoints 1:

Response rate and magnitude of serum antibody neutralization of autologous (HIV-1 BG505.664) and other heterologous tier 2 HIV-1 strains as measured by the TZM-bl assay 2 weeks after the second and after the optional third vaccination, if available

Response rate and magnitude of serum IgG binding antibodies to BG505 SOSIP.664 gp140 as measured by BAMA 2 weeks after the second and after the optional third vaccination, if available

Response rate and magnitude of IgG+ B cells binding to BG505 SOSIP.664 gp140 tetramers as measured by multiparameter flow cytometry 2 weeks after the second and after the optional third vaccination, if available

Percent HIV Env-specific CD4+ T cells in blood as measured by multiparameter flow cytometry 2 weeks after the second and after the optional third vaccination, if available

5.1.3 Exploratory objectives for Part A

Exploratory objective 1:

To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed in a subset of participants, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm

Exploratory objective 2:

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

5.2 Objectives and endpoints for Part B

5.2.1 Primary objectives and endpoints for Part B

Primary objective 1:

To evaluate the safety and tolerability of intramuscular (IM) administration of BG505 SOSIP.664 gp140 with the following adjuvants: CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum alone

Primary endpoint 1:

Signs and symptoms of local and systemic reactogenicity, laboratory measures of safety, adverse events (AEs), expedited AEs, and AEs of special interest (AESIs)

Primary objective 2:

To evaluate and compare the neutralizing antibody response induced by BG505 SOSIP.664 gp140 with different adjuvants (CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum alone)

Primary endpoint 2:

Response rate and magnitude of serum antibody neutralization of autologous (HIV-1 BG505) and other heterologous tier 2 HIV-1 strains as measured by TZM-bl assay 2 weeks after the second and third vaccinations

5.2.2 Secondary objectives and endpoints for Part B

Secondary objective 1:

To evaluate and compare the durability of neutralizing antibody responses elicited by BG505 SOSIP.664 gp140 with various adjuvants (CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum)

Secondary endpoint 1:

Response rate and magnitude of serum antibody neutralization of autologous (HIV-1 BG505) and other heterologous tier 2 HIV-1 strains as measured by TZM-bl assay 6 and 12 months post third vaccination

Secondary objective 2:

To evaluate and compare the peak and durability of serum binding antibody responses elicited by BG505 SOSIP.664 gp140 HIV-1 Env protein with various adjuvants (CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum)

Secondary endpoints 2:

Response rate, magnitude, and breadth of IgG binding antibody responses to HIV BG505 SOSIP.664 gp140, and HIV-1 gp120 and gp140 variant strains (to determine off-target non-neutralizing Abs) as measured by BAMA 2 weeks after the second vaccination and 2 weeks, 6 months, and 12 months after the third vaccination

Secondary objective 3:

To evaluate and compare the peak and durability of BG505 SOSIP.664 gp140-specific B cells elicited by BG505 SOSIP.664 gp140 with various adjuvants (CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum)

Secondary endpoint 3:

Response rate and magnitude of memory IgG+ B cells binding to BG505 SOSIP.664 gp140 tetramers as measured by multiparameter flow cytometry 2 weeks after the second vaccination and 2 weeks, 6 months, and 12 months after the third vaccination

Response rate and magnitude of BG505 SOSIP.664 gp140-specific plasmablasts as measured by multiparameter flow cytometry 1 week after the second and third vaccinations

Secondary objective 4:

To evaluate Env-specific CD4+ T-helper subpopulations, phenotypes and functions elicited by BG505 SOSIP.664 gp140 with various adjuvants (CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum)

Secondary endpoint 4:

Percent HIV Env-specific cytokine-expressing CD4+ T cells in blood by intracellular cytokine staining (ICS) multiparameter flow cytometry and

polyfunctional subset analysis 2 weeks after the second vaccination and 2 weeks, 6 months, and 12 months after the third vaccination

Secondary objective 5:

To evaluate innate immune responses and baseline predictors, compare the innate immune responses elicited by BG505 SOSIP.664 gp140 with various adjuvants (CpG 1018 + Alum, 3M-052-AF + Alum, GLA-LSQ, or Alum) after the first vaccination, and correlate with adaptive immune responses

Secondary endpoints 5:

Alterations in blood leukocyte populations during the innate response (day 1, 3 and 7) relative to prevaccine levels (day 0)

Alterations in RNAseq expression of leukocyte and/or immune cells (lymphocyte populations, natural killer (NK) cells, dendritic cell (DC) subsets, monocytes subsets, and granulocytes) (days 1, 3, and 7) relative to prevaccine levels (day 0)

Alterations of concentrations of immune cytokines and chemokines in serum samples postvaccination (days 1, 3, 7) relative to prevaccine levels (day 0)

Secondary objective 6:

To characterize systemic inflammatory markers among participants with moderate to severe reactogenicity after any vaccination

Secondary endpoint 6

Blood cell subpopulation dynamics by multiparameter flow cytometry, gene expression alterations by RNAseq/transcripts and soluble cytokine/inflammatory mediator alterations comparing day of last vaccination and day of visit for moderate to severe reactogenicity

5.2.3 Exploratory objectives for Part B

Exploratory objective 1:

To further characterize antibody responses (eg, IgA/IgG subclass binding, epitope mapping, Ab avidity, infected cell binding, linear peptide array)

Exploratory objective 2:

To characterize Fc-mediated antibody functions (eg, fragment crystallizable receptor [FcR] array, antibody-dependent cellular phagocytosis [ADCP], antibody-dependent neutrophil phagocytosis [ADNP], antibody-dependent cellular cytotoxicity [ADCC], and/or complement binding/deposition)

Exploratory objective 3:

To further characterize neutralizing antibody responses using diagnostic mutants of HIV-1 Env pseudotyped viruses in the TZM-bl assay

Exploratory objective 4:

To analyze the B cell repertoire including somatic hypermutation, affinity maturation, CDRH3 length, and other signatures of bnAb class epitopes

Exploratory objective 5:

To apply systems biology approaches (eg, RNAseq, LC-MS) to examine transcriptomic, and metabolomic and/or proteomic profiles of innate and adaptive immune responses

Exploratory objective 6:

To characterize Env-specific B and CD4+ T cells (including Tfh/pTfh) and antigen receptor sequences using samples such as PBMC, lymph node fine needle aspirates, bone marrow aspirates, or leukapheresis

Exploratory objective 7:

To assess polyclonal serum Ab (Fab) binding to Env epitopes using electron microscopic imaging

Exploratory objective 8:

To measure Env-specific antibody levels in mucosal secretions and tissues using a quantitative immunoassay and/or immunohistochemistry (IHC)

Exploratory objective 9:

To identify the regions and epitopes of the BG505 SOSIP.664 gp140 protein targeted by CD4+ T cells in response to vaccination

Exploratory objective 10:

To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed in a subset of participants, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm

Exploratory objective 11:

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

5.3 Objectives and endpoints for Part C

5.3.1 Primary objectives and endpoints for Part C

Primary objective 1:

To evaluate the safety and tolerability of IM administration of Trimer 4571 (BG505 SOSIP.664 DS gp140) with 3M-052-AF + Alum.

Primary endpoint 1:

Signs and symptoms of local and systemic reactogenicity and laboratory measures of safety, AEs, expedited AEs, and AESIs.

Primary objective 2:

To evaluate the neutralizing antibody response induced by Trimer 4571 (BG505 SOSIP.664 DS gp140) with 3M-052-AF + Alum.

Primary endpoint 2:

Response rate and magnitude of serum antibody neutralization of autologous (HIV-1 BG505) and other heterologous tier-2 HIV-1 strains, as measured by TZM-bl assay 2 weeks after the second and third vaccinations.

5.3.2 Secondary objectives and endpoints for Part C

Secondary objective 1:

To test whether a 3-mcg dose of 3M-052-AF + Alum is sufficient to generate autologous serum antibody neutralization (HIV-1 BG505) and other heterologous tier-2 HIV-1 strains, as measured by TZM-bl assay 2 weeks after the second and third vaccinations.

Secondary endpoint 1:

Response rate and magnitude of serum antibody neutralization of autologous (HIV-1 BG505) and other heterologous tier-2 HIV-1 strains, as measured by TZM-bl assay 2 weeks after the second and third vaccinations, comparing the 5-mcg dose of the 3M-052-AF + Alum with the 3-mcg dose of 3M-052-AF + Alum.

Secondary objective 2:

To evaluate and compare the peak and durability of serum binding antibody responses elicited by BG505 SOSIP.664 gp140 adjuvanted with 3 mcg of 3M-052-AF+Alum, and by Trimer 4571 adjuvanted with 5 mcg of 3M-052-AF+Alum with Part B groups

Secondary endpoints 2:

Response rate, magnitude, and breadth of IgG binding antibody responses to HIV BG505 SOSIP.664 gp140, Trimer 4571, and HIV-1 gp120 and gp140 variant strains (to determine off-target non-neutralizing Abs) as measured by BAMA 2 weeks after the second vaccination and 2 weeks, 6 months, and 12 months after the third vaccination

Secondary objective 3:

To evaluate and compare the peak and durability of BG505 SOSIP.664 gp140-specific B cells elicited by BG505 SOSIP.664 gp140 adjuvanted with 3 mcg of 3M-052-AF+Alum, and by Trimer 4571 adjuvanted with 5 mcg of 3M-052-AF+Alum with Part B groups

Secondary endpoint 3:

Response rate and magnitude of memory IgG+ B cells binding to BG505 SOSIP.664 gp140 tetramers as measured by multiparameter flow cytometry 2 weeks after the second vaccination and 2 weeks, 6 months, and 12 months after the third vaccination

Response rate and magnitude of BG505 SOSIP.664 gp140 antigen-specific plasmablasts as measured by multiparameter flow cytometry 1 week after the second and third vaccinations

Secondary objective 4:

To evaluate Env-specific CD4+ T-helper subpopulations, phenotypes and functions elicited by BG505 SOSIP.664 gp140 adjuvanted with 3 mcg of 3M-052-AF+Alum, and by Trimer 4571 adjuvanted with 5 mcg of 3M-052-AF+Alum

Secondary endpoint 4:

Percent HIV Env-specific cytokine-expressing CD4+ T cells in blood by intracellular cytokine staining (ICS) multiparameter flow cytometry and polyfunctional subset analysis 2 weeks after the second vaccination and 2 weeks, 6 months, and 12 months after the third vaccination

Secondary objective 5:

To evaluate innate immune responses and baseline predictors, compare the innate immune responses elicited by BG505 SOSIP.664 gp140 adjuvanted with 3 mcg of 3M-052-AF+Alum, and by Trimer 4571 adjuvanted with 5 mcg of 3M-052-AF+Alum after the first vaccination, and correlate with adaptive immune responses

Secondary endpoints 5:

Alterations in blood leukocyte populations during the innate response (day 1, 3 and 7) relative to prevaccine levels (day 0)

Alterations in RNAseq expression of leukocyte and/or immune cells (lymphocyte populations, natural killer (NK) cells, dendritic cell (DC) subsets, monocytes subsets, and granulocytes) (days 1, 3, and 7) relative to prevaccine levels (day 0)

Alterations of concentrations of immune cytokines and chemokines in serum samples postvaccination (days 1, 3, 7) relative to prevaccine levels (day 0)

Secondary objective 6:

To characterize systemic inflammatory markers among participants with moderate to severe reactogenicity after any vaccination

Secondary endpoint 6

Blood cell subpopulation dynamics by multiparameter flow cytometry, gene expression alterations by RNAseq/transcripts and soluble cytokine/inflammatory mediator alterations comparing day of last vaccination and day of visit for moderate to severe reactogenicity

5.3.3 Exploratory objectives for Part C

Exploratory objective 1:

To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed in a subset of participants, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm

Exploratory objective 2:

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

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 127 healthy, HIV-uninfected adult participants; 115 vaccinees and 12 placebo recipients.

Part A is a dose-escalation study; two doses of the 3M-052-AF adjuvant will be studied in 17 participants (5 vaccinees and 1 placebo in the low-dose group; 10 vaccinees and 1 placebo in the higher dose group). Enrollment to the higher dose group will be triggered when safety in the lower dose group is deemed acceptable. Part B is the adjuvant comparison study; 88 participants will be randomized to one of four groups, with 20 participants randomized to vaccine and 2 to placebo within each group. Section 11.3.2 of the protocol describes the criteria for advancing to Part B based on the Part A data. Part C augments Part B with data on a "medium" dose of the 3M-052 adjuvant (3 mcg as compared to 5 mcg studied in Part B) and with data on a next-generation BG505 trimer (Trimer 4571 versus BG505 SOSIP.664 studied in Part B). The sample-size rationale is described separately for Parts A, B, and C.

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 PBMCs or high assay background. Immunogenicity data from 17 phase 1 and 2 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of September 2014), indicate that 10% is a reasonable estimate for the rate of missing data at Month 2.5 and 6.5, and 15% is a reasonable estimate at Month 18. For this reason, the sample size calculations in Section 6.1.3 account for 10% and 15% of enrolled participants having missing data for the primary immunogenicity and durability endpoints, respectively.

6.1.1 Sample size calculations for safety (Part A)

The purpose of Part A is to select the 3M-052-AF adjuvant dose, by identifying potential 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 low-dose vaccine arm (n = 5), there is a 90% chance of observing at least 1 event if the true rate of such an event is 37% or more; and there is at least a 90% chance of observing no events if the true rate is 1% or less. For the high-dose vaccine arm (n = 10), there is at least a 90% chance of observing at least 1 event if the true rate of such an event is 21% or more; and there is a 90% chance of observing no events if the true rate is 1% or less. For the two vaccine arms combined (n = 15), there is at least a 90% chance of observing at least 1 event if the true rate of such an event is

14.3% or more; there is a 90% chance of observing no events if the true rate is 0.5% or less. As a reference, in HVTN vaccine trials from April 2008 through March 2018, about 1.74% of participants who received placebos experienced an SAE.

Binomial probabilities of observing 0, 1 or more, and 2 or more events among arms of size 5 and 10 are presented in Table 6-1 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of the Part A study design to identify potential safety problems with the 3M-052-AF–adjuvanted vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 5 and 10, for different true event rates

True event rate (%)	Pr(0/5)	Pr(1+/5)	Pr(2+/5)	Pr(0/10)	Pr(1+/10)	Pr(2+/10)
1	95.1	4.9	0.1	90.4	9.6	0.4
4	81.5	18.5	1.5	66.5	33.5	5.8
10	59	41	8.1	34.9	65.1	26.4
20	32.8	67.2	26.3	10.7	89.3	62.4
30	16.8	83.2	47.2	2.8	97.2	85.1
40	7.8	92.2	66.3	0.6	99.4	95.4

6.1.2 Sample size calculations for safety (Part B)

The goal of the safety evaluation for Part B of this study is to identify safety concerns associated with administration of one or more vaccine regimens. Specifically, for each vaccine arm of Part B (n = 20), there is a 90% chance of observing at least 1 event if the true rate of such an event is 11% or more; and there is a 90% chance of observing no events if the true rate is 0.5% or less. For the four vaccine arms combined (n = 80), there is a 90% chance of observing at least 1 event if the true rate of such an event is 2.9% or more; and there is a 90% chance of observing no events if the true rate is 0.1% or less.

Binomial probabilities of observing 0, 1 or more, and 2 or more events among arms of size 20 and 80 are presented in Table 6-2 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of the Part B study design to identify potential safety problems with the four vaccine regimens.

Table 6-2 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 20 and 80, for different true event rates

True event rate (%)	Pr(0/20)	Pr(1+/20)	Pr(2+/20)	Pr(0/80)	Pr(1+/80)	Pr(2+/80)
1	81.8	18.2	1.7	44.8	5.2	19.1
4	44.2	55.8	19.0	3.8	96.2	83.5
10	12.2	87.8	60.8	0	100	99.8
20	1.2	98.8	93.1	0	100	100
30	0.1	99.9	99.2	0	100	100

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 6-3 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method (84). If none of the 80 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 4.6%. For each individual vaccine arm (n = 20), the 2-sided upper confidence bound for this rate is 16.1%.

Table 6-3 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size n_1 and n_2

Observed event rate	95% Confidence interval (%)
0/20	(0%, 16.1%)
1/20	(0.9%, 23.6%)
2/20	(2.8%, 30.1%)
0/80	(0%, 4.6%)
1/80	(0.2%, 6.7%)
2/80	(0.7%, 8.7%)

6.1.3 Sample size calculations for immunogenicity (Part B)

Immunogenicity sample size calculations allow for a 10% rate of missing immunogenicity data at Month 2.5 and 6.5 (n = 18 for analysis) and 15% rate of missing immunogenicity data at Months 12 and 18 (n = 17 for analysis).

The first goal is to evaluate immune response rates based on data from the primary TZM-bl neutralizing antibody assay among vaccinees. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% confidence intervals for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-4. Calculations are done using the score test method (84).

Table 6-4 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the vaccinees (n = 18)

No. of responses	Observed response rate (%)	95% Confidence interval
4/18	22.2	(9.0, 45.2)
6/18	33.3	(16.3, 56.3)
8/18	44.4	(24.6, 66.3)
10/18	55.6	(33.7, 75.4)
12/18	66.7	(43.7, 83.7)
14/18	77.8	(54.8, 91.0)
15/18	83.3	(60.8, 94.2)
16/18	88.9	(67.2, 96.9)
17/18	94.4	(74.2, 99.0)

As shown in Table 6-5, there is limited power for a formal comparison of immunogenicity response rates between vaccine arms of size n = 20. For either 80% or 90% power, the sizes of differences that the trial is powered to detect are large. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05. However, the study is adequately powered to detect the differences seen in the CAVD 641 study in rhesus macaques led by Bali Pulendran. Rates of autologous tier 2 neutralizing antibody responses at week 26 (the peak timepoint) were compared between arms with Alum-3M-052 or GLA-LSQ adjuvant, both with BG505-SOSIP protein. The large difference in response rates—50% vs. 100%—could be detected with 97% power.

Table 6-5 Power for comparison of response rates between 2 arms (n1 = 18, n2 = 18)

True response rate Arm 1 (%)	Minimum true response rate in Arm 2 in order to detect a difference	
	80% power	90% power
10	58	65
20	71	77
30	81	87
40	89	94
50	95	99

Power for comparing the magnitude of immune responses between pairs of arms with different adjuvants is first calculated using binding antibody data from the aforementioned HEPLISAV study. Engerix, an HBV vaccine formulated with Alum, was compared to HEPLISAV, the same HBV vaccine formulated with CpG 1018. With n = 18 vaccinees per arm, the current study has 100% power to detect the difference in geometric mean immune response seen in the HEPLISAV study at Week 28 (the peak timepoint) (79.7 mIU/mL vs. 206.1 mIU/mL), assuming based on the HEPLISAV study that the group with higher geometric mean has a smaller standard deviation (51.38 mIU/mL vs. 6.04 mIU/mL), using a 0.025-level one-sided Wilcoxon rank sum test. In addition, power is also 100% for detecting the difference seen between adjuvants at Week 52 (the durability timepoint), based on the HEPLISAV data (geometric means 19.0 vs. 131.0 mIU/mL and standard deviations 34.57 vs. 6.92 mIU/mL).

In addition, “generic” power calculations inform the power of the study to compare immune response magnitudes between arms. These presume a continuous immune response, transformed to a 1-standard deviation scale, and a mean shift in scaled immune response between arms. Table 6-6 shows the power for comparing immune responses between pairs of arms with different adjuvants, separately at Months 2.5 and 6.5 (n = 18 per arm) and Months 12 or 18 (durability = 17 per arm) timepoints. Observe that the study is powered to detect moderate 1-standard deviation differences in immune responses between adjuvants at the early timepoints and 1.1-standard-deviation differences at the durability timepoints.

Table 6-6 Power for comparing the magnitude of a generic immune response between arms. Immune responses are compared using 0.025-level one-sided Wilcoxon rank sum tests. Continuous immune responses are assumed to follow a normal distribution, transformed to a 1-standard deviation scale, with a mean of zero in one arm and the same standard deviation in the two arms. To allow for missing immunogenicity data, analyses include 17 or 18 subjects in each arm. Power is based on 1000 simulations.

Mean difference between arms (in standard-deviation units)	Power, n = 18 per arm	Power, n = 17 per arm
0.8 SD	63%	61%
0.9 SD	71%	69%
1.0 SD	81%	78%
1.1 SD	85%	85%
1.2 SD	92%	90%
1.3 SD	97%	95%

In addition, we assessed the ability of the study to rank and select the adjuvant with the highest immune response at a given timepoint. Table 6-7 shows the probability of correctly selecting the adjuvant with the highest mean immune response at Month 6.5, as a function of the mean in the arm with next-lowest mean immune response. Calculations again assume that immune responses have been transformed to the 1-standard deviation scale. The results show that the study is sufficiently sized to correctly rank adjuvant arms, assuming the top two arms have a mean difference of at least 0.5 standard-deviations.

Table 6-7 Probability of correct ranking. Probability of correctly selecting the adjuvant with the highest mean immune response at Month 6.5, based on a generic standard-deviation-scaled continuous immune response. Probability is shown as a function of the mean immune response in the next-best arm. To allow for missing immunogenicity data, analyses include 18 subjects in each arm. Calculations assume all arms below the best have a mean immune response equal to that of the next-best arm and thus the results are conservative.

Mean difference, best – next-best	Probability of correct ranking
0.1 SD	0.38
0.2 SD	0.52
0.3 SD	0.65
0.4 SD	0.75
0.5 SD	0.82
0.6 SD	0.92

6.1.4 Sample size calculations for safety (Part C)

The goal of the safety evaluation for Part C of this study is to identify safety concerns associated with administration of 1 or more vaccine regimens studied in Part C. Specifically, for each vaccine arm of Part C (n = 10), there is a 90% chance of observing at least 1 event if the true rate of such an event is 20.6% or more and there is a 90% chance of observing no events if the true rate is 1% or less.

Binomial probabilities of observing 0 events, 1 or more events, and 2 or more events among arms of size 10 are presented in Table 6-8 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of the Part C study design to identify potential safety problems with the two vaccine regimens.

Table 6-8 Probability of observing 0 events, 1 or more events, and 2 or more events among arms of size 10 for different true event rates

True event rate (%)	Pr (0/10)	Pr (1+/10)	Pr (2+/10)
1	90.4	9.6	0.4
4	66.5	33.5	5.8
10	34.9	65.1	26.4
20	10.7	89.3	62.4
30	2.8	97.2	85.1

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval (CI) for the true rate of an adverse event based on the observed data. Table 6-9 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 (84). If none of the 10 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 27.8%.

Table 6-9 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size n_1 and n_2

Observed event rate	95% Confidence interval (%)
0/10	(0%, 27.8%)
1/10	(1.8%, 40.4%)
2/10	(5.7%, 51%)

6.1.5 Sample size calculations for immunogenicity (Part C)

Immunogenicity sample-size calculations allow for a 10% rate of missing immunogenicity data at Month 2.5 and 6.5 (n = 9 for analysis).

The first goal is to evaluate immune response rates based on data from the primary TZM-bl neutralizing antibody assay among vaccinees. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% CIs for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-10. Calculations are done using the score test method (84).

Table 6-10 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the vaccinees (n = 9)

No. of responses	Observed response rate (%)	95% Confidence interval
1/9	11.1	(2.0, 43.5)
2/9	22.2	(6.3, 54.7)
3/9	33.3	(12.1, 64.6)
4/9	44.4	(18.9, 73.3)
5/9	55.6	(26.7, 81.1)
6/9	66.7	(35.4, 87.9)
7/9	77.8	(45.3, 93.7)
8/9	88.9	(56.5, 98.0)
9/9	100.0	(70.1, 100.0)

As shown in Table 6-11and Table 6-12, there is limited power for a formal comparison of immunogenicity response rates between Parts B and C based on vaccine arms of size n = 10 (Parts A and C) and n = 20 (Part B). For either 80% or 90% power, the sizes of differences that the trial is powered to detect are large.

Table 6-11 Power for comparison of response rates between 2 arms (n1 = 9, n2 = 9)

True response rate Arm 1 (%)	Minimum true response rate in Arm 2 in order to detect a difference	
	80% power	90% power
10	83	91
20	92	99
30	99	N/A

Table 6-12 Power for comparison of response rates between 2 arms (n1 = 9, n2 = 18)

True response rate Arm 1 (%)	Minimum true response rate in Arm 2 in order to detect a difference	
	80% power	90% power
10	72	90
20	83	90
30	91	96
40	92	100

6.2 Randomization

A participant's randomization assignment will be computer generated and provided to the HVTN CRS pharmacist through a Web-based randomization

system. For Part A, B and C, participants within each dose group will be randomized to vaccine or placebo assignment. For Part C, Group 7 will be enrolled before Group 8. There will be no randomization for Groups 1 and 2 as they will be enrolled sequentially for dose escalation. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments (except in emergency situations as specified in the HVTN Manual of Operations [MOP]).

6.3 Blinding

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

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

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

6.4 Statistical analyses

This section describes the final study analyses, 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. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan (SAP) will address how to analyze the participant's safety data. Analyses are modified intent-to-treat in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

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

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

6.4.1 Analysis variables

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

6.4.2 Baseline comparability

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

6.4.3 Safety/tolerability analysis

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

6.4.3.1 Reactogenicity

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

6.4.3.2 AEs and SAEs

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

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

6.4.3.3 Local laboratory values

Box plots 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 box plot will show the first quartile, the median, and the third quartile. Outliers (values outside the box plot) will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

For each local laboratory measure, summary statistics will be presented by treatment 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 Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (see Section 11.2.2) 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 may be performed, limited to participants who received all scheduled injections per protocol. Assay results that are unreliable, from specimens collected outside of the visit window, or from HIV-infected participants postinfection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

Data from placebo arms will be pooled across groups (n = 8 for analysis in Part B). For analyses of immunogenicity after the second vaccination, data on the 3M-052-AF from Part A (with the MTD of the 3M-052-AF) and Part B will be

pooled. Otherwise, analyses of immunogenicity will be performed separately for Part A and Part B participants.

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% confidence interval estimates calculated using the score test method (84). Because of the small numbers of control participants in each group, no adjustment will be made to the vaccine arm estimates for the false positive rates (FPRs) in the control arms. Barnard or Fisher's exact tests, as specified in the SAP, will be used to compare the response rates of any 2 vaccine arms, with a significant difference declared if the 2-sided p-value is ≤ 0.05. In general Barnard's is preferred since under most circumstances it is more powerful than Fisher's (85).

For quantitative assay data (eg, percentage of positive cells from ICS assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be used for graphical display of all of the study arms. Typically, the results will be shown for each vaccine arm and for the pooled placebo group.

The difference between arms at a specific timepoint will be tested with a nonparametric Wilcoxon rank sum test if the data are not normally distributed and with a 2-sample t-test if the data appear to be normally distributed. Individual tests comparing the 6 pairs of vaccine arms will be performed unless prespecified. If rank-based tests are used, then the tests will be inverted to construct Hodges-Lehmann point estimates and 2-sided $(1\text{-}0.05/6) \times 100\%$ confidence intervals (CIs) about the differences in location centers of the 6 pair-wise comparisons of vaccine arms. If actual-value tests are used then the Dunnett's procedure will be used to construct simultaneous confidence intervals about the pairs of mean differences for the many-to-one comparisons (86) when multiple vaccine arms are each compared with the pooled placebo group. Given that all pair-wise comparisons between the multiple vaccine arms are of interest, the Tukey procedure (87) will be used. An appropriate data transformation (eg, $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance). Significance of the differences between pairs will be evaluated using two procedures, first based on whether the simultaneous 95% CIs exclude zero and secondly based on whether the nominal (unadjusted) 95% CIs exclude zero.

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, intracellular cytokine staining). If treatment arm differences for these assays are best summarized by a mixture model, then either Lachenbruch's test statistic (88) or an alternative two-part test (89) (as defined in the SAP) will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal

response distributions. Lachenbruch's test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

More sophisticated analyses employing repeated measures methodology (for example, linear mixed models or marginal mean models fit by generalized estimating equations) will be utilized to incorporate immune responses over several timepoints and to test for differences over time and differences in trajectories across treatment arms. For example, repeated measures analyses will be conducted to evaluate trajectories in innate immune responses over Days 1, 3, 7 relative to responses measured at Day 0. However, inference from such analyses would be limited by the small sample size of this study. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05$.

Based upon previous HVTN trials, missing 10% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed PBMCs. To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data are missing for an endpoint (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 evaluating repeated immunogenicity measurements over time, 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 (90) will be used, because they accommodate the censoring. In addition, secondary

analyses of repeated immunogenicity measurements may be done using weighted generalized estimating equation (GEE) (91) methods, which are valid under MAR. All of the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

Some immunogenicity endpoints are only measured in subset of participants. For example, fine needle lymph node aspirates, bone marrow aspirates, and leukapheresis will be performed only on participants consenting to these optional procedures, and immunogenicity endpoints based on these samples will only exist for those participants. For such endpoints, exploratory analyses will be conducted to assess the correlation of participant characteristics measured in (nearly) all participants with the resource-intensive endpoints. For example, if the same assay is performed on blood and fine needle lymph node aspirate samples, then a scatterplot and Spearman rank correlation coefficient (r) will be used to assess the correlation of responses. If at least moderate correlations exist (eg, $r \geq 0.3$), then the semiparametric efficient analysis method of Rotnitzky and Robins (92) will be used (described in Gilbert, Sato et al. for application to vaccine studies (93)) to estimate the mean of the resource-intensive endpoint for each group and to compare means between groups.

6.4.4.2 Multivariate display of immunogenicity endpoints

Data visualization techniques may be used to explore the relationship among immunogenicity readouts across assays, and across readouts for assays that produce high dimensional data. The set of readouts may be based on one of the primary endpoints (eg, neutralizing Abs), on the set of primary endpoints, or on immunogenicity endpoints that also include secondary or exploratory endpoints. To understand the relationship between pairs of readouts, scatter plots may be used when the number of readouts is small or for a larger number of readouts, a heatmap showing the degree of correlation between any two pairs. Principal component analysis (PCA) and associated ‘biplots’ of the scores and loadings are particularly useful to understand associations between readouts, especially when readouts are correlated (94). PCA is a method to reduce the dimensionality of the number of readouts to a smaller set of values (principal components) that are normalized linear combinations of the readouts in such a way that the first principal component accounts for the most variability in the data and subsequent components, while maximizing variability, are uncorrelated with each other. A ‘biplot’ displays the first and second principal component scores and principal component loadings. The x-axis is the value from the first principal component and the y-axis is the second principal component, where each axis label includes the percentage of variation in the total set of readouts captured by the principal component. The top axis is the first principal component loadings and the right axis is the second principal component loadings. An arrow is drawn for each immunogenicity readout (eg, Env-specific CD4+ T cell polyfunctionality score) from the origin to the point defined by its first two principal component loadings. The length of the arrow represents the amount of total variation of the set of readouts captured by the given readout. The direction of an arrow conveys the extent to which the variation of a readout is in the direction of the first or second

principal component. The angle between two arrows conveys information about the correlation of the two readouts, with a zero degree angle denoting perfect correlation and a 90 degree angle denoting no correlation. Each arrow on the biplot is labeled by the immunogenicity readout it represents. A biplot is annotated with key meta-information such as the treatment arm (most common application) or a demographic category. Depending on the application, K-means clustering and hierarchical clustering may also be applied for multivariate graphical display of immunogenicity readouts.

6.4.4.3 Primary analyses of neutralization data

The primary measure of antibody neutralization is the response to the vaccine-matched BG505.T332N isolate. As well, responses to a single highly neutralization-sensitive tier 1 virus (MW965.26) will be assessed. The mean magnitude of the response to the vaccine-matched isolate will be estimated for each arm and compared to the pooled placebo group. As well, the ratio of the response to the vaccine-matched antigen, relative to that to the neutralization-sensitive isolate, will be estimated for each arm.

If a tier 2 panel of viral isolates is used to assess the breadth of neutralizing antibody responses, the area-under-the-magnitude-breadth curve (AUC-MB) to a global panel of viral isolates (14) will be computed for each participant with evaluable neutralization data, as described in (95). 95% CIs about the four differences in mean AUC-MB for each vaccine regimen versus the pooled placebo groups (vaccine – placebo), will be calculated.

6.4.4.4 Analysis of CD4+ T cell responses as measured by the ICS assay

The analysis of CD4+ T cell response rates as measured by the ICS assay will be evaluated and compared as described under the general approach. For each T cell subset, the positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay. In general, the Mixture Models for Single-cell Assays (MIMOSA) statistical framework (96) and/or the Fisher's exact test-based positivity criteria will be used. Details of the positivity criteria will be discussed in the SAP. The magnitude of marginal response will be analyzed as described for quantitative data in the general approach section. For each T-cell subset, graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint. When 3 or more cytokines are being measured by the ICS assay, the polyfunctionality of ICS responses may also be analyzed as an exploratory endpoint. Besides descriptive plots of the magnitude of polyfunctional responses, the COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets) statistical framework (97) may also be used to perform joint modelling of multiple T-cell subsets of different cytokine combinations. For example, the functionality score (FS) and the polyfunctionality score (PFS) may be used to summarize the multi-parameter ICS responses.

6.4.4.5 Analysis of epitope mapping data

The minimal set of optimal length epitopes that can explain the observed T cells responses to a set of individually tested overlapping 15-mer peptides may be obtained to assess the breadth and depth of the T cell responses based on the epitope mapping data. When human leukocyte antigen (HLA) data are also available, computational HLA:peptide binding predictors (eg, NetMHCpan) may be used to more accurately identify each participant's T cell epitopes.

The set of epitopes may also be used to assess "coverage" of a representative set of circulating viruses by each participant's set of T-cell responses. The coverage provided by each epitope is defined by the fraction of viruses that match the sequence of the mapped epitope. The coverage provided by a participant's set of epitopes is defined by the fraction of viruses covered by at least one of the participant's epitopes.

6.4.4.6 Analysis of multiplexed immunoassay data

When a small panel of analytes (eg, ≤ 5) is being assessed in a multiplexed immunoassay, the analysis of response rates and response magnitudes will be evaluated and compared as described under the general approach. Details for calculating a positive response and response magnitude will be provided in the SAP. When a larger panel is being assessed, 3 approaches may be considered to evaluate the magnitude and breadth of these responses. First, Magnitude–Breadth (M-B) curves maybe employed to display individual- and group-level response breadth as a function of magnitude. Applied to tier 2 virus neutralizing antibody data, response breadth is defined as the number of isolates with magnitude above a positivity threshold. Two choices are to compare the M-B curves among vaccine arms, as follows: a non-parametric Wilcoxon rank sum test on the subject-specific AUC-MB or a Kolmogorov-Smirnov type test on the 2 group-average M-B curves. Simulations can be used to obtain 2-sided p-values for the latter test. Second, a weighted-average score-like variable may be constructed to account for the correlations between analytes as an integrate magnitude of responses to multiple analytes. Similar group comparison methods described in the first approach may be adopted. Third, machine-learning approaches such as super-learning (98) will be employed which achieve the goal of reducing the dimensionality of the assay readouts and simultaneously identifying individual responses and groups of responses that differ between treatment groups or over time. Details of either approach will be described in the SAP.

6.4.5 Analyses and data sharing prior to end of scheduled follow-up visits

Any analyses conducted prior to the end of the scheduled follow-up visits should not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments. In particular, early unblinded analyses by treatment assignment require careful consideration and should be made available on a need to know basis in accordance with Sections 6.4.5.1 and 6.4.5.2. Interim blinded safety and immunogenicity data should not be shared outside of

the SMB, HVTN 137 PSRT, the protocol team leadership, the HVTN Executive Management Team, the study product developer, and the study sponsor and/or its designee(s) for their regulatory reporting unless approved by the protocol leadership and the HVTN leadership.

6.4.5.1 Safety analyses

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study, as defined in Section 3, for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 137 PSRT. Refer to the process described in the HVTN Unblinding MOP for any requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity analyses

An unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after the primary immunogenicity endpoint (neutralizing antibody response) reaches the aforementioned threshold. The Laboratory Center will review the analysis report prior to distribution to the protocol chairs, DAIDS, study product developer, and other key HVTN members and investigators. Reports for distribution or presentation should use PubIDs and not participant ID (PTIDs) for individual responses. 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 information available at the time of enrollment, including results of screening laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions.

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

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

7.1 Inclusion criteria

General and Demographic Criteria

1. **Age** of 18 through 50 years, inclusive

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; 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 until after the final study contact.

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 as low risk for HIV acquisition per low-risk guidelines (see Appendix U) and agrees to avoid behavior associated with high risk of HIV exposure through the final study visit. Low risk may include persons stably taking PrEP as prescribed for 6 months or longer (see Appendix U).

Laboratory Inclusion Values

Hemogram/Complete blood count (CBC)

10. **Hemoglobin**

    - ≥ 11.0 g/dL for volunteers who were assigned female sex at birth
    - ≥ 13.0 g/dL for volunteers who were assigned male sex at birth and transgender males who have been on hormone therapy for more than 6 consecutive months
    - ≥ 12.0 g/dL for transgender females who have been on hormone therapy for more than 6 consecutive months
    - For transgender volunteers who have been on hormone therapy for less than 6 consecutive months, determine hemoglobin eligibility based on the sex assigned at birth.

11. **White blood cell count** = 2,500 to 12,000 cells/mm^3 with normal differential, or differential approved by Investigator of Record (IoR) or designee as not clinically significant

12. **Total lymphocyte count** ≥ 650 cells/mm^3 with normal differential, or differential approved by IoR or designee as not clinically significant

13. **Remaining differential** either within institutional normal range or with IoR or designee approval

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

Chemistry

15. **Alanine aminotransferase (ALT)** < 1.25 times the institutional upper limit of normal

16. **Creatinine** < 1.1 times the institutional upper limit of normal

Virology

17. **Negative HIV-1 and -2 blood test**: US volunteers must have a negative FDA-approved enzyme immunoassay (EIA) or chemiluminescent microparticle immunoassay (CMIA).

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

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

Urine

20. **Normal urine**:

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

Reproductive Status

21. **Volunteers who were assigned female sex at birth**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test at screening (ie, prior to randomization) and prior to study product administration. For any optional study procedure, pregnancy testing should be performed as per Section 9.5. Persons who are NOT of reproductive potential due to having undergone hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

22. **Reproductive status**: A volunteer who was assigned female sex at birth:

    - Must agree to use effective contraception for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment until 6 months after the final study vaccination. Effective contraception is defined as using the following methods:
        - Condoms (male or female) with or without a spermicide,
        - Diaphragm or cervical cap with spermicide,
        - Intrauterine device (IUD),
        - Hormonal contraception,
        - Tubal ligation, or
        - Any other contraceptive method approved by the HVTN 137 PSRT

  - Successful vasectomy in any partner assigned male sex at birth (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity postvasectomy);

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

- Or be sexually abstinent.

23. **Volunteers who were assigned female sex at birth must also agree not to seek pregnancy through alternative methods**, such as artificial insemination or in vitro fertilization until 6 months after the last vaccination.

7.2 Exclusion criteria

General

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

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

3. **Body mass index (BMI)** ≥ 40; or BMI ≥ 35 with 2 or more of the following: age > 45, systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, current smoker, known hyperlipidemia

4. **Intent to participate in another study** of an investigational research agent or any other study that requires non-HVTN HIV antibody testing during the planned duration of the HVTN 137 study

5. **Pregnant or breastfeeding**

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

Vaccines and other Injections

7. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 137 PSRT will determine eligibility on a case-by-case basis.

8. **Previous receipt of monoclonal antibodies (mAbs)**, whether licensed or investigational; the HVTN 137 PSRT will determine eligibility on a case-by-case basis

9. **Non-HIV experimental vaccine(s) received within the last 1 year** in a prior vaccine trial. Exceptions may be made by the HVTN 137 PSRT for vaccines that have subsequently undergone licensure or Emergency Use Authorization by the FDA or, if outside the United States, equivalent authorization by the national regulatory authority. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 137 PSRT will determine eligibility on a case-by-case basis. For volunteers who have received an experimental vaccine(s) greater than 1 year ago, eligibility for enrollment will be determined by the HVTN 137 PSRT on a case-by-case basis.

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

11. ACAM2000 vaccine for monkeypox received within 30 days prior to enrollment (note that enrollment of a participant coincides with receipt of study product) or scheduled within 30 days after injection.
    - ACAM2000 vaccine >30 days prior with a vaccination scab still present

12. **Any vaccines that are not live attenuated vaccines** and were received within 14 days prior to first vaccination or scheduled for 14 days after injection (eg, tetanus, pneumococcal, hepatitis virus A or B); replication incompetent vaccines such as the Jynneos monkeypox vaccine are not considered to be live vaccines

13. **Previous receipt of HEPLISAV, Shingrix, or RTS,S/AS01B/Mosquirix vaccine** received within 30 days prior to first vaccination or scheduled for 30 days after injection.

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

Immune System

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

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

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

18. **Autoimmune disease, current or history**

19. **AESIs:** Volunteers who currently have, or have a history of, any condition that could be considered an AESI for the product(s) administered in this protocol (representative examples are listed in Appendix V)

20. Immunodeficiency

Clinically significant medical conditions

21. **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.

22. **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

23. **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.

24. Current anti-tuberculosis (TB) prophylaxis or therapy

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

    Exclude a volunteer who:

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

26. **Diabetes mellitus** type 1 or type 2 (Not exclusionary: type 2 cases controlled with diet alone or a history of isolated gestational diabetes.)

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

28. **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 in this protocol 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.

29. **Bleeding disorder** (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

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

31. **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.

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

33. History of **generalized urticaria**, **angioedema**, or **anaphylaxis.** (Not exclusionary: angioedema or anaphylaxis to a known trigger with at least 5 years since last reaction to demonstrate satisfactory avoidance of trigger.)

7.3 Eligibility for optional procedures

Eligibility requirements for optional procedures are listed in Section 9.5.

7.4 Participant departure from vaccination schedule or withdrawal

This section concerns an individual participant's departure from the study product administration schedule. Pause rules for the trial are described in Section 11.4.

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
  - Receipt of allergy treatment with antigen injections
  - Receipt of ACAM2000 vaccine for monkeypox
    - After administration of ACAM2000 vaccine, a minimum of 30 days is required before administration of study vaccination if vaccination scab no longer present,
    - If a vaccination scab still present 30 days after receiving ACAM2000, delay study vaccination until the scab is no longer present;
- Within 14 days prior to any study injection
  - Receipt of any vaccines that are not live attenuated vaccines (eg, pneumococcal); replication incompetent vaccines such as the Jynneos monkeypox vaccine are not considered to be live vaccines

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

Vaccinations should not be administered outside the visit window period (see Appendix W).

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 or 3-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 137 PSRT)

- Clinically significant condition (ie, a condition that affects the immune system or for which continued vaccinations and/or blood draws may pose additional risk), including but not limited to the following:
  - Pregnancy (regardless of outcome) Vaccinations will be stopped while a participant is pregnant. If the participant is no longer pregnant and can be vaccinated within an appropriate visit window, vaccinations may resume
  - HIV infection
  - Any grade 4 local or systemic reactogenicity symptom, lab abnormality, or AE that is subsequently considered to be related to vaccination

  - Any grade 3 lab abnormality that is subsequently considered to be related to vaccination
  - Other grade 3 clinical AE that is subsequently considered to be related to vaccination with the exception of fever, and subjective local and systemic symptoms. For grade 3 injection site erythema and/or induration, upon review, the PSRT may allow continuation of vaccination
  - SAE that is subsequently considered to be related to vaccination
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 137 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination

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

Participants discontinuing study product for reasons other than HIV infection should be counseled on the importance of continuing with the study and strongly encouraged to participate in follow-up visits and protocol-related procedures per the protocol for the remainder of the trial, unless medically contraindicated (see *HVTN 137 Study Specific Procedures* (SSP)).

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

7.4.4 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,
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff), or
- Any condition where termination from the study is required by applicable regulations.

8 Study product preparation and administration

CRS pharmacists should consult the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for standard pharmacy operations. The protocol schema is shown in Table 3-1. See the Investigator's Brochure (IB) 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.

Part A:

Vaccination at month 6 for Group 1 and Group 2 of Part A is optional.

Group 1

Treatment 1 (T1): BG505 SOSIP.664 gp140, 100 mcg, admixed with 3M-052-AF, 1 mcg, and Aluminum Hydroxide Suspension (Alum), 500 mcg, to be administered as one 0.5 mL dose intramuscularly (IM) at months 0, 2, and 6 (optional).

Or

Placebo 1 (P1): Placebo (Tris-NaCl Buffer) to be administered as one 0.5 mL dose IM at months 0, 2, and 6 (optional).

Group 2

Treatment 2 (T2): BG505 SOSIP.664 gp140, 100 mcg, admixed with 3M-052-AF, 5 mcg, and Alum, 500 mcg, to be administered as one 0.5 mL dose IM at months 0, 2, and 6 (optional).

Or

Placebo 2 (P2): Placebo (Tris-NaCl Buffer) to be administered as one 0.5 mL dose IM at months 0, 2, and 6 (optional).

Part B:

Group 3

Treatment 3 (T3): BG505 SOSIP.664 gp140, 100 mcg, admixed with CpG 1018, 300 mcg, and Alum, 500 mcg, to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Or

Placebo 3 (P3): Placebo (Tris-NaCl Buffer) to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Group 4

Treatment 4 (T4): BG505 SOSIP.664 gp140, 100 mcg, admixed with 3M-052-AF, 5 mcg (highest tolerated dose from Part A), and Alum, 500 mcg, to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Or

Placebo 4 (P4): Placebo (Tris-NaCl Buffer) to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Group 5

Treatment 5 (T5): BG505 SOSIP.664 gp140, 100 mcg, admixed with GLA-LSQ (GLA 5 mcg, and QS-21 2 mcg) to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Or

Placebo 5 (P5): Placebo (Tris-NaCl Buffer) to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Group 6

Treatment 6 (T6): BG505 SOSIP.664 gp140, 100 mcg, admixed with Alum, 500 mcg, to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Or

Placebo 6 (P6): Placebo (Tris-NaCl Buffer) to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Part C:

Group 7

Treatment 7 (T7): BG505 SOSIP.664 gp140, 100 mcg admixed with 3M-052-AF, 3 mcg and Alum, 500 mcg to be administered as one 0.5-mL dose intramuscularly (IM) at months 0, 2, and 6.

Or

Placebo 7 (P7): Placebo (Tris-NaCl Buffer) to be administered as one 0.5 mL dose IM at months 0, 2, and 6.

Group 8

Treatment 8 (T8): Trimer 4571, 100 mcg admixed with 3M-052-AF, 5 mcg and Alum, 500 mcg to be administered as one 0.4-mL dose IM at months 0, 2, and 6.

Or

Placebo 8 (P8): Placebo (Tris-NaCl Buffer) to be administered as one 0.4 mL dose IM at months 0, 2, and 6.

8.2 Study product formulation

8.2.1 BG505 SOSIP.664 gp140

BG505 SOSIP.664 gp140 will be provided in 2 mL Type 1 glass vials with a rubber stopper and flip-off Aluminum seal. Each vial contains a fill volume of 0.6 ± 0.05 mL at a concentration of 2 mg/mL. BG505 SOSIP.664 gp140 is a clear to opalescent, colorless to slightly yellow liquid, without any visible particles. The product is stored at ≤ -65° C. The study product is described in further detail in the Investigator's Brochure (IB).

8.2.2 HIV-1 Trimer 4571 (VRC-HIVRGP096-00-VP)

Trimer 4571 will be provided as 3-mL glass vials with a 1.2 mL ± 0.1 mL fill volume at a concentration of 500 mcg/mL. Each vial contains a sterile, aqueous, buffered solution that is clear and colorless. Some small white or translucent particles may be present. Store Trimer 4571 frozen at -35ºC to -15ºC.

Vials should not be refrozen after thaw. Thawed vials can be stored at 2ºC to 8ºC for up to 48 hours or at 15ºC to 27ºC for up to 24 hours. Vials are intended for single use only and do not contain a preservative. A single vial may not be used for multiple dose preparations. The study product is described in further detail in the IB.

8.2.3 Tris-NaCl buffer (TBS) (Diluent for BG505 SOSIP.664 gp140 and Placebo)

Tris-NaCl buffer will be provided in 2 mL Type 1 glass vials with 13 mm rubber stoppers and 13 mm 6-Bridge, Flip-Off, Matte Top seals. Each vial contains a fill volume of 1.1 ± 0.1 mL. Tris-NaCl buffer is a clear to opalescent, colorless to slightly yellow liquid, without any visible particulates. The product is stored at 2-8° C. The study product is described in further detail in the IB.

8.2.4 3M-052-AF (Labeled as AP 60-702)

3M-052-AF will be provided in 2 mL Type 1 glass vials with a rubber stopper and flip-off Aluminum seal. Each vial contains a fill volume of 0.4 mL at a concentration of 50 mcg/mL. 3M-052-AF is a clear to slightly hazy, colorless liquid. The product is stored at 2-8° C. Do not freeze. The study product is described in further detail in the IB.

8.2.5 CpG 1018 (Labeled as CpG 1018 Drug Product)

CpG 1018 will be provided in 2 mL Type 1 glass vials. Each vial contains a fill volume of 0.7 mL at a concentration of 6 mg/mL. CpG 1018 is a colorless, clear liquid, free of visible particles. The product is stored at 2-8° C. The study product is described in further detail in the IB.

8.2.6 GLA-LSQ (Labeled as AP 10-602)

Glucopyranosyl Lipid A (GLA) is formulated in a liposomal composition with QS-21 to generate the adjuvant GLA-LSQ. GLA-LSQ will be provided in 2 mL Type I glass vials with stoppers and flip-off Aluminum seals. Each vial contains a fill volume of 0.4 mL at a concentration of 20 mcg/mL GLA and 8 mcg/mL QS-21. GLA-LSQ is a hazy, water-white, semi-transparent liquid. The product is stored at 2-8° C. Do not freeze. The study product is described in further detail in the IB.

8.2.7 Aluminum Hydroxide Suspension (Alum)

The Aluminum Hydroxide Suspension consists of Alhydrogel® 2% (Brenntag Biosector, Frederikssund, Denmark) and will be provided in 3 mL Type 1 glass vials. Each vial contains 0.7 mL fill volume at a concentration of 5 mg/mL Aluminum. Alum appears as an opaque, white gelatinous precipitate in aqueous suspension. The product is stored refrigerated at 2-8° C. Do not freeze. The study product is described in further detail in the IB.

8.3 Preparation of study products

Pharmacists must follow appropriate aseptic technique and sterile preparation procedures/guidance as outlined in USP <797> [medium risk], utilizing a pharmacy biosafety cabinet/isolator or better. Local regulations and site institutional policies and procedures for use of personal protective equipment, such as gloves, gowns, masks, and safety glasses, must be followed. Pharmacists should follow the requirements of their country, their institution, and their pharmacy regulatory authority regarding these procedures.

Any unused portion of study product will not be used for another participant. Empty vials, unused portion of entered vials, or unused prepared study product

should be discarded in a biohazard container and disposed of in accordance with institutional or pharmacy policy.

PART A:

8.3.1 BG505 SOSIP.664 gp140, 100 mcg, admixed with 3M-052-AF, 1 mcg, and Alum, 500 mcg (Group 1)

For all preparation steps, use a 1 mL Luer-Lok syringe with 0.01 mL graduations and a 22-27 gauge Luer-Lok needle.

1. Remove one vial of 3M-052-AF, one vial of TBS and one vial of Alum from the refrigerator. Resuspend Alum by inverting the vial for 30-60 seconds.

2. Remove one vial of BG505 SOSIP.664 gp140 from the freezer. Thaw BG505 SOSIP.664 gp140vial by holding in gloved hand. Once thawed, maintain the vial in upright position and gently swirl or tap the vial to mix the contents. Do not invert the vial.

Preparation of Adjuvants:

3. Remove one vial of Water for injection (WFI) from storage.

4. Withdraw 0.6 mL WFI and inject it into 3M-052-AF vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

5. Withdraw 0.1 mL of the mixed preparation and inject it into an empty sterile vial (mixing vial).

6. Withdraw 0.2 mL of Alum and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

7. Withdraw 0.6 mL TBS and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of BG505 SOSIP.664 gp140:

8. Withdraw 0.1 mL BG505 SOSIP.664 gp140 and add it to the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of the Admixed Vaccine:

9. Withdraw 0.5 mL of the mixed preparation for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.

10. The admixed product is stable for 4 hours at room temperature.

8.3.2 BG505 SOSIP.664, gp140 100 mcg, admixed with 3M-052-AF, 5 mcg, and Alum, 500 mcg (Group 2)

For all preparation steps, use a 1 mL Luer-Lok syringe with 0.01 mL graduations and a 22-27 gauge Luer-Lok needle.

1. Remove one vial of 3M-052-AF, one vial of TBS, and one vial of Alum from the refrigerator. Resuspend Alum by inverting the vial for 30-60 seconds.

2. Remove one vial of BG505 SOSIP.664 gp140 from the freezer. Thaw BG505 SOSIP.664 gp140 vial by holding in gloved hand. Once thawed, maintain the vial in upright position and gently swirl or tap the vial to mix the contents. Do not invert the vial.

Preparation of Adjuvants:

3. Withdraw 0.2 mL of 3M-052-AF and inject it into an empty sterile vial (mixing vial).

4. Withdraw 0.2 mL of Alum and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

5. Withdraw 0.5 mL TBS and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of BG505 SOSIP.664 gp140:

6. Withdraw 0.1 mL BG505 SOSIP.664 gp140 and add it to the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of the Admixed Vaccine:

7. Withdraw 0.5 mL of the mixed preparation for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.

8. The admixed product is stable for 4 hours at room temperature.

PART B:

8.3.3 BG505 SOSIP.664 gp140, 100 mcg, admixed with CpG 1018, 300 mcg, and Alum, 500 mcg (Group 3)

For all preparation steps, use a 1 mL Luer-Lok syringe with 0.01 mL graduations and a 22-27 gauge Luer-Lok needle.

1. Remove one vial of CpG 1018, one vial of TBS, and one vial of Alum from the refrigerator. Resuspend Alum by inverting the vial for 30-60 seconds.

2. Remove one vial of BG505 SOSIP.664 gp140 from the freezer. Thaw BG505 SOSIP.664 gp140 vial by holding in gloved hand. Once thawed, maintain the vial in upright position and gently swirl or tap the vial to mix the contents. Do not invert the vial.

Preparation of Adjuvants:

3. Withdraw 0.1 mL of CpG 1018 and inject it into an empty sterile vial (mixing vial).

4. Withdraw 0.2 mL of Alum and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

5. Withdraw 0.6 mL TBS and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of BG505 SOSIP.664 gp140:

6. Withdraw 0.1 mL BG505 SOSIP.664 gp140 and add it to the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of the Admixed Vaccine:

7. Withdraw 0.5 mL of the mixed preparation for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.

8. The admixed product is stable for 4 hours at room temperature.

8.3.4 BG505 SOSIP.664 gp140, 100 mcg, admixed with 3M-052-AF, 5 mcg, and Alum, 500 mcg (Group 4)

The highest tolerated dose of 3M-052-AF, 5 mcg, will be used in preparation of study product for administration to Group 4 participants. Follow preparation instructions in Section 8.3.2.

8.3.5 BG505 SOSIP.664 gp140, 100 mcg, admixed with GLA-LSQ, (GLA 5 mcg and QS-21 2 mcg) (Group 5)

For all preparation steps, use a 1 mL Luer-Lok syringe with 0.01 mL graduations and a 22-27 gauge Luer-Lok needle.

1. Remove one vial of GLA-LSQ and one vial of TBS from the refrigerator.

2. Remove one vial of BG505 SOSIP.664 gp140 from the freezer. Thaw BG505 SOSIP.664 gp140 vial by holding in gloved hand. Once thawed, maintain the vial in upright position and gently swirl or tap the vial to mix the contents. Do not invert the vial.

Preparation of BG505 SOSIP.664 gp140:

3. Withdraw 0.8 mL of TBS and inject it into an empty sterile vial (mixing vial).

4. Withdraw 0.2 mL BG505 SOSIP.664 gp140 and add it to the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of the Admixed Vaccine:

5. Withdraw 0.4 mL of the mixed preparation and add to GLA-LSQ vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

6. Withdraw 0.5 mL of the mixed preparation for study participant administration. To maintain study blind, wait 30 minutes prior to study product delivery to clinic staff. Prior to administration, gently invert the syringe to resuspend admixed product.

7. The admixed product is stable for 4 hours at room temperature.

8.3.6 BG505 SOSIP.664 gp140, 100 mcg, admixed with Alum, 500 mcg (Group 6)

For all preparation steps, use a 1 mL Luer-Lok syringe with 0.01 mL graduations and a 22-27 gauge Luer-Lok needle.

1. Remove one vial of TBS and one vial of Alum from the refrigerator. Resuspend Alum by inverting the vial for 30-60 seconds.

2. Remove one vial of BG505 SOSIP.664 gp140 from the freezer. Thaw BG505 SOSIP.664 gp140 vial by holding in gloved hand. Once thawed, maintain the vial in upright position and gently swirl or tap the vial to mix the contents. Do not invert the vial.

Preparation of the Admixed Vaccine:

3. Withdraw 0.2 mL of Alum and inject into an empty sterile vial (mixing vial).

4. Withdraw 0.7 mL TBS and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

5. Withdraw 0.1 mL BG505 SOSIP.664 gp140 and add it to the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

6. Withdraw 0.5 mL of the mixed preparation for study participant administration. To maintain study blind, wait 30 minutes prior to study product delivery to clinic staff. Prior to administration, gently invert the syringe to resuspend admixed product.

7. The admixed product is stable for 4 hours at room temperature.

PART C:

8.3.7 BG505 SOSIP.664 gp140, 100 mcg admixed with 3M-052-AF, 3 mcg and Alum, 500 mcg (Group 7)

For all preparation steps, use a 1-mL Luer-Lok syringe with 0.01-mL graduations and a 22-27 gauge Luer-Lok needle.

1 Remove one vial of 3M-052-AF, one vial of TBS, and one vial of Alum from the refrigerator. Resuspend Alum by inverting the vial for 30-60 seconds.

2 Remove one vial of BG505 SOSIP.664 gp140 from the freezer. Thaw BG505 SOSIP.664 gp140 vial by holding in gloved hand. Once thawed, maintain the vial in upright position and gently swirl or tap the vial to mix the contents. Do not invert the vial.

Preparation of Adjuvants:

3 Withdraw 0.12 mL of 3M-052-AF and inject it into an empty sterile vial (mixing vial).

4 Withdraw 0.2 mL of Alum and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

5 Withdraw 0.58 mL of TBS and inject into the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of BG505 SOSIP.664 gp140:

6 Withdraw 0.1 mL of BG505 SOSIP.664 gp140 and add it to the mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.

Preparation of the Admixed Vaccine:

7 Withdraw 0.5 mL of the mixed preparation for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.

8 The admixed product is stable for 4 hours at room temperature.

8.3.8 Trimer 4571, 100 mcg admixed with 3M-052-AF, 5 mcg and Alum, 500 mcg (Group 8)

For all preparation steps, use a 1-mL Luer-Lok syringe with 0.01-mL graduations and an 18-25 gauge Luer-Lok needle.

Preparation of Adjuvants:

1. Remove one vial of Alum from the refrigerator and equilibrate at ambient temperature (15°C to 27°C) for a minimum of 15 minutes.

2. Remove one vial of 3M-052-AF from the refrigerator and equilibrate at ambient temperature (15°C to 27°C) for a minimum of 15 minutes.

3. Mix the vial of 3M-052-AF gently by inverting 5 times. Withdraw 0.2 mL of 3M-052-AF and inject it into an empty sterile vial (mixing vial).

4. Mix the vial of Alum by gently inverting 5 times.

5. Withdraw 0.2 mL of Alum and inject into the mixing vial.

6. Mix and wait for 30 minutes. During this time, proceed to Step 7.

Preparation of Trimer 4571:

7. During the incubation period of the mixing vial, thaw one vial of Trimer 4571 at ambient temperature (15ºC to 27ºC) for a minimum of 30 minutes. Vial should NOT be moved directly from the freezer to the refrigerator to thaw.

8. Swirl the thawed, equilibrated Trimer 4571 vial for about 30 seconds with sufficient force to mix the solution, yet avoid foaming. Do NOT shake the vial. If some white to translucent particles are observed, the vial may still be used.

9. Withdraw 0.4 mL of Trimer 4571 and add it to the mixing vial. The final volume in the mixing vial will be 0.8 mL.

Preparation of the Admixed Vaccine:

10. Invert mixing vial gently 5 times to mix. Withdraw 0.4 mL of the mixed preparation for study participant administration. Immediately prior to administration, gently invert the syringe 10 times to mix.

11. The admixed product may be stored for up to 8 hours at 2°C to 8°C and/or up to 4 hours at ambient temperature (15°C to 27°C), including dose administration time.

8.3.9 Placebo (Tris-NaCl buffer) (Placebo 1-8)

1. Remove one vial of TBS from the refrigerator.

2. For Groups 1-7, use a 1 mL Luer-Lok syringe and a 22-27 gauge needle to withdraw 0.5 mL of TBS for study participant administration. For Group 8, use a 1 mL Luer-Lok syringe and an 18-25 gauge needle to withdraw 0.4 mL of TBS for study participant administration. To maintain study blind, wait 30 minutes prior to study product delivery to clinic staff.

3. For Groups 1-7, the placebo is stable for 4 hours at room temperature. For Group 8, the placebo is stable for up to 8 hours at 2°C to 8°C and/or up to 4 hours at ambient temperature (15°C to 27°C), including dose administration time.

8.3.10 Labeling of Study Product

Label the study product as follows and apply an overlay/tape to each syringe to ensure blinding is maintained:

- Participant identifier(s)
- Study Product Name in blinded manner, for example:
    - Protein (BG505 SOSIP.664 gp140 or Trimer 4571) + Adjuvant(s) or Placebo
- Final volume (mL)
- Route (IM)
- Beyond use date and time
- Any additional information required by jurisdiction

8.4 Administration

All injections are to be given IM in the deltoid, using standard IM injection technique.

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.

Any administrator of study product will be blinded to the individual participant's treatment assignment.

8.5 Acquisition of study products

BG505 SOSIP.664 gp140 and Tris-NaCl buffer were manufactured by Ajinomoto Althea, Inc (San Diego, CA, USA) and will be provided by the International AIDS Vaccine Initiative (IAVI, New York, NY, USA).

Trimer 4571 was manufactured by Leidos Biomed (Vaccine Research Center, Frederick, MD, USA) and will be provided by DAIDS, NIAID, NIH (Rockville, MD, USA).

Alum was manufactured by Leidos Biomed (Vaccine Research Center, Frederick, MD, USA) and will be provided by DAIDS, NIAID, NIH (Rockville, MD, USA).

3M-052-AF (labeled as AP 60-702) is manufactured and provided by Access to Advanced Health Institute (AAHI, Seattle, WA, USA).

GLA-LSQ (labeled as AP 10-602) was manufactured by the Access to Advanced Health Institute (AAHI) (AAHI, Seattle, WA, USA) and will be provided by DAIDS, NIAID, NIH (Rockville, MD, USA)

CpG 1018 (labeled as CpG 1018 Drug Product) was manufactured by Aji Bio-Pharma (Ajinomoto Althea, Inc., San Diego, CA, USA) and will be provided by DAIDS, NIAID, NIH (Rockville, MD, USA).

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 outlined 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

For US clinical research sites, all unused study products must be returned to the CRPMC after the study is completed or terminated unless otherwise instructed by the study sponsor. For non-US clinical research sites, all unused study products must be destroyed after the study is completed or terminated unless otherwise instructed by the study sponsor. The procedures are included in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

9 Clinical procedures

The schedules of clinical procedures are shown in Appendix Q, Appendix R Appendix S, and Appendix T.

9.1 Informed consent

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

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

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

Note: As defined in the DAIDS Protocol Registration Manual, an RE is "Any entity/body that has the power to regulate which includes authorities that review submitted clinical data and those that conduct inspections. These are sometimes referred to as competent authorities. These are entities/bodies whose approval/authorization/acknowledgment of a clinical trial is required for conducting a clinical trial. Any organization whose approval is required prior to a CRS's participation in DAIDS funded and/or Sponsored Clinical Trial. Includes but not limited to approvals from state/national health systems and administrative bodies, drug agencies etc." CRSs are responsible for knowing the requirements of their applicable REs.

9.1.1 Screening consent form

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

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

9.1.2 Protocol-specific consent forms

The protocol-specific consent forms describe the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. Sample protocol-specific consent forms for Parts A, B, and C of the main study are located in Appendix A, Appendix B, and Appendix C. A separate sample consent form for other uses of specimens is located in Appendix G. An addendum consent form for participation in the Part A Optional Second Boost is located in Appendix D (Sample addendum to informed consent form for Part A with Optional Third Vaccination participants only).

Each HVTN CRS is responsible for developing a protocol-specific consent form(s) for local use, based on the sample protocol-specific consent forms in Appendix A, Appendix B, Appendix C, Appendix D and Appendix G. The consent form(s) must be developed in accordance with requirements of the following:

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

Study sites are strongly encouraged to have their local CABs review their 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 forms include instructions for developing specific content.

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 whether the volunteer is at low risk for HIV infection (see Appendix U)
- 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; skin (and pelvic and/or rectal exam for volunteers agreeing to provide mucosal samples)
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products,

alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots

- Laboratory tests, including:
  - Screening HIV test
  - HBsAg
  - Anti-HCV Abs
  - CBC with differential and platelets
  - Chemistry panel (ALT, creatinine)
  - Urine dipstick
  - Urine or serum pregnancy test (volunteers who were assigned female sex at birth); Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.
- Administration of behavioral risk assessment (BRA) questionnaire
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html)
- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.7
- Discussion of pregnancy prevention. A pregnant or breastfeeding person may not be enrolled in this trial. Specific criteria and assessment of contraception and pregnancy status are described in study inclusion criteria. Discussion of pregnancy prevention includes advising a participant who was assigned female sex at birth and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.
- Assessment of volunteer eligibility for optional procedures

9.2.1 Use of screening results from another HVTN study

If a participant screens for an HVTN study at the same HVTN CRS but then does not join that study, screening results from that effort may be applied to the screening for this protocol, as long as the screening was done under participant

consent, the participant has signed a consent form to begin screening for this study, and the tests were conducted within the time periods specified in the eligibility criteria (see Sections 7.1 and 7.2).

9.3 Enrollment and vaccination visits

Once a volunteer has consented to trial participation and is found to meet all eligibility criteria (see Sections 7.1 and 7.2), the HVTN CRS requests the randomization assignment via a Web-based randomization system. Enrollment is simultaneous with first vaccination. In general, the time interval between randomization and enrollment should not exceed 4 working days. However, circumstances may require a participant's enrollment visit to be changed. This may exceed the 4-day randomization time limit.

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

- Abbreviated physical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of baseline reactogenicity parameters;
- Assessment of concomitant medications (as described in Section 9.2);
- Assessment of any new or unresolved AEs/intercurrent illnesses; and
- Urine or serum pregnancy test (for participants who were assigned female sex at birth). Persons who are NOT of reproductive potential due to having undergone hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. For pregnant participants, see Section 9.13.

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

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

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

- Risk reduction counseling (as described in Section 9.7);

- Contraception status assessment (as described in Section 9.2 and 9.8). During follow-up in persons who are confirmed pregnant, contraception status assessment is not required; 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 and clinical laboratory tests will be performed **at vaccination visits** as specified in Appendix J, Appendix K, Appendix L, Appendix M, Appendix N, Appendix O, Appendix P, Appendix Q, Appendix R, Appendix S, and Appendix T:

- Specimen collection (including optional rectal and/or vaginal secretion and/or biopsy samples)

- Pelvic and/or rectal exam (as appropriate) for volunteers agreeing to provide optional mucosal samples

- Gonorrhea and chlamydia (for participants providing any biopsy samples)

- Trichomonas vaginalis (for participants providing cervicovaginal secretion and/or vaginal biopsy, only when clinically indicated)

- Bacterial vaginosis (for participants providing cervicovaginal secretion and/or vaginal biopsy samples, only when clinically indicated)

- Yeast (for participants providing cervicovaginal secretion and/or vaginal biopsy samples, only when clinically indicated)

9.4 Follow-up visits

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

- Risk reduction counseling (as described in Section 9.7);

- Contraception status assessment (as described in Section 9.2 and 9.8). During follow-up in persons who are confirmed pregnant, contraception status assessment is not required; 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);
- Assessment of new or unresolved AEs/intercurrent illnesses; and
- Specimen collection.

Additional procedures will be performed **at scheduled follow-up visits** as specified in Appendix Q, Appendix R, Appendix S and Appendix T:

- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;
- HIV infection assessment including pretest counseling and HIV testing. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- 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;
- Pelvic and/or rectal exam (as appropriate) for volunteers agreeing to provide optional mucosal samples

Clinical laboratory tests will be performed **at scheduled follow-up visits** as specified in, Appendix J, Appendix K, Appendix L, Appendix M, Appendix N, Appendix O, and Appendix P, including:

- CBC with differential
- Chemistry panel (see Section 9.2)
- Urine dipstick (urinalysis if appropriate; see Section 9.9)
- Gonorrhea and chlamydia (for participants providing biopsy samples)

- Trichomonas vaginalis (for participants providing cervicovaginal secretion and/or vaginal biopsy samples, only when clinically indicated)
- Bacterial vaginosis (for participants providing cervicovaginal secretions and/or vaginal biopsy samples, only when clinically indicated)
- Yeast (for participants providing cervicovaginal secretions and/or vaginal biopsy samples, only when clinically indicated)
- Urine or serum pregnancy test (for participants who were assigned female sex at birth). Persons who are NOT of reproductive potential due to having undergone hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. During follow-up in persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

Additional optional procedures may be performed **at scheduled follow-up visits** as specified in Appendix K, Appendix L, Appendix M, Appendix N, and Appendix O. These procedures may not be performed at all sites. At sites where the procedures are performed, eligible participants have the option of participating in none, any, or all available procedures.

- Collection of rectal and/or cervicovaginal secretion and/or biopsy samples
- Leukapheresis
- Lymph node (LN) fine needle aspiration (FNA)
- Bone marrow aspiration

9.5 Optional procedures

Mucosal secretion and biopsy samples, leukapheresis samples, lymph node fine needle aspirates, and bone marrow aspirates will be collected at timepoints indicated in Appendix K, Appendix M, Appendix N, and Appendix O from study participants who agree (see Appendix B) and who are eligible for these procedures. Participants assigned female sex at birth may provide cervicovaginal secretions and/or vaginal biopsies.

Rectal and vaginal samples

Small (~2-4 mm) tissue biopsies are obtained from the vagina using a speculum and from the rectum by anoscopy by experienced clinicians. At the timepoints specified in Appendix K, Appendix L, Appendix M, Appendix N, and Appendix O, the following are collected: 5 biopsies per mucosal compartment (rectal and vaginal), one cervicovaginal secretion sample, and one rectal secretion sample;

however, fewer samples may be taken based on the judgment of the clinician performing the procedure.

Eligibility for rectal and vaginal sampling:

- Participants who can become pregnant must have a negative pregnancy test on the same day prior to any rectal mucosal sampling (secretions or biopsies) or vaginal biopsy sampling. For cervicovaginal secretion sampling, a pregnancy test must be done on the same day but can be performed prior to or after sampling.

- Cervicovaginal secretion or vaginal biopsy sampling will not be performed (or may be deferred to a later date within the visit window) if a participant has an active ulcerative genital lesion, is known to have an active genital tract infection (GTI) at the scheduled timepoint, or has any condition noted during pelvic exam via speculum or in medical history that in the opinion of the clinician represents a contraindication to mucosal sampling (eg, bacterial vaginosis).

- Participants will be instructed to refrain from vaginal sex and to avoid using anything in or around their vagina, including tampons, spermicide, lubricants, or medications (eg, topical yeast treatments) for 2 days prior to cervicovaginal secretion or vaginal biopsy sampling. Cervicovaginal sampling may be deferred to a later date within the visit window if a participant is known to have not followed these instructions.

- Cervicovaginal secretion or vaginal biopsy sampling will be deferred to a later date if a participant is menstruating.

- Rectal secretion or biopsy sampling will not be performed (or may be deferred to a later date within the visit window) if a participant has an anorectal condition, such as an active infection or inflammation of the colorectal area (eg, an HSV-2 outbreak or inflamed hemorrhoids or colitis/diarrhea), internal hemorrhoids, or any other condition noted during screening rectal exam via anoscope or in medical history that in the opinion of the clinician represents a contraindication to rectal secretion or biopsy sampling.

- Participants will be instructed to refrain from receptive anal intercourse and to avoid inserting anything into their anus (including cleaning products, lubricant, enemas, or douches (including water) for 2 days prior to rectal secretion or biopsy sampling. Rectal sampling may be deferred to a later date within the visit window if a participant is known to have not followed these instructions.

Additional considerations for biopsies:

- Participants must not have taken antithrombotic medications (except acetylsalicylic acid [ASA] and non-steroidal anti-inflammatory drug [NSAIDs]) for 5 days prior to the procedure. If a participant is taking these medications for medical reasons, biopsies should not be collected and these medications should not be interrupted.

- Participant should not currently be taking anticoagulants. For participants with recent anticoagulant use, a CRS clinician must consult with the HVTN 137 PSRT for approval prior to collecting any tissue biopsies.

- Participants should not have receptive anal sex and/or insert any foreign object or substance into the rectum for **5 days** after rectal biopsy samples have been collected.

- Participants should not have receptive vaginal sex and/or insert any foreign object or substance into the vagina for **7 days** after vaginal biopsy samples have been collected.

Leukapheresis:

Collection of PBMC via leukapheresis will be performed in accordance with the standard practices of the participating apheresis center. Typically, in this procedure, approximately 12 liters of blood will be processed over about 3 to 4 hours using peripheral veins for venous access. The blood will be anti-coagulated in accordance with standard practice of the apheresis center.

Post-procedural safety assessments will be performed in accordance with the standard practices of the participating apheresis center. Additionally, the participant will be advised to contact the study site if they experience any adverse events following the procedure.

Eligibility for leukapheresis:

- Prior to leukapheresis, participant must meet all apheresis center requirements for this procedure.

- Participants who can become pregnant must have a negative pregnancy test within 48 hours prior to the procedure.

Bone marrow aspiration:

Collection of bone marrow will be performed in accordance with the standard practices of the participating provider and/or facility. Using a sterile needle and sterile technique, up to 20 cc of marrow will be aspirated following a single puncture of the skin and periosteum at the posterior iliac crest. The entire

aspiration procedure will take approximately 15 minutes, with the entire visit taking approximately 45 minutes.

Post-procedural safety assessments will be performed in accordance with the standard practices of the participating provider and/or facility. The CRS will contact the participant approximately 7 days after the procedure for a post-procedure safety check (see HVTN 137 SSP). The participant will be advised to contact the study site if they experience pain or numbness for greater than 48 hours following the procedure or fever or other evidence of infection (inflammation and/or pus) at the aspiration site. In general, a participant who reports any post-procedure reaction greater than mild is seen by a clinician within 48 hours, unless the reaction is improving and/or has completely resolved.

Eligibility for bone marrow aspiration:

- Participant may not be currently taking warfarin, oral antithrombin equivalents (including, but not limited to, apixiban, rivaroxiban, dabigatran), enoxaparin injections, or other medications that would increase the risk of bleeding as assessed by the clinician performing the procedure.

- No evidence of localized infection directly superior to aspiration site

- No history of allergy to local anesthesia (eg, Novocaine, Lidocaine)

- No other contraindication to procedure as assessed by the clinician performing the procedure

- Participants who can become pregnant must have a negative pregnancy test within 48 hours prior to the procedure.

Lymph node FNA:

Tissue sampling of an axillary lymph node will be carried out percutaneously by fine needle aspiration (FNA). This procedure will be performed in accordance with the standard practices of the participating provider and/or facility. The procedure involves tissue retrieval with a needle via a small skin incision under sonographic guidance. Approximately 2-4 passes will be made to retrieve cytologic material.

Post-procedural safety assessments will be performed in accordance with the standard practices of the participating provider and/or facility. The participant will be advised to contact the study site if they experience severe pain, fever or other evidence of infection (inflammation and/or pus) at the aspiration site, or arm numbness or weakness.

Eligibility for lymph node FNA:

- Participant may not be currently taking warfarin, oral antithrombin equivalents (including, but not limited to, apixiban, rivaroxiban, dabigatran), enoxaparin injections, or other medications that would increase the risk of bleeding as assessed by the clinician performing the procedure.
- No evidence of localized infection directly superior to aspiration site
- No history of allergy to local anesthesia (eg, Novocaine, Lidocaine)
- No other contraindication to procedure as assessed by the clinician performing the procedure
- Participants who can become pregnant must have a negative pregnancy test within 48 hours prior to the procedure.

9.6 AESI contact for Part A

CRS staff will contact Part A study participants 14 months after enrollment to collect the information listed below; Part A Optional Second Boost participants will provide this information at a visit 12 months after the third vaccination (see Appendix J). Clinic visits will only be required if HIV confirmatory testing is necessary (see Section 9.7.1); however, a clinic visit may be arranged for other reasons (eg, AESI assessment and referral).

- Confirmation of vital status; if deceased, attempt to learn cause and date of death;
- If participant is alive, record the following events:
  - New AEs related to study product(s);
  - Medically attended adverse events (MAAE), defined as any adverse events leading to an unscheduled visit to a healthcare professional (see HVTN 137 SSP);
  - SAEs;
  - AEs of special interest (AESI, see Section 11.2.2). A sample list of AESI is provided in Appendix V. AESI are reported regardless of relationship to study product(s);
  - New diagnosis of HIV infection; and
  - Pregnancies and outcomes, including congenital anomalies/birth defects.

All such events will be recorded.

9.6.1 Interim contacts

CRSs may report safety information obtained at a contact other than the AESI contact. These contacts are reported as interim visits.

9.7 HIV counseling and testing

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

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

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

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

9.7.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

The study product may elicit an antibody 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 Q, Appendix R, and Appendix T. 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 J, Appendix K, Appendix L, Appendix M, Appendix N, Appendix O, and Appendix P). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see *HVTN 137 Study Specific Procedures* (SSP)), which is able to distinguish vaccine-induced antibody responses from actual HIV infections.

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

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

9.7.2 VISP registry

Experimental HIV vaccines may induce antibody production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP) (see Section 9.7.2). In order to provide poststudy HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of poststudy testing and counseling services to HIV vaccine recipients. The registry contains the names of all study participants, unless they request that their names be removed.

9.8 Contraception status

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

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

9.9 Urine testing

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

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

Follow-up visit dipstick testing should be deferred if a participant is experiencing non-urinary bleeding (eg, menstruation), but should be performed as soon as possible. If a follow-up visit dipstick is abnormal due to a participant's non-urinary bleeding (eg, menstruation), document in the comment section of the case report form (CRF) and repeat the dipstick once the participant is no longer experiencing non-urinary bleeding. A micro-urinalysis is not required. If a follow-up visit dipstick or micro-urinalysis is abnormal due to infection, provide appropriate treatment and/or referral and document this in the participant's source documentation. See the Urine Testing MOP for further details.

9.10 Assessments of reactogenicity

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

for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, except as noted in Section 11.2.2.

The reactogenicity assessment period is 7 full days for Part A and Part B and 14 full days for Part C following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record symptoms using a Participant Diary. Contacts between the participant and the site staff should take place at least once between 1-3 days postvaccination. In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 (Parts A & B) or day 14 (Part C) to resolution.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, and vaccine-related lesions. Events not listed on a CRF, or with an onset after the reactogenicity assessment period (day of vaccination and 7 full days after for Part A and Part B and 14 full days after for Part C), or those meeting SAE/adverse events requiring expedited reporting according to DAIDS criteria, are recorded on an adverse event log form.

Table 9-1 Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Baseline: before vaccination	HVTN CRS clinician
	Early: 25-60 minutes after vaccination	HVTN CRS clinician
	Between early assessment and 11:59pm day 0	HVTN CRS clinician or participant
1-7[b]	Between 12:00am and 11:59pm on the respective day	HVTN CRS clinician or participant
1-14[c]	Between 12:00am and 11:59pm on the respective day	HVTN CRS clinician or participant

[a] Day of vaccination
[b] New or unresolved reactogenicity symptoms present on day 7 are followed until resolution for Parts A and B
[c] New or unresolved reactogenicity symptoms present on day 14 are followed until resolution for Part C

9.10.1 Assessment of systemic and local symptoms

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

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

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

Participants with evidence of moderate to severe reactogenicity may be asked to come to the clinic for additional assessment and/or blood sampling.

9.10.2 Assessment of injection site

Typical injection site reactions are erythema/redness and induration/swelling. The maximum diameter for all injection site reactions is recorded.

All injection site reactions are monitored until resolution. Reactions with diameters greater than 5 cm are followed daily; otherwise, the frequency of follow-up is based on clinician judgment. See HVTN 137 SSP for detail.

9.11 Visit windows and missed visits

Visit windows are included in Appendix W. The procedures for documenting missed visits and out of window visits are described in the HVTN 137 SSP. 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. Except for 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. Detail will be provided in the HVTN 137 SSP.

If a missed visit required vaccination, please refer to Section 7.4.2 and Section 7.4.3 for resolution.

9.12 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, and chemistry panel), pregnancy testing (note: for persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated), social impact assessment, and HIV test. For participants who have a confirmed diagnosis of HIV infection, see Section 9.14.

9.13 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given. During pregnancy, no more STI testing, mucosal collections or additional optional collections will be conducted. Remaining visits and study procedures should be completed unless medically contraindicated or applicable regulations require termination from the study. During follow-up in persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome. Pregnancies and pregnancy outcomes will be reported as described in the HVTN 137 SSP section on Pregnancy Management and Reporting. If the participant is no longer pregnant, refer to Section 7.4.3.

If the participant is no longer pregnant (as defined by two consecutive negative tests) or breast-feeding and study product administration and mucosal and additional optional collections can be performed within an appropriate visit window, these may resume with unanimous consent of the HVTN 137 PSRT.

9.14 HIV infection during the study

If a participant becomes HIV-infected during the course of the study, no additional study product will be administered. Participants will be encouraged to continue scheduled study visits for up to 12 weeks following their last study product administration. Follow-up duration for participants diagnosed with HIV infection may be adjusted in consultation with the CRS investigator and the HVTN 137 PSRT (eg, to avoid interference with participant initiation of HIV treatment). At postinfection follow-up visits, only specimens required for protocol-specified safety laboratory tests, urinalysis, and pregnancy tests will be collected (note: for persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated); in addition, some clinic procedures may be modified or discontinued (see Appendix Q, Appendix R, and Appendix T).

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN 137 Site Lab Instructions and SSP provide further guidelines for operational issues concerning the clinical and processing laboratories. These documents include guidelines for general specimen collection, special considerations for phlebotomy, specimen labeling, whole blood processing, HIV screening/diagnostic testing, and general screening and safety testing.

Tube types for blood collection are specified in Appendix J, Appendix K, Appendix L, Appendix M, Appendix N, Appendix O, and Appendix P. For tests performed locally, the local lab may assign appropriate tube types.

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

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

10.2 Total blood volume

Required blood volumes per visit are shown in Appendix J, Appendix K, Appendix L, Appendix M, Appendix N, Appendix O, and Appendix P. 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.

The preferred laboratory specimen tube types for research samples are shown in Appendix J, Appendix K, Appendix L, Appendix M, Appendix N, Appendix O, and Appendix P. Alternate tube types may be used under certain circumstances (eg, ACD tube shortages) upon approval of the HVTN Laboratory Center. Refer to the HVTN 137 Specimen Collection SSP for more information.

10.3 Primary immunogenicity timepoint

The primary immunogenicity timepoints in this study occur 2 weeks after the last vaccination for groups receiving 2 vaccinations and 1 and 2 weeks after the second and third vaccinations for the groups receiving 3 vaccinations. For the

group receiving 3 vaccinations, primary timepoints for innate immunity and inflammation assays are baseline, and days 1, 3, and 7 post first vaccination.

Endpoint assays for humoral and cellular responses are performed on specimens collected from participants at the primary immunogenicity timepoint and may be performed on samples collected at baseline. Depending on the initial results, assays for humoral, cellular, innate immunity, and inflammatory responses may be performed on samples collected from participants at other timepoints; the schedules are shown in Appendix J, Appendix K, Appendix L, Appendix M, Appendix N, Appendix O, and Appendix P.

10.4 Endpoint assays: cellular

10.4.1 Intracellular cytokine staining (ICS) assay

Flow cytometry will be used to examine vaccine-specific CD4+ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the HIV-1 envelope protein. ICS parameters will include cytokines such as interferon gamma (IFN-γ), interleukin (IL)-2, and TNF-α, and may include other cytokines (such as cytokines relevant to Th2 and Th17 responses) to identify T cells of specific functionality. Data will be reported as percentages of CD4+ T cells responding to a specific peptide pool. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

10.4.2 CD4+ T follicular helper cells (Tfh/pTfh)

Multicolor flow cytometry may be used to identify, phenotype and isolate circulating CD3+ T cells in the peripheral blood (PBMC) and LN FNAs. Peripheral Tfh may be characterized based on expression of lineage markers including CXCR5, PD-1, and ICOS on CD4+ T cells. Functionality of Tfh cells might be tested by in vitro stimulation of cells with synthetic HIV peptides that span the HIV-1 envelope protein to identify, enumerate and immunophenotype antigen-specific Tfh cells.

10.4.3 Env-specific B cell and plasmablast phenotyping

HIV-1 Env-specific B cells and plasmablasts induced by vaccination will be identified and characterized using fluorescently-labeled recombinant Env proteins (including BG505 SOSIP.664) in combination with a flow cytometry antibody panel. In particular, Env-reactive B cells and plasmablasts will be enumerated and may be further characterized for expression of memory, activation, inhibitory or other markers of interest.

10.4.4 BCR repertoire analysis

Single or bulk populations of memory B cells and plasmablasts may be sorted for BCR sequencing and gene expression analysis. Env-specific B cells may instead

be expanded for detection of and functional testing of secreted antibodies by enzyme-linked immunosorbent assay (ELISA) or microneutralization assays, and BCR sequencing. The resulting VH and VL genes may be cloned into an IgG backbone for antibody expression and characterization of the binding, epitope-specificity, and neutralization.

10.4.5 Epitope Mapping

Epitope mapping will be performed using either the ICS or IFN-γ ELISpot assays. PBMCs will be stimulated with synthetic peptides that span the BG505 SOSIP.664 gp140. Epitope mapping will be performed using BG505 SOSIP.664 gp140 15-mer peptides overlapping by 11 aa. Once positive responders are identified using peptide pools, the specific responses will be mapped to the single 15 amino acid peptides.

10.5 Endpoint assays: humoral

10.5.1 Binding antibody multiplex assay (BAMA)

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

10.5.2 Antibody avidity

Antibody avidity may be measured using BAMA with the addition of a dissociation step to calculate the antibody avidity index (BAMA-AI). Biolayer Interferometry (BLI) and/or Surface Plasmon Resonance (SPR) technologies may also be used to define antibody avidity.

10.5.3 Antibody-dependent cellular cytotoxicity assay (ADCC)

ADCC activity may be assessed using serum samples from study participants taken at the primary immunogenicity timepoints. Specimens from the baseline and other timepoints may also be analyzed at the discretion of the HVTN Laboratory Center, which may be contingent on the results of the primary immunogenicity timepoints. For the Luciferase-based cytotoxicity assay, participant sera are incubated with infectious molecular clone (IMC)-infected cells and percent killing is measured through the use of Viviren luminescence.

10.5.4 Antibody-dependent cellular phagocytosis (ADCP)

To assess the ability of vaccine-elicited antibodies to engage cellular FcR for potential antiviral function, ADCP may be measured using serum samples from

study participants taken at baseline and at a primary immunogenicity timepoint. ADCP is measured by assessing the ability of vaccine elicited antibodies to mediate monocyte phagocytosis of HIV-1 antigen coated fluorescent beads by flow cytometry (99, 100). An array of antigens or viruses in addition to specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Center, which may be contingent on the results of the primary immunogenicity timepoint.

10.5.5 Antibody-dependent neutrophil phagocytosis (ADNP)

To assess the ability of vaccine-elicited antibodies to engage cellular FcR for potential antiviral function, ADNP may be measured using serum samples from study participants at a primary immunogenicity timepoint and compared to placebo controls. ADNP is measured by assessing the ability of vaccine elicited antibodies to mediate neutrophil cell line phagocytosis of HIV-1 antigen coated fluorescent beads by flow cytometry (101). An array of antigens or viruses in addition to specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Center, which may be contingent on the results of the primary immunogenicity timepoint.

10.5.6 Complement assays

The capacity of antibodies to bind complement will be assessed either through a direct binding assay or a complement deposition assay (102, 103). The complement deposition assay is a cell-based method. The binding assay is a bead-based multiplexed method that measures engagement of antigen specific antibody with complement.

10.5.7 Infected cells antibody binding assay (ICABA)

The capacity of vaccine elicited antibodies to recognize epitopes exposed on the surface of infected cells may be assessed using serum samples from study participants taken at baseline and the primary immunogenicity timepoint. Specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Center contingent upon the results of the primary immunogenicity timepoint. This assay measures the capacity of vaccine elicited antibodies to recognize HIV envelope on the surface of infected cells (ie, the first step in mediating antibody Fc effector function) with a readout by flow cytometry.

10.5.8 Fc receptor binding

The ability of vaccine elicited antibodies to bind to cellular Fc receptors enables characterization of the antibody Fc profile that results in antiviral function (ie, includes subclass and glycans). HIV-specific antibody binding to FcγR proteins will be assessed by the FcR BAMA. The FCR BAMA is a modification of the binding assay where fluorescently labeled FcR proteins are utilized as the detection reagent for serum antibodies bound to HIV proteins on microspheres.

The readout may include the magnitude and/or avidity for an array of Fc receptors involved in mediating antiviral activity.

10.5.9 Peptide microarray

Linear epitope specificities of purified serum IgG will be examined by peptide microarray using an Env peptide library, which contains 15-mer peptides that overlap by 12 amino acids and cover consensus Env strains (gp160) and vaccine strains (gp120).

10.5.10 Neutralizing antibody assay

HIV-1–specific nAb assays will be performed on serum samples from study participants taken at the primary immunogenicity timepoint(s). Specimens from the baseline and other timepoints may also be analyzed at the discretion of the HVTN Laboratory Center, which may be contingent on the results of the primary immunogenicity timepoints. The TZM-bl assay will test neutralization of the vaccine strain (BG505.T332N) and a single highly neutralization-sensitive tier 1 virus (MW965.26). The global panel and/or subtype-specific panels may be used to assess heterologous tier 2 neutralization (14, 15).

10.5.11 Polyclonal serum Ab (Fab) binding

A comprehensive probe of polyclonal antibody responses will be performed on sera. Single particle electron microscopy will be conducted on IgGs that have been isolated and digested into Fabs and made into complexes with immunogens. This technique will be applied to reveal the epitopes that are targeted after immunization with stabilized, prefusion conformation HIV-1 envelope glycoprotein BG505 SOSIP gp140 in the presence of different adjuvants. If neutralization breadth is detected, then complexes will also be made with SOSIP trimers with corresponding sequences to map the cross-reactive antibody responses.

10.6 Innate immunity and inflammation assays

10.6.1 Soluble factors in serum or plasma

Multiplex cytokine detection assays (such as meso-scale discovery [MSD]) or ELISA may be used to measure soluble cytokines, chemokines, and other immunomodulatory factors in the serum or plasma. Analytes may include IFN-γ, IL-6, TNF- α, IL-10, IFN-γ inducible protein 10 (IP-10), and/or monocyte chemoattractant protein 1 (MCP-1). Other analytes may also be included.

10.6.2 Enumeration and phenotyping of cell populations

Phenotyping of DCs, monocytes, NK cells, B cells, T cells, or other leukocytes for lineage, maturation, and activation markers may be performed on fresh or

cryopreserved PBMC. Trucount tubes with whole blood will be used when possible for direct enumeration of major leukocyte populations in the blood, including DC, by flow cytometry. Data will be reported as cell concentrations per microliter of blood and/or as percentage of live cells.

10.6.3 Gene expression

Bulk PBMC or whole blood will be cryopreserved in an RNA protection reagent. RNA may be isolated and used for analysis by RNA sequencing and/or real-time PCR. Signatures of gene expression changes will be analyzed over time after vaccination.

10.6.4 Metabolomic profiles

Metabolomics profiling in response to study product may be performed on serum during innate responses. Liquid chromatography–mass spectrometry (LC-MS) analysis will be performed and mass spectral data will be collected using reversed phase chromatography with both positive and negative electrospray ionization. Metabolite confirmation will be performed with tandem MS-MS experiments with additional mass spectrometers. To identify vaccine-derived metabolites, the vaccine will be analyzed with the same metabolomics protocol as for serum.

10.6.5 Proteomics

Proteomics profiling in response to study product may be performed on serum or plasma to identify and quantify peptides in human subjects. The proteomics method uses a modified higher-energy collisional dissociation (HCD) Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method. Quantitation of proteins will be performed using summed peptide intensities given by MaxQuant.

10.7 Mucosal assays

Env-specific antibodies in mucosal samples will be assessed using quantitative immunoassays and/or IHC. For immunoassays using mucosal secretions and/or tissue samples, antibody levels may be normalized relative to total protein and/or total IgG. Hemoglobin measurements may be used to perform quality control of mucosal secretions. For IHC using mucosal tissue samples, the distribution of Env-specific antibodies will be described qualitatively or semi-quantitatively relative to the mucosal epithelium, stroma and/or lamina propria.

10.8 Lab assay algorithm

The Lab Assay Algorithm lists assays to characterize cellular, humoral, and innate immune responses as well as host genetics that may be conducted to determine endpoints in HVTN vaccine trials. The type of assay(s) employed will be

dependent on the response obtained by the primary immunogenicity assays at relevant timepoints. Please note that the Lab Assay Algorithm will be updated periodically to include new assays.

10.9 Exploratory studies

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

10.10 Specimen storage and other use of specimens

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

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

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

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

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

10.11 Biohazard containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all personnel in the drawing of blood and

shipping and handling of all specimens for this study, as currently recommended by the CDC and the NIH or other applicable agencies.

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

11 Safety monitoring and safety review

11.1 Safety monitoring and oversight

11.1.1 HVTN 137 PSRT

The HVTN 137 PSRT is composed of the following members:

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

The clinician members of HVTN 137 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, clinical data manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 137 PSRT meetings.

11.1.2 HVTN SMB

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

The SMB reviews safety data, unblinded as to treatment arm, approximately every 4 months. The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS. The SMB conducts additional special reviews at the request of the HVTN 137 PSRT.

Study sites will receive SMB summary minutes and are responsible for forwarding them to their IRB/EC and any applicable RE.

11.1.3 SDMC roles and responsibilities in safety monitoring

The roles and responsibilities of the SDMC in relation to safety monitoring include:

- Maintaining a central database management system for HVTN clinical data;
- Providing reports of clinical data to appropriate groups such as the HVTN 137 PSRT and HVTN SMB (see Section 11.1.2);

11.1.4 HVTN Core roles and responsibilities in safety monitoring

- Daily monitoring of clinical data for events that meet the safety pause and HVTN 137 PSRT AE review criteria (see Section 11.4);
- Notifying HVTN CRSs and other groups when safety pauses or planned holds are instituted and lifted (see Section 11.4);
- Querying HVTN CRSs for additional information regarding reported clinical data; and
- Providing support to the HVTN 137 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

Site staff must submit all safety forms (eg, reactogenicity, adverse experience, urinalysis, local lab results, and concomitant medications) before the end of the next business day, excluding federal or bank holidays. The forms should not be held in anticipation of additional information at a later date. If additional information is received at a later date, the forms should be updated and resubmitted before the end of the next business day after receiving the new information. For the case of a longer site holiday closure, site staff must submit the data by the end of the 5th day (local time) after receiving the information even if this day is a holiday.

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

11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs are graded according to the Division of AIDS

(DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables, except:

- Unintentional Weight Loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health (see HVTN 137 Study Specific Procedures);

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

- Creatinine is required to be reported as an AE only if it is gradable per the increase from local lab ULN parameter. Do not grade elevated creatinine based on the change from the baseline parameter.

All 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 DAIDS (see Section 11.2.3) and (2) if the AE meets the criteria for a safety pause/prompt AE review (see Section 11.4).

During the main study period (see Section 3), all 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 DAIDS (see Section 11.2.3), (2) if the AE meets the criteria for a safety pause/prompt AE review (see Section 11.4), (3) if the AE is a medically attended adverse event (MAAE), and (4) if the AE is a potential immune-mediated disease that may be listed as an AESI. A sample list of AESI is provided in Appendix V.

After the main study period in Part A, report the subset of AEs bulleted in Section 9.6 until the AESI health contact (see Section 3) is complete.

Sites are expected to notify HVTN clinical safety staff of any serious safety concern requiring their attention (Table 11-1). Telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn137). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

In the case of email notification, clinical safety staff will reply within one business day. Serious events that meet pause rule criteria will be addressed immediately (as outlined in Table 11-1). If email service is not available, the CRS should notify clinical safety staff of the event by telephone, and 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 adverse events to DAIDS

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

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

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

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

The study products for which expedited reporting are required are:

- BG505 SOSIP.664 gp140
- Trimer 4571
- CpG 1018
- 3M-052-AF
- GLA-LSQ
- Alum adjuvant
- Tris-NaCl Diluent
- In addition to the expedited Reporting Category identified above, other AEs that must be reported in an expedited manner are all autoimmune diseases.

Throughout the duration of the study, the SAE Reporting Category will be used.

After a Part A participant has completed the month 14 AESI health contact and is off study, sites must report SUSARs as defined in Version 2.0 of the DAIDS EAE Manual if the study site staff becomes aware of the events on a passive basis (eg, from publicly available information).

For Part B and C participants, after completion of scheduled study visits, only SUSARs must be reported to DAIDS, if the study staff become aware of the events.

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

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

11.3 Safety reviews

11.3.1 Initial safety evaluation and dose escalation

Enrollment in Group 1 across all participating HVTN CRSs will be restricted to a maximum of 1 participant per day until 6 participants have been enrolled. The HVTN 137 PSRT will review cumulative safety data available on all participants in Group 1 up to and including the 2-week visit after the first vaccination to determine whether dose escalation (ie, enrollment in Group 2) may begin. The HVTN 137 PSRT may consult with the HVTN SMB on an ad hoc basis for these evaluations.

Enrollment in Group 2 across all participating HVTN CRSs will be restricted to a maximum of 1 participant per day until 5 participants have been enrolled. The HVTN 137 PSRT will review the cumulative safety data including at minimum local and systemic reactogenicity data reported for the first 72 hours postvaccination on each of these 5 participants and will determine whether it is safe to proceed with full enrollment in that group.

11.3.2 Safety evaluation for moving from Part A to Part B

In addition to monitoring participant safety throughout the study period, the HVTN 137 PSRT will review all cumulative safety data available from groups 1

and 2 up to and including the 2-week visit after the second vaccination. Based on the assessment of these safety data, the HVTN 137 PSRT will make a decision regarding the appropriateness of moving to part B as well as regarding the recommended (maximum safe) dose of 3M-052-AF to be used in part B. The HVTN SMB may perform an additional unblinded review of this safety data to make the final determination based on safety for proceeding to part B.

11.3.3 Safety evaluation for Group 8

Enrollment in Group 8 across all participating HVTN CRSs will be restricted to a maximum of 1 participant per day until 5 participants have been enrolled. Enrollment will then be held. The HVTN 137 PSRT will review cumulative safety data available on all participants in Group 8 up to and including the 2-week visit after the first vaccination to determine whether it is safe to proceed with full enrollment in that group.

11.4 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 137 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 137 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.4.2.

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

Event and relationship to study products/procedures	Severity	HVTN CRS action[a]	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and submit forms immediately	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately	Immediate PSRT notification
SAE, related	Grade 3, 2, or 1	Email and submit forms immediately	Immediate PSRT notification and prompt PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and submit forms immediately	Immediate PSRT notification and prompt PSRT AE review to consider pause

[a] Phone numbers and email addresses are found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn137).

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

For all safety pauses, HVTN Core notifies the HVTN 137 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS

Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and participating HVTN CRSs. When an immediate safety pause is triggered, HVTN Core notifies the SMB.

Once a trial is paused, the HVTN 137 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the SMB if necessary. HVTN Core notifies the participating HVTN CRSs, HVTN Regulatory Affairs, DAIDS PAB, DAIDS RAB, and DAIDS SPT of the decision regarding resumption or discontinuation of study vaccinations. Based on the HVTN 137 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 137 PSRT notification or prompt HVTN 137 PSRT AE review is triggered, HVTN Core notifies the HVTN 137 PSRT as soon as possible during working hours (local time)—or, if the information was received during off hours, by the morning of the next workday. If a prompt HVTN 137 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

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

In addition, all other AEs are reviewed routinely by the HVTN 137 PSRT (see Section 11.5.2).

11.5 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.

11.5.1 Daily review

Blinded daily safety reviews are routinely conducted by HVTN Core for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 137 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the HVTN 137 PSRT reviews clinical safety reports on a weekly basis and conducts calls to review the data as

appropriate. After the injections and the final 2-week safety visits are completed, less frequent reporting and safety reviews may be conducted at the discretion of the HVTN 137 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.

11.5.3 AESI health contact quarterly review

After the main study period, a monitoring team reviews safety reports quarterly during the annual health contacts period. This monitoring team comprises a DAIDS Medical Officer, Core medical monitor, and an HVTN clinical safety staff member.

11.6 Study termination

This study may be terminated early by the determination of the HVTN 137 PSRT, the US FDA, NIH, Office for Human Research Protections (OHRP), or study product 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 (on-site or remote) and compliance;
- Advocacy and assistance to participants regarding negative social impacts associated with the vaccine trial;
- Risk reduction counseling;
- Specimen collection, processing, and analysis;
- Exploratory and ancillary studies and sub-studies, and
- Destruction of specimens.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 137 *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 Emergency communication with study participants

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

When such communication is needed, the CRS will request that its IRB/EC and any applicable RE expedite review of the message. If this review cannot be completed in a timeframe consistent with the urgency of the required communication, the site can contact the participant without IRB/EC approval if such communication is necessary to avoid imminent harm to the study participant. The CRS must notify the IRB/EC and any applicable RE of the matter as soon as possible.

13 Version history

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

The version history of, and modifications to, Protocol HVTN 137 are described below.

Protocol history and modifications

Date: September 26, 2022

Protocol version: Version 5.0
Protocol modification: Full Protocol Amendment 4

Item 1 Revised throughout the protocol: Title to reflect changes in study design

Item 2 Added in Title page; Section 3, *Overview*; Section 4, *Background*; Section 5, *Objectives and endpoints*; Section 6, *Statistical considerations*; Section 8, *Study product preparation and administration*; Section 11, *Safety monitoring and safety review*; a new Appendix C, *Sample informed consent form for Part C*; Appendix F, *Approved birth control methods for persons assigned female sex at birth (for Part B and Part C sample informed consent form)*; Appendix H, *Tables of procedures (for sample informed consent form)*; Appendix P, *Laboratory procedure for Part B (no optional procedures) and Part C*; Appendix T, *Procedures at HVTN CRS for Part B (no lymph node FNA) and Part C*; and Appendix W, *Visit Windows*: Information for new Part C justifying recruitment of additional participants in order to optimize neutralizing antibody titers observed in Part A

Item 3 Updated Section 3.1, *Protocol Team*

Item 4 Updated Section 4.1.5, *Clinical studies*: Title of Table 4-2

Item 5 Updated Section 4.5.3, *Clinical studies of 3M-052*: safety data from HVTN 300 study

Item 6 Added in Section 7.2, Exclusion criteria; and Section 7.4.1, Delaying vaccinations for a participant: considerations for timing of receipt of vaccines for Monkeypox

Item 7 Revised in Section 9.10, *Assessments of reactogenicity*; Appendix T, *Procedures at HVTN CRS for Part B (no lymph node FNA) and Part C*: reactogenicity period increased from 7 to 14 days

Item 8 Added in Section 12, *Protocol conduct*: Stipulation for Protocol monitoring

Item 9 Updated Appendix U, *HVTN low risk guidelines for the US*: Sub-heading and PrEP language

Item 10 Updated in Title page; Section 13, *Version history*; Section 15, *Acronyms and abbreviations*; and Section 16, *Literature cited*: Contents of this amendment

Item 11 Updated throughout the Protocol: Name of the study product provider

Item 12 Corrected throughout the Protocol: Minor errors in grammar, typography, formatting

Item 13 Updated throughout the Protocol: Section numbering and cross-references

Item 14 Updated per Protocol Version 4.0, Letter of Amendment 1, dated May 12, 2021

Item 15 Updated per Protocol Version 4.0, Letter of Amendment 2, dated August 02, 2021

Item 16 Updated per Protocol Version 4.0, Clarification Memo 1, dated August 16, 2022

Date: August 16, 2022

Protocol version: Version 4.0
Protocol modification: Clarification Memo 1

Item 1 Added in Section 10.2, Total blood volume: alternate laboratory specimen tube types allowed for research samples upon HVTN laboratory center approval

Date: August 02, 2021

Protocol version: Version 4.0
Protocol modification: Letter of Amendment 2

Item 1 Revised Section 7.1, *Inclusion criteria*, and Appendix S, *HVTN low risk guidelines for the US*: include persons stably taking PrEP

Date: May 12, 2021

Protocol version: Version 4.0
Protocol modification: Letter of Amendment 1

Item 1 Corrected in Appendix I, *Laboratory procedures for Part A with Optional Second Boost:* Safety labs collection timepoint

Date: March 10, 2021

Protocol version: Version 4.0
Protocol modification: Full Protocol Amendment 3

Item 1 Revised the protocol to expand the scientific rationale for the optional third vaccination in HVTN 137 Part A and inform participants

Item 2 Revised the volume of bone marrow aspirate in Section 9.5, *Optional Procedures* and Appendix *B*, *SICF for Part B*, Item 14

Item 3 Updated Section 3.1, *Protocol Team*: protocol team membership

Item 4 Updated Title page, Table of contents, and Section 13, *Version history*: date, version number and contents of this amendment

Date: February 10, 2021

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

Item 1 Added a third vaccination as an optional second boost to HVTN 137 Part A

Item 2 Corrected minor document errors in Protocol Version 2, Letter of Amendment 4, dated December 21, 2020, Item 3 and Item 6: misplaced Appendix K (renamed to Appendix L in this Full Protocol Amendment), clarified footnotes referring to pregnancy testing, Appendix S (renamed to Appendix U) Visit Windows for HVTN 137 Part B~~A~~

Item 3 Updated per Clarification Memo 1 version 2.0, dated July 16, 2020

Item 4 Updated per Letter of Amendment 4, version 2.0, dated December 21, 2020

Item 5 Updated per Letter of Amendment 3, version 2.0, dated September 16, 2020

Item 6 Updated per Letter of Amendment 2, version 2.0, dated July 16, 2020

Item 7 Updated per Letter of Amendment 1, version 2.0, dated June 11, 2020

Item 8 Updated per Clarification Memo 3 version 2.0, dated March 24, 2020

Item 9 Updated per Clarification Memo 2 version 2.0, dated January 27, 2020

Item 10 Updated per Clarification Memo 1 version 2.0, dated December 12, 2019

Item 11 Corrected minor document errors

Item 12 Updated Title page, Table of contents, and Section 13, *Version history*: date, version number and contents of this amendment

Date: December 21, 2020

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 4

Item 1 Revised in Section 7.1, Inclusion criteria: language referring to pregnancy testing for optional study procedures in inclusion criterion 21

Item 2 Revised in Section 9.5, Optional procedures: clarified timing of negative pregnancy test prior to rectal and vaginal sampling and removed requirement that pregnancy test must be performed on urine samples prior to leukapheresis, bone marrow aspiration, and lymph node FNA as blood samples can also be used

Item 3 Appendices I - L, Laboratory procedures for Part B: consolidated footnotes for pregnancy testing of participants that will provide optional procedure samples and updated table footnotes

Item 4 Removed in Appendix B, SICF for Part B, item 4: manufacturer of the Alum adjuvant

Item 5 Revised in Appendices I and J, Laboratory procedures for Part B: blood collection volumes at Visit 9

Item 6 Updated in Appendix S, Visit Windows: footnote 1 to HVTN 137 Part A Visit Windows table, referring to visit target dates

Item 7 Updated in Section 3.1, Protocol team: Membership

Date: September 16, 2020

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 3

Item 1 Removed in *Section 6.2, Randomization*: stratified randomization and requirement for at least half of the participants in Part B to consent to the fine needle aspiration procedure

Item 2 Removed in Laboratory Procedure Tables for Part B (Appendices I, J, K, and L): Prothrombin time / INR testing

Item 3 Corrected in Appendix B, SICF for Part B, item 15: participant instructions for rectal tissue collection

Item 4 Updated in Section 3.1, *Protocol team*: Membership

Item 5 Corrected a document error in Protocol Version 2, Letter of Amendment 2, dated July 16, 2020: upper allowable visit window for the Final Visit (Visit 14.0) in Part A

Date: July 16, 2020

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 2

Item 1 Updated *Appendix S* Visits Windows to expand the upper allowable window for Visit 7/Vaccination 2 from 120 days to 180 days

Date: June 11, 2020

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

Item 1 Updated with changes described in Protocol Version 2, Clarification Memo 3, dated March 24, 2020

Item 2 Updated in Section 3.1, Protocol team: Membership

Date: March 24, 2020

Protocol version: Version 2.0

Protocol modification: Clarification Memo 3

Item 1 Clarified in Appendix S, Visit windows: Allowable visit windows for Part A

Item 2 Clarified throughout protocol: “Clinic visits”

Date: January 27, 2020

Protocol version: Version 2.0
Protocol modification: Clarification Memo 2

Item 1 Reconciled in Section 7.2, *Exclusion criteria*: Exclusion for autoimmune conditions

Date: December 12, 2019

Protocol version: Version 2.0
Protocol modification: Clarification Memo 1

Item 1 Clarified in Section 6.3, *Blinding*: Treatment assignment but not group assignment blinded throughout trial

Item 2 Clarified in Inclusion criterion 23 in Section 7.1: No alternative pregnancy methods until 6 months after last vaccination

Item 3 Clarified in Appendices H through M: Lab procedures table footnotes regarding optional blood draws and blood draw totals

Item 4 Corrected in Section 13: *Version history*

Date: October 30, 2019

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

Item 1 IND number added to title page

Item 2 Age eligibility clarified

Item 3 SAE and MAAE reporting added throughout protocol duration

Item 4 Safety experience of CpG 1018 revised in Section 4.5.1 and Appendix B

Item 5 Fill volumes clarified in Sections 8.2.1 and 8.2.2

Item 6 Extraneous text removed from Appendix B

Date: August 28, 2019

Protocol version: 1.0
Protocol modification: NA

Original Protocol

14 Document references (other than literature citations)

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

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines:
  - Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf
  - Revised Guidelines for HIV Counseling, Testing, and Referral. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5019a1.htm
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures
- Division of AIDS Protocol Registration Manual. Available at https://www.niaid.nih.gov/sites/default/files/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Corrected Version 2.1, July 2017. Available at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 137 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 137 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN 137 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 https://www.iata.org/publications/dgr/Pages/index.aspx

- Lab assay algorithm (available upon request)

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

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

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

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

- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf

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

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

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

15 Acronyms and abbreviations

A/G	albumin/globulin (ratio)
AAHI	Access to Advanced Health Institute
Ab	antibody
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
ADNP	antibody-dependent neutrophil phagocytosis
AE	adverse event
AESI	adverse event of special interest
AF	aqueous formulation
ALT	alanine aminotransferase
AMI	acute myocardial infarction
APC	antigen presenting cell
APTT	activated partial thromboplastin time
ART	antiretroviral therapy
ASA	Acetylsalicylic acid
AST	aspartate aminotransferase
AUC-MB	area-under-the-curve-magnitude-breadth
β-HCG	beta human chorionic gonadotropin
BAMA	binding antibody multiplex assay
BAMA-AI	BAMA avidity index
BCR	B cell receptor
BLI	biolayer interferometry
BM	bone marrow
BMI	body mass index
bnAb	broadly neutralizing antibody
BRA	behavioral risk assessment
CAB	Community Advisory Board
CAVD	Collaboration for AIDS Vaccine Discovery
CAVIMC	Comprehensive Antibody Vaccine Immune Monitoring Consortium
CBC	complete blood count
CD4bs	CD4 binding site
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practice
CHIL	Cape Town HIV Immunology Laboratory
CI	confidence interval
CMIA	chemiluminescent microparticle immunoassay

COMPASS	Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets
CpG	cytidine phosphoguanosine
CRF	case report form
CRP	C-reactive protein
CRPMC	NIAID Clinical Research Products Management Center
CRS	clinical research site
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS (US NIH)
DC	dendritic cell
DHHS	(US) Department of Health and Human Services
DSMB	(NIAID) Data and Safety Monitoring Board
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EMH	extramedullary hematopoiesis
Env	HIV envelope
Fab	fragment antigen-binding
Fc	fragment crystallizable
FcR	Fc receptor
FDA	(US) Food and Drug Administration
FNA	fine needle aspiration
FPR	false positive rate
Fred Hutch	Fred Hutchinson Cancer Research Center (FHCRC)
FS	functionality score
GCLP	Good Clinical Laboratory Practice
GCP	Good Clinical Practice
GEE	generalized estimating equation
GLA	glucopyranosyl lipid A
GPA	granulomatosis with polyangiitis
GSK	Glaxo Smith Kline
GTI	genital tract infection
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCD	higher-energy collisional dissociation
HCDR3	heavy chain complementarity-determining region 3
HCV	hepatitis C virus
Hg	mercury

HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HVTN	HIV Vaccine Trials Network
IAVI	International AIDS Vaccine Initiative
IB	Investigator's Brochure
ICABA	infected cells antibody binding assay
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
IFN-α	interferon alpha
IFN-γ	interferon gamma
IgA	immunoglobulin subtype A
IgG	immunoglobulin subtype G
IHC	immunohistochemistry
IL	interleukin
IM	intramuscular (injection)
IMC	infectious molecular clone
IND	Investigational New Drug
IoR	Investigator of record
IP-10	IFN-γ inducible protein 10
IRB	Institutional Review Board
IRM	immune response modifier
ISCOM	immune stimulating complex
IUD	intrauterine device
kDa	kilodalton
LC	Laboratory Center
LC-MS	liquid chromatography mass spectrometry
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LN	lymph node
MAAE	medically attended adverse event
mAb	monoclonal antibody
MAR	missing at random
M-B	magnitude-breadth
MCAR	missing completely at random
MCP-1	monocyte chemoattractant protein 1
mDC	myeloid dendritic cell
MDP	muramyl dipeptide
MedDRA	Medical Dictionary for Regulatory Activities

MIMOSA	Mixture Models for Single-cell Assays
mIU/mL	mill-international units per milliliter
MMR	measles, mumps, and rubella
MOP	Manual of Operations
MPER	membrane proximal external region
MPL	monophosphoryl lipid A
MS	mass spectrometry
MS-MS	tandem mass spectrometry
MSD	meso-scale discovery
MTD	maximum tolerated dose
MTP-PE	muramyl tripeptide phosphatidylethanolamine
nAb	neutralizing antibody
NAEPP	National Asthma Education and Prevention Program
NFL	native flexibly-linked
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NIH	(US) National Institutes of Health
NK	natural killer
NOD	nucleotide-binding oligomerization domain
NSAID	non-steroidal anti-inflammatory drug
ODN	oligodeoxynucleotide
OHRP	US Office for Human Research Protections
OPV	oral polio vaccine
PAB	(DAIDS) Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PCA	principal component analysis
PCR	polymerase chain reaction
pDC	plasmacytoid dendritic cell
PFS	polyfunctionality score
PI	Principal Investigator
PLGA	Poly(lactic-co-glycolic acid)
PSRT	Protocol Safety Review Team
pTfh	peripheral T follicular helper cells
PTID	participant ID
QS-21	*Quillaja saponaria* fraction 21
RAB	(DAIDS) Regulatory Affairs Branch
RBC	red blood cells
RE	regulatory entity
RSC	(DAIDS) Regulatory Support Center

SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SE	stable emulsion
SHIV	simian-human immunodeficiency virus
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPR	surface plasmon resonance
SPT	(DAIDS) Safety and Pharmacovigilance Team
SSP	Study Specific Procedures
STI	sexually transmitted infection
SVA-MLV	simian virus amphotropic murine leukemia virus
TB	tuberculosis
TBS	Tris-NaCl buffer
TCR	T cell receptor
Tfh	T follicular helper
TLR	toll-like receptor
TNF-α	tumor necrosis factor alpha
UCA	unmutated common ancestor
UFO	uncleaved prefusion-optimized
USP	United States Pharmacopeia
UW-VSL	University of Washington Virology Specialty Laboratory
VISC	Vaccine Immunology Statistical Center
VISP	vaccine induced seropositivity
VLP	virus-like particle
VRC	Vaccine Research Center (NIAID)
WHC	white blood cells
WFI	water for injection

16 Literature cited

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Appendix A Sample informed consent form for Part A

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV uninfected adults

HVTN protocol number: HVTN 137

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.

Key information

These are some of the things you should know about this study:

The purpose of the study is to test an experimental HIV vaccine. The study vaccine is made from two parts: a man-made piece of protein that looks like a protein found on the outside of HIV and an adjuvant. An adjuvant is a substance that helps the immune system respond better. The study will see if the protein and two different amounts of the same adjuvant are safe and if people can get them without being too uncomfortable. The study will also test how people's immune systems respond to the vaccine made with two different amounts of the same adjuvant. This is the first study to give this vaccine to people.

If you join the study, you will have about 5 clinic visits over about 8 months. Clinic visits will involve physical exams, collection of blood and urine for routine laboratory tests (including pregnancy tests if you can get pregnant), HIV testing at some visits, and collection of blood samples to look at your immune responses to the vaccine. Some visits will involve questionnaires, some of which ask very personal questions. You will get vaccine injections at two visits. After your clinic visits are over, you will get a phone call to check on your health about 6 months later.

Some of the risks of getting vaccines are fever, chills, rash, aches and pains, nausea, headache, dizziness, feeling tired, and pain, redness, swelling, or itching where you get the injection. On rare occasions, a vaccine can cause an allergic reaction. These can even be life threatening. Very rarely, a vaccine causes a person to have an autoimmune disease or makes an autoimmune disease worse.

Because this vaccine has not been given to people yet, we do not know what all of the risks may be. We think the risks will be similar to these general risks. There may be other side effects that we don't yet know, even serious ones.

We do not expect the study vaccine to benefit you in any way. You do not have to join, and you are free to leave at any time.

The rest of this form provides a more complete description of the study. Please read it carefully.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test an HIV vaccine. HIV is the virus that causes AIDS. The study vaccine is made from two parts: a protein and an adjuvant. Adjuvants are substances that help the immune system respond better. (Your immune system protects you from disease.) In this study we will test one protein with different adjuvants.

About 105 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.

This study is divided into two parts, part A and part B. About 17 people will be in part A. In part A, we will test 2 different doses (amounts) of the same adjuvant. The study vaccine we give to the first people to join will contain a low dose of the adjuvant. We will only give the study vaccine which contains the higher dose of the adjuvant after we know that there were no serious health problems in people who got the lower dose.

After we see the results from part A, we will decide what dose of the adjuvant to include in the study vaccine in part B. In part B, 88 more people will join. In part B, the study vaccine will include either the adjuvant tested in part A or 1 of 3 other adjuvants.

You are being invited to join part A of the study.

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

   - Is the study vaccine safe to give to people?
   - Are people able to take the study vaccine without becoming too uncomfortable?
   - How do people's immune systems respond to the study vaccine?
   - Does the study vaccine have different effects at different doses?

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

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

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

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

Several studies have tested whether HIV vaccines can reduce the risk of getting HIV from another person. In some studies, people who got the vaccine seemed to have the *same* risk of getting HIV as people who did not get the vaccine. In one study, people who got the vaccine seemed to have a *lower* risk of getting HIV than people who did not get the vaccine. In studies with a different vaccine, some people who got the vaccine had a *higher* risk of getting HIV than people who did not get the vaccine. This study differs from the studies in which people who got the vaccine had a higher or lower risk of getting HIV. We can tell you about the differences. We do not know whether the vaccine 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. **This study vaccine is experimental.**

   The study vaccine in Part A of this study is BG505 SOSIP.664 gp140 + 3M-052-AF. It includes two parts. BG505 SOSIP.664 gp140 is a man-made piece of protein that looks like a protein found on the outside of HIV. From here on we will call it "the protein." The second part of the study vaccine is the adjuvant called 3M-052-AF + Alum. (An adjuvant is something that helps the immune system respond better.) From here on, we will call it "the adjuvant." We will use the term "the study vaccine" to refer to the combination of the protein and the adjuvant. The study vaccine is an experimental HIV vaccine. That means we do not know if it will be safe to use in people, or if it will work to prevent HIV infection. This study vaccine is used only in research studies.

   The protein (BG505 SOSIP.664 gp140):

   The protein was designed to look like a piece of protein on HIV. This piece of protein has been engineered to stay in a shape much like that in HIV. In this way it is different from proteins in some other HIV study vaccines. The protein was developed by John Moore at Cornell Weill Medical Center in New York, New

York, USA. It is being provided for this study by the International AIDS Vaccine Initiative (IAVI) in New York, New York, USA.

The adjuvant (3M-052-AF + Alum):

3M-052 is one of many similar products developed by 3M to treat skin conditions, tumors, and to make vaccines more effective. These products are designed to stimulate parts of the immune system that recognize viral and bacterial invaders. In this study, 3M-052 is dissolved in water and is mixed with Alum. Alum has been used in many commercial vaccines for more than 90 years.

3M-052 was developed by 3M Corporation in St. Paul, Minnesota, USA. In this study, the 3M-052 is dissolved in water and this mixture is being provided by the Access to Advanced Health Institute (AAHI) in Seattle, Washington, USA. The Alum was manufactured by Brenntag Biosector in Frederikssund, Denmark.It is being provided by the Division of AIDS (DAIDS) at the US National Institutes of Health in Rockville, Maryland, USA.

The 3M-052 adjuvant has been given to 52 participants with advanced cancer in one experimental study. The cancer study differed from this vaccine study in important ways. The cancer study participants were very sick. The 3M-052 was mixed with sesame oil. It was injected directly into tumors at higher doses and for a different purpose. In the cancer study, one participant died four days after getting his second injection into a liver tumor. The exact cause of death could not be determined but was felt to be related to the 3M-052 adjuvant and the injection procedure. Two other participants in the cancer study developed a condition called cytokine release syndrome, which can be severe and require hospitalization. Both participants recovered with medical care. We don’t expect to see these outcomes due to the many differences described above. We can discuss this more with you if you would like.

Risks of the protein and adjuvant:

The protein has been tested for safety and for immune responses in rats, rabbits, monkeys, and cows. It is currently being tested with a different adjuvant in a study with people in Seattle, USA. The study is planned to include Boston, USA and Kenya. As of August 9, 2019, 6 people have joined this study. Reactions to the vaccine have so far been mild to moderate. There have not been any serious health problems.

This combination of protein and adjuvant has been tested for safety and/or immune response in mice, rats, guinea pigs, rabbits, and monkeys. The adjuvant has also been tested for safety in animal studies of other vaccines, such as an experimental vaccine against influenza. In these studies, animals that got these products had symptoms consistent with the general risks of other vaccines (as described below). This combination of protein and adjuvant has not been tested in humans before. Even if something looks like it is safe or works in animals, it may not be true for people.

General risks of vaccines:

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

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

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.

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 get a study product. Being in more than one study may not be safe.

   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 want 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 and hepatitis C. We will ask you about medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy.

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

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

7. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here for free.**

   For the care that we cannot give, we will explain how we will help you get care elsewhere. For health problems that are unrelated to the study, we will not pay for care.

8. **If you were assigned female sex at birth and can become pregnant, you must agree to use birth control to join this study.**

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

You should not become pregnant during the study because we do not know how the study vaccine could affect the developing baby. You must agree to use effective birth control from 21 days (3 weeks) before your first injection until 6 months after your last study injection. We will talk to you about effective birth control methods. They are listed on a handout that we will give you.

Being in the study

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

9. **You will come to the clinic for scheduled visits about [#] times over [Insert period of time].**

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

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

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

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

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

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

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

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

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

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

11. **We will give you either the study vaccine or a placebo.**

    Not everyone in this study will get the study vaccine. Some people will get a placebo, a substance that does not contain vaccine. In this study, the placebo is a combination of salt water and a preservative (to keep the solution from becoming too acidic).

    You have a 15-in-17 chance of getting the study vaccine. Whether you get the study vaccine or the placebo is completely random, like flipping a coin.

    We have no say in whether you get the study vaccine or the placebo. We will not know which one you are getting, and neither will you. Only the pharmacist at this clinic will have this information while the study is going on.

    You will have to wait until everyone in Part B completes their final study visits to find out whether you got the study vaccine or the placebo. 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.

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

    You will be in one of 2 groups. Group 1 will get a low dose of the study vaccine. If there are no serious health problems with Group 1, then Group 2 will get a higher dose (with more adjuvant in it). You will get 2 injections during the study into the muscle of your upper arm.

Group	Number of participants	Injection schedule	
		At enrollment	At 2 months
1	5	protein + low dose of adjuvant	protein + low dose of adjuvant
	1	Placebo	Placebo
2	10	protein + higher dose of adjuvant	protein + higher dose of adjuvant
	1	Placebo	Placebo

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 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. *Site: Customize the next sentence based on how you collect reactogenicity information.* You will bring this information back to the clinic at your next visit. Within 3 days of each injection, we will also need to be in contact with you to ask how you are doing. Contact the clinic staff if you have any issues or concerns after getting an injection. If you have a problem, we will continue to check on you until it goes away.

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

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

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 20 mL and 200 mL (a little more than 1 tablespoon to a little less than 1 cup). Your body will make new blood to replace the blood we take out.

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

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

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

14. **We will counsel you about protecting yourself from HIV.**

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

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

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

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

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

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

    These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

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

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

16. **When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.**

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

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

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

What information is shared with HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with the HVTN, other researchers, or with anyone else who does not need to know your name. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, sex, health information from the study, and HIV status. We

may share information about the study product you received and how your body responded to the study product.

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

Researchers may also do genetic testing on your samples.

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

If you agree, your samples could also be used for genome-wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

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

People who may see your information are:

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

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

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

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

Your study records and samples will be kept in a secure location. We will label all of your samples and most of your records with a code number, not your name or

other personal information. However, it is possible to identify you, if necessary. We will not share your name with the lab that does the tests on your samples, or with anyone else who does not need to know your name.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs. You can remove the box around the text.

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

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

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

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

- The US National Institutes of Health and its study monitors,
- The US Food and Drug Administration,
- Any regulatory agency that reviews clinical trials,
- [Insert name of local IRB/EC] ,
- DAIDS, IAVI, AAHI and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board (SMB) and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. If you are found to have a medical condition that we are required to report by law, then some of your information may be shared. 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.) If your site does not have public health or legal reporting requirements, you may delete the last sentence in the paragraph above, along with the bullets below.

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

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

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

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

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

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

18. **There are several reasons why we may stop your injections. We may stop them even if you want to stay in the study and even if you were scheduled for more injections.**

    We will stop your injections if you become pregnant. We will encourage you to stay in the study if you choose. We will discuss your study options with you. If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery.

    We will stop your injections if you get HIV. We will also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study for up to 12 weeks after your last injection if you choose. We will discuss your

study options with you. We will counsel you about having HIV and about telling your partner(s). *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

We will stop your injections if you enroll in another study where you receive a study product.

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

19. **We may take you out of the study at any time.**

We may take you out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you, or
- the study is stopped for any reason.

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, a sore, bleeding, and (rarely) muscle damage or infection where you got the injection. Taking blood can cause a low blood cell count (anemia), making you feel tired.

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 have 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 vaccine may cause you to test positive on some types of HIV antibody tests, even if you

do not have HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccine, a routine HIV test done outside this clinic may 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 vaccine.

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

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

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

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

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

Embarrassment/anxiety:

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

Risks of disclosure of your personal information:

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

Risks of genetic testing:

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

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

Unknown risks:

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

We do not know if getting this study vaccine will affect how you respond to any future approved HIV vaccine. Currently, no HIV vaccine has been approved for use.

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

Benefits

21. **The study may not benefit you.**

We do not expect the study vaccine to 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 vaccine later becomes 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

Sites: Approval from HVTN Regulatory Affairs (at vtn.core.reg@hvtn.org) is needed for any change (other than those that the instructions specifically request or those previously approved by HVTN Regulatory Affairs) to the boxed text. You can remove the box around the text.

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

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

If you become sick or injured in this study, the HVTN has a process to decide if it is related to the study products and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

Some of the study product providers may pay medical costs for study-related injuries that are determined to be caused by their own study products. If provider funds are not available or are not enough, or if the injury is determined to be

caused by study procedures, the HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale- appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Health contact

25. **After your clinic visits end, we will contact you to check on your health.**

We will contact you by phone, email, or text message *[Site: Modify mode of contact as appropriate; consult IRB/EC if necessary]* about 12 months after your last injection to ask some questions about your health. If you prefer to answer these questions in person, you can come to the clinic to do this.

If we have any concerns about your health, we may need to have more contact with you. You are welcome to contact us at any time if you have concerns about your health related to being in the study.

If we ask you to come to the clinic, we will give you
[Site: Insert compensation amount] for each visit. This amount is to cover the costs of [Site: Insert text].

If someone outside this study clinic told you that you have HIV, we will ask you to come back to the clinic for another HIV test. We will draw about 15 mL (1 tablespoon) of blood. We may ask you to come back more than once for this testing.

Because we will want to contact you, please tell us if your contact information changes, if you are moving away, or if you do not want us to contact you anymore.

You can tell us at any time that you don't want any more contacts. If you do so, you will not lose any benefits or rights you would normally have.

All other information that is discussed earlier in this consent also applies to this contact.

Questions

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

    If you have questions about this study, contact
    [name 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/EC] , at the committee.

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

Your permissions and signature

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

27. **In Section 16 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used. You can change your mind after signing this form.**

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

OR

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

OR

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

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

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

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

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

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

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

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

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

Appendix B Sample informed consent form for Part B

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV uninfected adults

HVTN protocol number: HVTN 137

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.

Key information

These are some of the things you should know about this study:

The purpose of the study is to test 4 experimental HIV vaccines. The study vaccines are made from two parts: a man-made piece of protein that looks like a protein found on the outside of HIV and an adjuvant. Adjuvants are substances that help the immune system respond better. The study vaccines all have the same protein but different adjuvants. The study will see if the study vaccines are safe for people and how people's immune systems respond to them. This is the first study to give these study vaccines to people.

If you join the study, you will have about 15 clinic visits over about a year and a half. Clinic visits will involve physical exams, collection of blood and urine for routine laboratory tests (including pregnancy tests if you are capable of getting pregnant), HIV testing at some visits, and collection of blood samples to look at your immune responses to the study vaccines. Some visits will involve questionnaires, some of which ask very personal questions. You will get injections at three visits.

Some of the risks of getting vaccines are fever, chills, rash, aches and pains, nausea, headache, dizziness, feeling tired, and pain, redness, swelling, or itching where you get the injection. On rare occasions, a vaccine can cause an allergic reaction. These can even be life threatening. Very rarely, a vaccine causes a person to have an autoimmune disease or makes an autoimmune disease worse. Because this protein and these adjuvants have not been given to many people yet, we do not know what all of the risks may be. We think the risks will be similar to these general risks. There may be other side effects that we don't yet know, even serious ones.

We do not expect the study vaccine to benefit you in any way. You do not have to join, and you are free to leave at any time.

The rest of this form provides a more complete description of the study. Please read it carefully.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test HIV vaccines. HIV is the virus that causes AIDS. The study vaccines are made from two parts: a protein and an adjuvant. Adjuvants are substances that help the immune system respond better. (Your immune system protects you from disease.) In this study we will test one protein with different adjuvants.

About 105 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.

This study is divided into two parts, part A and part B. About 17 people will be in part A. In part A, we tested 2 different doses (amounts) of 1 adjuvant. We may be recruiting people for Part B before we know the results from Part A. After we see the results from part A, we will decide whether or not to do part B of the study. If we decide to do part B, 88 more people will join. The results from part A will also help us decide on the dose of that 1 adjuvant to use in part B. In part B, we will test 4 study vaccines. In each of them, the protein will be the same but the adjuvant will be different.

1. **You are being invited to join part B of the study. We are doing part B of 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?

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

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

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

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

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

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

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

4. **These study vaccines are experimental.**

We are testing 4 study vaccines. The study vaccines are made up of two parts, the protein and the adjuvant. (An adjuvant is something that helps the immune system respond better.) The protein is BG505 SOSIP.664 gp140. This is a man-made piece of protein that looks like a protein found on the outside of HIV. From here on we will call it "the protein." The protein will be included in all 4 study vaccines. The second part of the study vaccine, the adjuvant, will be different in each study vaccine. The adjuvants are Alum, CpG 1018 + Alum, 3M-052-AF + Alum, and GLA-LSQ.

We are calling each of these combinations of protein and adjuvant "a study vaccine." These are experimental HIV vaccines. That means we do not know if the vaccines will be safe to use in people, or if they will work to prevent HIV infection. These vaccines are used only in research studies.

The protein (BG505 SOSIP.664 gp140):

The protein was designed to look like a piece of protein on HIV. This piece of protein has been engineered to stay in a shape much like that in HIV. In this way it is different from proteins in some other HIV study vaccines. The protein was developed by John Moore at Cornell Weill Medical Center in New York, New York, USA. It is being provided for this study by the International AIDS Vaccine Initiative (IAVI) in New York, New York, USA.

The adjuvants:

Alum:

Alum alerts the immune system and attracts immune cells to the injection site. It is being provided for this study by the Division of AIDS (DAIDS) at the US National Institutes of Health in Rockville, Maryland, USA.

CpG 1018 + Alum:

The CpG 1018 adjuvant was developed by Dynavax Corporation in Berkeley, California, USA and is being provided for this study by DAIDS. It includes short man-made bits of DNA that stimulate a part of the immune system that recognizes invading viruses and bacteria. This adjuvant has been tested in clinical trials of vaccines to prevent flu, anthrax, and malaria. CpG 1018 is used in an FDA-approved vaccine against hepatitis B. Before it was approved by the FDA, that vaccine was tested in more than 9500 people. Overall the side effects were similar to the side effects seen with other vaccines. In one study, people who got the vaccine with CpG 1018 had a heart attack more often than people who got a different vaccine against hepatitis B. The heart attacks were rare. After careful review by experts, no relationship between heart attack and the vaccine with CpG 1018 was found. In this study, CpG 1018 is mixed with Alum. The FDA-approved vaccine does not contain Alum.

3M-052-AF + Alum:

The 3M-052 is one of many similar products developed by 3M to treat skin conditions, tumors, and to make vaccines more effective. These products are designed to stimulate parts of the immune system that recognize viral and bacterial invaders. In this study, 3M-052 is dissolved in water and is mixed with Alum.

3M-052 was developed by 3M Corporation in St. Paul, Minnesota, USA. In this study, the 3M-052 is dissolved in water and this mixture is being provided by the Access to Advanced Health Institute (AAHI) in Seattle, Washington, USA.

The 3M-052 adjuvant has been given to 52 participants with advanced cancer in one experimental study. The cancer study differed from this vaccine study in important ways. The cancer study participants were very sick. The 3M-052 was mixed with sesame oil. It was injected directly into tumors at higher doses and for a different purpose. In the cancer study, one participant died four days after getting his second injection into a liver tumor. The exact cause of death could not be determined but was felt to be related to the 3M-052 adjuvant and the injection procedure. Two other participants in the cancer study developed a condition called cytokine release syndrome, which can be severe and require hospitalization. Both participants recovered with medical care. We don’t expect to see these outcomes due to the many differences described above. We can discuss this more with you if you would like.

HVTN 137 Part A tested 2 different doses (amounts) of the 3M-052-AF + Alum adjuvant. As there were no serious health problems in Part A participants, the decision was made to use the higher dose of adjuvant in the study vaccine in Part B of the study.

Two participants in Part A did have reactions to their study injections that we want to tell you about. Both had redness and swelling over a large area on their arm where they got the injection that started about 1 week after a study injection. The redness lasted for about 2 to 3 days. For one of these people, the swelling went away within 2 to 3 days. The second person had significant swelling for about 3 days. The swelling went down but it took about 5 weeks to completely go away. They had mild pain which did not prevent them from going to work. Because the study is ongoing and is blinded, we do not know if these people got the study vaccine or placebo.

GLA-LSQ:

The GLA-LSQ adjuvant was developed by AAHI and is being provided for this study by DAIDS. It simulates parts of the immune system that recognize the cell walls of invading bacteria. GLA-LSQ has been tested in clinical trials of vaccines against TB, malaria, and some tropical diseases

Risks of the protein and adjuvants:

For more than 90 years, Alum has been used as an adjuvant in commercial vaccines. Hundreds of millions of people have gotten vaccines containing Alum.

The protein has been tested for safety and immune response in rats, rabbits, monkeys, and cows. It is currently being tested with a different adjuvant in people in Seattle, USA. The study is planned to include Boston, USA and Kenya. As of August 9, 2019, 6 people have joined this study. Reactions to the vaccine have so far been mild to moderate. There have not been any serious health problems.

All the study vaccines have been tested for safety in rats. Some of them have been tested for safety in rabbits and monkeys. All of the study vaccines have been tested for immune response in rats, guinea pigs, rabbits, and monkeys. In these studies, animals that got the study vaccines had symptoms consistent with the general risks of other vaccines (as described below). The study vaccines have not been tested in humans before. Even if something looks like it is safe or works in animals, it may not be true for people.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Vaccines can also cause pain, redness, swelling, or itching where you got the injection. Most people can still do their planned

activities after getting a vaccine. Rarely, people have side effects that limit their normal activities or make them go to the doctor.

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

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.

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 get a study product. Being in more than one study may not be safe.

   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 want 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 and hepatitis C. We will ask you about

medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy.

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

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

7. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here for free.**

   For the care that we cannot give, we will explain how we will help you get care elsewhere. For health problems that are unrelated to the study, we will not pay for care.

8. **If you were assigned female sex at birth and can become pregnant, you must agree to use birth control to join this study.**

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

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 21 days (3 weeks) before your first injection until 6 months after your last study injection. We will talk to you about effective birth control methods. They are listed on a handout that we will give you.

Being in the study

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

9. **You will come to the clinic for scheduled visits about [#] times over [Insert period of time].**

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

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

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

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

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

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

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

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

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

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

11. **We will give you either the study vaccines or a placebo.**

    Not everyone in this study will get the study vaccines. Some people will get a placebo, a substance that does not contain vaccine. In this study, the placebo is a combination of salt water and a preservative (to keep the solution from becoming too acidic). We will compare the results from people who got the different study vaccines. We will also compare results from people who got the placebo with results from people who got the study vaccines.

    You have a 10-in-11 chance of getting the study vaccines. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture*. Whether you get one of the study vaccines or the placebo is completely random, like flipping a coin.

    We have no say in whether you get the study vaccines or the placebo. We will not know which one you are getting, and neither will you. Only the pharmacist at this clinic 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 placebo. 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.

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

    You will be in one of 4 groups. You will get 3 injections into the upper arm during the study.

Group	Number of participants	Injection schedule		
		At enrollment	**At 2 months**	**At 6 months**
3	20	protein + (CpG 1018 + Alum)	protein + (CpG 1018 + Alum)	protein + (CpG 1018 + Alum)
	2	Placebo	Placebo	Placebo
4	20	protein + (3M-052-AF + Alum)	protein + (3M-052-AF + Alum)	protein + (3M-052-AF + Alum)
	2	Placebo	Placebo	Placebo
5	20	protein + GLA-LSQ	protein + GLA-LSQ	protein + GLA-LSQ
	2	Placebo	Placebo	Placebo
6	20	protein + Alum	protein + Alum	protein + Alum
	2	Placebo	Placebo	Placebo

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 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. *Site: Customize the next sentence based on how you collect reactogenicity information.* You will bring this information back to the clinic at your next visit. Within 3 days of each injection, we will also need to be in contact with you to ask how you are doing. Contact the clinic staff if you have any issues or concerns after getting an injection. If you have a problem, we will continue to check on you until it goes away.

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

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

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 17 mL and 325 mL (about 1½ tablespoon to a little

more than ⅔ of a pint). Your body will make new blood to replace the blood we take out.

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

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

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

14. **If you agree, we may collect other samples to test your immune responses.**

Immune responses to vaccines involve different parts of the body. These include the cells in blood, in lymph nodes near the injection site, and in bone marrow. We would like to collect samples from these parts of the body to learn more about how the immune system responds to the study vaccines. Every additional sample provides more information. Not every type of sample will be collected by every clinic participating in the study. Some of the sample collection will be done at other locations. Those institutions may require additional forms of consent.

At the end of this form, we will ask if you agree to each of these sampling procedures. You can agree now and change your mind later. Even if you agree to give these samples, you may not be eligible to do so but you will remain in the study. You can decide not to give any of these samples and still be in the study.

Site: Of the following procedures, include only those available to your participants and edit the information as needed to reflect how they will be done at the site/procedure facility.

Site: Images of the optional procedures in this section have been provided in a separate protocol appendix. You may insert then next to the narrative descriptions below or provide them as a separate document if it is helpful for your study participants. You are not required to do so.

Leukapheresis procedure:

This procedure collects large amounts of white blood cells. Blood is made up of red cells that carry oxygen, white cells that fight infection, platelets that help form clots, and plasma, the fluid left over when all the cells are removed. During leukapheresis, white blood cells are removed and the rest of the blood is put back into the body.

The leukapheresis procedure will be done at *[site: insert location]*. Your eligibility to have this procedure will be assessed by staff at our clinic and at the procedure facility.

For the procedure, a clinician will insert a sterile needle into a vein in each of your arms. The needles are attached to tubes. Your blood will go out of your body through one tube and into a machine that separates the blood and takes out the white blood cells. When the white blood cells are taken out, the rest of the blood will go back into your body through the tube going into your other arm. Sometimes the fluid lost during the procedure is replaced by a sterile salt solution or a solution containing a protein called albumin. This protein is normally found in human blood. An anticoagulant called ACD-A may be added to your blood during the procedure. Anticoagulants prevent blood from clotting.

It is normal to feel tired for up to 24 hours after leukapheresis.

If you agree to have leukapheresis, it will happen about 2 weeks after your second injection. It will take about [site insert timeframe] for the procedure.

We will give you [Site: Insert compensation] for having leukapheresis. This amount is to cover the costs of [Site: Insert text].

Risks of leukapheresis

Generally, the risks of leukapheresis include nausea, vomiting, fainting or dizziness, pain, bruising or swelling at the needle sites, low blood pressure, increased pulse rate, seizures, blood loss, and infection. Rarely, albumin can cause an allergic reaction. If the leukapheresis procedure has to be stopped, it could result in the loss of as much as 1 cup of blood. Your body makes new blood within 2 weeks.

The side effects of ACD-A can include muscle cramping, tingling sensations around the mouth, feeling chilled, numbness, or a feeling that the body is vibrating.

If you notice any symptoms during leukapheresis, please let the nurse know immediately. Usually the symptoms can be reversed quickly by adding fluid or by slowing the procedure. If there are any problems, the staff will use the appropriate medical procedures to treat you.

Lymph node cell collection procedure

This procedure collects cells from lymph nodes. Lymph nodes are one of the key places where immune responses develop. In this procedure, a doctor will use a very thin needle guided by ultrasound to collect cells from a lymph node near the injection site.

The lymph node cell collection procedure will be done at *[site: insert location]*. Your eligibility to have this procedure will be assessed by staff at our clinic and at the procedure facility.

For the procedure, a clinician will locate the appropriate lymph node by applying a cold gel and pressing a hand-held ultrasound device against your skin. If necessary, a small area near the lymph node may be shaved. A cleaning solution will be applied to the skin in that area. The area will be numbed by an injection of a local anesthetic, such as Lidocaine, which is like Novocaine. The doctor will insert a very thin needle and use the images from the ultrasound to guide it into the lymph node. The needle may be moved up and down to collect cells from the lymph node. Fluid may also be collected through the needle into a syringe or bottle. Each cell collection lasts about 10-15 seconds. It usually takes about 4 separate cell collections to get enough cells.

After the procedure is done, the area will be cleaned with warm water. You will be given a band-aid to cover the needle site.

If you agree to have lymph node cell collection, it will happen about 3 weeks after your second injection. It will take about [site insert timeframe] for the procedure.

We will give you [Site: Insert compensation] for having a lymph node cell collection. This amount is to cover the costs of [Site: Insert text].

Risks of lymph node cell collection

The main risks of this procedure include pain and bruising where the needle is inserted and infection at that spot. The cleaning solution, or shaving the area, may cause irritation. Injecting the local anesthetic may sting or burn for a little while until the numbing takes effect. Very rarely, damage to tissues, nerves, or blood vessels may happen. Examining the lymph node area may be uncomfortable or cause embarrassment. There may be bleeding during or after the procedure. If there is an infection, antibiotic treatment may be needed.

If discomfort, problems, or side effects happen during or after lymph node cell collection, the staff will use the appropriate medical procedures to treat you.

Bone marrow cell collection procedure

This procedure collects cells that make antibodies for a long time and cells that remember immune responses to the vaccine. Cell collection involves removing some marrow from your hip bone. The bone marrow cell collection procedure will be done at *[site: insert location]*. Your eligibility to have this procedure will be assessed by staff at our clinic and at the procedure facility.

For the procedure, a clinician will locate the back of your hip bone just below your waist near your spine. A cleaning solution will be applied to the skin in that area. The area will be numbed by an injection of a local anesthetic, such as

Lidocaine, which is like Novocaine. This makes the procedure less uncomfortable. The doctor will insert a single needle into your hip bone to get up to 20 cc (up to ¾ of an ounce or a little less than 1 ½ tablespoons) of bone marrow.

Once the bone marrow has been removed, a sterile dressing will be applied. A nurse will instruct you on how to care for this area. Your hip bone may be sore for a few days. You may feel tired for several days until your body can replace the bone marrow that was taken out.

If you agree to bone marrow cell collection, it will happen about a year after your last injection.

We will give you [Site: Insert compensation] for having bone marrow cell collection. This amount is to cover the costs of [Site: Insert text].

Risks of bone marrow cell collection

Removing bone marrow may cause pain and bruising that may last 1 to 3 days. It may cause tiredness for a few days. Rarely, people have sweating, nausea, lightheadedness, or fainting. Very rarely there are more serious side effects. These include damage to normal blood vessels or bone structures or an infection where the bone marrow was removed. The cleaning solution may cause irritation. Injecting the local anesthetic may sting or burn for a little while until the numbing takes effect. Very rarely, damage to tissues, nerves, or blood vessels may happen. Examining the hip bone area may be uncomfortable or cause embarrassment. There may be bleeding during or after the procedure. If there is an infection, antibiotic treatment may be needed.

If discomfort, problems, or side effects happen during or after bone marrow cell collection, the staff will use appropriate medical procedures to treat you.

15. **If you agree, we may also collect rectal and vaginal fluid and tissue.**

We want to see how the study vaccines affect the parts of the body where people may be exposed to HIV: their rectum and vagina. We would like to collect rectal fluid and tissue from all participants and vaginal fluid and tissue from participants assigned female sex at birth. We will collect these samples at our clinic.

At the end of this form we will ask if you agree to provide these samples. You can agree now and change your mind later. Even if you agree to give these samples, you may not be eligible to do so but you will remain in the study. You can decide not to give any of these samples and still be in the study.

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

If you were assigned female sex at birth, we may test you for trichomonas vaginalis, yeast and/or bacterial vaginosis if clinically indicated when we collect these samples. If you are providing tissue samples we will test you for gonorrhea and chlamydia when we collect them.

We will give you your test results. If you need care, we will tell you about the care we can give you here. We will also tell you about care we can help you get elsewhere.

Site: localize measurement units throughout this section as needed

Rectal fluid collection (for all participants)

We will perform a rectal exam to make sure it is safe to collect rectal fluids. We will collect rectal fluid by first placing a plastic tube about 2 cm wide (a little less than an inch) into your rectum to hold it open. The tube will go in about 6½ cm (about 2½ inches). A small balloon will be placed through the tube and into the rectum. The balloon will stay in for less than a minute. The balloon will be inflated to about half the size of a chicken egg after it is inside your rectum and deflated before it is removed. We will collect rectal fluid at 2 visits.

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

- Do not have receptive anal sex.
- 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 steroid or other anti-inflammatory creams in or around your anus.

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

We will give you [Site: Insert compensation] for rectal fluid collection. This amount is to cover the costs of [Site: Insert text].

Rectal tissue collection (for all participants)

We will perform a rectal exam to make sure it is safe to collect rectal tissue samples. We will collect small samples of tissue about the size of half a grain of rice from the lining of your rectum. These are called rectal biopsies. We will collect up to 5 biopsies at 2 visits. To take the samples, we will place a plastic tube about 2 cm wide (a little less than an inch) into the anus to view the lower part of the rectum. You may feel some discomfort, but the biopsies are almost always painless. It will take 5 to 10 minutes.

You may see blood in your first few stools. This is normal after a biopsy. If you think the bleeding is excessive, contact us immediately.

For the 2 days before we collect your rectal tissue, we will ask you to follow these instructions:

- Do not have receptive anal sex.
- 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 steroid or other anti-inflammatory creams in or around your anus.

For the 5 days after we collect your rectal tissue, we will ask you to follow these instructions:

- Do not have receptive anal sex.
- Do not put anything into your anus, including cleaning products (creams, gels, lotions, pads, etc.), lubricant, enemas or douches (even with water).

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

We will give you [Site: Insert compensation] for rectal tissue collection. This amount is to cover the costs of [Site: Insert text].

Vaginal fluid collection (for participants assigned female sex at birth)

We will perform a pelvic exam to make sure it is safe to collect vaginal fluids. To collect vaginal fluid, a disposable menstrual cup will be inserted into your vagina. You will wear the cup for 1 to 6 hours and remove it at the clinic. The study staff will explain how to insert and remove the cup, or they can do it for you here. We will collect vaginal fluid at 2 visits.

For the 2 days before we collect your vaginal fluid, we will ask you to follow these instructions:

- Do not have vaginal sex or insert anything into your vagina, including tampons.
- Do not douche or use anything in or around your vagina with spermicide, lubricants, or medication (such as topical yeast infection treatments).

We will not collect vaginal fluid if you are menstruating or pregnant or if we think you may have a cervical or vaginal infection. If you are menstruating, we may ask you to return to collect this sample.

We will give you [Site: Insert compensation] for vaginal fluid collection. This amount is to cover the costs of [Site: Insert text].

Vaginal tissue collection (for participants assigned female sex at birth)

We will perform a pelvic exam to make sure it is safe to collect vaginal tissue samples. We will collect small samples of tissue about the size of half a grain of rice from your vagina. These are called vaginal biopsies. We will collect up to 5 biopsies at 2 visits. We will insert a speculum into your vagina. A speculum is a metal or plastic tool that looks like a bird's beak. It is used to help open your vagina a few inches. After the speculum is put into your vagina, the vaginal wall will be cleaned with a clean cotton ball or swab.

Biopsies will be taken with clean forceps. Forceps are a metal tool to help get the tissue from inside your vagina. You may feel cramping, pain, or discomfort. We will check to make sure that there is no bleeding from where the biopsies are taken. If there is bleeding, we will use a medication to stop it. One type of medication, silver nitrate, has a gray color. You may see gray flecks in your vaginal discharge after the biopsy. This is normal. The procedure will take about 10 minutes.

For the 2 days before and the 7 days after we collect your vaginal tissue, we will ask you to follow these instructions:

- Do not have vaginal sex or insert anything into your vagina, including tampons.

- Do not douche or use anything in or around your vagina with spermicide, lubricants, or medication (such as topical yeast infection treatments).

We will not collect vaginal tissue if you are menstruating or pregnant or if we think you may have a cervical or vaginal infection. If you are menstruating, we may ask you to return to collect this sample.

We will give you [Site: Insert compensation] for vaginal tissue collection. This amount is to cover the costs of [Site: Insert text].

Risks of rectal and vaginal fluid and tissue collection

We will ask you to stop some activities before and after we collect these samples. You may find this inconvenient. These sample collections may cause some anxiety, temporary discomfort, and embarrassment. We will try to make you as comfortable as possible.

All biopsies may cause a small amount of bleeding, which usually stops on its own. In rare cases, excess bleeding or infection may occur from a biopsy. If you need care, we will tell you about the care we can give you here. We will also tell you about care we can help you get elsewhere. Until the areas where the biopsies

were taken heal, you may be at increased risk for HIV or other sexually transmitted infection (STI) infection if you are exposed. Most people heal within 5 to 14 days, but some may take longer.

16. **We will counsel you about protecting yourself from HIV.**

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

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

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

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

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

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

    These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

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

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

18. **When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.**

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

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

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

What information is shared with HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with the HVTN, other researchers, or with anyone else who does not need to know your name. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, sex, health information from the study, and HIV status. We

may share information about the study product you received and how your body responded to the study product.

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

Researchers may also do genetic testing on your samples.

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

If you agree, your samples could also be used for genome-wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

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

People who may see your information are:

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

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

19. **We will do our best to protect your private information.**

US sites: Check HIPAA authorization for conflicts with this section

Site: Explain any personal information sharing between your site and any institution(s) to which you will be referring participants for optional sample

collection(s) either here or in the section(s) where the sample collection(s) are described.

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.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs. You can remove the box around the text.

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

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

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

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

- The US National Institutes of Health and its study monitors,
- The US Food and Drug Administration,
- Any regulatory agency that reviews clinical trials,
- [Insert name of local IRB/EC] ,
- IAVI, Dynavax, AAHI, DAIDS, and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board (SMB) and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. If you are found to have a medical condition that we are required to report by law, then some of your information may be shared. 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.) If your site does not have public health or legal reporting requirements, you may delete the last sentence in the paragraph above, along with the bullets below.

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

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

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

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

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

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

20. **There are several reasons why we may stop your injections. We may stop them even if you want to stay in the study and even if you were scheduled for more injections.**

    We will stop your injections if you become pregnant. We will encourage you to stay in the study if you choose. We will discuss your study options with you. If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery.

We will stop your injections if you get HIV. We will also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study for up to 12 weeks after your last injection if you choose. We will discuss your study options with you. We will counsel you about having HIV and about telling your partner(s). *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

We will stop your injections if you enroll in another study where you receive a study product.

In the unlikely event that you get a bad reaction to a study injection we may stop your injections.

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

21. **We may take you out of the study at any time.**

We may take you out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you, or
- the study is stopped for any reason.

Other Risks

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

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

Risks of routine medical procedures:

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

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

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

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

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

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

Embarrassment/anxiety:

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

Risks of disclosure of your personal information:

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

Risks of genetic testing:

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

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

Unknown risks:

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

We do not know if getting these study vaccines will affect how you respond to any future approved HIV vaccine. Currently, no HIV vaccine has been approved for use.

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

Benefits

23. **The study may not benefit you.**

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

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

Your rights and responsibilities

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

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

Leaving the study

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

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

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

Injuries

Sites: Approval from HVTN Regulatory Affairs (at vtn.core.reg@hvtn.org) is needed for any change (other than those that the instructions specifically request or those previously approved by HVTN Regulatory Affairs) to the boxed text. You can remove the box around the text.

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

Your health is important to us. *(Sites: adjust the following 2 sentences if applicable to the care available at your site)* We will tell you about the care that

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

If you become sick or injured in this study, the HVTN has a process to decide if it is related to the study products and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

Some of the study product providers may pay medical costs for study-related injuries that are determined to be caused by their own study products. If provider funds are not available or are not enough, or if the injury is determined to be caused by study procedures, the HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale- appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Questions

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

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

    If you have any symptoms that you think may be related to this study, 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/EC] , at the committee.

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

Your permissions and signature

28. **In Section 14 of this form, we told you about 3 other optional sample collection procedures. Please write your initials or make your mark in the boxes next to the procedure(s) that you agree to have done. You can change your mind after signing this form.**

Site: Include only boxes below for the procedures available to your participants.

☐ I agree to leukapheresis.

☐ I agree to lymph node cell collection.

☐ I agree to bone marrow cell collection.

29. **In section 15 of this form, we told you about 4 other types of optional sample collections. Please write your initials or make your mark in the boxes next to the sample(s) you agree to provide. You can change your mind after signing this form.**

☐ I agree to provide rectal fluid.

☐ I agree to provide rectal tissue.

☐ I agree to provide vaginal fluid.

☐ I agree to provide vaginal tissue.

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

30. **In Section 18 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how**

your samples and information can be used. You can change your mind after signing this form.

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

OR

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

OR

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

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

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

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

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

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

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

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

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

Appendix C Sample informed consent form for Part C

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV uninfected adults

HVTN protocol number: HVTN 137

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.

Key information

These are some of the things you should know about this study:

The purpose of the study is to test 2 experimental HIV vaccines. The study vaccines are made from two parts: a man-made piece of protein that looks like a protein found on the outside of HIV and an adjuvant. An adjuvant is a substance that helps the immune system respond better. The study will see if the study vaccines are safe and if people can get them without being too uncomfortable. The study will also test how people's immune systems respond to the study vaccines. This is the first study to give these study vaccines to people. If you join the study, you will have about 15 clinic visits over about a year and a half. Clinic visits will involve physical exams, collection of blood and urine for routine laboratory tests (including pregnancy tests if you are capable of getting pregnant), HIV testing at some visits, and collection of blood samples to look at your immune responses to the study vaccines. Some visits will involve questionnaires, some of which ask very personal questions. You will get injections at three visits.

Some of the risks of getting vaccines are fever, chills, rash, aches and pains, nausea, headache, dizziness, feeling tired, and pain, redness, swelling, or itching where you get the injection. On rare occasions, a vaccine can cause an allergic reaction. These can even be life threatening. Very rarely, a vaccine causes a person to have an autoimmune disease or makes an autoimmune disease worse. Because these proteins and these doses (amounts) of the adjuvant have not been given to many people yet, we do not know what all of the risks may be. We think the risks will be similar to these general risks. There may be other side effects that we don't yet know, even serious ones.

We do not expect the study vaccine to benefit you in any way. You do not have to join, and you are free to leave at any time.

The rest of this form provides a more complete description of the study. Please read it carefully.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test HIV vaccines. HIV is the virus that causes AIDS. The study vaccines are made from two parts: a protein and an adjuvant. Adjuvants are substances that help the immune system respond better. (Your immune system protects you from disease.)

About 127 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.

This study is divided into three parts: part A, part B, and part C. In part A, we tested 2 different doses (amounts) of the adjuvant with a protein. In part B, we tested the same protein as part A but with different adjuvants. 17 people were enrolled in part A and 88 were enrolled in Part B. We have completed enrollment for parts A and B. In part C of this study, we will test two proteins with 2 different doses of an adjuvant. About 22 people will be enrolled in part C. You are being invited to join part C of the study.

1. **We are doing part C of 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?

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

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

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

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

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

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

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

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

4. **These study vaccines are experimental.**

   The study vaccines are made up of two parts, the protein and the adjuvant. (An adjuvant is something that helps the immune system respond better.) We are testing 2 study vaccines in part C.

   The first study vaccine is BG505 SOSIP.664 gp140 + 3M-052-AF + Alum. It includes two parts. BG505 SOSIP.664 gp140 is a man-made piece of protein that looks like a protein found on the outside of HIV. From here on we will call it "protein 1." The second part of this study vaccine is the adjuvant called 3M-052-AF + Alum. From here on, we will call it "the adjuvant."

   The second study vaccine is Trimer 4571 + 3M-052-AF + Alum. It also includes two parts. Trimer 4571 is a man-made piece of protein that looks like a protein found on the outside of HIV. From here on we will call it "protein 2." The second part of this study vaccine is the same adjuvant used in the first study vaccine but a different dose (amount) of it. This is the first study to give this study vaccine to people.

   These study vaccines are experimental HIV vaccines. That means we do not know if they will be safe to use in people, or if they will work to prevent HIV infection. These study vaccines are used only in research studies.

   Protein 1 (BG505 SOSIP.664 gp140):

   Protein 1 is a first-generation product that was designed to look like a piece of protein on HIV and engineered to stay in a shape much like that in HIV. In this way it is different from proteins in some other HIV study vaccines. The protein was developed by John Moore at Cornell Weill Medical Center in New York, New York, USA. It is being provided for this study by the International AIDS Vaccine Initiative (IAVI) in New York, New York.

Protein 2 (Trimer 4571):

Protein 2 is like Protein 1 product but includes small changes to keep it fixed in one shape. Researchers think that this will lead to better antibodies when compared to proteins that don't have these changes. The protein was developed and is being provided by the Dale and Betty Bumpers Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID).

The adjuvant:

3M-052-AF + Alum:

The 3M-052 is one of many similar products developed by 3M to treat skin conditions, tumors, and to make vaccines more effective. These products are designed to stimulate parts of the immune system that recognize viral and bacterial invaders. In this study, 3M-052 is dissolved in water and is mixed with Alum.

Alum is made from Aluminum Hydroxide. Alum is an adjuvant with a long-standing safety record that has been used in approved vaccines for many years.

3M-052 was developed by 3M Corporation in St. Paul, Minnesota, USA. In this study, the 3M-052 is dissolved in water and this mixture is being provided by the Access to Advanced Health Institute (AAHI) in Seattle, Washington, USA.

The 3M-052 adjuvant has been given to 52 participants with advanced cancer in one experimental study. The cancer study differed from this vaccine study in important ways. The cancer study participants were very sick. The 3M-052 was mixed with sesame oil. It was injected directly into tumors at higher doses and for a different purpose. In the cancer study, one participant died four days after getting his second injection into a liver tumor. The exact cause of death could not be determined but was felt to be related to the 3M-052 adjuvant and the injection procedure. Two other participants in the cancer study developed a condition called cytokine release syndrome, which can be severe and require hospitalization. Both participants recovered with medical care. We don't expect to see these outcomes due to the many differences described above. We can discuss this more with you if you would like.

HVTN 137 Part A tested a high dose (amount) and a low dose of the 3M-052-AF + Alum adjuvant. As there were no serious health problems in Part A participants, the decision was made to use the high dose of adjuvant in the study vaccines in part B of the study. There have been no serious health problems in Part B participants. In Part C, we will be testing the high dose and a medium dose of the adjuvant with two different proteins. We want to see if the medium dose can produce the same response as the high dose.

Risks of the study vaccines:

Protein 1 + the adjuvant (at a higher and lower dose) have been tested in Part A and Part B of this study and there have been no serious health problems. Two participants in Part A who received the higher dose did have reactions to their study injections that we want to tell you about. Both had redness and swelling over a large area on their arm where they got the injection that started about 1 week after a study injection. The redness lasted for about 2 to 3 days. For one of these people, the swelling went away within 2 to 3 days. The second person had significant swelling for about 3 days. The swelling went down but it took about 5 weeks to completely go away. They had mild pain which did not prevent them from going to work. Because the study is ongoing and is blinded, we do not know if these people got the study vaccine or placebo. Redness and swelling are common reactions shortly after an injection but usually start within 1-2 days and not usually a week after the injection. No participants in Part B have had this type of delayed reaction. However, we will ask Part C participants to watch for reactions for a longer period of time than we did in Part A & B.

Protein 2 mixed with another adjuvant was given to people as part of two different ongoing studies. In one study, 16 people got the vaccine mixed with the Alum adjuvant alone. Each person got 3 injections of the vaccine at the same dose or higher than the dose we will use in this study. The injections did not cause any serious health problems.

Most people in that study had at least one of the side effects listed in the *General risks of vaccines* section below. They said the side effects were mild, and they went away within about a week. One person showed a slightly decreased number of one type of white blood cell at 7 days after their second injection. However, their count went back up to normal levels by the 8th day without any health concerns.

In the second study, 3 people got a single dose of this protein vaccine combined with Alum at a higher dose than the one we will use in this study. These injections did not cause any serious health problems. Because this protein vaccine has only been given to a small number of people, we do not know all the side effects. There may be side effects, even serious or life-threatening ones, that we do not know about yet.

Risks of the study adjuvant:

For more than 90 years, Alum has been used as an adjuvant in commercial vaccines. Hundreds of millions of people have gotten vaccines containing Alum.

Another protein vaccine and 3M-052-AF + Alum adjuvant combination is being tested for the first time in people in HVTN 300. Parts A and B of this study (HVTN 137) have also tested 3M-052-AF+ Alum with Protein 1. As of June 2022, in these 2 studies, 48 people have had at least 1 study injection and at least

41 people have had 3 injections containing 3M-052-AF + Alum as an adjuvant. These injections have not caused any serious health problems.

HVTN 300 enrolled 13 people. The most common side effects experienced by participants were headache and feeling unwell or tired.

All people had some side effects that they described as mild to moderate. One (1) person had severe pain/tenderness in both the right and left injection sites 3 days following the fourth vaccination, though it lasted only one day. Five (5) people had severe side effects, including chills, headache, muscle aches, and generally feeling unwell. These severe side effects went away within 2 days. Of the 5 people who had severe side effects, 2 decided to stop getting study injections because they felt generally unwell after injections, and it was affecting their daily lives. Another 2 of the 5 people who had severe side effects got more injections; one of them had more severe side effects while the other did not. And one (1) of the 5 people who had severe side effects has not yet received another injection.

Two more people also decided to stop getting the study injections and dropped out of the study. One stopped because they experienced a panic attack after the first injection. However, this person had a history of panic attacks before being part of this study. The other did not return for their next injection visit and is no longer in the study. We do not know the reason for this.

Given the side effects experienced in HVTN 300 and HVTN 137, we can expect that many people in this study will have some side effects. The side effects we expect would be similar to those listed in the *General risks of vaccines* section below.

These are the side effects we know about. There may be others that we don't know about. We will tell you if we learn about new side effects that could affect your willingness to stay in the study.

General risks of vaccines:

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

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

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.

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 get a study product. Being in more than one study may not be safe.

   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 want 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 and hepatitis C. We will ask you about medications you are taking, including HIV pre-exposure prophylaxis (PrEP). We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy.

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

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

7. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here for free.**

   For the care that we cannot give, we will explain how we will help you get care elsewhere. For health problems that are unrelated to the study, we will not pay for care.

8. **If you were assigned female sex at birth and can become pregnant, you must agree to use birth control to join this study.**

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

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 21 days (3 weeks) before your first injection until 6 months after your last study injection. We will talk to you about effective birth control methods. They are listed on a handout that we will give you.

Being in the study

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

9. **You will come to the clinic for scheduled visits about [#] times over [Insert period of time].**

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

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

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

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

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

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

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

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

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

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

11. **We will give you either the study vaccines or a placebo.**

    Not everyone in part C of this study will get the study vaccines. Some people will get a placebo, a substance that does not contain vaccine. In this study, the placebo is a combination of salt water and a preservative (to keep the solution from becoming too acidic). We will compare the results from people who got the different study vaccines. We will also compare results from people who got the placebo with results from people who got the study vaccines.

    Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.

    You have a 10-in-11 chance of getting the study vaccines. Whether you get one of the study vaccines or the placebo is completely random, like flipping a coin.

    We have no say in whether you get the study vaccines or the placebo. We will not know which one you are getting, and neither will you. Only the pharmacist at this clinic 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 placebo. 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.

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

    You will be in one of 2 groups. Group 7 will get protein 1 with medium dose of adjuvant or the placebo and Group 8 will get protein 2 with high dose of adjuvant or the placebo. You will get 3 injections into the upper arm during the study.

    The protein 2 + adjuvant study vaccine is being given to people for the first time in this study, to participants in Group 8. We will enroll only 1 participant per day in this group for the first 5 participants. If there are no serious health problems with these 5, we will enroll the remaining 5 participants in Group 8.

Group	Number of participants	Injection schedule		
		At enrollment	**At 2 months**	**At 6 months**
7	10	protein 1 + medium dose of adjuvant	protein 1 + medium dose of adjuvant	protein 1 + medium dose of adjuvant
	1	Placebo	Placebo	Placebo
8	10	protein 2 + high dose of adjuvant	protein 2 + high dose of adjuvant	protein 2 + high dose of adjuvant
	1	Placebo	Placebo	Placebo

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 14 more days, you will need to keep track of how you are feeling and if you have any symptoms. *Site: Customize the next sentence based on how you collect reactogenicity information.* You will bring this information back to the clinic at your next visit. Within 3 days of each injection, we will also need to be in contact with you to ask how you are doing. Contact the clinic staff if you have any issues or concerns after getting an injection. If you have a problem, we will continue to check on you until it goes away.

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

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

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 17 mL and 325 mL (about 1½ tablespoon to a little more than ⅔ of a pint). Your body will make new blood to replace the blood we take out.

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

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

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

14. **We will counsel you about protecting yourself from HIV.**

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

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

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

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

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

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

    These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

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

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

16. **When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.**

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

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

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

What information is shared with HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with the HVTN, other researchers, or with anyone else who does not need to know your name. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, sex, health information from the study, and HIV status. We

may share information about the study product you received and how your body responded to the study product.

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

Researchers may also do genetic testing on your samples.

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

If you agree, your samples could also be used for genome-wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

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

People who may see your information are:

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

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

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

US sites: Check HIPAA authorization for conflicts with this section

Site: Explain any personal information sharing between your site and any institution(s) to which you will be referring participants for optional sample

collection(s) either here or in the section(s) where the sample collection(s) are described.

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.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs. You can remove the box around the text.

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

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

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

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

- The US National Institutes of Health and its study monitors,
- The US Food and Drug Administration,
- Any regulatory agency that reviews clinical trials,
- [Insert name of local IRB/EC] ,
- IAVI, Dynavax, AAHI, DAIDS, and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board (SMB) and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. If you are found to have a medical condition that we are required to report by law, then some of your information may be shared. 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.) If your site does not have public health or legal reporting requirements, you may delete the last sentence in the paragraph above, along with the bullets below.

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

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

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

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

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

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

18. **There are several reasons why we may stop your injections. We may stop them even if you want to stay in the study and even if you were scheduled for more injections.**

    We will stop your injections if you become pregnant. We will encourage you to stay in the study if you choose. We will discuss your study options with you. If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery.

We will stop your injections if you get HIV. We will also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study for up to 12 weeks after your last injection if you choose. We will discuss your study options with you. We will counsel you about having HIV and about telling your partner(s). *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

We will stop your injections if you enroll in another study where you receive a study product.

In the unlikely event that you get a bad reaction to a study injection we may stop your injections.

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

19. **We may take you out of the study at any time.**

We may take you out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you, or
- the study is stopped for any reason.

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, a sore, bleeding, and (rarely) muscle damage or infection where you got the injection. Taking blood can cause a low blood cell count (anemia), making you feel tired.

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

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

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

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

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

Embarrassment/anxiety:

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

Risks of disclosure of your personal information:

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

Risks of genetic testing:

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

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

Unknown risks:

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

We do not know if getting these study vaccines will affect how you respond to any future approved HIV vaccine. Currently, no HIV vaccine has been approved for use.

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

Benefits

21. **The study may not benefit you.**

We do not expect the study vaccines to 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

Sites: Approval from HVTN Regulatory Affairs (at vtn.core.reg@hvtn.org) is needed for any change (other than those that the instructions specifically request or those previously approved by HVTN Regulatory Affairs) to the boxed text. You can remove the box around the text.

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

Your health is important to us. *(Sites: adjust the following 2 sentences if applicable to the care available at your site)* We will tell you about the care that

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

If you become sick or injured in this study, the HVTN has a process to decide if it is related to the study products and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

Some of the study product providers may pay medical costs for study-related injuries that are determined to be caused by their own study products. If provider funds are not available or are not enough, or if the injury is determined to be caused by study procedures, the HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale- appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Questions

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 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/EC] , at the committee.

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

Your permissions and signature

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

26. **In Section 18 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used. You can change your mind after signing this form.**

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

OR

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

OR

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

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 entire discussion of this consent form.

Appendix D Sample addendum to informed consent form for Part A with Optional Third Vaccination participants only

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV uninfected adults

HVTN protocol number: HVTN 137

Site: [Insert site name]

1.**Key information**

- Enrollment in Part A is complete with 17 participants and injections have been completed. We are now adding an optional third injection of the same study product already received for all Part A participants. Getting a third study injection is optional.
- Participants who do not agree to have a third injection will not sign this consent form. They will continue on in the study according to the consent form they signed when they joined it.
- Participants who agree to have a third injection will need to sign this consent form. They will have the same study procedures as were described in the original consent form but their schedule of study visits will change. Their study duration will also change. They will be in the study for 12 months after getting the third injection
- Because this is the first study to give this study vaccine to people, the risks of a third injection of study vaccine are unknown at this time. However, we expect the risks will be the same as we told you in the main study informed consent form.

1. Study progress update

In HVTN 137 Part A, we are testing 2 different doses (amounts) of the same adjuvant. Enrollment in Part A is complete with 17 participants and injections have been completed. The study vaccine we gave to the first people to join contained a low dose of the adjuvant. There were no serious health problems in those people, so as planned, we gave people who later joined the study the vaccine with the higher dose of the adjuvant. As there were no serious health problems in those people either, the decision was made to use the higher dose of adjuvant in the study vaccine in Part B of the study, which is now open and enrolling participants.

Two participants in Part A did have reactions to their study injections that we want to tell you about. Both had redness and swelling over a large area on their arm where they got the injection that started about 1 week after a study injection. The redness lasted for about 2 to 3 days. For one of these people, the swelling went away within 2 to 3 days. The second person had significant swelling for about 3 days. The swelling went down but it took about 5 weeks to completely go away. They had mild pain which did not prevent them from going to work. Because the study is ongoing and is blinded, we do not know if these people got the study vaccine or placebo.

2. New optional study plan for Part A participants

The researchers would like to offer all Part A participants the option to receive a third injection of the same dose and combination of study products they already received. This study opened just before the COVID-19 pandemic started and is taking longer than planned because of it. The delay allowed the researchers to take an early look at Part A participants' immune responses to their study injections and they found that some were very good. The researchers would like to see if these responses can be improved with one more injection. The researchers would also like to make up for the delay and quickly learn more about the adjuvant given in Part A.

Getting a third study injection is optional. Participants who agree to it will follow a revised study visit schedule. We are calling the third injection and revised study visit schedule 'the Part A Optional Third Vaccination'. The optional third injection will be about 6 to 12 months after your first injection. If you join the Part A Optional Third Vaccination portion of the study, the study procedures will be the same but your study visit schedule will change. Your study duration will also change. You will be in the study for 12 months after you get the third injection. We will tell you more about this below.

As we told you in the main study informed consent form, we do not know whether the study vaccine 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. If you get HIV, we do not know how the study vaccine might affect your HIV infection or how long it takes to develop AIDS. We do not know if getting this study vaccine will affect how you respond to any future approved HIV vaccine.

Please review this information carefully. You are free to ask questions at any time.

3. You are free to choose whether or not to join the Part A Optional Third Vaccination.

2.If you do not want to join the Part A Optional Third Vaccination, you will not sign this consent form and you will continue on in the study following the consent

form you signed when you joined it. If you decide you want to join the Part A Optional Third Vaccination, we will ask you to sign this consent form. We will give you a copy to keep.

4. **If you join the Part A Optional Third Vaccination, the study procedures will be the same but your study visit schedule and study duration will change.**

   You will be in the study for 12 months after you get the third injection. You will come to the clinic for scheduled visits about 4 times over the 12 months following the third injection.

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

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

5. **Many things described in the informed consent form you signed previously remain the same.**

   These include:

   - The study procedures we will do;
   - The potential risks and benefits of being in the study;
   - Your rights and responsibilities in the study;
   - How your samples will be used;
   - What we will do if we find you have a health problem;
   - How we will protect your private information and who can access your study records;
   - Reasons we might take you out of the study;
   - There may be some restrictions on blood or tissue donation; and
   - What will happen if you get sick or injured during the study.

   As before, there is no cost to you for being in the study. Participants who agree to have a third injection will be given [Site: Insert additional compensation] for each study visit completed for the optional third vaccination.

   In the consent form you signed when you joined this study, you chose whether the HVTN could use your extra samples and information in other studies. The HVTN will continue to honor the choice you made in that consent form. You can change your mind if you want. Your decision will not affect your being in this study or have any negative consequences here.

As stated in the Key Information section, the potential additional risks of a third injection of study vaccine are unknown at this time.

6. **If you have questions or problems at any time during 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/EC], at the committee.

   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].

7. **If you agree to join the Part A Optional Third Vaccination, 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 Part A Optional Third Vaccination is about and what will happen to you if you join it. 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 the Part A Optional Third Vaccination.

   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 E Approved birth control methods for persons assigned female sex at birth (for Part A sample informed consent form)

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

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

You must agree to use effective birth control from 21 days before your first injection until 6 months after your last study injection.

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

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

You do not have to use birth control if:

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

- You are sexually abstinent (no sex at all).

Remember: If you are having sex, male and female condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

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

Appendix F Approved birth control methods for persons assigned female sex at birth (for Part B and Part C sample informed consent form)

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

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 21 days before your first injection until 6 months after your last study injection.

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

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

You do not have to use birth control if:

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

- You are sexually abstinent (no sex at all).

Remember: If you are having sex, male and female condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

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

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

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV uninfected adults

HVTN protocol number: HVTN 137

Site: [Insert site name]

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

Key information

These are some of the things you should know about the use of your samples and information for other studies:

- The extra samples will be labeled with a code number and some personal information. They will not be labeled with your name. The extra samples are stored in a secure place. At your request, the HVTN will destroy all your extra samples. You can still join the main study even if you do not agree to use of your extra samples in other studies.
- Researchers may do genetic testing on your samples, which could include genome wide studies. It is unlikely, but these tests could show you may be at risk for certain diseases. In the very unlikely event that others found out, this could lead to discrimination or other problems.
- You will not be paid or otherwise benefit from allowing your extra samples to be used in other studies.

The rest of this form gives more information about use of your extra samples for other studies. Please read it carefully.

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. Your samples will be stored in the HVTN repository in the United States.

3. How long will the samples be stored?

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

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

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

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

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

6. Will the HVTN sell my samples and information?

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

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

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

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

The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with the HVTN, other researchers, or with anyone else who does not need to know your name. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

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

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

Researchers may also do genetic testing on your samples.

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

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

10. What are the risks of genetic testing?

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

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

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

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- The researcher’s Institutional Review Board or Ethics Committee

- Any regulatory agency that reviews clinical trials
- The people who work with the researcher

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

Questions

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

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

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

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

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

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

OR

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

OR

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

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

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

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

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

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

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

Part A

		Time after first injection visit					
Procedure	Screening visit(s)	First Injection visit	2 weeks	2 months	2½ months	8 months	14 months**
Injection		√		√			
Medical history	√						
Complete physical	√					√	
Brief physical		√	√	√	√		
Urine test	√		√		√		
Blood drawn	√	√	√		√	√	
Pregnancy test (participants assigned female sex at birth)*	√	√		√		√	
HIV testing and pretest counseling	√				√	√	
Risk reduction counseling	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√

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

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

** Phone contact about one year after last injection to check on certain aspects of participant health.

Part A with Optional Third Vaccination

	Time after first injection visit									
Procedure	Screening visit(s)	Injection visit	2 weeks	2 months	2½ months	6 months	6¼ months	6½ months	12 months	18 months
Injection		√		√		√				
Medical history	√									
Complete physical	√									√
Brief physical		√	√	√	√	√	√	√	√	
Urine test	√		√		√			√		
Blood drawn	√	√	√		√	√	√	√	√	√
Pregnancy test (participants assigned female sex at birth)*	√	√		√		√			√	
HIV testing and pretest counseling	√				√	√			√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√

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

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

Part B

		Time after first injection visit													
Procedure	Screening visit(s)	First injection visit	1 day	3 days	1 week	2 weeks	2 months	2¼ months	2½ months	2¾ months	6 months	6¼ months	6½ months	12 months	18 months
Injection		√					√				√				
Medical history	√														
Complete physical	√														√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	
Urine test	√	√**				√							√		
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Rectal and vaginal swabs		√**											√**		
Pregnancy test (participants assigned female sex at birth)*	√	√					√		√**	√**	√		√**	√	√**
HIV testing and pretest counseling	√								√		√			√	√
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Site: Include only rows below for the procedures available to your participants															
Leukapheresis (optional)									√						
Lymph node cell collection (optional)										√					
Bone marrow cell collection (optional)															√
Vaginal and/or rectal fluid collection (optional) ***		√											√		
Vaginal and/or rectal biopsy (optional) ***		√											√		

Greyed out visit is only applicable for participants doing lymph node cell collection.

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

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

** For participants having any of the optional procedures at these visits.

*** Procedure involves a rectal and/or pelvic exam, as appropriate.

Part C

	Time after first injection visit															
Procedure	Screening visit(s)	First injection visit	1 day	3 days	1 week	2 weeks	2 months	2¼ months	2½ months	2¾ months	6 months	6¼ months	6½ months	12 months	18 months
Injection		√					√				√				
Medical history	√														
Complete physical	√														√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	
Urine test	√					√							√		
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pregnancy test (participants assigned female sex at birth)*	√	√					√				√			√	
HIV testing and pretest counseling	√								√		√			√	√
Risk-reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

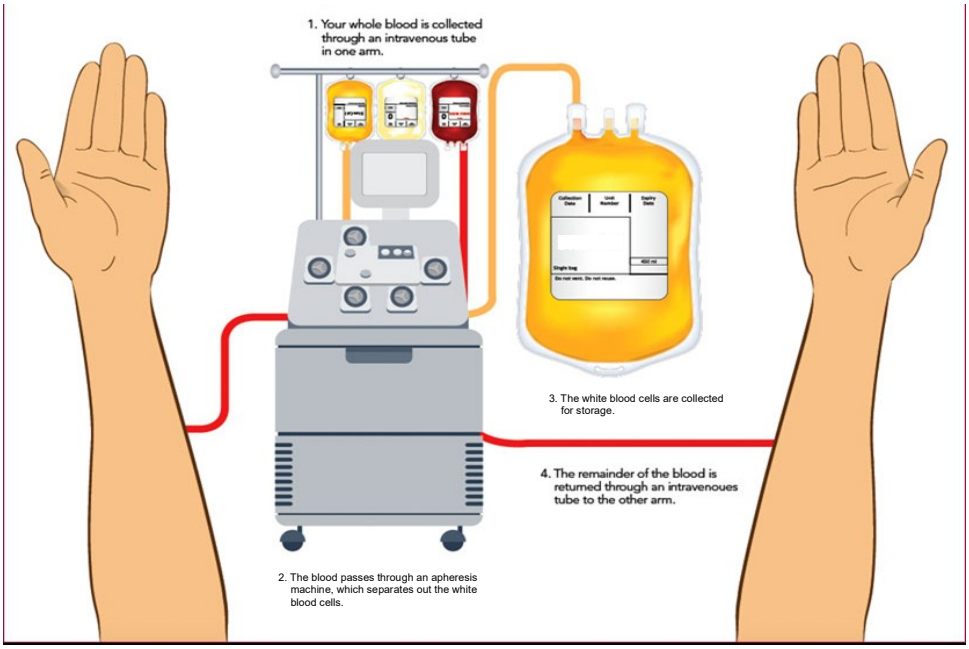
Grayed out visit are not applicable.

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

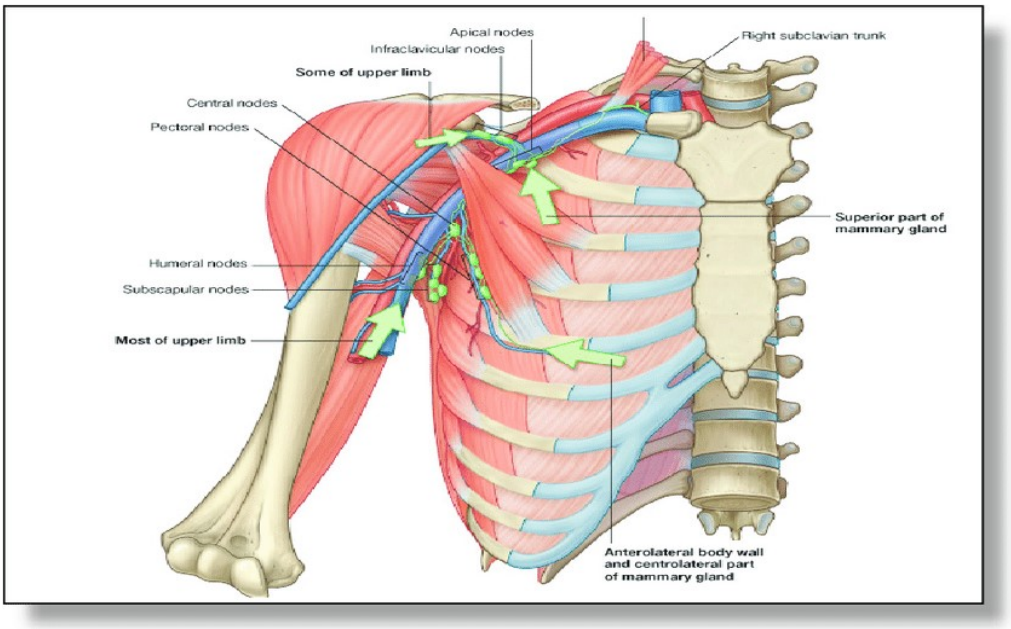
* Persons who had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) are not required to have a pregnancy test.

Appendix I Optional procedure images for Part B Sample informed consent form

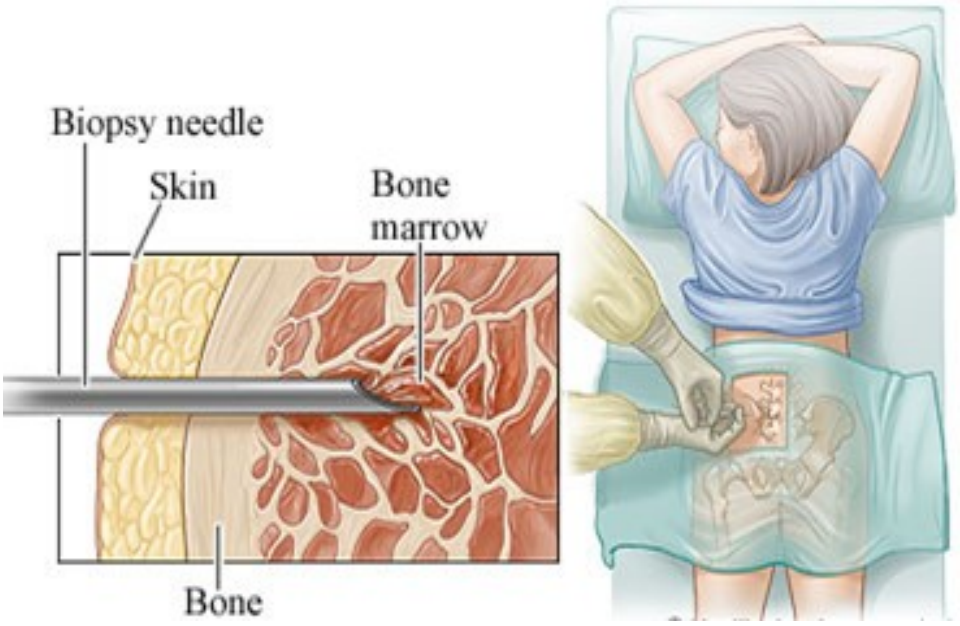
Leukapheresis procedure



Lymph node cell collection



Bone marrow cell collection



Appendix J Laboratory procedures for Part A

Procedure	Ship to[1]	Assay location[2]	Tube type[3]	(vol. capacity)	Visit: 1	2[10]	3	4	5	6	7	8	9	10	11	12	13	14	AESI[9]	Total
					Day: Screening visit[4]	D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D425	
					Week:	W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W61	
					Month:	M0			M0.25	M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M14	
						VAC1					VAC2									
BLOOD COLLECTION																				
Screening/Diagnostic																				
HIV screening test	Local lab	Local lab	SST	5mL	5	—				—	—		—					—	—	**5**
HBsAg/ anti-HCV	Local lab	Local lab	SST	5mL	5	—				—	—		—					—	—	**5**
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL	—	—				—	—		10					20	—	**30**
Safety labs																				
CBC/ Diff/ Platelets	Local lab	Local lab	EDTA	5mL	5	—				5	—		5					5	—	**20**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—				5	—		5					5	—	**20**
Immunogenicity assays																				
Cellular assays																				
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	34				34	—		34					34	—	**136**
ICS	CSR	HVTN Labs	ACD	8.5mL	—	34				34	—		34					34	—	**136**
Humoral assays																				
Neutralizing Ab assay	CSR	HVTN Labs	SST	8.5mL	—	8.5				8.5	—		8.5					8.5	—	**34**
Binding Ab assay	CSR	HVTN Labs	SST	8.5mL	—	8.5				8.5	—		8.5					8.5	—	**34**
STORAGE																				
PBMC	CSR	—	ACD	8.5mL	—	34				34	—		34					34	—	**136**
Serum	CSR	—	SST	8.5mL	—	25.5				25.5	—		25.5					25.5	—	**102**
Visit total					**20**	**145**				**155**	**0**		**165**					**175**	**0**	**658**
56-Day total					**20**	**165**				**319**	**319**		**319**					**175**	**0**	
URINE COLLECTION																				
Urine dipstick[6]	Local lab	Local lab			X					X			X							
Pregnancy test[7]	Local lab	Local lab			X	X					X							X		

Grayed out visits are not applicable to these groups.

[1] CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment procedures) and Section 9.4 (follow-up visits).

[6] And microscopy if needed.

[7] For participants assigned female sex at birth. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing

[8] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 14 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14)

[9] For information concerning the AESI contact, see Section 9.6. Clinic visits are not required except that any participant reporting a diagnosis of HIV infection from testing outside of the HVTN will be asked to come to the clinic to collect specimens for HIV testing with HVTN HIV diagnostic algorithms.

[10] Specimens indicated for Day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination.

Appendix K Laboratory procedures for Part A with Optional Second Boost

Procedure	Ship to[1]	Assay location[2]	Tube type[3]	(vol. capacity)	Visit:	1	2[9]	3	4	5	6	7	8	9	10	11[10]	12	13	14	15	16	Total
					Day:	Screening visit[4]	D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
					Week:		W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
					Month:		M0			M0.25	M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
							VAC1					VAC2				BOOST						
BLOOD COLLECTION																						
Screening/Diagnostic																						
HIV screening test	Local lab	Local lab	SST	5mL		5	—				—	—		—		—	—	—	—	—	—	**5**
HBsAg/ anti-HCV	Local lab	Local lab	SST	5mL		5	—				—	—		—		—	—	—	—	—	—	**5**
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL		—	—				—	—		10		10	—	—	—	10	20	**50**
Safety labs																						
CBC/ Diff/ Platelets	Local lab	Local lab	EDTA	5mL		5	—				5	—		5		—	—	5	—	5	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—				5	—		5		—	—	5	—	5	—	**25**
Immunogenicity assays																						
Cellular assays																						
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	34				34	—		34		—	—	34	—	34	34	**204**
ICS	CSR	HVTN Labs	ACD	8.5mL		—	34				34	—		34		—	—	34	—	34	34	**204**
Humoral assays																						
Neutralizing Ab assay	CSR	HVTN Labs	SST	8.5mL		—	8.5				8.5	—		8.5		8.5	—	8.5	—	8.5	8.5	**60**
Binding Ab assay	CSR	HVTN Labs	SST	8.5mL		—	8.5				8.5	—		8.5		8.5	—	8.5	—	8.5	8.5	**60**
STORAGE																						
PBMC	CSR	—	ACD	8.5mL		—	34				34	—		34		—	68	68	—	34	42.5	**315**
Serum	CSR	—	SST	8.5mL		—	25.5				25.5	—		25.5		—	—	25.5	—	25.5	25.5	**153**
Visit total						**20**	**145**				**155**	**0**		**165**		**27**	**68**	**189**		**165**	**173**	**1105**
56-Day total						**20**	**165**				**319**	**319**		**319**		**27**	**95**	**284**		**165**	**173**	
URINE COLLECTION																						
Urine dipstick[6]	Local lab	Local lab				X	—				X	—		X		—	—	X		—	—	
Pregnancy test[7]	Local lab	Local lab				X	X				—	X		—		X	—	—		X	—	

Grayed out visits are not applicable to these groups.

[1] CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment procedures) and Section 9.4 (follow-up visits).

[6] And microscopy if needed.

[7] For participants assigned female sex at birth. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[8] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 16 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14).

[9] Specimens indicated for Day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination.

[10] For participants who agree to undergo boost vaccination, collection of Month 8 samples (as shown in the Lab procedures table for Part A, see Appendix J) will not occur. Participants who have already undergone sample collection at Month 8 will be placed on the Part A with Optional Second Boost schedule at Visit 11.

Appendix L Laboratory procedures for Part B with leukapheresis and lymph node FNA

Procedure	Ship to[1]	Assay location[2]	Tube type[3]	(vol. capacity)	Visit: 1	2[13]	3	4	5	6	7	8	9	10[11]	11	12	13	14	15	16	Total
					Day:	D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
					Week: Screening visit[4]	W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
					Month:	M0			M0.25	M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
						VAC1					VAC2				VAC3						
BLOOD COLLECTION																					
Screening/Diagnostic																					
HIV screening test	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—		—	—	**5**
HBsAg/ anti-HCV	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—		—	—	**5**
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	—	—	—	—	10	—	10	—	—		10	20	**50**
Safety labs																					
CBC/ Diff/ Platelets	Local lab	Local lab	EDTA	5mL	5	—	—	—	—	5	—	—	5	—	—	—	5		5	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	—	—	5	—	—	5	—	—	—	5		5	—	**25**
Immunogenicity assays																					
Cellular assays																					
Tfh/pTfh phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	25.5	—	—	25.5	—	—	25.5	—	25.5	—	25.5	—		—	25.5	**153**
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	34	—	—	***z***	34	—	—	***34***		***34***	17	**170**
Ag-specific Plasmablast phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	17	—	—	***34***	—	—	—	***34***	—		—	34	**119**
B-cell repertoire analysis	CSR	HVTN Labs	ACD	8.5mL	—	34	—	—	—	—	—	—	z	—	—	—	34		34	34	**136**
ICS	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	—	—	—	—	***z***	—	—	—	***34***		***34***	34	**102**
Humoral assays																					
Binding Ab assay	CSR	HVTN Labs	SST	8.5mL	—	***8.5***	—	—	—	—	—	—	***8.5***	—	—	—	***8.5***		***8.5***	8.5	**43**
Neutralizing Ab assay	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	—	—	***8.5***	—	—	—	***8.5***		***8.5***	8.5	**43**
Fc-mediated Ab functions[9]	CSR	HVTN Labs	SST	8.5mL	—	*y*	—	—	—	—	—	—	*y*	—	—	—	*y*		*y*	*y*	
Innate immunity and inflammation assays																					
Phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	***8.5***	***8.5***	***8.5***	***8.5***	—	8.5	—	—	—	8.5	—	—		—	—	**51**
RNA gene expression	CSR	HVTN Labs	Tempus	3mL	—	***3***	***3***	***3***	***3***	—	3	—	3	—	3	—	3		—	—	**24**
Serum cytokines/chemokines	CSR	HVTN Labs	SST	5mL	—	***5***	***5***	***5***	***5***	—	5	—	—	—	5	—	—		—	—	**30**
STORAGE																					
PBMC	CSR	—	ACD	8.5mL	—	34	—	—	17	68	—	34	z	—	—	68	153		102	42.5	**519**
Serum[17]	CSR	—	SST	8.5mL	—	25.5	8.5	8.5	8.5	25.5	—	—	17	—	—	—	25.5		17	17	**153**
Visit total					**20**	**170**	**25**	**25**	**85**	**138**	**17**	**94**	**57**	**60**	**27**	**128**	**311**		**258**	**241**	**1652**
56-Day total[10]					**20**	**190**	**215**	**240**	**324**	**462**	**478**	**332**	**305**	**227**	**27**	**154**	**465**		**258**	**241**	
URINE COLLECTION																					
Urine dipstick[6]	Local lab	Local lab			X	—	—	—	—	X	—	—	—	—	—	—	X		—	—	
Pregnancy test[7]	Local lab	Local lab			X	X	—	—	—	—	X	—	X^{12}	X^{12}	X	—	X^{12}		X	X^{12}	
Chlamydia/Gonorrhea[14]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
RECTAL SWAB COLLECTION																					
Chlamydia/Gonorrhea[14]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
CERVICAL/VAGINAL SWAB COLLECTION																					
Chlamydia/Gonorrhea[14]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
Trichomonas vaginalis[15]	Local lab	Local lab			—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	
Bacterial vaginosis[16]	Local lab	Local lab			—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	
Yeast[16]	Local lab	Local lab			—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	
OTHER COLLECTIONS (OPTIONAL)																					
Lymph node FNA	CSR	HVTN Labs			—	—	—	—	—	—	—	—	—	X	—	—	—		—	—	
Bone marrow aspirate	CSR	HVTN Labs			—	—	—	—	—	—	—	—	—	—	—	—	—		—	X	
Leukapheresis	CSR	HVTN Labs			—	—	—	—	—	—	—	—	X	—	—	—	—		—	—	
MUCOSAL SECRETION COLLECTION (OPTIONAL)																					
Cervicovaginal secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
Rectal secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
MUCOSAL BIOPSY COLLECTION (OPTIONAL)																				**Total Biopsies**	
Vaginal biopsies	CSR	HVTN Labs			—	5	—	—	—	—	—	—	—	—	—	—	5		—		10
Colorectal biopsies	CSR	HVTN Labs			—	5	—	—	—	—	—	—	—	—	—	—	5		—		10

Grayed out visits are not applicable to this subgroup.

[1] CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment procedures) and Section 9.4 (follow-up visits).

[6] And microscopy if needed.

[7] For participants assigned female sex at birth. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[8] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 16 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14).

[9] Fc-mediated Ab functions may include FcR binding assay, ADCC, ADNP ADCP, and complement assay.

[10] The 56-day total blood volume does not include up to 16.5 mL blood collected for a reaction (see Section 9.10.1); however, the 56-day limit is not exceeded at any visit by this possible collection.

[11] Visit 10 will only apply to participants who are providing lymph node FNA collections.

[12] Pregnancy testing at indicated visit is only required of participants who were assigned female sex at birth and are providing optional procedure samples (leukapheresis, lymph node FNA, bone marrow aspirate, and rectal or vaginal mucosal secretion or biopsy). See Section 9.5 for the timing of the pregnancy test in order to meet eligibility requirements for the optional procedures.

[13] Specimens indicated for Day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination.

[14] Chlamydia/gonorrhea testing will only be performed for participants providing biopsy samples. In males, chlamydia/gonorrhea testing will be done on both rectal swabs and urine. In females, chlamydia/gonorrhea testing will be done on rectal swabs and on urine or vaginal swabs.

[15] Trichomonas testing will be done with cervical/vaginal swabs or urine and will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples.

[16] Cervical/vaginal swabs for yeast and bacterial vaginosis testing will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples

[17] Antibody avidity, peptide microarray, metabolomic profiles, and proteomics assays may be done with serum collected for storage.

y = SST blood collected for the Binding Ab and Neutralizing Ab assays will also cover specimen needs for the Fc-mediated Ab function assays; no separate blood draw is needed.

z = Leukapheresis samples collected will cover specimen needs for the Ag-specific B-cell phenotyping, B-cell repertoire analysis and ICS assays; no separate blood draw is needed.

Appendix M Laboratory procedures for Part B with leukapheresis (no lymph node FNA)

Procedure	Ship to[1]	Assay location[2]	Tube type[3]	(vol. capacity)	Visit:	1	2[12]	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
					Day:	Screening visit[4]	D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
					Week:		W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
					Month:		M0			M0.25	M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
							VAC1					VAC2				VAC3						
BLOOD COLLECTION																						
Screening/Diagnostic																						
HIV screening test	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—		—	—	—		—	—	**5**
HBsAg/ anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—		—	—	—		—	—	**5**
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL		—	—	—	—	—	—	—	—	10		10	—	—		10	20	**50**
Safety labs																						
CBC/ Diff/ Platelets	Local lab	Local lab	EDTA	5mL		5	—	—	—	—	5	—	—	5		—	—	5		5	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—	—	—	—	5	—	—	5		—	—	5		5	—	**25**
Immunogenicity assays																						
Cellular assays																						
Tfh/pTfh phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	25.5	—	—	25.5	—	—	25.5	—		—	25.5	—		—	25.5	**128**
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	17	—	—	—	34	—	—	***z***		—	—	***34***		***34***	17	**136**
Ag-specific Plasmablast phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	—	—	—	17	—	—	***34***	—		—	***34***	—		—	34	**119**
B-cell repertoire analysis	CSR	HVTN Labs	ACD	8.5mL		—	34	—	—	—	—	—	—	z		—	—	34		34	34	**136**
ICS	CSR	HVTN Labs	ACD	8.5mL		—	—	—	—	—	—	—	—	***z***		—	—	***34***		***34***	34	**102**
Humoral assays																						
Binding Ab assay	CSR	HVTN Labs	SST	8.5mL		—	***8.5***	—	—	—	—	—	—	***8.5***		—	—	***8.5***		***8.5***	8.5	**43**
Neutralizing Ab assay	CSR	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	—	—	***8.5***		—	—	***8.5***		***8.5***	8.5	**43**
Fc-mediated Ab functions[9]	CSR	HVTN Labs	SST	8.5mL		—	*y*	—	—	—	—	—	—	*y*		—	—	*y*		*y*	*y*	
Innate immunity and inflammation assays																						
Phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	***8.5***	***8.5***	***8.5***	***8.5***	—	8.5	—	—		8.5	—	—		—	—	**51**
RNA gene expression	CSR	HVTN Labs	Tempus	3mL		—	***3***	***3***	***3***	***3***	—	3	—	3		3	—	3		—	—	**24**
Serum cytokines/chemokines	CSR	HVTN Labs	SST	5mL		—	***5***	***5***	***5***	***5***	—	5	—	—		5	—	—		—	—	**30**
STORAGE																						
PBMC	CSR	—	ACD	8.5mL		—	34	—	—	17	68	—	34	z		—	68	153		102	42.5	**519**
Serum[16]	CSR	—	SST	8.5mL		—	25.5	8.5	8.5	8.5	25.5	—	—	17		—	—	25.5		17	17	**153**
Visit total						**20**	**170**	**25**	**25**	**85**	**138**	**17**	**94**	**57**		**27**	**128**	**311**		**258**	**241**	**1592**
56-Day total[10]						**20**	**190**	**215**	**240**	**324**	**462**	**478**	**332**	**305**		**27**	**154**	**465**		**258**	**241**	
URINE COLLECTION																						
Urine dipstick[6]	Local lab	Local lab				X	—	—	—	—	X	—	—	—		—	—	X		—	—	
Pregnancy test[7]	Local lab	Local lab				X	X	—	—	—	—	X	—	X[11]		X	—	X[11]		X	X[11]	
Chlamydia/Gonorrhea[13]	Local lab	Local lab				—	X	—	—	—	—	—	—	—		—	—	X		—	—	
RECTAL SWAB COLLECTION																						
Chlamydia/Gonorrhea[13]	Local lab	Local lab				—	X	—	—	—	—	—	—	—		—	—	X		—	—	
CERVICAL/VAGINAL SWAB COLLECTION																						
Chlamydia/Gonorrhea[13]	Local lab	Local lab				—	X	—	—	—	—	—	—	—		—	—	X		—	—	
Trichomonas vaginalis[14]	Local lab	Local lab				—	—	—	—	—	—	—	—	—		—	—	—		—	—	
Bacterial vaginosis[15]	Local lab	Local lab				—	—	—	—	—	—	—	—	—		—	—	—		—	—	
Yeast[15]	Local lab	Local lab				—	—	—	—	—	—	—	—	—		—	—	—		—	—	
OTHER COLLECTIONS (OPTIONAL)																						
Bone marrow aspirate	CSR	HVTN Labs				—	—	—	—	—	—	—	—	—		—	—	—		—	X	
Leukapheresis	CSR	HVTN Labs				—	—	—	—	—	—	—	—	X		—	—	—		—	—	
MUCOSAL SECRETION COLLECTION (OPTIONAL)																						
Cervicovaginal secretions	CSR	HVTN Labs				—	X	—	—	—	—	—	—	—		—	—	X		—	—	
Rectal secretions	CSR	HVTN Labs				—	X	—	—	—	—	—	—	—		—	—	X		—	—	
MUCOSAL BIOPSY COLLECTION (OPTIONAL)																					**Total Biopsies**	
Vaginal biopsies	CSR	HVTN Labs				—	5	—	—	—	—	—	—	—		—	—	5		—		10
Colorectal biopsies	CSR	HVTN Labs				—	5	—	—	—	—	—	—	—		—	—	5		—		10

Grayed out visits are not applicable to this subgroup.

[1] CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment procedures) and Section 9.4 (follow-up visits).

[6] And microscopy if needed.

[7] For participants assigned female sex at birth. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing

[8] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 16 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14).

[9] Fc-mediated Ab functions may include FcR binding assay, ADCC, ADNP ADCP, and complement assay.

[10] The 56-day total blood volume does not include up to 16.5 mL blood collected for a reaction (see Section 9.10.1); however, the 56-day limit is not exceeded at any visit by this possible collection.

[11] Pregnancy testing at indicated visit is only required of participants who were assigned female sex at birth and are providing optional procedure samples (leukapheresis, bone marrow aspirate, and rectal or vaginal mucosal secretion or biopsy). See Section 9.5 for the timing of the pregnancy test in order to meet eligibility requirements for the optional procedures.

[12] Specimens indicated for Day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination.

[13] Chlamydia/gonorrhea testing will only be performed for participants providing biopsy samples. In males, chlamydia/gonorrhea testing will be done on both rectal swabs and urine. In females, chlamydia/gonorrhea testing will be done on rectal swabs and on urine or vaginal swabs.

[14] Trichomonas testing will be done with cervical/vaginal swabs or urine and will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples.

[15] Cervical/vaginal swabs for yeast and bacterial vaginosis testing will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples.

[16] Antibody avidity, peptide microarray, metabolomic profiles, and proteomics assays may be done with serum collected for storage.

y = SST blood collected for the Binding Ab and Neutralizing Ab assays will also cover specimen needs for the Fc-mediated Ab function assays; no separate blood draw is needed.

z = Leukapheresis samples collected will cover specimen needs for the Ag-specific B-cell phenotyping, B-cell repertoire analysis and ICS assays; no separate blood draw is needed.

Appendix N Laboratory procedures for Part B with lymph node FNA (no leukapheresis)

Procedure	Ship to[1]	Assay location[2]	Tube type[3]	(vol. capacity)	Visit: 1	2[13]	3	4	5	6	7	8	9	10[11]	11	12	13	14	15	16	Total
					Screening visit[4]	D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
						W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
						M0			M0.25	M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
						VAC1					VAC2				VAC3						
BLOOD COLLECTION																					
Screening/Diagnostic																					
HIV screening test	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—		—	—	**5**
HBsAg/ anti-HCV	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—		—	—	**5**
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	—	—	—	—	10	—	10	—	—		10	20	**50**
Safety labs																					
CBC/ Diff/ Platelets	Local lab	Local lab	EDTA	5mL	5	—	—	—	—	5	—	—	5	—	—	—	5		5	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	—	—	5	—	—	5	—	—	—	5		5	—	**25**
Immunogenicity assays																					
Cellular assays																					
Tfh/pTfh phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	25.5	—	—	25.5	—	—	25.5	—	25.5	—	25.5	—		—	25.5	**153**
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	34	—	—	***34***	34	—	—	***34***		***34***	17	**204**
Ag-specific Plasmablast phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	17	—	—	***34***	—	—	—	***34***	—		—	34	**119**
B-cell repertoire analysis	CSR	HVTN Labs	ACD	8.5mL	—	34	—	—	—	—	—	—	34	—	—	—	34		34	34	**170**
ICS	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	—	—	—	—	***34***	—	—	—	***34***		***34***	34	**136**
Humoral assays																					
Binding Ab assay	CSR	HVTN Labs	SST	8.5mL	—	***8.5***	—	—	—	—	—	—	***8.5***	—	—	—	***8.5***		***8.5***	8.5	**43**
Neutralizing Ab assay	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	—	—	***8.5***	—	—	—	***8.5***		***8.5***	8.5	**43**
Fc-mediated Ab functions[9]	CSR	HVTN Labs	SST	8.5mL	—	*y*	—	—	—	—	—	—	*y*	—	—	—	*y*		*y*	*y*	
Innate immunity and inflammation assays																					
Phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	***8.5***	***8.5***	***8.5***	***8.5***	—	8.5	—	—	—	8.5	—	—		—	—	**51**
RNA gene expression	CSR	HVTN Labs	Tempus	3mL	—	***3***	***3***	***3***	***3***	—	3	—	3	—	3	—	3		—	—	**24**
Serum cytokines/chemokines	CSR	HVTN Labs	SST	5mL	—	***5***	***5***	***5***	***5***	—	5	—	—	—	5	—	—		—	—	**30**
STORAGE																					
PBMC	CSR	—	ACD	8.5mL	—	34	—	—	17	68	—	34	68	—	—	68	153		102	42.5	**587**
Serum[17]	CSR	—	SST	8.5mL	—	25.5	8.5	8.5	8.5	25.5	—	—	17	—	—	—	25.5		17	17	**153**
Visit total					**20**	**170**	**25**	**25**	**85**	**138**	**17**	**94**	**227**	**60**	**27**	**128**	**311**		**258**	**241**	**1822**
56-Day total[10]					**20**	**190**	**215**	**240**	**324**	**462**	**478**	**332**	**475**	**397**	**27**	**154**	**465**		**258**	**241**	
URINE COLLECTION																					
Urine dipstick[6]	Local lab	Local lab			X	—	—	—	—	X	—	—	—	—	—	—	X		—	—	
Pregnancy test[7]	Local lab	Local lab			X	X	—	—	—	—	X	—	—	X[12]	X	—	X[12]		X	X[12]	
Chlamydia/Gonorrhea[14]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
RECTAL SWAB COLLECTION																					
Chlamydia/Gonorrhea[14]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
CERVICAL/VAGINAL SWAB COLLECTION																					
Chlamydia/Gonorrhea[14]	Local lab	Local lab			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
Trichomonas vaginalis[15]	Local lab	Local lab			—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	
Bacterial vaginosis[16]	Local lab	Local lab			—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	
Yeast[16]	Local lab	Local lab			—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	
OTHER COLLECTIONS (OPTIONAL)																					
Lymph node FNA	CSR	HVTN Labs			—	—	—	—	—	—	—	—	—	X	—	—	—		—	—	
Bone marrow aspirate	CSR	HVTN Labs			—	—	—	—	—	—	—	—	—	—	—	—	—		—	X	
MUCOSAL SECRETION COLLECTION (OPTIONAL)																					
Cervicovaginal secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
Rectal secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	X		—	—	
MUCOSAL BIOPSY COLLECTION (OPTIONAL)																				**Total Biopsies**	
Vaginal biopsies	CSR	HVTN Labs			—	5	—	—	—	—	—	—	—	—	—	—	5		—		10
Colorectal biopsies	CSR	HVTN Labs			—	5	—	—	—	—	—	—	—	—	—	—	5		—		10

Grayed out visits are not applicable to this subgroup.

[1] CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment procedures) and Section 9.4 (follow-up visits).

[6] And microscopy if needed.

[7] For participants assigned female sex at birth. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing

[8] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 16 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14).

[9] Fc-mediated Ab functions may include FcR binding assay, ADCC, ADNP ADCP, and complement assay.

[10] The 56-day total blood volume does not include up to 16.5 mL blood collected for a reaction (see Section 9.10.1); however, the 56-day limit is not exceeded at any visit by this possible collection.

[11] Visit 10 will only apply to participants who are providing lymph node FNA collections.

[12] Pregnancy testing at indicated visit is only required of participants who were assigned female sex at birth and are providing optional procedure samples (lymph node FNA, bone marrow aspirate, and rectal or vaginal mucosal secretion or biopsy). See Section 9.5 for the timing of the pregnancy test in order to meet eligibility requirements for the optional procedures.

[13] Specimens indicated for Day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination.

[14] Chlamydia/gonorrhea testing will only be performed for participants providing biopsy samples. In males, chlamydia/gonorrhea testing will be done on both rectal swabs and urine. In females, chlamydia/gonorrhea testing will be done on rectal swabs and on urine or vaginal swabs.

[15] Trichomonas testing will be done with cervical/vaginal swabs or urine and will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples.

[16] Cervical/vaginal swabs for yeast and bacterial vaginosis testing will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples.

[17] Antibody avidity, peptide microarray, metabolomic profiles, and proteomics assays may be done with serum collected for storage.

y = SST blood collected for the Binding Ab and Neutralizing Ab assays will also cover specimen needs for the Fc-mediated Ab function assays; no separate blood draw is needed.

Appendix O Laboratory procedures for Part B (no leukapheresis and no lymph node FNA)

Procedure	Ship to[1]	Assay location[2]	Tube type[3]	(vol. capacity)	Visit: 1	2[12]	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
					Day: Screening visit[4]	D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
					Week:	W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
					Month:	M0			M0.25	M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
						VAC1					VAC2				VAC3						
BLOOD COLLECTION																					
Screening/Diagnostic																					
HIV screening test	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—		—	—	—		—	—	**5**
HBsAg/ anti-HCV	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—		—	—	—		—	—	**5**
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	—	—	—	—	10		10	—	—		10	20	**50**
Safety labs																					
CBC/ Diff/ Platelets	Local lab	Local lab	EDTA	5mL	5	—	—	—	—	5	—	—	5		—	—	5		5	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	—	—	5	—	—	5		—	—	5		5	—	**25**
Immunogenicity assays																					
Cellular assays																					
Tfh/pTfh phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	25.5	—	—	25.5	—	—	25.5	—		—	25.5	—		—	25.5	**128**
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	17	—	—	—	34	—	—	***34***		—	—	***34***		***34***	17	**170**
Ag-specific Plasmablast phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	17	—	—	***34***	—		—	***34***	—		—	34	**119**
B-cell repertoire analysis	CSR	HVTN Labs	ACD	8.5mL	—	34	—	—	—	—	—	—	34		—	—	34		34	34	**170**
ICS	CSR	HVTN Labs	ACD	8.5mL	—	—	—	—	—	—	—	—	***34***		—	—	***34***		***34***	34	**136**
Humoral assays																					
Binding Ab assay	CSR	HVTN Labs	SST	8.5mL	—	***8.5***	—	—	—	—	—	—	***8.5***		—	—	***8.5***		***8.5***	8.5	**43**
Neutralizing Ab assay	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	—	—	***8.5***		—	—	***8.5***		***8.5***	8.5	**43**
Fc-mediated Ab functions[9]	CSR	HVTN Labs	SST	8.5mL	—	*y*	—	—	—	—	—	—	*y*		—	—	*y*		*y*	*y*	
Innate immunity and inflammation assays																					
Phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	***8.5***	***8.5***	***8.5***	***8.5***	—	8.5	—	—		8.5	—	—		—	—	**51**
RNA gene expression	CSR	HVTN Labs	Tempus	3mL	—	***3***	***3***	***3***	***3***	—	3	—	3		3	—	3		—	—	**24**
Serum cytokines/chemokines	CSR	HVTN Labs	SST	5mL	—	***5***	***5***	***5***	***5***	—	5	—	—		5	—	—		—	—	**30**
STORAGE																					
PBMC	CSR	—	ACD	8.5mL	—	34	—	—	17	68	—	34	68		—	68	153		102	42.5	**587**
Serum[16]	CSR	—	SST	8.5mL	—	25.5	8.5	8.5	8.5	25.5	—	—	17		—	—	25.5		17	17	**153**
Visit total					**20**	**170**	**25**	**25**	**85**	**138**	**17**	**94**	**227**		**27**	**128**	**311**		**258**	**241**	**1762**
56-Day total[10]					**20**	**190**	**215**	**240**	**324**	**462**	**478**	**332**	**475**		**27**	**154**	**465**		**258**	**241**	
URINE COLLECTION																					
Urine dipstick[6]	Local lab	Local lab			X	—	—	—	—	X	—	—	—		—	—	X		—	—	
Pregnancy test[7]	Local lab	Local lab			X	X	—	—	—	—	X	—	—		X	—	X[11]		X	X[11]	
Chlamydia/Gonorrhea[13]	Local lab	Local lab			—	X	—	—	—	—	—	—	—		—	—	X		—	—	
RECTAL SWAB COLLECTION																					
Chlamydia/Gonorrhea[13]	Local lab	Local lab			—	X	—	—	—	—	—	—	—		—	—	X		—	—	
CERVICAL/VAGINAL SWAB COLLECTION																					
Chlamydia/Gonorrhea[13]	Local lab	Local lab			—	X	—	—	—	—	—	—	—		—	—	X		—	—	
Trichomonas vaginalis[14]	Local lab	Local lab			—	—	—	—	—	—	—	—	—		—	—	—		—	—	
Bacterial vaginosis[15]	Local lab	Local lab			—	—	—	—	—	—	—	—	—		—	—	—		—	—	
Yeast[15]	Local lab	Local lab			—	—	—	—	—	—	—	—	—		—	—	—		—	—	
OTHER COLLECTIONS (OPTIONAL)																					
Bone marrow aspirate	CSR	HVTN Labs			—	—	—	—	—	—	—	—	—		—	—	—		—	X	
MUCOSAL SECRETION COLLECTION (OPTIONAL)																					
Cervicovaginal secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—		—	—	X		—	—	
Rectal secretions	CSR	HVTN Labs			—	X	—	—	—	—	—	—	—		—	—	X		—	—	
MUCOSAL BIOPSY COLLECTION (OPTIONAL)																				**Total Biopsies**	
Vaginal biopsies	CSR	HVTN Labs			—	5	—	—	—	—	—	—	—		—	—	5		—		10
Colorectal biopsies	CSR	HVTN Labs			—	5	—	—	—	—	—	—	—		—	—	5		—		10

Grayed out visits are not applicable to this subgroup.

[1] CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment procedures) and Section 9.4 (follow-up visits).

[6] And microscopy if needed.

[7] For participants assigned female sex at birth. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[8] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 16 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14).

[9] Fc-mediated Ab functions may include FcR binding assay, ADCC, ADNP ADCP, and complement assay.

[10] The 56-day total blood volume does not include up to 16.5 mL blood collected for a reaction (see Section 9.10.1); however, the 56-day limit is not exceeded at any visit by this possible collection.

[11] Pregnancy testing at indicated visit is only required of participants who were assigned female sex at birth and are providing optional procedure samples (bone marrow aspirate and rectal or vaginal mucosal secretion or biopsy). See Section 9.5 for the timing of the pregnancy test in order to meet eligibility requirements for the optional procedures.

[12] Specimens indicated for Day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination.

[13] Chlamydia/gonorrhea testing will only be performed for participants providing biopsy samples. In males, chlamydia/gonorrhea testing will be done on both rectal swabs and urine. In females, chlamydia/gonorrhea testing will be done on rectal swabs and on urine or vaginal swabs.

[14] Trichomonas testing will be done with cervical/vaginal swabs or urine and will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples.

[15] Cervical/vaginal swabs for yeast and bacterial vaginosis testing will only be performed when clinically indicated for participants who agree to provide cervicovaginal secretion or vaginal biopsy samples.

[16] Antibody avidity, peptide microarray, metabolomic profiles, and proteomics assays may be done with serum collected for storage.

y = SST blood collected for the Binding Ab and Neutralizing Ab assays will also cover specimen needs for the Fc-mediated Ab function assays; no separate blood draw is needed.

Appendix P Laboratory procedures for Part B (no optional procedures) and Part C

Procedure	Ship to[1]	Assay location[2]	Tube type[3]	Tube size (vol.	Visit:	1	2[11]	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
					Day:	Screening visit[4]	D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
					Week:		W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
					Month:		M0			M0.25	M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
							VAC1					VAC2				VAC3						
BLOOD COLLECTION																						
Screening/Diagnostic																						
HIV screening test	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—		—	—	—		—	—	**5**
HBsAg/ anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—		—	—	—		—	—	**5**
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL		—	—	—	—	—	—	—	—	10		10	—	—		10	20	**50**
Safety labs																						
CBC/ Diff/ Platelets	Local lab	Local lab	EDTA	5mL		5	—	—	—	—	5	—	—	5		—	—	5		5	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—	—	—	—	5	—	—	5		—	—	5		5	—	**25**
Immunogenicity assays																						
Cellular assays																						
Tfh/pTfh phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	25.5	—	—	25.5	—	—	25.5	—		—	25.5	—		—	25.5	**128**
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	17	—	—	—	34	—	—	***34***		—	—	***34***		***34***	17	**170**
Ag-specific Plasmablast phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	—	—	—	17	—	—	***34***	—		—	***34***	—		—	34	**119**
B-cell repertoire analysis	CSR	HVTN Labs	ACD	8.5mL		—	34	—	—	—	—	—	—	34		—	—	34		34	34	**170**
ICS	CSR	HVTN Labs	ACD	8.5mL		—	—	—	—	—	—	—	—	***34***		—	—	***34***		***34***	34	**136**
Humoral assays																						
Binding Ab assay	CSR	HVTN Labs	SST	8.5mL		—	***8.5***	—	—	—	—	—	—	***8.5***		—	—	***8.5***		***8.5***	8.5	**43**
Neutralizing Ab assay	CSR	HVTN Labs	SST	8.5mL		—	8.5	—	—	—	—	—	—	***8.5***		—	—	***8.5***		***8.5***	8.5	**43**
Fc-mediated Ab functions[9]	CSR	HVTN Labs	SST	8.5mL		—	*y*	—	—	—	—	—	—	*y*		—	—	*y*		*y*	*y*	
Innate immunity and inflammation assays																						
Phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	***8.5***	***8.5***	***8.5***	***8.5***	—	8.5	—	—		8.5	—	—		—	—	**51**
RNA gene expression	CSR	HVTN Labs	Tempus	3mL		—	***3***	***3***	***3***	***3***	—	3	—	3		3	—	3		—	—	**24**
Serum cytokines/chemokines	CSR	HVTN Labs	SST	5mL		—	***5***	***5***	***5***	***5***	—	5	—	—		5	—	—		—	—	**30**
STORAGE																						
PBMC	CSR	—	ACD	8.5mL		—	34	—	—	17	68	—	34	68		—	68	153		102	42.5	**587**
Serum[12]	CSR	—	SST	8.5mL		—	25.5	8.5	8.5	8.5	25.5	—	—	17		—	—	25.5		17	17	**153**
Visit total						**20**	**170**	**25**	**25**	**85**	**138**	**17**	**94**	**227**		**27**	**128**	**311**		**258**	**241**	**1762**
56-Day total[10]						**20**	**190**	**215**	**240**	**324**	**462**	**478**	**332**	**475**		**27**	**154**	**465**		**258**	**241**	
URINE COLLECTION																						
Urine dipstick[6]	Local lab	Local lab				X	—	—	—	—	X	—	—	—		—	—	X		—	—	
Pregnancy test[7]	Local lab	Local lab				X	X	—	—	—	—	X	—	—		X	—	—		X	—	

Grayed out visits are not applicable to this subgroup.

[1] CSR = Central Specimen Repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2] HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5] Chemistry panels are defined in Section 9.2 (pre-enrollment procedures) and Section 9.4 (follow-up visits).

[6] And microscopy if needed.

[7] For participants assigned female sex at birth. Pregnancy test may be performed on blood specimens. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing

[8] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 16 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.14).

[9] Fc-mediated Ab functions may include FcR binding assay, ADCC, ADNP ADCP, and complement assay.

[10] The 56-day total blood volume does not include up to 16.5 mL blood collected for a reaction (see Section 9.10.1); however, the 56-day limit is not exceeded at any visit by this possible collection.

[11] Specimens indicated for Day 0 may be obtained within the 14 days prior to vaccination, except for a pregnancy test which must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination.

[12] Antibody avidity, peptide microarray, metabolomic profiles, and proteomics assays may be done with serum collected for storage.

y = SST blood collected for the Binding Ab and Neutralizing Ab assays will also cover specimen needs for the Fc-mediated Ab function assays; no separate blood draw is needed.

Appendix Q Procedures at HVTN CRS for Part A

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11	12	13	14	AESI[2]	Post
Day:		D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D425	
Week:		W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W61	
Month:		M0				M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M14	
Procedure	Scr.	VAC1					VAC2									
Study procedures																
Signed screening consent (if used)	✓															
Assessment of understanding	✓															
Protocol consent	✓															
Medical history	✓															
Complete physical exam	✓													✓		
Contraception status assessment[3]	✓	✓				✓	✓		✓					✓		
Behavioral risk assessment questionnaire[4]	✓													✓		
Risk reduction counseling[5]	✓	✓				✓	✓		✓					✓		
Confirm eligibility	✓															
Obtain demographics	✓															
Randomize	✓															
Concomitant medications	✓	✓				✓	✓		✓					✓		
HIV infection assessment[6]	✓								✓					✓		
Abbreviated physical exam		✓				✓	✓		✓							
Intercurrent illness/adverse experience		✓				✓	✓		✓					✓		
Social impact assessment		✓				✓	✓		✓					✓		
Social impact assessment questionnaire														✓		
Outside testing and belief questionnaire														✓		
AESI contact															✓	
Specimen collection[7]	✓	✓				✓	✓		✓					✓		
Vaccination procedures[8]																
Vaccination[9]		✓					✓									
Reactogenicity assessments[10]		✓					✓									
Poststudy																
Unblind participant																✓

Grayed out visits are not applicable to Part A.

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

[2] See section 9.6 (AESI health contact)

[3] Contraception status assessment is required only for participants assigned female sex at birth who are sexually active in a way that could cause pregnancy. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[4] Not applicable to HIV-infected participants. Conduct **before** Risk Reduction Counseling if both occur at same visit.
[5] Includes brief risk screening and targeted risk reduction counseling, if indicated. Includes transmission risk reduction counseling for HIV-infected participants. Conduct **after** BRA questionnaire if both occur at same visit.
[6] Includes pretest steps and HIV testing. A subsequent follow-up contact is conducted to provide post-test steps and to report results to participant. If a participant has a confirmed diagnosis of HIV infection, do not perform HIV infection assessment.
[7] For specimen collection requirements, see Appendix J. For participants with a confirmed diagnosis of HIV infection, specimens listed under "Safety labs" in Appendix J, urinalysis, and urine pregnancy tests will be collected per the protocol schedule.
[8] Not applicable to HIV-infected participants.
[9] Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated. See Appendix J.
[10] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.10).

Appendix R Procedures at HVTN CRS for Part A with Optional Second Boost

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11[10]	12	13	14	15	16	Post
Day:		D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
Week:		W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
Month:		M0				M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
Procedure	Scr.	VAC1					VAC2				BOOST						
Study procedures																	
Signed screening consent (if used)	✓																
Assessment of understanding	✓																
Protocol consent	✓										✓						
Medical history	✓																
Complete physical exam	✓															✓	
Contraception status assessment[2]	✓	✓				✓	✓		✓		✓	✓	✓		✓	✓	
Behavioral risk assessment questionnaire[3]	✓															✓	
Risk reduction counseling[4]	✓	✓				✓	✓		✓		✓	✓	✓		✓	✓	
Confirm eligibility	✓																
Obtain demographics	✓																
Randomize	✓																
Concomitant medications	✓	✓				✓	✓		✓		✓	✓	✓		✓	✓	
HIV infection assessment[5]	✓								✓		✓				✓	✓	
Abbreviated physical exam		✓				✓	✓		✓		✓	✓	✓		✓		
Intercurrent illness/adverse experience		✓				✓	✓		✓		✓	✓	✓		✓	✓	
Social impact assessment		✓				✓	✓		✓		✓	✓	✓		✓	✓	
Social impact assessment questionnaire											✓						
Outside testing and belief questionnaire											✓						
Specimen collection[6]	✓	✓				✓	✓		✓		✓	✓	✓		✓	✓	
Vaccination procedures[7]																	
Vaccination[8]		✓					✓				✓						
Reactogenicity assessments[9]		✓					✓				✓						
Poststudy																	
Unblind participant																	✓

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

[2] Contraception status assessment is required only for participants assigned female sex at birth who are sexually active in a way that could cause pregnancy. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[3] Not applicable to HIV-infected participants. Conduct **before** Risk Reduction Counseling if both occur at same visit.

[4] Includes brief risk screening and targeted risk reduction counseling, if indicated. Includes transmission risk reduction counseling for HIV-infected participants. Conduct **after** BRA questionnaire if both occur at same visit.

[5] Includes pretest steps and HIV testing. A subsequent follow-up contact is conducted to provide post-test steps and to report results to participant. If a participant has a confirmed diagnosis of HIV infection, do not perform HIV infection assessment.
[6] For specimen collection requirements, see Appendix K. For participants with a confirmed diagnosis of HIV infection, specimens listed under "Safety labs" in Appendix K, urinalysis, and urine pregnancy tests will be collected per the protocol schedule.
[7] Not applicable to HIV-infected participants.
[8] Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated. See Appendix K.
[9] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.10).
[10] For participants who agreed to undergo boost vaccination collection of Month 8 samples (see Appendix J and Appendix Q) will not occur. If a participant has already undergone sample collection at Month 8 they will be placed on the boost schedule.

Appendix S Procedures at HVTN CRS for Part B (with lymph node FNA)

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	Post
Day:		D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
Week:		W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
Month:		M0				M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
Procedure	Scr.	VAC1					VAC2				VAC3						
Study procedures																	
Signed screening consent (if used)	✓																
Assessment of understanding	✓																
Protocol consent	✓																
Medical history	✓																
Complete physical exam	✓															✓	
Contraception status assessment[2]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
Behavioral risk assessment questionnaire[3]	✓															✓	
Risk reduction counseling[4]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
Confirm eligibility	✓																
Obtain demographics	✓																
Randomize	✓																
Concomitant medications	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
HIV infection assessment[5]	✓								✓		✓				✓	✓	
Abbreviated physical exam		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		
Intercurrent illness/adverse experience		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
Social impact assessment		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
Social impact assessment questionnaire							✓				✓						
Outside testing and belief questionnaire											✓						
Pelvic and/or rectal exam[6]		✓											✓				
Specimen collection[7]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
Vaccination procedures[8]																	
Vaccination[9]		✓					✓				✓						
Reactogenicity assessments[10]		✓					✓				✓						
Poststudy																	
Unblind participant																	✓

Grayed out visits are not applicable.

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

[2] Contraception status assessment is required only for participants assigned female sex at birth who are sexually active in a way that could cause pregnancy. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[3] Not applicable to HIV-infected participants. Conduct **before** Risk Reduction Counseling if both occur at same visit.

[4] Includes brief risk screening and targeted risk reduction counseling, if indicated. Includes transmission risk reduction counseling for HIV-infected participants. Conduct **after** BRA questionnaire if both occur at same visit.
[5] Includes pretest steps and HIV testing. A subsequent follow-up contact is conducted to provide post-test steps and to report results to participant. If a participant has a confirmed diagnosis of HIV infection, do not perform HIV infection assessment.
[6] As appropriate for participants providing rectal and/or vaginal mucosal samples (see Section 9.5).
[7] For specimen collection requirements, see Appendix L, and Appendix N. For participants with a confirmed diagnosis of HIV infection, specimens for urinalysis and urine pregnancy tests listed under "Safety labs" in Appendix L, and Appendix N will be collected per the protocol schedule.
[8] Not applicable to HIV-infected participants.
[9] Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated. See Appendix L and Appendix N.
[10] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.10).

Appendix T Procedures at HVTN CRS for Part B (no lymph node FNA) and Part C

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	Post
Day:		D0	D1	D3	D7	D14	D56	D63	D70	D77	D168	D175	D182	D224	D364	D546	
Week:		W0			W1	W2	W8	W9	W10	W11	W24	W25	W26	W32	W52	W78	
Month:		M0				M0.5	M2	M2.25	M2.5	M2.75	M6	M6.25	M6.5	M8	M12	M18	
Procedure	Scr.	VAC1					VAC2				VAC3						
Study procedures																	
Signed screening consent (if used)	✓																
Assessment of understanding	✓																
Protocol consent	✓																
Medical history	✓																
Complete physical exam	✓															✓	
Contraception status assessment[2]	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	
Behavioral risk assessment questionnaire[3]	✓															✓	
Risk reduction counseling[4]	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	
Confirm eligibility	✓																
Obtain demographics	✓																
Randomize	✓																
Concomitant medications	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	
HIV infection assessment[5]	✓								✓		✓				✓	✓	
Abbreviated physical exam		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓		
Intercurrent illness/adverse experience		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	
Social impact assessment		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	
Social impact assessment questionnaire							✓				✓						
Outside testing and belief questionnaire											✓						
Pelvic and/or rectal exam[6]		✓											✓				
Specimen collection[7]	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	
Vaccination procedures[8]																	
Vaccination[9]		✓					✓				✓						
Reactogenicity assessments[10]		✓					✓				✓						
Poststudy																	
Unblind participant																	✓

Grayed out visits are not applicable.

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

[2] Contraception status assessment is required only for participants assigned female sex at birth who are sexually active in a way that could cause pregnancy. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[3] Not applicable to HIV-infected participants. Conduct **before** Risk Reduction Counseling if both occur at same visit.

[4] Includes brief risk screening and targeted risk reduction counseling, if indicated. Includes transmission risk reduction counseling for HIV-infected participants. Conduct **after** BRA questionnaire if both occur at same visit.
[5] Includes pretest steps and HIV testing. A subsequent follow-up contact is conducted to provide post-test steps and to report results to participant. If a participant has a confirmed diagnosis of HIV infection, do not perform HIV infection assessment.
[6] As appropriate for participants providing rectal and/or vaginal mucosal samples (see Section 9.5).
[7] For specimen collection requirements, see Appendix M, Appendix O, and Appendix P. For participants with a confirmed diagnosis of HIV infection, specimens for urinalysis and urine pregnancy tests listed under "Safety labs" in Appendix M, Appendix O, and Appendix P will be collected per the protocol schedule.
[8] Not applicable to HIV-infected participants.
[9] Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated. See Appendix M, Appendix O, and Appendix P.
[10] Reactogenicity assessments performed daily for at least 7 days postvaccination for Part B. Reactogenicity assessments performed daily for at least 14 days postvaccination for Part C (see Section 9.10).

Appendix U HVTN low risk guidelines for the US

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

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

A. For US volunteers NOT on stable Pre-exposure prophylaxis (PrEP)

1 SEXUAL BEHAVIORS

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

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

AND

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

In the **last 6 months**:

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

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

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

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

- Had 2 or fewer partners for anal or vaginal intercourse, and had no unprotected anal or vaginal sex, OR

- Had unprotected anal or vaginal intercourse sex with 1 partner only within a monogamous relationship lasting at least 12 months (during which neither partner had any other partners). If the monogamous relationship ended, may then have had protected anal or vaginal sex with 1 other partner (total 2 or fewer partners in the last 12 months).

AND

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

2 NON-SEXUAL BEHAVIORS

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

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

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

Acquired an STI (ie new infection) in the **last 12 months**:

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

B. For US volunteers on Pre-exposure prophylaxis (PrEP)

1. PrEP ASSESSMENT

- Reports 6 months (180 days) or more of protective PrEP use
  - For daily oral PrEP use:
    - For persons AMAB who have sex with persons AMAB: Reports equal to or greater than 70% when asked the following: "*Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications?"*
    - For people with a vagina having intravaginal intercourse: Reports equal to or greater than 90% when asked the following: "*Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications?"*
  - For event-driven (on-demand or "2-1-1") PrEP use[1] in persons AMAB who have sex with persons AMAB: Reports use consistent with the guidance of the professional education organization, the International Antiviral Society–USA (IAS-USA):
    - For individuals with frequent use (> 15 pills per month): At least 80% of condomless sex acts are covered with on-demand PrEP at the recommended dose schedule
    - For individuals with less frequent use (≤ 15 pills per month):
      - A past history of high adherence (> 90%)
      - Commitment to use on-demand PrEP for *all* condomless sex acts at the recommended dose schedule
  - Emtricitabine/tenofovir alafenamide (FTC/TAF, Descovy) and emtricitabine/tenofovir disoproxil fumarate (FTC/TDF, Truvada) when taken as PrEP do not interfere with pseudoneutralization assays. However, other antiretroviral medications may potentially interfere with immunogenicity assays. For PrEP medications other than FTC/TAF or FTC/TDF, refer to the SSP for details.
  - Commits to maintaining protective PrEP use throughout trial

[1] See Study-specific Procedures (SSP) for additional guidance on on-demand PrEP.

2. SEXUAL BEHAVIORS

- Persons stably taking PrEP as prescribed above for 6 months or longer are considered low risk of HIV infection, regardless of any sexual behavior that might otherwise be associated with high risk of HIV exposure.

3. NON-SEXUAL BEHAVIORS

In the **last 12 months** did <u>not</u>:

- Inject drugs or other substances without a prescription

- Use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment, rendered the participant at greater than low risk for acquiring HIV infection.

The investigator's judgment should consider local epidemiologic information about HIV prevalence in the area and community networks.

Appendix V Adverse events of special interest

AEs of special interest (AESI) for this protocol include but are not limited to potential immune-mediated diseases; representative examples of AESI are listed below. Updates to AESI will be provided as an appendix to the *HVTN 137 Study Specific Procedures.*

<table>
<tr><th>Neuroinflammatory disorders</th><th>Musculoskeletal disorders</th><th>Skin disorders</th></tr>
<tr>
<td>
• Cranial nerve disorders, including paralyses/paresis (eg Bell’s palsy)
• Optic neuritis
• Multiple sclerosis
• Transverse myelitis
• Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
• Acute disseminated encephalomyelitis, including site specific variants: eg non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis
• Myasthenia gravis, including Lambert-Eaton myasthenic syndrome
• Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy).
• Narcolepsy
</td>
<td>
• Systemic lupus erythematosus and associated conditions
• Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome
• Idiopathic inflammatory myopathies, including dermatomyositis
• Polymyositis
• Antisynthetase syndrome
• Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still’s disease
• Polymyalgia rheumatica
• Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
• Psoriatic arthropathy
• Relapsing polychondritis
• Mixed connective tissue disorder
</td>
<td>
• Psoriasis
• Vitiligo
• Erythema nodosum
• Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)
• Alopecia areata
• Lichen planus
• Sweet’s syndrome
• Localized Scleroderma (Morphea)
• Cutaneous lupus erythematosus
Metabolic disorders
• Addison’s disease
• Autoimmune thyroiditis (including Hashimoto thyroiditis)
• Diabetes mellitus type I
• Grave's or Basedow’s disease
</td>
</tr>
<tr><th>Vasculitides</th><th>Blood disorders</th><th>Others</th></tr>
<tr>
<td>
• Large vessels vasculitis including: giant cell arteritis such as Takayasu’s arteritis and temporal arteritis.
• Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener’s granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger’s disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet’s syndrome, leukocytoclastic vasculitis.
</td>
<td>
• Autoimmune hemolytic anemia
• Autoimmune thrombocytopenia
• Antiphospholipid syndrome
• Pernicious anemia
• Autoimmune aplastic anemia
• Autoimmune neutropenia
• Autoimmune pancytopenia
Gastrointestinal disorders
• Celiac disease
• Crohn’s disease
• Ulcerative colitis
• Ulcerative proctitis
Liver disorders
• Autoimmune cholangitis
• Autoimmune hepatitis
• Primary biliary cirrhosis
• Primary sclerosing cholangitis
</td>
<td>
• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
• Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy)
• Autoimmune myocarditis/cardiomyopathy
• Sarcoidosis
• Stevens-Johnson syndrome
• Sjögren’s syndrome
• Idiopathic pulmonary fibrosis
• Goodpasture syndrome
• Raynaud’s phenomenon
</td>
</tr>
</table>

Appendix W Visit Windows

HVTN 137 Part A Visit Windows

Visit Number	Visit Type	Lower Allowable Window (-)	Lower Target Window (-)	Target Day	Upper Target Window (+)	Upper Allowable Window (+)
01.0	Screening	-56	-	0	-	+0
02.0	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
03.0						
04.0						
05.0						
06.0	2 weeks postvaccination 1	-	-4	14	+4	+7
07.0	**Vaccination 2**	**-**	**-7**	**56**	**+9**	**+180**
08.0						
09.0	2 weeks postvaccination 2 *Primary Immunogenicity*	-	-4	70	+4	+7
10.0						
11.0						
12.0						
13.0						
14.0	Final Visit *(Should be scheduled ~6 months post-Vaccination 2)*	-28	-14	224	+14	+120
AESI	AESI Contact *(Should be scheduled ~12 months post-Vaccination 2)*	-28	-14	425	+14	120

All target dates are relative to Day 0, with the exception of the postvaccination visits, visits 6.0 and 9.0, which are relative to the vaccination immediately preceding the visit.

The highlighted (blue) visits are not required for Part A participants.

HVTN 137 Part A with Optional Second Boost Visit Windows

Visit Number	Visit Type	Lower Allowable Window (-)	Lower Target Window (-)	Target Day	Upper Target Window (+)	Upper Allowable Window (+)
01.0	Screening	-56	-	0	-	+0
02.0	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
03.0						
04.0						
05.0						
06.0	2 weeks postvaccination 1	-	-4	14	+4	+7
07.0	**Vaccination 2**	**-**	**-7**	**56**	**+9**	**+180**
08.0						
09.0	2 weeks postvaccination 2 *Primary Immunogenicity*	-	-4	70	+4	+7
10.0						
11.0	**Vaccination 3**	**-**	**-7**	**168**	**+9**	**+365**
12.0	7 days postvaccination 3	-1	-	175	-	+1
13.0	2 weeks postvaccination 3 *Primary Immunogenicity*	-	-4	182	+4	+7
14.0						
15.0	Follow-up Visit	-84	-14	364	+14	+84
16.0	Final Visit	-56	-14	546	+14	+84

All target dates are relative to Day 0, with the exception of the postvaccination visits 9.0, 12.0, 13.0, 15.0, and 16.0, which are relative to the vaccination immediately preceding the visit.

The highlighted (blue) visits are not required for any Part A Boost participants.

HVTN 137 Part B and Part C Visit Windows

Visit Number	Visit Type	Lower Allowable Window (-)	Lower Target Window (-)	Target Day[1]	Upper Target Window (+)	Upper Allowable Window (+)
01.0	Screening	-56	-	0	-	+0
02.0	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
03.0	1 day postvaccination 1	-0	-	1	-	+0
04.0	3 days postvaccination 1	-0	-	3	-	+0
05.0	7 days postvaccination 1	-1	-	7	-	+1
06.0	2 weeks postvaccination 1	-	-4	14	+4	+7
07.0	**Vaccination 2**	**-**	**-7**	**56**	**+9**	**+14**
08.0	7 days postvaccination 2	-1	-	63	-	+1
09.0	2 weeks postvaccination 2 *Primary Immunogenicity*	-	-4	70	+1	+4
10.0[2]	FNA visit	-3	-1	77	+4	+7
11.0	**Vaccination 3**	**-**	**-7**	**168**	**+9**	**+14**
12.0	7 days postvaccination 3	-1	-	175	-	+1
13.0	2 weeks postvaccination 3 *Primary Immunogenicity*	-	-4	182	+4	+7
14.0[3]						
15.0	Follow-up Visit	-84	-14	364	+14	+84
16.0	Final Visit	-56	-14	546	+14	+28

[1] All target dates are relative to Day 0, with the exception of the postvaccination visits, visits 8.0, 9.0, 10.0, 12.0, and 13.0, which are relative to the vaccination immediately preceding the visit.

[2] The green highlighted visit is only required for participants who are providing lymph node FNA collections in Part B.

[3] The highlighted (blue) visit is not required for any Part B or Part C participants.

Appendix X Protocol Signature Page

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or Alum adjuvants and VRC HIV Env Trimer 4571 and 3M-052-AF with Alum in healthy, HIV-uninfected adults

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 (U.S.) 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 (eg, U.S. National Institutes of Health, Division of AIDS) and institutional policies

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

DAIDS Protocol Number: HVTN 137

DAIDS Protocol Version: Version 5.0

Protocol Date: September 26, 2022