HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 133

A phase 1 clinical trial to evaluate the safety and immunogenicity of an HIV-1 gp41 MPER-656 liposome vaccine in healthy, HIV-uninfected adult participants

DAIDS DOCUMENT ID 12047

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CLINICAL TRIAL SPONSORED BY

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

STUDY PRODUCT(S) PROVIDED BY

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

March 13, 2019
Final
HVTN 133
Version 1.0

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 9). Safety is monitored daily by HVTN Core and routinely by the HVTN 133 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

2.6 Protect privacy/confidentiality

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

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

research participants or potential research participants as individuals whereas the term “confidentiality” is used to refer to the treatment of information about those individuals. This protocol respects the privacy of participants by informing them about who will have access to their personal information and study data (see Appendix A). The privacy of participants is protected by assigning unique identifiers in place of the participant’s name on study data and specimens. In the United States, research participants in HVTN protocols are protected by a Certificate of Confidentiality from the US NIH, which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form. In addition, each staff member at each study site in this protocol signs 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 an HIV-1 gp41 MPER-656 liposome vaccine in healthy, HIV-uninfected adult participants

Primary objective(s)

Primary objective 1

- To evaluate the safety and tolerability of different doses of a prime-boost regimen of MPER-656 liposomes in HIV-uninfected healthy adults

Primary endpoint 1

- Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and adverse events (AEs) and serious adverse event (SAEs)

Primary objective 2

- To evaluate binding antibody responses elicited by different doses of the MPER-656 liposome vaccine

Primary endpoint 2

- MPER-peptide-specific immunoglobulin G (IgG) binding antibody (Ab) responses as assessed by binding Ab multiplex assay 2 weeks after the 3rd and 4th vaccination with MPER-656 liposome vaccine

Study products and routes of administration

- MPER-656 liposomes at 0.5 mg/mL with 500 mcg alum (from 5 mg/mL suspension) for two 0.5 mL doses in two sites (total dose of 500 mcg in 1 mL) administered by IM injection in the deltoid muscles (one injection into each deltoid)

- MPER-656 liposomes at 2 mg/mL with 500 mcg alum (from 5 mg/mL suspension) for two 0.5 mL doses in two sites (total dose of 2000 mcg in 1 mL) administered by IM injection in the deltoid muscles (one injection into each deltoid)

- Sodium Chloride for Injection USP, 0.9% administered for two 0.5 mL doses in two sites by IM injection in the deltoid muscles (one injection into each deltoid)

Table 3-1 Schema

Study arm	N	Month 0 (Day 0)	Month 2 (Day 56)	Month 6 (Day 168)	Month 12 (Day 364)
Group 1	5	500 mcg peptide	500 mcg peptide	500 mcg peptide	500 mcg peptide
	1	Placebo	placebo	placebo	placebo
Group 2	15	2000 mcg peptide	2000 mcg peptide	2000 mcg peptide	2000 mcg peptide
	3	Placebo	placebo	placebo	placebo
Total	24 (20/4)				

Note:
Safety data for Group 1 through 2 weeks after the first vaccination will inform whether to proceed with escalation to the higher dose (Group 2).

Participants

24 healthy, HIV-1–uninfected volunteers aged 18 to 50 years; 20 vaccinees, 4 placebo recipients

Design

Multicenter, randomized, dose escalation, placebo-controlled, double-blind trial

Duration per participant

18 months of scheduled clinic visits per participant (main study), followed by an adverse event of special interest (AESI) health contact at month 24

Estimated total study duration

30 months (includes enrollment, planned safety holds, follow-up, and AESI health contact)

Investigational New Drug (IND) sponsor

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

Study product provider

- MPER-656 liposomes: International AIDS Vaccine Initiative, (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)

HIV diagnostic laboratory

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

Endpoint assay laboratories

- Duke University Medical Center (Durham, North Carolina, USA)
- Fred Hutch/University of Washington (Seattle, Washington, USA)

Study sites

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

Safety monitoring

HVTN 133 PSRT; HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

Protocol leadership

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4 Background

4.1 Rationale for trial concept

Elicitation of broadly neutralizing antibodies (bnAbs) against the HIV-1 envelope (Env) is likely to be central to a successful protective HIV-1 vaccine (4). There are multiple conserved sites on Env that are targets for bnAbs but no current vaccination regimens elicit these kinds of antibodies. A subset of chronically infected subjects make bnAbs, and this has allowed identification of several characteristics shared by all bnAbs (5).

One highly conserved and well-studied vaccine target in Env is gp41 near the viral membrane proximal external region (MPER) where several bnAbs bind (6). One of these bnAbs, 2F5, has been shown to protect against vaginal simian/human immunodeficiency virus (SHIV) transmission (7) when given passively suggesting that, if induced in sufficient titers, such bnAbs may be effective for prevention of HIV-1 infection. The MPER of gp41 is a highly conserved region, rich in aromatic residues, and its role in HIV-1 fusion is evident from studies showing that mutation of tryptophan residues in the MPER inhibits cell fusion and viral infectivity (8). However, MPER-specific bnAbs are rarely made in natural HIV-1 infection (9) and as yet are not made following HIV-1 envelope protein vaccination (10, 11).

Immunological tolerance mechanisms (12, 13), which cause profound clonal deletion of B cells expressing autoreactive bnAbs like 2F5 (14), have been postulated to limit induction of MPER gp41 bnAbs. Verkoczy and Haynes have recently demonstrated in mice that although most precursors of MPER bnAbs are deleted at the first tolerance checkpoint in bone marrow, a small population (5%) of bnAb precursors emigrate from the bone marrow and exist in peripheral immune organs as anergic B cells that can be activated by an appropriate immunogen to produce 2F5 bnAbs in plasma (15). These residual anergic clones are competent to undergo class-switching, activation and expansion in response to immunization resulting in potent serum IgG neutralizing responses. Thus, in principle, overcoming tolerance of self-reactive bnAb-expressing B cells by immunization is a feasible part of an antibody-based HIV-1 vaccine strategy.

Despite these data showing tolerance control of this class of antibodies, there are data that suggest it might be possible to elicit such antibodies by vaccination. While most individuals develop bnAbs only after several years of infection, Sather et al. described two HIV-1–infected persons who developed MPER-directed bnAb activity one year after infection (16) suggesting that it may be possible to develop MPER-directed bnAb activity relatively quickly compared with other bnAb epitopes (17). Rhesus macaques immunized using MPER peptides displayed on liposomes (described in Section 4.2) developed antibodies that recognized the 2F5-epitope (18, 19), and monoclonal antibodies isolated from these macaques demonstrated a maturation pattern similar to that of 2F5-including broad neutralization in an assay using TZM-bl cells that express CD64 (FcγR1)

(19). Thus, MPER peptide liposomes are an immunogen that have been shown to initiate the development of 2F5-like bnAb precursors in rhesus macaques, providing a rationale for the testing of this immunogen in human participants. This study will determine if 2F5-like bnAb precursors can be elicited in humans utilizing an MPER peptide thus opening the path to exploiting B cell lineage development as a mechanism to elicit bnAbs.

4.2 GTH1-656 MPER peptide liposome

Rationale for gp41 MPER peptide liposome immunogen design

Data suggest that MPER residues are oriented or presented differently on a lipid bi-layer compared to free peptides (20-22). MPER peptides alone as immunogens generally are ineffective for the induction of bnAbs (23). MPER peptides in micelles or in liposomes with membrane anchor tags are likely to be less flexible and more likely to adopt a relatively ordered conformation (20, 21). It has been shown that stable scaffold structures of MPER peptides can induce antibody responses that target the 2F5-bound peptide conformation (23). Studies using synthetic peptide-liposome conjugates have shown that membrane anchoring is important for the presentation of 2F5 epitope structure (24); the N and C termini of the gp41 MPER are differentially exposed on the membrane; and the stable docking of monoclonal antibodies (mAbs) 2F5 to peptide-liposomes is influenced by the extent of membrane immersion of the binding epitopes (21).

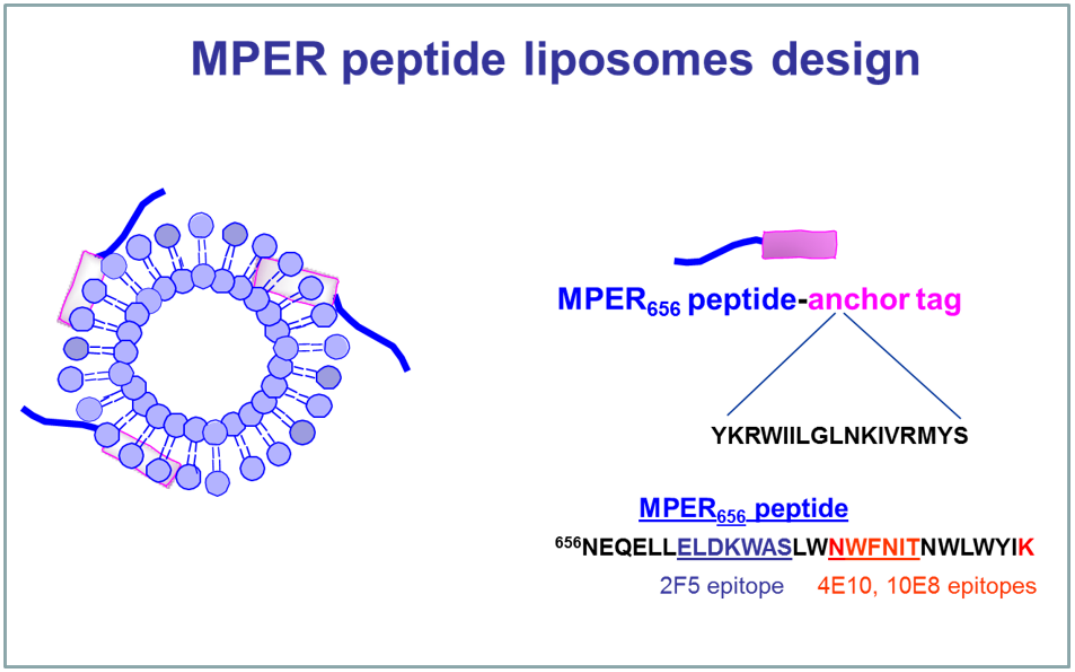

Figure 4-1 Design of the MPER peptide liposome

Figure 4-1 shows the general design of the MPER peptide-liposome. The MPER-656 liposomes immunogen includes the relevant gp41 MPER component and a

lipid component to induce membrane binding. The MPER-656 peptide sequence (^{656}NEQELLELDKWASLWNWFNITNWLWYIK^{683}) is derived from the membrane proximal region of HIV-1 gp41 and includes the core epitopes of the broadly neutralizing mAbs 2F5 (^{662}ELDKWAS), 4E10 (^{671}NWFNIT), and 10E8 (^{671}NWF^{676}T^{683}K). The MPER-656 peptide was synthesized with a C-terminal hydrophobic membrane anchor tag (YKRWIILGLNKIVRMYS) which facilitates anchoring of the peptide to synthetic liposomes and presentation of the optimal peptide conformation for MPER bnAb binding, while occluding the binding of non-neutralizing MPER antibodies (21, 24). The gp41 MPER-656 peptide liposome immunogen has been shown to be able to induce triggering of anergic 2F5 bnAb cells in 2F5 knock-in mice, resulting in high levels of plasma bnAb. MPER-656 peptide adjuvanted liposomes were constructed as described (21, 24, 25).

4.3 Alhydrogel® adjuvant

Alhydrogel® adjuvant, aluminum hydroxide (alum), is a commonly used vaccine adjuvant and will be administered with this peptide vaccine.

4.4 Trial design rationale

This study will evaluate the safety and immunogenicity of a novel gp41 MPER peptide-liposome vaccine in healthy HIV-1–uninfected adults. The study will evaluate 2 different dose levels of the vaccine (500 and 2,000 mcg) in a dose escalation trial. Safety and tolerability will be assessed after the first vaccination and safety parameters met before advancing to the higher dose level. Vaccination will be given by needle and syringe intramuscularly. Each dose will be split in two and given at 2 separate injection sites.

Preclinical testing of this and other HIV-1 vaccine candidates suggests that sequential immunization with immunogens, perhaps over a prolonged period of time, may be required to induce bnAb production. This vaccine will be given at 4 timepoints over a period of 12 months to determine the degree to which MPER-directed bnAb precursors can be elicited and matured by this vaccine candidate. Safety, tolerability, and immunogenicity data will be collected after each vaccination.

Preclinical trials have given up to 2 mg of peptide-liposome vaccine without significant toxicity. However, a human mAb targeting the epitope included in this vaccine has been shown to react with cardiolipin histones and centromere B autoantigens (26). Participants in this study will be monitored for vaccine-induced toxicities. This vaccine is designed to elicit 2F5-like bnAb precursors, and human trials of passive immunization with up to 5 grams of 2F5 antibody did not result in clinical prolongation of clotting times (activated partial thromboplastin time [aPTT], prothrombin time [PT] and dilute Russell's viper venom time [DRVVT]) and no binding to cardiolipin was seen in clinical samples. In human studies

administering combinations of mAbs 2F5, 4E10, and 2G12, slight rises in binding to phosphatidylserine and cardiolipin were seen in plasma along with a mild, transient prolongation of aPTT in 4/12 participants (27). Because of this possible autoreactivity, volunteers will be screened for pre-existing autoimmune disorders and safety labs will include evaluation of clotting times as well as induction of anti-cardiolipin antibody and anti-nuclear antibody. The full MPER neutralizing antibody (nAb) epitope, ELDKWA, is present in the host tryptophan catabolism pathway enzyme kynureninase (KYNU). It is not expected that antibodies against this enzyme will have clinically apparent adverse effects, and studies in mice and non-human primates (NHPs) have shown that immunization with MPER peptide liposome vaccine did not alter tryptophan metabolism (18).

4.4.1 Dose selection

Based on preclinical studies (see Section 4.7 below) two doses have been selected to be given in a 4-dose regimen over 12 months. This will allow assessment of a dose response, including up to the maximum studied in the NHP model, to determine if bnAbs can be elicited via this approach.

4.4.2 Schedule

At present it is not known what the optimal vaccine interval is for eliciting HIV-1 bnAbs. Natural history studies show that most persons who develop breadth do so only after years of infection (28, 29), and to date no vaccine candidate has reliably elicited bnAbs, making the rational choice of a vaccine interval challenging. Data from individuals who have been found to make MPER-directed bnAbs vary, with some individuals making bnAbs after only 1 year of infection (16) while others appear to have required a longer period of time (30). Trials with Env immunogens that did not elicit bnAbs but that were associated with protection (ie, RV144 and the follow-on RV305 study) suggest that immunogenicity and lineage maturation may be enhanced/improved when boosting occurs after a prolonged rest (31). The length of time between immunizations to increase bnAb development is not known and must be inferred based on other analyses such as the expansion of B cell lineages or B cell precursors of bnAb lineages. For these reasons, a longer interval of 4 months (vaccine #3) and 6 months (vaccine #4) is proposed.

4.4.3 Control

Placebo used will be Sodium Chloride for Injection USP, 0.9%

4.5 Plans for future product development and testing

These data will inform our ability to elicit bnAbs via vaccination, and in particular, this trial will determine whether this immunogen can elicit and expand MPER-directed bnAb precursors in humans. Success in this trial would include the elicitation of neutralizing antibodies directly or the initiation of bnAb lineages that could be further matured using additional vaccine strategies. If successful, an

important avenue of HIV-1 vaccine development will be opened up and follow-on studies to increase the efficiency of antibody elicitation and breadth of the immune response will be pursued.

4.6 Preclinical safety studies

4.6.1 Evaluation of kynureninase cross-reactivity

4.6.1.1 Mouse #059

The HIV MPER gp41 bnAb epitope ELDKWA is present in enzyme kynureninase (KYNU), which is in the tryptophan catabolism pathway and the 2F5 bnAb cross-reacts in vitro with monomeric KYNU (18). Because MPER-peptide liposomes are designed to elicit 2F5-like antibodies which can cross-react with KYNU, it is possible that immunization with this vaccine will have an autoimmune effect targeting KYNU enzyme activity. This risk of autoimmunity was assessed in 2F5 knock-in (KI) mice which were engineered to express the broadly and potently neutralizing mature HIV-1 antibody 2F5 that binds to gp41 MPER (15). MPER-656 liposomes were administered in combination with Glucopyranosyl Lipid Adjuvant (GLA: a synthetic toll-like receptor 4 agonist, that has been used as an adjuvant in investigational vaccine studies) or alum (19). In addition to MPER binding antibodies, KYNU-reactive antibodies were detected by enzyme-linked immunosorbent assay (ELISA). In an in vitro enzymatic assay of KYNU activity, serum from all MPER-656 liposome-immunized mouse groups had no impact on KYNU activity. Additionally, immunization had no effect on levels of tryptophan, kynurenine and kynurenic acid in serum and the brain measured by liquid chromatography-mass spectrometry. Histopathology of brain and spleen tissue revealed no pathological changes as a result of immunization (19). These results indicated KYNU-reactive antibodies elicited by MPER-656 liposomes had no impact on the KYNU metabolic pathway metabolites in serum or brain nor had any impact on brain or spleen histopathology. KYNU reactivity was further evaluated in rhesus macaques as described in Section 4.6.1.2.

4.6.1.2 NHP #204/#205

The effect of MPER-656 liposome immunization on KYNU activity was also evaluated in NHP studies 204 and 205. Immunogenicity outcomes are summarized in Section 4.7.1.4. While KYNU-reactive, MPER gp41-reactive antibodies were elicited by MPER-656 liposomes, those antibodies had no detectable impact on in vitro KYNU activity, and histopathology of spleen tissue was normal in all groups. 4E10-like antibodies, which would pose a greater risk of autoreactive pathogenicity, were not detected in 4E10 blocking assays, indicating that these antibodies are not elicited by MPER-656 liposomes. Rhesus macaques in Studies 204 and 205 were also monitored for changes in comprehensive clinical chemistry and hematology assays. No significant abnormalities deemed related to study treatment were observed, although mild

anemia was noted throughout the study in all groups, as well as transient low phosphorous measurements (19). The anemia was likely a result of the blood draw volumes and the physiological stress of study procedures, despite adherence to safe blood volume limits. In combination with the 2F5 mouse data in Section 4.6.1.1, these data suggest that MPER-binding antibodies that are KYNU cross-reactive will not impair host tryptophan metabolism in human subjects.

4.6.2 NHP #208

A dose study in rhesus macaques of MPER-656 liposomes mixed with alum (Alhydrogel®) tested the three vaccine doses planned for the clinical study. Immunogenicity results are summarized in Section 4.7.1.5. There were no adverse events in this study thought to be related to the vaccine, although 2 of 20 animals were anemic at all timepoints after the initial immunization, both of which were in the empty liposome and alum control group. As with NHP #204/205, the anemia was likely the result of blood draws and the stress of study procedures. One animal was necropsied at week 11 due to more profound anemia (total hemoglobin of 4.9 mg/dL) and found to have erythroid hypoplasia on bone marrow but with no obvious association to the study treatment. As with NHP #204/#205, autoreactive 4E10-blocking antibodies were not detected. These results support the safety of the use of MPER-656 liposomes with alum at the planned clinical dose range.

4.6.3 GLP Rabbit Toxicology Study CRL505285

A GLP toxicology study in New Zealand White Rabbits was designed to determine the potential toxicity and local tolerance of MPER-656 liposomes when administered by IM injection with alum once every two weeks for 10 weeks (total 6 injections) and to evaluate the potential reversibility of any findings. Three groups of twenty animals were dosed as indicated in Table 4-1.

Table 4-1 Repeat dose toxicity schedule

Gp	Test article	Dosing Days	Animals/Group	
			Primary Necropsy SD 74	Recovery Necropsy SD 92
1	Vehicle control	1, 15, 29, 43, 57, 71	5M/5F	5M/5F
2	2 mg peptide liposome + 500 mcg alum*	1, 15, 29, 43, 57, 71	5M/5F	5M/5F
3	0.2 mg peptide liposome + 500 mcg alum*	1, 15, 29, 43, 57, 71	5M/5F	5M/5F

The toxicity study demonstrated an acceptable safety profile for use in human subjects at the highest dose planned for clinical use.

4.7 Preclinical immunogenicity studies

Table 4-2 Summary of preclinical immunogenicity studies

Study number	Animal	N/group	Dose (mg) groups	Route	Schedule (wks)
VMU #029	2F5 Mature KI mice	3	0.025	IP	0, 2, 4, 6, 8, 10
VMU #048	2F5 Mature UA mice	4	0.025	IP	0, 2, 4, 6, 8, 10
NHP #22	Rhesus macaques	4	0.35 mucosal; 1.0 IM	mucosal; IM	JRFL gp140 prime and boost with MPER-liposomes: mucosal at wks 12, 16, 20, 24 and IM. at wks 41, 45, 50, 88, 92, 129, 135, 137, 142, 148
NHP #204	Rhesus macaques	8	1.0	IM	0, 6, 12, 18, 24, 30
NHP #205	Rhesus macaques	7	1.0	IM	0, 6, 12, 18, 24, 30
NHP #208		4	2.0	IM	0, 5, 8
R25	Rabbits	3	2.0	IM	0, 3, 6, 9, 12

IM = intramuscular, IP = intraperitoneal.

4.7.1 Immunogenicity of HIV-1 MPER peptide-liposome

4.7.1.1 Study VMU #029

Initial proof-of-concept data were generated using a prototype of the MPER-656 liposomes formulation made at the Duke Human Vaccine Institute. 2F5 KI mice were engineered to express the broadly and potently neutralizing mature HIV-1 antibody 2F5 that binds to gp41 MPER and is also autoreactive (15). In these mice, the majority of the 2F5 B cells are deleted in the bone marrow, leaving a small population of anergic B cells in the periphery (19). The 2F5 KI model has provided a system to determine if an MPER-656 liposome designed to optimally bind to the 2F5 antibody can selectively target and/or reverse functional silencing of residual autoreactive B-cell subsets with B-cell receptor (BCR) dual reactivity (ie, lipid/MPER) required for 2F5's neutralization ability (32).

In the first proof-of-concept study, 2F5 mature heavy chain variable region (VH) + light chain variable region (VL) KI mice received an HIV-1 JRFL gp140 Env prime followed by 6 biweekly immunizations with MPER-656 liposomes manufactured with monophosphoryl lipid A (MPLA) and mixed with the adjuvants Emulsigen and CpG before injection. Class-switching of 2F5 bnAb B cells was observed, and high levels of plasma 2F5 antibody were produced in mature 2F5 KI mice, thus demonstrating that when B cells with bnAb BCRs are present in sufficient numbers, the MPER-656 liposome vaccine is antigenic and immunogenic for their activation (15). Despite the immunological tolerance that results in deletion of the majority of 2F5-recognizing B cells in bone marrow

from 2F5 KI mice, the small population of 2F5 BCR-bearing B cells that emerge as anergic B cells can be activated to produce 2F5 bnAbs in plasma, and are competent to undergo class-switching, activation and expansion in response to immunization resulting in potent serum IgG neutralizing responses (15).

Thus, in principle, overcoming tolerance of self-reactive bnAb-expressing B cells by immunization is a feasible part of an antibody-based HIV-1 vaccine strategy. Hence, a major goal of the human clinical trial of MPER-656 liposomes is to determine if the germline-targeting immunogen can expand MPER-targeted bnAb precursors in humans.

4.7.1.2 VMU #048

In a second study, a related strain of 2F5 KI mice were used, that expressed the VH and VL of the germline unmutated ancestor (UA) of the 2F5 antibody. These 2F5-UA mice manifested even greater B cell deletion than the 2F5-mature VH + VL KI mice (32). After six immunizations of these 2F5-UA mice with MPER-656 liposomes, the remaining anergic B cells were capable of being activated by germline-binding immunogens to make gp41-reactive immunoglobulin M (IgM), but experienced limited class-switching to IgG and limited somatic mutations required for neutralizing activity (32). This study demonstrated that immunization with MPER-656 liposomes can at least partially rescue anergic bnAb precursors by inducing activation and expansion of the population of 2F5 UA BCR-bearing naïve B cells.

4.7.1.3 NHP Study #22

An early study in rhesus macaques evaluated the ability of MPER-656 liposomes to initiate gp41-specific B cell lineages. This study utilized an immunization regimen of a prime with vaccinia virus-expressed HIV-1 JRFL gp140 Env and a combination of HIV-1 JRFL gp140 Env protein and MPER-656 liposome boosts, using MPLA, R848 and ODN10103 (CpG) as the MPER-656 liposome adjuvant, followed by multiple MPER-656 liposome boosts as described in Zhang et al, 2016 (19). Immunized macaques made B cell clonal lineages targeted to the 2F5 bnAb epitope, but 2F5-like antibodies were either deleted or did not attain sufficient affinity for gp41-lipid complexes to achieve the neutralization potency of 2F5 (19). This limitation of neutralization potency was shown to be due to MPER-reactive B cell control by tolerance mechanisms preventing mutations to generate the needed hydrophobic HCDR3 regions needed for bnAb breadth (19).

4.7.1.4 NHP Study #204 and #205

Two NHP studies were designed to evaluate the effects of the adjuvant regimens (GLA or alum) on the immunogenicity of the MPER-656 liposome product. NHP 204 tested the immunogenicity of the MPER-656 liposome product formulated with GLA or GLA plus alum (n=8 per group), and NHP 205 tested the MPER-656 liposome vaccine with alum alone (n=7), as described by Bradley et al. (18). Rhesus macaques were immunized with 1 mg of MPER-656 liposomes six times,

six weeks apart, formulated with either 25 mcg of GLA, or 500 mcg alum, or a combination of both adjuvants.

After 2 immunizations in rhesus macaques, MPER-656-specific antibodies were present in all animals in the alum alone and GLA plus alum arms but not in the GLA-alone adjuvant arm. Sequential vaccinations did not further boost the immune response above that observed following the 2nd injection, perhaps due to hyperimmunization at 6-week intervals (18), a period shorter than that proposed for the current study.

These NHP studies demonstrated that the MPER peptide contained within liposomes was antigenic and can be used to generate antibodies against the 2F5 epitope in immunized NHPs after >1 immunization. The data also support the use of alum as an adjuvant in this phase 1 clinical study. A neutralizing antibody response was not detected in these NHPs, likely due to the immune tolerance control mechanisms indicated above for NHP 22 study (Section 4.7.1.3), but data from that study did indicate that the vaccine regimen could begin the maturation process for 2F5-like antibodies, and this phase 1 study will determine whether the same initiation of bnAb lineages can occur in humans.

4.7.1.5 NHP Study #208

A dose study was performed in Rhesus macaques (NHP 208) to test three vaccine doses of MPER-656 liposomes mixed with alum (Alhydrogel®). This study covered the dose range used for the preclinical GLP toxicity study and the proposed HVTN clinical study (0.2, 1.0, and 2.0 mg MPER-656 liposomes, all with 0.5 mg alum adjuvant). There were also 2 control groups—2.0 mg MPER liposomes without alum, and empty liposomes plus 0.5 mg alum (N=4/ group). All animals were immunized at weeks 0, 4, and 8 (three immunizations) and bled on the immunization days, two weeks after each immunization, and a final bleed was collected at 12 weeks (four weeks after the last immunization) for antibody binding titers. Plasma antibody binding results at the week 12 timepoint showed that adjuvant was required to achieve the highest MPER-binding titers in this study, but there was no significant difference in MPER antibody titers between the tested doses (Figure 4-2).

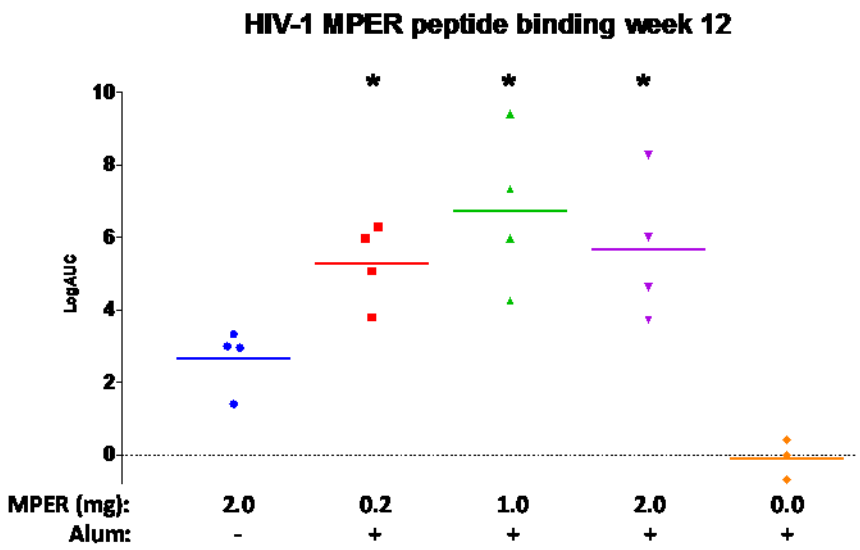

Figure 4-2 Alum adjuvant significantly enhances MPER-specific antibodies in rhesus macaques. At week 12, four weeks after the final immunization, MPER-binding plasma antibodies were detected by ELISA. Data are shown as the log-transformed area under the curve (AUC) values with each data point representing a single animal. Alum significantly enhanced antibodies levels at all MPER-peptide liposome doses, and there were no significant differences between the three doses ($P < 0.05$; Wilcoxon Mann-Whitney compared to no adjuvant).

There is only a 4-fold difference between the lowest and highest doses planned for the clinical trial. There was no significant difference in MPER-specific binding antibodies tested at week 12 between any of the treatment groups in this NHP study.

4.7.1.6 Rabbit study R25

An early immunogenicity study was performed in rabbits to confirm relevance of this animal model for use in the toxicity study. Only the high dose was used in this study, 2.0 mg of MPER-656 liposomes containing 1.0 mg alum. The data demonstrated that the MPER-656 liposome vaccine was immunogenic, supporting the rabbit as an appropriate model for the GLP toxicity study, and no safety concerns were observed.

Three rabbits received an IM immunization every 2 weeks (for a total of 5 immunizations) with 2 mg of peptide in MPER-656 liposome vaccine formulated with alum (1.0 mg of total aluminum). Bleeds were taken 10 days after each immunization. Results (measured by direct-binding ELISA) for longitudinal binding of serum antibodies from R25 rabbits to the MPER-656 (MPER 656-GTH1) and the 2F5 bnAb epitope (sp62 peptide QQEKNEQELLELDKWASLWN) are shown in Figure 4-3.

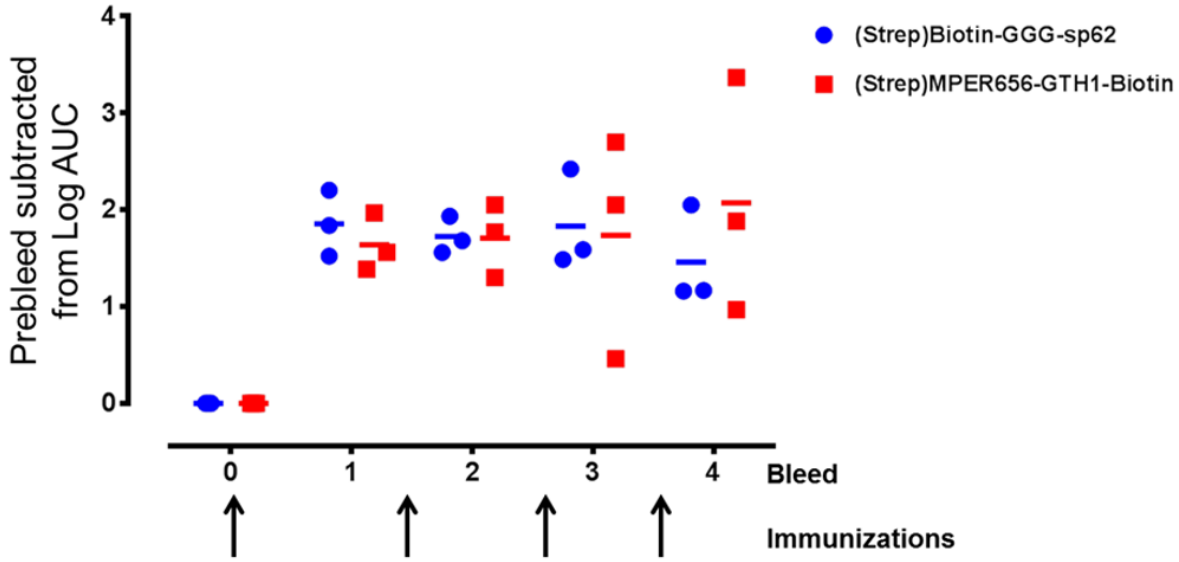

Figure 4-3 Longitudinal binding by direct-binding ELISA of serum antibodies from R25 rabbits to the MPER-peptide (MPER656-GTH1) and the 2F5 bnAb epitope (sp62). Animals were immunized every 3 weeks, as indicated by arrows. AUC: area under the curve. Horizontal lines indicate the geometric mean.

Immunization with 2 mg of MPER-656 liposome vaccine formulated with alum (1.0 mg total aluminum) induced antibodies in rabbits that bound to the GTH1-656 MPER peptide and the 2F5 bnAb epitope peptide sp62. Sequential vaccinations did not further boost the immune response above that observed following the 1st injection, possibly due to hyperimmunization at 3-week intervals.

4.8 Clinical studies

4.8.1 Rationale for human trials

Preclinical trials have shown that immunization with HIV-1 MPER peptide-liposome vaccines can initiate bnAb lineages against HIV-1 gp41 (15) in mice and non-human primate models (25). The purpose of this trial will be to determine if MPER-directed bnAb precursors can be expanded in humans. The primary objective of this phase 1 study will be to determine the safety of the MPER peptide liposome vaccine in healthy adults. In previous studies with small animals (guinea pigs) an MPER peptide immunogen dose of 100 mcg per animal induced antibodies that bound to peptides that included the bnAb 2F5 epitope (25). A higher immunogen dose of 1 mg administered to NHP induced antibodies that mapped to the core (^{664}DKW) 2F5 epitope and also bound avidly to the conformationally relevant gp41-inter and 2F5 scaffold proteins (25). Thus, in human vaccinees, we will evaluate doses of 0.5 mg and 2 mg of peptide in a dose escalation fashion. Preclinical trials utilizing doses as high as 2 mg peptide in liposomes have shown no adverse effects. This study will evaluate two doses over a longer period of time (4 immunizations over a 12-month period). Safety and

tolerability will continue to be the primary endpoints but immunogenicity, including binding antibody that maps to the 2F5-epitope, expansion of antibodies with characteristics of MPER-directed bnAb B-cell lineages, and evaluation of sera for the production of bnAbs will be important endpoints.

The MPER peptide included in the vaccine includes epitopes for both 2F5 and 4E10 bnAbs. Despite the inclusion of the 4E10 epitope, antibodies to this area of the peptide have not been elicited in any preclinical trials (15). This is felt to be due to the fact that the 4E10 epitope is located adjacent to the anchoring tag and thus is likely not well presented. For this reason, the immunological endpoints in this study will be directed to the 2F5-epitope. As noted in Section 4.4, some MPER bnAbs including 2F5 and 4E10 monoclonal antibodies (mAbs) have autoreactivity. The preclinical data presented above demonstrate that this immunogen does not elicit pathological antibodies supporting the safe use of the MPER-656 liposome vaccine in humans. We do not anticipate problems with those kinds of antibodies in this trial, but our monitoring plan is designed to capture those problems should they arise.

Development of bnAbs appears to be a lengthy process in HIV-1–infected persons, and vaccines to elicit bnAbs will likely require sequential immunogens over a long period of time. This trial will evaluate whether MPER peptide liposomes can expand 2F5-epitope directed bnAb precursor B cell pools in humans, a critical first step in the development of a vaccine to elicit MPER-directed bnAbs.

4.9 Potential risks of study products and administration

Table 4-3 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 Primary objectives and endpoints

Primary objective 1:

To evaluate the safety and tolerability of different doses of a prime-boost regimen of MPER-656 liposomes in HIV-uninfected healthy adults

Primary endpoint 1:

- Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and AEs and SAEs

Primary objective 2:

To evaluate binding antibody responses elicited by different doses of the MPER-656 liposome vaccine

Primary endpoint 2:

- MPER-peptide-specific IgG binding Ab responses as assessed by binding Ab multiplex assay 2 weeks after the 3rd and 4th vaccination with MPER-656 liposome vaccine

5.2 Secondary objectives and endpoints

Secondary objective 1:

To assess other vaccine elicited antibodies including the mapping of MPER-specific binding antibody responses and lipid-specific antibody responses

Secondary endpoint 1:

- MPER-peptide specific antibody responses as assessed by binding Ab multiplex assay and/or other binding assays using an array of antigens that identify epitope- and lipid-specific responses to the vaccine 2 weeks after the 3rd and 4th vaccinations with MPER-656 liposomes

Secondary objective 2:

To evaluate the ability of different doses of the vaccine regimen to elicit HIV-specific nAbs

Secondary endpoint 2:

- nAb responses-against HIV-1 viral isolates 2 weeks after the 3rd and 4th vaccinations with MPER-656 liposomes

5.3 Exploratory objectives

Exploratory objective 1:

To determine the frequency and phenotype of MPER-specific B cells

Exploratory objective 2:

To isolate single B cells with desired specificities and determine lineage characteristics. Monoclonal antibodies may be evaluated for binding and neutralization including tier 2 virus bnAb activity and 2F5 and 10E8 binding site binding. Serum antibodies may be evaluated as well.

Exploratory objective 3:

To evaluate HIV-specific T-cell responses induced by the MPER-656 liposome vaccine

Exploratory objective 4:

To assess vaccine-induced blood follicular helper T-cell (Tfh) responses

Exploratory objective 5:

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

Exploratory objective 6:

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

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 24 healthy, HIV-uninfected adult participants aged 18 to 50 years old at low risk of HIV infection in regions where clade B is the predominant clade.

Enrollment will be concurrent with receiving the first study vaccination, thus all participants will provide some safety data. For immunogenicity analyses, however, it is possible that data may be missing for various reasons such as participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). Immunogenicity data from 11 phase 1 and 1 phase 2a HVTN trials, which began enrolling after June 2005 (data as of June 2011), indicate that 10% is a reasonable estimate for the rate of missing data. For this reason, the sample size calculations in Section 6.1.2 account for 10% of enrolled participants having missing data for the primary immunogenicity endpoint.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to identify SAEs can be expressed by the true event rate above which at least 1 event would likely be observed and the true event rate below which no events would likely be observed. Specifically, in the Group 1 vaccine arm (n = 5), there is at least a 90% chance of observing at least one event, if the true rate is 37% or more, and there is at least a 90% chance of observing no events, if the true rate is 1% or less. In the Group 2 vaccine arm (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, and there is at least a 90% chance of observing no events if the true rate is 0.6% or less. In all vaccine arms of the study combined (n = 20), there is at least a 90% chance of observing at least one event, if the true rate is 10.9% or more, and there is at least 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 conducted in the US, about 1.7% 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 15 and 5 are presented in Table 6-1 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 15 and 5, for different true event rates

True event rate (%)	Pr(0/15)	Pr(1+/15)	Pr(2+/15)	Pr(0/5)	Pr(1+/5)	Pr(2+/5)
1	86	14	1	95.1	4.9	0.1
5	46.3	53.7	17.1	77.4	22.6	2.3
10	20.6	79.4	45.1	59	41	8.1
20	3.5	96.5	83.3	32.8	67.2	26.3
30	0.5	99.5	96.5	16.8	83.2	47.2
40	0	100	99.5	7.8	92.2	66.3

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 6-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method (33). If none of the 20 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 16%. For the Group 1 vaccine arm (n = 5), the 2-sided upper confidence bound for this rate is 43%. For the Group 2 vaccine arm (n = 15), the 2-sided upper confidence bound for this rate is 20%.

Table 6-2 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints for arms of size 5, 15 and 20

Observed event rate	95% Confidence interval (%)
0/5	[0, 43.4]
1/5	[3.6, 62.4]
2/5	[11.8, 76.9]
0/15	[0, 20.4]
1/15	[1.2, 29.8]
2/15	[3.7, 37.9]
0/20	[0, 16.1]
1/20	[0.9, 23.6]
2/20	[2.8, 30.1]

6.1.2 Sample size calculations for immunogenicity

The main goals of this trial regarding immunogenicity outcomes involve a preliminary estimation of rates of "response" (defined as binding response positivity to the 2F5 epitope) based on data from immune assays among vaccinees. To address binding antibody endpoints, the analysis will descriptively summarize binding response positivity call rates, and for the comparison of Group 1 vs 2, test superiority of the rate of responses targeting the 2F5 binding site by Barnard's exact test. No adjustment for multiple comparisons will be made for the use of multiple assays. 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 6-3. Calculations are done using the score test method (33). The n = 4, n = 13, and n = 18 (for the two arms combined) assume a 10% rate of missing immunogenicity data.

Table 6-3 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the vaccinees (n = 4, n = 13, and n = 18)

No. of responses	Observed response rate (%)	95% Confidence interval
1/4	25	[4.6, 69.9]
2/4	50	[15, 85]
3/4	75	[30.1, 95.4]
1/13	7.7	[1.4, 33.3]
3/13	23.1	[8.2, 50.3]
5/13	38.5	[17.7, 64.5]
7/13	53.8	[29.1, 76.8]
9/13	69.2	[42.4, 87.3]
3/18	17	[5.8, 39.2]
6/18	33	[16.3, 56.3]
9/18	50	[29, 71]
12/18	67	[43.7, 83.7]
15/18	83	[60.8, 94.2]

An alternative approach is to consider the probability of observing 0, 1 or more, and 50% or more responses for groups of sizes 4, 13 and 18; presented in Table 6-4 for a range of possible true response rates. If the true rate is 20%, then there is a 59% chance of observing any responses in the Group 1 vaccine arm, a 94.5% chance of observing any responses in the Group 2 vaccine arm and a 98% chance of observing at least one response across the two arms. In all cases, the probability of observing 50% or more positive responses is in excess of 80% if the true positivity rate is at least 60%, but when the true rate is below 40%, there is at least a 50% chance of observing fewer than half respond. If the true rate is 50%, then there is a 59% chance of observing at least half (2) respond in the n = 4 Group 1 vaccine arm, a 71% chance of observing at least half (6, rounding down) respond in the n = 13 Group 2 vaccine arm and a 59% chance of observing at least half (9) respond across the two arms combined (n = 18).

Table 6-4 Probability of observing 0 events, 1 or more events, and 50% or more events, among groups of size 4, 13 and 18, for different true event rates

True rate (%)	Pr(0/4)	Pr(1+/4)	Pr(2+/4)	Pr(0/13)	Pr(1+/13)	Pr(6+/13)	Pr(0/18)	Pr(1+/18)	Pr(9+/18)
10	65.6	34.4	5.2	25.4	74.6	0.1	15	85	0
20	41	59	18.1	5.5	94.5	3	1.8	98.2	0.4
30	24	76	34.8	1	99	16.5	0.2	99.8	6
40	13	87	52.5	0.1	99.9	42.6	0	100	26.3
50	6.2	93.8	68.8	0	100	70.9	0	100	59.3
60	2.6	97.4	82.1	0	100	90.2	0	100	86.5
70	0.8	99.2	91.6	0	100	98.2	0	100	97.9
80	0.2	99.8	97.3	0	100	99.9	0	100	99.9
90	0	100	99.6	0	100	100	0	100	100

As shown in Table 6-5, there is limited power for a formal comparison of immunogenicity response rates between vaccine arms of size n = 13 vs n = 4. For either 80% or 90% power, the sizes of differences that the trial is powered to detect are fairly large. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05.

Table 6-5 Power for comparison of response rates between 2 arms (n1 = 13, n2 = 4)

	Minimum true response rate in Arm 2 in order to detect a difference	
True response rate Arm 1 (%)	80% power	90% power
0	89	97
1	90	97
2	91	98
3	92	99
4	92	99
5	93	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. 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 participant treatment arm assignments (eg, vaccine or control). 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 systemic symptoms will be calculated. Wilcoxon rank sum tests will be used to test for differences in severity between arms.

6.4.3.2 AEs and SAEs

AEs will be summarized using 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 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 Dose Escalation

All available safety data through 2 weeks after the first vaccination for Group 1 participants will be evaluated by the PSRT to inform whether to proceed with escalation to the higher dose (Group 2).

6.4.3.5 Reasons for vaccination discontinuation and early study termination

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

6.4.4 Immunogenicity analysis

6.4.4.1 General approach

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

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method (33). 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 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 (34).

In addition to response rate estimates for each timepoint, the probability of observing at least 1 positive response by a given timepoint and the probability of observing more than 1 positive response by a given timepoint will be estimated, with corresponding confidence intervals, for each vaccine arm using maximum likelihood-based methods (35).

For quantitative assay data (eg, binding antibody multiplex assay [BAMA]), 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 set of control arms pooled into one group.

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 data [ie, the observed data are just a random sample of all the potential data]). 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 with methods such as targeted minimum loss-based estimation (TMLE), using parametric generalized linear models fit by maximum likelihood based on appropriate modeling assumptions for the endpoint and adjusted using covariate adjustment, weighting methods or combined with imputation of missing data. 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 only depends upon the observed responses and upon observed covariates, but not upon any unobserved factors. Thus, this assumption is less stringent than the MCAR assumption. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right- censored, then the linear mixed effects models of Hughes (36) 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) (37) 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.

6.4.4.2 Multivariate display of immunogenicity endpoints

Data visualization techniques may be used to explore the relationship among immunogenicity readouts. The set of readouts may be based on one of the primary endpoints (eg, intracellular cytokine staining [ICS]), 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 (38). 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, Env-specific CD8+ T cell total magnitude) 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 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, two 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. Two choices are to compare the M-B curves among vaccine arms, as follows: a nonparametric Wilcoxon rank sum test on the

subject-specific area-under-the-magnitude-breadth (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. 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 133 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 for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 133 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. The Laboratory Program 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 de-identified publication identification numbers (PubIDs) and not participant identification numbers (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 to 50 years

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

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

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

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

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

HIV-Related Criteria:

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

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

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

Laboratory Inclusion Values

Hemogram/Complete blood count (CBC)

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

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

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

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

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

Chemistry

15. **Chemistry panel: alanine aminotransferase (ALT)** < 1.25 times the institutional upper limit of normal; creatinine ≤ 1.1 times the institutional upper limit of normal

Clotting and autoantibodies

16. **Anticardiolipin IgG antibodies** below the upper limit of normal

17. Negative **antinuclear antibodies**

Virology

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

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

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

Urine

21. **Normal urine**:

    - Negative or trace urine protein, and

    - Negative, trace, or 1+ blood urine hemoglobin (if +1 hemoglobin is present on dipstick, a microscopic urinalysis with red blood cells levels within institutional normal range)

Reproductive Status

22. **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 on the day of study product administration.. 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.

23. **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 through the last required protocol clinic visit

    - 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 133 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.

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

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 133 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 133 PSRT will determine eligibility on a case-by-case basis.

8. **Previous receipt of monoclonal antibodies (mAbs)**, whether licensed or investigational; the HVTN 133 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 133 PSRT for vaccines that have subsequently undergone licensure by the FDA. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 133 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 133 PSRT on a case-by-case basis.

10. **Live attenuated vaccines** received within 30 days before first vaccination or scheduled within 14 days after injection (eg, measles, mumps, and rubella

[MMR]; oral polio vaccine [OPV]; varicella; yellow fever; live attenuated influenza vaccine)

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

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

Immune System

13. **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)

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

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

16. **Autoimmune disease, current or history, (**Not exclusionary: mild, well-controlled psoriasis)

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

18. **Immunodeficiency**

Clinically significant medical conditions

19. **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.

20. **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

21. **Any contraindication that would preclude injections into both left and right deltoids**

22. **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.

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

24. **Asthma exclusion criteria:**

    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 had 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.

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

26. **Thyroidectomy, or thyroid disease** requiring medication during the last 12 months (Not exclusionary: well-controlled non-autoimmune thyroid disease)

27. **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.

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

29. **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)

30. **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.

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

32. 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 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.

7.3.1 Delaying vaccinations for a participant

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

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

- Within 30 days prior to any study injection

  - Receipt of live attenuated vaccines
  - Receipt of allergy treatment with antigen injections

- Within 14 days prior to any study injection
  - Receipt of any vaccines that are not live attenuated vaccines (eg, pneumococcal)

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

Vaccinations should not be administered outside the visit window period.

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 2-week interval between a study vaccination and completion of the 2 weeks postvaccination follow-up visit.

7.3.2 Participant departure from vaccination schedule

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

7.3.3 Discontinuing vaccination for a participant

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

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 133 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)
  - 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, vomiting, 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 133 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 133 SSP).

Participants diagnosed with HIV infection during the study should be encouraged to participate in follow-up visits as indicated in Section 9.13.

7.3.4 Participant termination from the study

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

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

8 Study product preparation and administration

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

8.1 Vaccine regimen

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

Group 1

Treatment 1 (T1): MPER-656 liposomes, 500 mcg, admixed with Aluminum Hydroxide Suspension, to be administered as two 0.5 mL doses intramuscularly (IM) at months 0, 2, 6, and 12.

Or

Control 1 (C1): Placebo for MPER-656 liposomes (Sodium Chloride for Injection USP, 0.9%) to be administered as two 0.5 mL doses IM at months 0, 2, 6, and 12.

Group 2

Treatment 2 (T2): MPER-656 liposomes, 2000 mcg, admixed with Aluminum Hydroxide Suspension, to be administered as two 0.5 mL doses IM at months 0, 2, 6, and 12.

Or

Control 2 (C2): Placebo for MPER-656 liposomes (Sodium Chloride for Injection USP, 0.9%) to be administered as two 0.5 mL doses IM at months 0, 2, 6, and 12.

8.2 Study product formulation

8.2.1 MPER-656 Liposomes

MPER-656 liposomes will be provided as a frozen liquid in 2 mL borosilicate glass vials. Each vial contains 1.1 mL of MPER liposomes at a nominal concentration of 2.2 mg/mL. MPER-656 liposomes are a homogenous translucent off-white liquid with consistent visual density and without visible phase separation. MPER liposomes are formulated as a sterile, aqueous buffered solution that is filled in single-use glass vials for IM injection.

The product is stored at -25°C to -10°C. The study product is described in further detail in the IB.

8.2.2 Aluminum Hydroxide Suspension (Alhydrogel®)

The Aluminum Hydroxide Suspension will be provided in 3 mL glass vials. Each vial contains 0.7 mL fill volume at a concentration of 5 mg/mL aluminum. The Aluminum Hydroxide Suspension appears as an opaque, white gelatinous precipitate in aqueous suspension.

The product is stored refrigerated at 2° to 8° C. The study product is described in further detail in the MPER-656 liposomes delivered with Alhydrogel® IB.

8.2.3 Placebo for MPER-656 Liposomes (Sodium Chloride for Injection USP, 0.9%)

Sodium Chloride for Injection USP, 0.9% will be used as the placebo for MPER-656 liposomes. The product must be stored as directed by the manufacturer.

8.3 Preparation of study products

Pharmacists should keep in mind that the preparation instructions below are considered medium risk per USP 38 General Chapter Physical Tests / <797> Pharmaceutical Compounding - Sterile, and should follow the requirements of their country, their institution, and their pharmacy regulatory authority regarding these procedures.

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.

8.3.1 MPER-656 Liposomes, 500 mcg, admixed with Aluminum Hydroxide Suspension (Group 1)

1. Remove one vial of MPER-656 Liposomes from freezer and equilibrate to room temperature for approximately 30-60 minutes.

2. Remove one vial of Aluminum Hydroxide Suspension from refrigerator and equilibrate to room temperature for at least 5 minutes.

3. Using aseptic technique, withdraw 0.5 mL MPER-656 Liposomes and dispense into a sterile empty glass vial.

4. Using aseptic technique, withdraw 1.4 mL Sodium Chloride for Injection USP, 0.9% and dispense into the vial from Step 3.

5. Resuspend Aluminum Hydroxide Suspension by inverting the vial for 30-60 seconds.

6. Using aseptic technique, withdraw 0.2 mL of Aluminum Hydroxide Suspension and inject it into the vial from Step 4. Gently swirl the vial to mix. Allow formulation to incubate at room temperature for 60 minutes with intermittent gentle swirling or inverting of vial every 15-20 minutes.

7. Using aseptic technique, withdraw 0.5 mL of the mixed preparation into a 1 mL syringe with a 23-25 gauge needle attached. Repeat this step to prepare a second syringe. Prior to administration, gently invert syringes to resuspend admixed product.

8. The admixed product is stable at 2-8° C for four hours, after the 60 minute incubation is complete. Each admixed MPER-656 liposomes and alum dose should be kept refrigerated or on ice and injected within four hours after preparation. The admixed product should be kept on ice during transportation from the pharmacy to the clinic and up until the point of administration.

8.3.2 MPER-656 Liposomes, 2000 mcg, admixed with Aluminum Hydroxide Suspension (Group 2)

1. Remove one vial of MPER-656 Liposomes from freezer and equilibrate to room temperature for approximately 30-60 minutes.

2. Remove one vial of Aluminum Hydroxide Suspension from refrigerator and equilibrate to room temperature for at least 5 minutes.

3. Resuspend Aluminum Hydroxide Suspension by inverting the vial for 30-60 seconds.

4. Using aseptic technique, withdraw 0.1 mL of Aluminum Hydroxide Suspension and inject it into the MPER-656 Liposomes vial. Gently swirl the vial to mix. Allow formulation to incubate at room temperature for 60 minutes with intermittent gentle swirling or inverting of vial every 15-20 minutes.

5. Using aseptic technique, withdraw 0.5 mL of the mixed preparation into a 1 mL syringe with a 23-25 gauge needle attached. Repeat this step to prepare a second syringe. Prior to administration, gently invert syringes to resuspend admixed product.

6. The admixed product is stable at 2-8° C for four hours, after the 60 minute incubation is complete. Each admixed MPER-656 liposomes and alum dose should be kept refrigerated or on ice and injected within four hours after preparation. The admixed product should be kept on ice during transportation from the pharmacy to the clinic and up until the point of administration.

8.3.3 Placebo for MPER-656 Liposomes (Sodium Chloride for Injection USP, 0.9%) (Control 1 and Control 2)

1. Remove one vial of Sodium Chloride for Injection USP, 0.9% from storage.

2. Using aseptic technique, withdraw 0.5 mL of Sodium Chloride for Injection USP, 0.9% into a 1 mL syringe with a 23-25 gauge needle attached. Repeat this step to make a second syringe.

3. The placebo should be kept on ice during transportation from the pharmacy to the clinic and up until the point of administration

8.3.4 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)
- HVTN 133 Study Product 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. As there are two injections per visit, one injection will be given in the right deltoid and the other injection will be given in the left deltoid. At sites where registered pharmacists are legally authorized to administer injections, the HVTN CRS may choose to have the pharmacist administer vaccinations.

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

If an injection is administered in the contralateral deltoid due to a medical contraindication, the appropriate study staff should document this clearly. Under

this circumstance, this is NOT a protocol violation. Two injections administered into the same deltoid should be documented in the participant's study record.

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

8.5 Acquisition of study products

MPER-656 liposome will be provided by the International AIDS Vaccine Initiative (IAVI, New York, New York, USA).

Aluminum Hydroxide Suspension will be provided by the NIH, NIAID, Vaccine Research Center (VRC, Bethesda, MD).

Sodium Chloride for Injection USP, 0.9% will not be provided through the protocol and must be obtained by the site. Once an HVTN CRS is protocol registered, the pharmacist can obtain study products from the NIAID Clinical Research Products Management Center (CRPMC) by following the ordering procedures 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 schedule of clinical procedures is shown in Appendix D and Appendix F.

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 (ICF) 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 ICFs.

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 RE for human subjects protection review and approval.

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

9.1.1 Screening consent form

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

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

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

9.1.2 Protocol-specific consent forms

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

Each HVTN CRS is responsible for developing a protocol-specific consent form(s) for local use, based on the sample protocol-specific consent forms in Appendix A and Appendix C. 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 ICFs 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 ICF 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 G).
- Complete physical examination, including height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots
- Laboratory tests, including:
  - Screening HIV test
  - HBsAg
  - Anti-HCV abs
  - Syphilis test
  - CBC with differential
  - Chemistry panel (ALT, AST, alkaline phosphatase, creatinine)

  - Urine dipstick
  - Urine or serum pregnancy test (volunteers who were born female); Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing
  - Anticardiolipin IgG antibodies
  - Antinuclear antibodies
- Administration of behavioral risk assessment questionnaire
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html)
- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.6
- Contraception status assessment. 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 contraception status includes advising a participant who was assigned female sex at birth and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.

9.2.1 Use of screening results from another HVTN study

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

9.3 Enrollment and vaccination visits

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 9.12.

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 9.9).

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

- Risk reduction counseling (as described in Section 9.6);
- Contraception status assessment (as described in Section 9.2 and 9.7). 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 will be performed **at scheduled visits as specified** in Appendix E and Appendix F:

- Administration of behavioral risk assessment questionnaire
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;
- HIV infection assessment including pretest counseling and HIV testing. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate; and
- Specimen collection (should be completed prior to vaccination)

9.4 Follow-up visits

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

- Risk reduction counseling (as described in Section 9.6);
- Contraception status assessment (as described in Section 9.2 and 9.7). 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); and
- Assessment of new or unresolved AEs/intercurrent illnesses.

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

- 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;
- Behavioral risk assessment
- 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;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Specimen collection;
- Clinical laboratory tests including:
  - CBC with differential,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.8); 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. During follow-up in persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.
- AESI health contact (see HVTN 133 SSP).

9.5 AESI health contact

CRS staff will contact study participants 12 months after last vaccination to collect the information listed below. Clinic visits will only be required if HIV

confirmatory testing is necessary (see Section 9.6.1); 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)
  - AEs of special interest (AESI, see Section 11.2.2). A sample list of AESI is provided in Appendix H. AESI are reported regardless of relationship to study product(s);
  - New diagnosis of HIV infection; and
  - Pregnancies and outcomes, including congenital anomalies/birth defects.

9.5.1 Interim contacts

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

9.6 HIV counseling and testing

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

Participants will be counseled routinely during the trial on the avoidance of HIV infection and on the potential negative social impacts of testing 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.

9.6.1 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 F. 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 E and Appendix F). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see HVTN 133 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.

9.6.2 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 9.6.1). In order to provide poststudy HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of poststudy testing and counseling services to HIV vaccine recipients. The registry contains the names of all study participants, unless they request that their names be removed.

9.7 Contraception status

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

9.8 Urine testing

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

If the screening dipstick is transiently abnormal due to 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.

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

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, 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), or those meeting SAE/adverse events requiring expedited reporting according to DAIDS criteria, are recorded on an adverse experience log form.

Table 9-1 Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Baseline: before vaccination	HVTN CRS 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

[a] Day of vaccination

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

9.9.1 Assessment of systemic and local symptoms

Systemic symptoms include increased body temperature, malaise and/or fatigue, myalgia, headache, chills, arthralgia, 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 nonaxillary thermometry. This includes temperatures taken in the clinic, as well as temperatures taken by participants during the reactogenicity period.

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

9.9.2 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 133 SSP for details.

9.10 Visit windows and missed visits

Visit windows are included in Appendix I. Visits conducted outside of the allowable visit windows are considered protocol deviations. Further information about visit windows are described in the HVTN 133 SSP. If a missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

Procedures performed at an interim visit are usually toxicity/safety assessments (including local safety labs) and HIV testing. With the exception of HIV testing, these procedures are performed only if they were required at the missed visit or if

clinically indicated. HIV testing may be performed as deemed appropriate by the study staff. Blood samples for immunogenicity assays are not typically collected at interim visits.

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

9.11 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, chemistry panel, antinuclear antibodies, anticardiolipin IgG antibodies), 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 9.13.

9.12 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given, but 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. If the participant is no longer pregnant, refer to Section 7.3.3.

9.13 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 18 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 133 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 E and Appendix F).

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN 133 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 E. For tests performed locally, the local lab may assign appropriate tube types.

Of note, all assays described below are performed as research assays 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 E. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated, or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 500 mL in any 56-day (8-week) period.

10.3 Primary immunogenicity timepoints

The primary immunogenicity timepoints in this study occur 2 weeks after the third and fourth vaccinations. Endpoint assays may be performed on specimens collected from participants at the primary immunogenicity timepoints and samples collected at baseline and other timepoints; the schedule is shown in Appendix E.

10.4 Endpoint assays: cellular

10.4.1 Flow cytometry: intracellular cytokine staining (ICS)

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

Additional cell surface markers, cytokines, or functional markers may also be analyzed.

10.4.2 Flow cytometry: antigen-specific B cell phenotyping assay

Antigen-specific B cells induced by vaccination will be identified and characterized using fluorescently-labeled recombinant protein probes in combination with a flow cytometry phenotyping panel. In particular, MPER-specific B cells will be enumerated and may be further characterized for expression of memory, activation, inhibitory or other markers by protein and/or gene expression. B cells may also be sorted for further analysis by BCR sequencing or gene expression analysis.

10.4.3 B-cell lineage analysis

MPER-specific B cells may be single cell or bulk sorted. Individual MPER-reactive memory B cells or plasmablasts may be sorted into individual wells of culture plates, expanded in short-term cultures and screened for binding or in vitro neutralization before PCR and VH and VL sequencing. VH and VL genes may be amplified and cloned into an antibody expression backbone and tested for Env binding and HIV neutralization. Finally, next generation sequencing for all VH and VL families may be performed on memory B cells or plasmablasts for VH and VL genes for either single-cells or bulk sorted MPER-specific B cells.

10.4.4 Peripheral T follicular helper cells (pTfh)

Flow cytometry may be used to identify and phenotype T cells in the peripheral blood. pTfh may be characterized based on expression of lineage markers including CXCR5, PD-1, and ICOS on CD4+ T cells. In addition, serum may be used to determine the level of CXCL13 in circulation to monitor the germinal center reaction upon vaccination. The flow cytometry panels might also include additional markers.

10.5 Endpoint assays: humoral

10.5.1 Binding antibody multiplex assay (BAMA)

HIV-1–specific IgG antibodies to MPER epitopes will be assessed on serum samples from study participants taken at the primary immunogenicity timepoints and baseline by Binding Antibody Multiplex Assay (BAMA). 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 assays.

10.5.2 Lipid binding assays

Binding antibodies to lipid antigens will be assessed by binding assays using serum samples from study participants taken at baseline and at the primary immunogenicity timepoints. Specimens from other timepoints may also be assayed based on the results of the initial assays.

10.5.3 Antibody avidity

MPER epitopes that map specificity and kynureninase may be included as part of the binding and avidity index measurements (BAMA-AI). Biolayer Interferometry (BLI) and/or Surface Plasmon Resonance (SPR) technologies may also be utilized to define antibody avidity to the MPER and MPER-liposomes.

10.5.4 Neutralizing antibody assay

HIV-1–specific nAb assays will be performed on serum samples from study participants taken at the primary immunogenicity timepoints. Specimens from the baseline and other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoints. The TZM-bl and TZM-bl/FcγRI assays will test neutralization of three clade B strains (HXB2, W61D.TCLA.71 and SC422661.8) that are highly neutralization-sensitive to antibodies induced in immunized 2F5 knock-in mice. The global panel and/or clade-specific panels may be used to assess Tier 2 neutralization breadth in greater detail (39, 40).

10.6 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.

10.7 Exploratory studies

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

10.8 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 ICF for the main study (see 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 ICF, 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.

10.9 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 133 PSRT

The HVTN 133 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 133 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 133 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 during the main study, as defined in Section 3. 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 133 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 133 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 133 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 133 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 133 SSP);

- 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.

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) and (2) if the AE meets the criteria for a safety pause/prompt AE review (see Section 11.3), and (3) if the AE is listed as an AESI. A list of AESI to be reported in this protocol is provided in Appendix H.

After the main study period, report the subset of AEs bulleted in Section 9.5 until the 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/hvtn133). 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:

- MPER-656 liposomes with 500 mcg alum
- Sodium Chloride for Injection USP, 0.9%

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

After completion of the main study period through the completion of the AESI health contact (see Section 3) the Suspected, Unexpected Serious Adverse Reactions (SUSAR) Reporting Category will be used.

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

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 Safety considerations for dose escalation

In addition to monitoring participant safety throughout the study period, the HVTN 133 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 may occur. The HVTN 133 PSRT may consult with the HVTN SMB on an ad hoc basis for these evaluations.

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 133 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 133 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

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

Event and relationship to study products	Severity	HVTN CRS action[a]	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and submit forms immediately	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately	Immediate 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/hvtn133).

[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 133 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 133 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 133 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 133 PSRT notification or prompt HVTN 133 PSRT AE review is triggered, HVTN Core notifies the HVTN 133 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 133 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 133 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 133 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the HVTN 133 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 133 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.

11.6 Study termination

This study may be terminated early by the determination of the HVTN 133 PSRT, a pertinent national regulatory authority, the 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 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 133 SSP.

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 133 are described below.

Protocol history and modifications

Date: March 13, 2019

Protocol version: 1.0
Protocol modification:

Original protocol

14 Document references (other than literature citations)

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

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines:
  - Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf
  - Revised Guidelines for HIV Counseling, Testing, and Referral. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5019a1.htm
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures
- Division of AIDS Protocol Registration Manual. Available at https://www.niaid.nih.gov/sites/default/files/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Corrected Version 2.1, July 2017. Available at 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 133 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 133 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN 133 Site Lab Instructions. Accessible through the HVTN protocol-specific website.
- HVTN Manual of Operations. Accessible through the HVTN website.

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

- Lab assay algorithm (available upon request).

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

- NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research. Available at 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

Ab	antibody
AE	adverse event
AESI	adverse event of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
ART	antiretroviral therapy
AST	aspartate aminotransferase
AUC	area under the curve
AUC-MB	area-under-the-magnitude-breadth
β-HCG	beta human chorionic gonadotropin
BAMA	binding antibody multiplex assay
BCR	B-cell receptor
BMI	body mass index
bnAb	broadly neutralizing antibodies
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	confidence intervals
CIOMS	Council for International Organizations of Medical Sciences
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS	clinical research site
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
DRVVT	dilute Russell's viper venom time
EAE	expedited adverse event
EC	Ethics Committee
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
Env	HIV-1 envelope
FDA	US Food and Drug Administration
Fred Hutch	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
GLA	Glucopyranosyl Lipid Adjuvant

HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HVTN	HIV Vaccine Trials Network
IAVI	International AIDS Vaccine Initiative
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
IDRI	Infectious Disease Research Institute
IFN-γ	interferon gamma
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin
IM	intramuscular
IND	Investigational New Drug
IP	intraperitoneal
IRB	Institutional Review Board
IUD	intrauterine device
KI	knock-in
KYNU	kynureninase
mAb	monoclonal antibody
MAR	missing at random
M-B	Magnitude–Breadth
MCAR	missing completely at random
MedDRA	Medical Dictionary for Regulatory Activities
MMR	measles, mumps, and rubella
MOP	Manual of Operations
MPER	membrane proximal external region
MPLA	monophosphoryl lipid A
nAb	neutralizing antibody
NAEPP	National Asthma Education and Prevention Program
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NIH	US National Institutes of Health
OHRP	US Office for Human Research Protections
OPV	oral polio vaccine

PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PCA	principal component analysis
PCR	polymerase chain reaction
PI	Principal Investigator
PSRT	Protocol Safety Review Team
PTE	prothrombin time
pTfh	Peripheral T follicular helper cells (pTfh)
PTID	participant identification number
PubID	de-identified publication identification number
RAB	DAIDS Regulatory Affairs Branch
RE	Regulatory Entity
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SAP	Statistical Analysis Plan
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SHIV	simian/human immunodeficiency virus
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
SSP	Study Specific Procedures
SUSAR	Suspected, Unexpected Serious Adverse Reactions
TB	tuberculosis
Tfh	follicular helper T-cell
TNF	tumor necrosis factor
UA	unmutated ancestor
UW-VSL	University of Washington Virology Specialty Laboratory
VH	heavy chain variable region
VISP	Vaccine induced seropositivity
VL	light chain variable region
VRC	Vaccine Research Center (NIAID)

16 Literature cited

1. UNAIDS. Ethical considerations in biomedical HIV prevention trials. 2007 7/2007.

2. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research. 1979 4/18/1979.

3. Council for International Organizations of Medical Sciences (CIOMS). International ethical guidelines for biomedical research involving human subjects. Bull Med Ethics. 2002(182):17-23.

4. Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. Annu Rev Immunol. 2010;28:413-44.

5. McElrath MJ, Haynes BF. Induction of immunity to human immunodeficiency virus type-1 by vaccination. Immunity. 2010;33(4):542-54.

6. Stiegler G, Kunert R, Purtscher M, Wolbank S, Voglauer R, Steindl F, et al. A potent cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of human immunodeficiency virus type 1. AIDS Res Hum Retroviruses. 2001;17(18):1757-65.

7. Hessell AJ, Rakasz EG, Tehrani DM, Huber M, Weisgrau KL, Landucci G, et al. Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. J Virol. 2010;84(3):1302-13.

8. Salzwedel K, West JT, Hunter E. A conserved tryptophan-rich motif in the membrane-proximal region of the human immunodeficiency virus type 1 gp41 ectodomain is important for Env-mediated fusion and virus infectivity. J Virol. 1999;73(3):2469-80.

9. Chen Y, Zhang J, Hwang KK, Bouton-Verville H, Xia SM, Newman A, et al. Common tolerance mechanisms, but distinct cross-reactivities associated with gp41 and lipids, limit production of HIV-1 broad neutralizing antibodies 2F5 and 4E10. J Immunol. 2013;191(3):1260-75.

10. Cambier JC, Gauld SB, Merrell KT, Vilen BJ. B-cell anergy: from transgenic models to naturally occurring anergic B cells? Nat Rev Immunol. 2007;7(8):633-43.

11. Uccellini MB, Busconi L, Green NM, Busto P, Christensen SR, Shlomchik MJ, et al. Autoreactive B cells discriminate CpG-rich and CpG-poor DNA

and this response is modulated by IFN-alpha. J Immunol. 2008;181(9):5875-84.

12. Verkoczy L, Kelsoe G, Moody MA, Haynes BF. Role of immune mechanisms in induction of HIV-1 broadly neutralizing antibodies. Curr Opin Immunol. 2011;23(3):383-90.

13. Verkoczy L, Diaz M, Holl TM, Ouyang YB, Bouton-Verville H, Alam SM, et al. Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. Proc Natl Acad Sci U S A. 2010;107(1):181-6.

14. Verkoczy L, Chen Y, Bouton-Verville H, Zhang J, Diaz M, Hutchinson J, et al. Rescue of HIV-1 broad neutralizing antibody-expressing B cells in 2F5 VH x VL knockin mice reveals multiple tolerance controls. J Immunol. 2011;187(7):3785-97.

15. Verkoczy L, Chen Y, Zhang J, Bouton-Verville H, Newman A, Lockwood B, et al. Induction of HIV-1 broad neutralizing antibodies in 2F5 knock-in mice: selection against membrane proximal external region-associated autoreactivity limits T-dependent responses. J Immunol. 2013;191(5):2538-50.

16. Sather DN, Carbonetti S, Malherbe DC, Pissani F, Stuart AB, Hessell AJ, et al. Emergence of broadly neutralizing antibodies and viral coevolution in two subjects during the early stages of infection with human immunodeficiency virus type 1. J Virol. 2014;88(22):12968-81.

17. Liao HX, Lynch R, Zhou T, Gao F, Alam SM, Boyd SD, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature. 2013;496(7446):469-76.

18. Bradley T, Yang G, Ilkayeva O, Holl TM, Zhang R, Zhang J, et al. HIV-1 Envelope Mimicry of Host Enzyme Kynureninase Does Not Disrupt Tryptophan Metabolism. J Immunol. 2016;197(12):4663-73.

19. Zhang R, Verkoczy L, Wiehe K, Munir Alam S, Nicely NI, Santra S, et al. Initiation of immune tolerance-controlled HIV gp41 neutralizing B cell lineages. Sci Transl Med. 2016;8(336):336ra62.

20. Shen X, Dennison SM, Liu P, Gao F, Jaeger F, Montefiori DC, et al. Prolonged exposure of the HIV-1 gp41 membrane proximal region with L669S substitution. Proc Natl Acad Sci U S A. 2010;107(13):5972-7.

21. Dennison SM, Stewart SM, Stempel KC, Liao HX, Haynes BF, Alam SM. Stable docking of neutralizing human immunodeficiency virus type 1 gp41 membrane-proximal external region monoclonal antibodies 2F5 and 4E10 is

dependent on the membrane immersion depth of their epitope regions. J Virol. 2009;83(19):10211-23.

22. Sun ZY, Oh KJ, Kim M, Yu J, Brusic V, Song L, et al. HIV-1 broadly neutralizing antibody extracts its epitope from a kinked gp41 ectodomain region on the viral membrane. Immunity. 2008;28(1):52-63.

23. Montero M, van Houten NE, Wang X, Scott JK. The membrane-proximal external region of the human immunodeficiency virus type 1 envelope: dominant site of antibody neutralization and target for vaccine design. Microbiol Mol Biol Rev. 2008;72(1):54-84, table.

24. Alam SM, McAdams M, Boren D, Rak M, Scearce RM, Gao F, et al. The role of antibody polyspecificity and lipid reactivity in binding of broadly neutralizing anti-HIV-1 envelope human monoclonal antibodies 2F5 and 4E10 to glycoprotein 41 membrane proximal envelope epitopes. J Immunol. 2007;178(7):4424-35.

25. Dennison SM, Sutherland LL, Jaeger FH, Anasti KM, Parks R, Stewart S, et al. Induction of antibodies in rhesus macaques that recognize a fusion-intermediate conformation of HIV-1 gp41. PLoS ONE. 2011;6(11):e27824.

26. Haynes BF, Fleming J, St Clair EW, Katinger H, Stiegler G, Kunert R, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005;308(5730):1906-8.

27. Vcelar B, Stiegler G, Wolf HM, Muntean W, Leschnik B, Mehandru S, et al. Reassessment of autoreactivity of the broadly neutralizing HIV antibodies 4E10 and 2F5 and retrospective analysis of clinical safety data. AIDS. 2007;21(16):2161-70.

28. Gray ES, Madiga MC, Hermanus T, Moore PL, Wibmer CK, Tumba NL, et al. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. J Virol. 2011;85(10):4828-40.

29. Landais E, Huang X, Havenar-Daughton C, Murrell B, Price MA, Wickramasinghe L, et al. Broadly Neutralizing Antibody Responses in a Large Longitudinal Sub-Saharan HIV Primary Infection Cohort. PLoS Pathog. 2016;12(1):e1005369.

30. Gray ES, Madiga MC, Moore PL, Mlisana K, Abdool Karim SS, Binley JM, et al. Broad neutralization of human immunodeficiency virus type 1 mediated by plasma antibodies against the gp41 membrane proximal external region. J Virol. 2009;83(21):11265-74.

31. Easterhoff D, Moody MA, Fera D, Cheng H, Ackerman M, Wiehe K, et al. Boosting of HIV envelope CD4 binding site antibodies with long variable

heavy third complementarity determining region in the randomized double blind RV305 HIV-1 vaccine trial. PLoS Pathog. 2017;13(2):e1006182.

32. Alam SM, Morelli M, Dennison SM, Liao HX, Zhang R, Xia SM, et al. Role of HIV membrane in neutralization by two broadly neutralizing antibodies. Proc Natl Acad Sci U S A. 2009;106(48):20234-9.

33. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat. 1998;52(2):119-26.

34. Lydersen S, Fagerland MW, Laake P. Recommended tests for association in 2 x 2 tables. Stat Med. 2009;28(7):1159-75.

35. Hudgens MG. Estimating cumulative probabilities from incomplete longitudinal binary responses with application to HIV vaccine trials. Statistics in Medicine. 2003;22:463-79.

36. Hughes JP. Mixed effects models with censored data with application to HIV RNA levels. Biometrics. 1999;55(2):625-9.

37. Rotnitzky A, Robins J. Analysis of semi-parametric regression models with non-ignorable non-response. Stat Med. 1997;16(1-3):81-102.

38. James G, Witten D, Hastie T, Tibshirani R. An introduction to statistical learning with applications in R. New York: R. Springer; 2013 2013.

39. Seaman M, Janes H, Hawkins N, randpre L, Devoy C, Giri A, et al. Tiered Categorization of a Diverse Panel of HIV-1 Env Pseudoviruses for Assessment of Neutralizing Antibodies. J Virol. 2010;84(3):1439-52.

40. DeCamp A, Hraber P, Bailer RT, Seaman MS, Ochsenbauer C, Kappes J, et al. Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. J Virol. 2014;88(5):2489-507.

Appendix A Sample informed consent form

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of an HIV-1 gp41 MPER-656 liposome vaccine in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 133

Site: [Insert site name]

Thank you for your interest in our research study. Please read this consent form or ask someone to read it to you. If you decide to join the study, we will ask you to sign or make your mark on this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test an HIV vaccine. HIV is the virus that causes AIDS.

About 24 people will take part in this study at multiple sites. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

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

- 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? (Your immune system protects you from disease.)
- Does the 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 is called MPER-656 liposomes. From here on out, we will just call it the study vaccine.

The study vaccine has not been given to people before. It is an experimental HIV vaccine. That means we do not know if the study vaccine will be safe to use in people, or if it will work to prevent HIV infection.

The study vaccine has been tested in animals and appears safe. Even if something looks like it is safe or works in animals, it may not be true for people. The study vaccine is used only in research studies.

The study vaccine was developed by Duke University, working with the Infectious Disease Research Institute (IDRI). This study vaccine has man-made, short pieces of a protein called a peptide. This peptide looks like part of a protein found in HIV. The peptide in this study vaccine is combined with a tiny bit of fat called a liposome. The liposome helps keep the peptide in a shape that might make it easier for your immune system to respond to it. If you want to know more about the study vaccine, ask the study staff.

The study vaccine is mixed with an adjuvant. An adjuvant is a substance added to the vaccine to help the immune system respond better. The adjuvant in this study is called aluminum hydroxide or alum. Aluminum has been used in vaccines for more than 60 years.

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.

Risks of the study vaccine:

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

After getting the study vaccine, you could make antibodies that recognize parts of cells in humans. These are called autoantibodies. In animal studies, these autoantibodies did not show effects on the health of the animals.

Liposomes have been used for decades in cancer vaccines, animal vaccines, and drug treatments. When injected, liposomes can cause symptoms like other vaccines. The most common are redness and swelling where you got the injection and fever.

The adjuvant, alum, is the most widely used vaccine adjuvant. It has been used in licensed vaccines given to hundreds of millions of people all over the world. People can have the same kinds of side effects from the adjuvant as they do with vaccines, such as pain where they got the injection, muscle aches, or a fever.

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, which will tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also do tests to see if you currently have autoantibodies. We will test for syphilis, 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 could become pregnant, you must agree to use birth control to join this study.

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

You should not become pregnant during the study because we do not know how the study vaccine could affect the developing baby. You must agree to use effective birth control from at least 21 days before your first injection through the last required protocol clinic visit. We will talk to you about effective birth control methods. They are listed on a handout that we will give to you.

Being in the study

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

9. You will come to the clinic for scheduled visits about [#] times over about a year and a half.

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 will contact you 6 months after your last scheduled visit to the clinic to check on your health. (For example, we will ask you if you had any side effects that you think may be related to the study vaccine or any of the study procedures.)

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

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

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

US sites: Include the following paragraph. 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. We will compare the results from people who got the placebo with results from people who got the study vaccine. In this study, the placebo is sterile salt water.

You have a 5-in-6 chance of getting the study vaccine. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.*

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 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 two groups. You will get two injections, one into each of your upper arms at four visits during the study. Group 1 will be enrolled first, and then Group 2 will be enrolled.

		Injection Schedule			
	Group	First injection	2 months	6 months	12 months
5 people	1 Low dose (Study vaccine at 0.5 mg/mL with 500 mcg alum)	MPER with alum adjuvant	MPER with alum adjuvant	MPER with alum adjuvant	MPER with alum adjuvant
1 person		Placebo	Placebo	Placebo	Placebo
15 people	2 Higher dose (Study vaccine at 2 mg/mL with 500 mcg alum)	MPER with alum adjuvant	MPER with alum adjuvant	MPER with alum adjuvant	MPER with alum adjuvant
3 people		Placebo	Placebo	Placebo	Placebo
Total 24 people (20 study vaccine recipients and 4 placebo recipients)					

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 10 mL and 340 mL (1 tablespoon to a little less than 1 and a half cups). Your body will make new blood to replace the blood we take out.

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

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

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

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. 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. *[Site: choose one of the following two sentences. African sites*

should choose the sentence referencing the repository in South Africa. All other sites should choose the sentence referencing the repository in the United States.] Your samples will be stored in the HVTN repository in South Africa. Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

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

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

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

Researchers may also do genetic testing on your samples.

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

If you agree, your samples could also be used for genome-wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers

compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

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

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- 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] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- The Division of AIDS and people who work for them,
- The HVTN and people who work for them,
- Infectious Disease Research Institute,
- Duke University,
- The Safety Monitoring Board and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. 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 18 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.

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,
- you enroll in a different research study where you get another study product, 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.

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

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, there is a process to decide if it is related to the [study product(s)] and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

The study product provider may agree to pay medical costs for study-related injuries that are determined to be caused by the study product.

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

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

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

Questions

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.

Your permissions and signature

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

26. In Section 15 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 B Approved birth control methods for persons assigned female sex at birth (for 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 at least 21 days before your first injection through the last required protocol clinic visit.

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 C 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 an HIV-1 gp41 MPER liposome vaccine in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 133

Site: [Insert site name]

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

1. Do I have to agree?

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

2. Where are the samples stored?

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

3. How long will the samples be stored?

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

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

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

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

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

6. Will the HVTN sell my samples and information?

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

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

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

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

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

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

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

Researchers may also do genetic testing on your samples.

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

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

10. What are the risks of genetic testing?

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

US Sites, include the following paragraph

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

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

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- 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 D Table of procedures (for sample informed consent form)

			Time after first injection visit									
Procedure	Screening visit(s)	First injection visit	2 weeks	2 months	2½ months	6 months	6½ months	9 months	12 months	12¼ months	12½ 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.

Appendix E Laboratory procedures

					Visit:	1	2	3	4	5	6	7	8	9	10	11	12	
					Day:	Screening	D0	D14	D56	D70	D168	D182	D273	D364	D371	D378	D546	
					Month:	visit[3]	M0	M0.5	M2	M2.5	M6	M6.5	M9	M12	M12.25	M12.5	M18	
							VAC1		**VAC2**		**VAC3**			**VAC4**				
Procedure	**Ship to[1]**	**Assay Location[2]**	**Tube[4]**	**Tube size (vol. capacity)[4]**			**MPER or Placebo**		**MPER or Placebo**		**MPER or Placebo**			**MPER or Placebo**				**Total**
BLOOD COLLECTION																		
Screening or diagnostic assays																		
Screening HIV test	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	5
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	5
Syphilis	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	5
Anticardiolipin antibodies (ACA), IgG	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	5
Antinuclear antibodies (ANA)	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	—	—	—	—	—	—	5
HIV diagnostics[9]	UW-VSL	UW-VSL	EDTA	10mL		—	—	—	—	10	10	—	10	10	—	—	20	60
Safety labs[10]																		
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
Autoantibody assays																		
Antinuclear antibodies (ANA)	CSR	Non-HVTN Labs at Duke-DHVI	SST	8.5mL		—	y	y	—	y	—	y	—	—	—	y	y	0.0
Anticardiolipin antibodies (ACA), IgG	CSR	Non-HVTN Labs at Duke-DHVI	SST	8.5mL		—	y	y	—	y	—	y	—	—	—	y	y	0.0
Host genetics[7]	CSR	HVTN Labs	ACD	8.5mL		—	17	—	—	—	—	—	—	—	—	—	—	17
Immunogenicity assays[6]																		
Humoral assays																		
HIV-1 binding Ab	CSR	HVTN Labs	SST	8.5mL		—	***8.5***	8.5	—	8.5	—	***8.5***	—	—	—	***8.5***	8.5	51
Lipid binding Ab	CSR	HVTN Labs	SST	8.5mL		—	y	y	—	y	—	***y***	—	—	—	***y***	y	0
Ab avidity	CSR	HVTN Labs	SST	8.5mL		—	y	y	—	y	—	***y***	—	—	—	***y***	y	0
Neutralizing Ab	CSR	HVTN Labs	SST	8.5mL		—	8.5	8.5	—	8.5	—	***8.5***	—	—	—	***8.5***	8.5	51
Cellular assays																		
HIV-specific ICS	CSR	HVTN Labs	ACD	8.5mL		—	—	—	—	42.5	—	42.5	—	—	—	42.5	42.5	170
Phenotyping (pTfh)	CSR	HVTN Labs	ACD	8.5mL		—	42.5	—	—	—	—	—	—	42.5	42.5	—	42.5	170
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL		—	42.5	42.5	—	42.5	—	42.5	—	—	42.5	42.5	42.5	297.5
B-cell lineage	CSR	HVTN Labs	ACD	8.5mL		—	34	34	—	34	—	34	—	—	34	34	34	238
Specimen storage																		
PBMC	CSR		ACD	8.5mL		—	51	51	—	85	—	85	—	—	25.5	85	85	467.5
Serum	CSR		SST	8.5mL		—	17	17	—	17	—	17	—	8.5	—	17	17	110.5
Visit total						**35**	**221.0**	**172**	**0**	**258.0**	**10**	**248.0**	**10**	**61**	**145**	**248.0**	**301**	**1707.5**
56-Day total						**35**	**256.0**	**427.5**	**427.5**	**429.5**	**10**	**258.0**	**10**	**61**	**206**	**454**	**301**	
URINE COLLECTION[10]																		
Urine dipstick[11]	Local lab	Local lab				X	—	X	—	—	—	—	—	—	—	X	—	
Pregnancy test[8]	Local lab	Local lab				X	X	—	X	—	X	—	—	X	—	X	—	

[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). Non-HVTN laboratories: Duke Human Vaccine Institute (Durham, North Carolina, USA)

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

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

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

[6] Immunogenicity assays will be performed at M0 (for binding Ab assay), M6.5, and M12.5. Depending on the initial results, assays for humoral and cellular responses may be performed on samples collected from participants at other timepoints. Immunogenicity assays at M6.5 may begin as samples become available, and are not contingent on data from the primary immunogenicity timepoint at M12.5.

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

[8] For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens within 24 hours of vaccination with negative results received prior to vaccination. Persons who have undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[9] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.11), blood should be drawn for HIV diagnostic testing, as shown for visit 12 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.13).

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

[11] And microscopy if needed.

y = SST collected for serum storage will also cover specimen needs for autoantibody assays, Ab avidity, and lipid binding Ab assays; no separate blood draw is needed.

Appendix F Procedures at HVTN CRS

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11	12	13	Post
Day:		D0	D14	D56	D70	D168	D182	D273	D364	D371	D378	D546	D728	
Month:		M0	M0.5	M2	M2.5	M6	M6.5	M9	M12	M12.25	M12.5	M18	M24	
Procedure	Scr.	VAC1		VAC2		VAC3			VAC4				AESI	
Study procedures[2]														
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Signed protocol consent	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	X	—	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	—	—	—
Risk reduction counseling[3]	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Contraception status assessment[4]	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Behavioral risk assessment[5]	X	—	—	—	—	X	—	—	X	—	—	X	—	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	X	X	X	—	—
Social impact assessment questionnaire	—	—	—	X	—	X	—	—	—	—	—	X	__	—
Outside testing and belief questionnaire	—	—	—	—	—	X	—	—	—	—	—	X	__	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	—	—
HIV infection assessment[6]	X	—	—	—	X	X	—	X	X	—	—	X	—	—
Confirm HIV test results provided to participant	—	X	—	—	—	X	X	—	X	X	—	X	—	—
Specimen collection (see Appendix E)[7]	X	X	X		X	X	X	X	X	X	X	X		
Vaccination procedures [5]														
Vaccination[8]	—	X	—	X	—	X	—	—	X	—	—	—	—	—
Reactogenicity assessments[9]	—	X	—	X	—	X	—	—	X	—	—	—	—	—
AESI health contact[10]														
AESI assessment	—	—	—	—	—	—	—	—	—	—	—	—	X	—
Poststudy														
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	X

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

[2] For specimen collection requirements, see Appendix E.

[3] Includes transmission risk reduction counseling for HIV-infected participants. Conduct **after** BRA questionnaire if both occur at same visit.

[4] Contraception status assessment is required only for participants who were assigned female sex at birth and are capable of becoming pregnant. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[5] Not applicable to HIV-infected participants. Conduct **before** Risk Reduction Counseling if both occur at same visit.

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

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

[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.

[9] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.9).

[10] Clinic visits are not required unless participant indicates symptoms that require further assessment. See Appendix H.

Appendix G 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 meets these guidelines:

1. Sexual behaviors

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

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

AND

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

In the **last 6 months**:

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

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

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

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

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

AND

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

2. Non-sexual behaviors

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

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

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

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

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

Appendix H Adverse events of special interest

Adverse events of special interest (AESIs) for this protocol include but are not limited to unexpected induction of autoimmune or auto-inflammatory diseases; representative examples of AESIs are listed below.

Gastrointestinal disorders	Liver disorders	Metabolic diseases
• Celiac disease • Crohn's disease • Ulcerative colitis • Ulcerative proctitis	• Autoimmune cholangitis • Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis	• Addison's disease • Autoimmune thyroiditis (including Hashimoto thyroiditis) • Diabetes mellitus type I • Grave's or Basedow's disease
Neuroinflammatory disorders	**Musculoskeletal disorders**	**Skin disorders**
• Acute disseminated encephalomyelitis, including site specific variants (eg, non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis) • Cranial nerve disorders, included paralyses/paresis (eg, Bell's palsy) • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy • Multiple sclerosis • Narcolepsy • Optic neuritis • Transverse Myelitis • Myasthenia gravis, including Eaton-Lambert syndrome	• Antisynthetase syndrome • Dermatomyositis • Juvenile chronic arthritis (including Still's disease) • Mixed connective tissue disorder • Polymyalgia rheumatic • Polymyositis • Psoriatic arthropathy • Relapsing polychondritis • Rheumatoid arthritis • Scleroderma, including diffuse systemic form and CREST syndrome • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Systemic lupus erythematosus • Systemic sclerosis	• Alopecia areata • Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis • Cutaneous lupus erythematosus • Erythema nodosum • Morphoea • Lichen planus • Psoriasis • Sweet's syndrome • Vitiligo
Vasculitides	**Others**	
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis	• Antiphospholipid syndrome • Autoimmune hemolytic anemia • Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Autoimmune myocarditis/cardiomyopathy • Autoimmune thrombocytopenia • Goodpasture syndrome • Idiopathic pulmonary fibrosis • Pernicious anemia • Raynaud's phenomenon • Sarcoidosis • Sjögren's syndrome • Stevens-Johnson syndrome • Uveitis	

Appendix I HVTN 133 Visit Windows

Visit Number	Visit Type	Lower Allowable Window	Lower Target Day	Target Day	Upper Target Day	Upper Allowable Window
01.0	Screening	-56	-		-	
02.0	**Enrollment**		-	0	-	
03.0	2 Weeks Post-Vaccination		-4	14	+4	+7
04.0	**Vaccination 2**		-7	56	+9	+14
05.0	2 Weeks Post-Vaccination		-4	70	+4	+7
06.0	**Vaccination 3**	-28	-14	168	+14	+28
07.0	2 Weeks Post-Vaccination		-4	182	+4	+7
08.0	Follow-up	-28	-14	273	+14	+28
09.0	**Vaccination 4**	-28	-14	364	+14	+28
10.0	1 Week Post-Vaccination	-2	-1	371	+1	+2
11.0	2 Weeks Post-Vaccination		-3	378	+4	+7
12.0	Follow-up	-28	-14	546	+14	+28
13.0	6 Month Health Contact	-28	-14	728	+14	+28

1. Target dates are relative to Day 0 (Enrollment), with the exception of post-vaccination visits 3.0, 5.0, 7.0, 10.0, 11.0 which are relative to the prior vaccination visit.
2. See Section 9.10.

Appendix J Protocol Signature Page

A phase 1 clinical trial to evaluate the safety and immunogenicity of an HIV-1 gp41 MPER-656 liposome vaccine in healthy, HIV-uninfected adult participants

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (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 133

DAIDS Protocol Version: Version 1.0

Protocol Date: March 13, 2019