HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 303

A Phase 1, Open-Label Clinical Trial to Evaluate Safety, Tolerability, and Immunogenicity of Adjuvanted HIV-1 Fusion Peptide Conjugate Vaccine (VRC-HIVVCP0108-00-VP) Alone or in Prime-Boost Regimens with Adjuvanted HIV-1 Envelope Trimer 4571 (VRC-HIVRGP096-00-VP) and HIV-1 Trimer 6931 (VRC-HIVRGP0106-00-VP) Vaccines in Healthy Adults

DAIDS DOCUMENT ID 38835

IND 028412 HELD BY DAIDS

CLINICAL TRIAL SPONSORED BY

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

STUDY PRODUCTS PROVIDED BY

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

September 21, 2023
Final
HVTN 303
Version 2.0

HVTN 303 enrolled (vaccinated) its first participant on August 15, 2022, with the goal to enroll 60 participants in the study. As of Dec 20, 2022, 44 participants were enrolled (no enrollments occurred after this date). Part A (Groups 1, 2, 3, 4, and 5) completed enrollment of 15 participants from August 15 to October 14, 2022.

Part B enrolled 29 participants from November 3 to December 20, 2022: Group 6 enrolled 10 ppts, all of whom received 1 vaccination; Group 7 enrolled 10 participants, 5 of whom received 2 vaccinations; Group 8 enrolled 9 participants, 6 of whom received 2 vaccinations. No participants received a 3rd vaccination.

Over time, the PSRT observed trends in local and systemic reactogenicity (grade 3 fever, grade 3 injection site erythema, and/or induration/swelling), unsolicited adverse events (urticaria, rash, and serum sickness), and temporary increases in absolute eosinophil counts (AECs) post-vaccination. Certain of these events resulted in pauses in all vaccinations, and the PSRT requested the HVTN Safety Monitoring Board (SMB) perform an ad hoc review of all safety data. Following this review on January 11, 2023, the SMB recommended that all vaccinations in the study be permanently discontinued, and that participants be continued in safety follow-up, and these recommendations were implemented.

On January 13, 2023, a protocol memo was distributed informing the clinical research sites that all vaccinations in HVTN 303 were permanently discontinued. Vaccinations in Part A were completed on October 14, 2022, and specimen collection for safety labs was completed 4 weeks after the vaccination on November 10, 2022. Discontinuation of vaccinations applied to Part B only. On March 13, 2023, FDA informed DAIDS that the IND has been placed on Clinical Hold and confirmed that all protocol-related activities must cease except for any participant safety follow-up assessments. Safety follow-up assessments, including specimen collection for safety labs, have been conducted for at least 7 months after last vaccination for all participants in Part B and no safety concerns have been identified during this follow-up period.

All participants have been informed using “Dear Participant” letters.

HVTN 303 Protocol sections 1, 2, 3, 6, 7 and 8 have been revised to include language in prior protocol modifications and protocol changes that resulted from the permanent discontinuation of vaccinations in the study. Procedures specified for remaining follow-up visits have been revised.

- For participants in Groups 7 and 8 that received two doses of their respective vaccines, Leukapheresis or 204 ml of blood draw has been added at 10 months +/- 2 month past their last vaccination to investigate immunological outcomes.

- The revised study procedures for Part A participants are shown in new Appendix B and for Part B participants are shown in new Appendix D.

- The revised visit windows for participants are shown in new Appendix F. New Appendix H and Appendix J contains addendums to the sample consent forms for Part A and Part B participants respectively that updates study information and new tables of procedures.

Protocol Signature Page

A Phase 1, Open-Label Clinical Trial to Evaluate Safety, Tolerability, and Immunogenicity of Adjuvanted HIV-1 Fusion Peptide Conjugate Vaccine (VRC-HIVVCP0108-00-VP) Alone or in Prime-Boost Regimens with Adjuvanted HIV-1 Envelope Trimer 4571 (VRC-HIVRGP096-00-VP) and HIV-1 Trimer 6931 (VRC-HIVRGP0106-00-VP) Vaccines in Healthy Adults

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US 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, US National Institutes of Health, Division of AIDS) and institutional policies

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

DAIDS Protocol Number: HVTN 303

DAIDS Protocol Version: Version 2.0

Protocol Date: September 21, 2023

Acronyms and abbreviations

Ab	antibody
AE	adverse event
AEC	absolute eosinophil count
AESI	adverse events of special interest
Ag	antigen
ALT	Alanine aminotransferase
AoU	Assessment of Understanding
ART	antiretroviral therapy
AUC-MB	area-under-the-magnitude breadth curve
β-HCG	beta human chorionic gonadotropin
BAMA	binding antibody multiplex assay
bnAb	broadly neutralizing antibody
BP	blood pressure
CAB	community advisory board
CBC	complete blood count
cGMP	current Good Manufacturing Practice
CHO	Chinese hamster ovary
CI	confidence interval
CMIA	chemiluminescent microparticle immunoassay
ConC	consensus clade C sequence
COVID-19	coronavirus disease 2019
CRF	case report form
CRPMC	Clinical Research Products Management Center
CRS	clinical research site
CSS	clinical safety specialist
CTL	CD8+ T lymphocyte(s)
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS
DHHS	Department of Health and Human Services
d/v	dose per volume
DS	disulfide mutation
EAE	expedited adverse event
EC	ethics committee
eGFR	estimated glomerular filtration rate
EIA	enzyme immunoassay
ELICA	electrochemiluminescence
Env	HIV-1 envelope glycoprotein

EUA	emergency use authorized/authorization
EUL	emergency use listing
FDA	Food and Drug Administration
FP	fusion peptide
FP8v1-rTTHC	fusion peptide conjugate vaccine
FP8v2	eight N-terminal FP residues
FS	functionality score
GCP	Good Clinical Practice
GEE	generalized estimating equations
GLP	good laboratory practice
GPP	Good Participatory Practices
HA	hemagglutinin
HCV	hepatitis C virus
ICH	international council for harmonization
IB	investigator's brochure
ICS	intracellular cytokine staining assay
IDR	immunodominant region
IM	intramuscular/intramuscularly
IND	investigational new drug (application)
IRB	institutional review board
IV	intravenous
KLH	keyhole limpet hemocyanin
MAAE	medically attended adverse events
MAR	missing at random
MCAR	missing completely at random
mcg	microgram
mg	milligram
mL	milliliter
MOP	manual of procedures
MPL	monophosphoryl lipid A
MSD	Meso Scale Discovery
MSM	person born male with partner(s) born male
nAb	neutralizing antibody
NAT	nucleic acid test
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PAB	Pharmaceutical Affairs Branch

PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCA	principal component analysis
PCR	polymerase chain reaction
PEF	peak expiratory flow
PFS	polyfunctionality score
PI	Principal Investigator
PIMMC	potential immune-mediated medical condition
PrEP	pre-exposure prophylaxis
PSRT	Protocol Safety Review Team
RAB	Regulatory Affairs Branch
RE	Regulatory Entity
RSC	Regulatory Support Center
rTTHC	recombinant tetanus toxoid heavy chain fragment C
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SAS	statistical analysis system
SC	subcutaneous/subcutaneously
SICF	sample informed consent form
SMB	safety monitoring board
SOP	Standard Operating Procedure
SPT	Safety and Pharmacovigilance Team
SSP	study specific procedures
SUSAR	suspected unexpected serious adverse reaction
ULN	upper limit of normal
VCMP	Vaccine Clinical Materials Program
VISP	vaccine-induced seropositivity
VRC	Vaccine Research Center
WBC	white blood cell
WHO	World Health Organization

Contents

1 Executive summary

1.1 Title

A Phase 1, Open-Label Clinical Trial to Evaluate Safety, Tolerability, and Immunogenicity of Adjuvanted HIV-1 Fusion Peptide Conjugate Vaccine (VRC-HIVVCP0108-00-VP) Alone or in Prime-Boost Regimens with Adjuvanted HIV-1 Envelope Trimer 4571 (VRC-HIVRGP096-00-VP) and HIV-1 Trimer 6931 (VRC-HIVRGP0106-00-VP) Vaccines in Healthy Adults.

1.2 Design

This is a phase 1, open-label, dose-escalation study to evaluate the dose, safety, tolerability and immunogenicity of adjuvanted HIV-1 Fusion Peptide (FP) conjugate vaccine (FP8v1-rTTHC) alone or in prime-boost regimens with adjuvanted HIV-1 Trimer 4571 ~~and adjuvanted HIV-1 Trimer 6931~~. The primary hypothesis is that FP8v1-rTTHC, HIV-1 Trimer 4571, and HIV-1 Trimer 6931 adjuvanted vaccines are safe and tolerable when administered alone and when co-administered with HIV-1 Trimer 4571, in prime-boost regimens.

1.3 Study products

- FP8v1-rTTHC (FP conjugate vaccine) is an HIV-1 fusion peptide conjugated to recombinant tetanus toxoid heavy chain fragment C (rTTHC) via sulfo-SIAB chemical linker. FP8v1 corresponds to the amino-terminal eight residues of the most prevalent HIV fusion peptide sequence. FP conjugate vaccine will be provided at a 1-milligram (mg)/milliliter (mL) concentration in 3-mL glass vials filled to 0.7 mL.

- HIV-1 Trimer 4571 (Trimer 4571) is a synthetic soluble HIV-1 envelope product that consists of an HIV-1 envelope (Env) trimer variant, derived from clade A, strain BG505. Trimer 4571 is provided at a 500-mcg/mL concentration in 3-mL glass vials filled to 1.2 mL

- HIV-1 Trimer 6931 (Trimer 6931) is a synthetic soluble HIV-1 envelope product that consists of an HIV-1 envelope (Env) trimer variant, derived from consensus clade C sequence (ConC). Trimer 6931 will be provided at a 1-mg/mL concentration in 3-mL glass vials filled to 1.2 mL.

- Adjuplex is the adjuvant and will be provided in a sterile, pyrogen-free, homogeneous suspension at 0.7 mL in 3-mL glass vials. Adjuplex will be mixed with study products in the pharmacy during preparation prior to vaccination at a 20% dose by volume.

- PBS (labeled as Phosphate Buffered Saline pH 7.2): Diluent.

1.4 Study population

Healthy adults aged 18 to 50 years, inclusive.

1.5 Study plan and schema table

This study has two parts. Part A will evaluate the safety, tolerability, and immunogenicity of single doses of the FP conjugate, Trimer 4571 and Trimer 6931 vaccines, in a dose-escalation design. Each product must be assessed as safe prior to use in Part B. Trimer 4571 with alum adjuvant has been previously evaluated in humans but will be tested in Part A with Adjuplex. Part B will evaluate the safety, tolerability, and immunogenicity of FP conjugate prime, Trimer 4571 prime, or an FP plus Trimer 4571 prime, all followed by subsequent doses of Trimer 4571, ~~Trimer 6931 and both Trimers combined~~. Study vaccines will be administered intramuscularly (IM) via needle and syringe in two injection sites. The study schema is in Table 1-1 below:

Table 1-1 Schema

Part A: Dose Escalation**											
Group	**N**	**W0**									
1	3	25 mcg FP conjugate vaccine									
2	3	200 mcg FP conjugate vaccine									
3	3	100 mcg Trimer 6931									
4	3	200 mcg Trimer 6931									
5	3	200 mcg Trimer 4571									
Part A Total	**15***										
Part B: Prime Boost Regimen**											
Group	**N**	**W0**	**W4**	**W8*** **	**W12*** **	**W20*** **	**W24*** **	**W32*** **	**W36*** **	**W44*** **	**W48*** **
6	~~15~~ 10	200 mcg Trimer 4571			~~200 mcg Trimer 4571~~		~~200 mcg Trimer 6931~~		~~100 mcg Trimer 4571 ± 100 mcg Trimer 6931~~		~~100 mcg Trimer 4571 ± 100 mcg Trimer 6931~~
7	~~15~~ 10	200 mcg FP conjugate vaccine	200 mcg FP conjugate vaccine	~~200 mcg FP conjugate vaccine~~	~~200 mcg Trimer 4571~~		~~200 mcg Trimer 4571~~		~~200 mcg Trimer 6931~~		~~100 mcg Trimer 4571 ± 100 mcg Trimer 6931~~
8	~~15~~ 9	200 mcg FP conjugate vaccine + 200 mcg Trimer 4571	200 mcg FP conjugate vaccine + 200 mcg Trimer 4571	~~200 mcg FP conjugate vaccine ± 200 mcg Trimer 4571~~		~~200 mcg Trimer 6931~~		~~100 mcg Trimer 4571 ± 100 mcg Trimer 6931~~		~~100 mcg Trimer 4571 ± 100 mcg Trimer 6931~~	
Part B total	**~~45~~$** 29**										
Overall Total	**~~60~~‡ 44**										

Table 1-1 Footnotes:
**Adjuplex adjuvant will be mixed with all study products in Part A and Part B at 20% by volume in the pharmacy during product preparation for all vaccinations. Once mixed, all study injections will be divided into 2 syringes, and each syringe will be administered intramuscularly to one of the deltoids.

* In Part A, up to 20 participants may be enrolled if needed for safety evaluations. Additional participants may be enrolled to ensure the availability of 2-week safety data from at least 3 participants per group. Refer to the HVTN 303 Study Specific Procedures (SSP) for further details.

§ In Part B, up to 50 participants may be enrolled if needed for safety evaluations. Additional participants may be enrolled to ensure the availability of 2-week safety data from at least 15 participants per group. Refer to the HVTN 303 SSP for further details.

† Total up to 70 participants can be enrolled if needed for safety evaluations. Refer to the HVTN 303 SSP for further details.
Notes:

***Vaccination in Part B starting from Week 8 onwards did not take place.

Actual Ns for Group 6, 7 and 8 were 10, 10 and 9 respectively. In Part B total 29 participants were enrolled.

Part A of the study may begin with direct enrollment of participants into the following groups simultaneously:
- Group 1 with no more than 1 participant enrolled per day for the 3 participants.
- Group 3 with no more than 1 participant enrolled per day for the 3 participants.

After Groups 1 and 3 have been fully enrolled, the study will be placed on a safety hold. No additional enrollments will proceed until the Protocol Safety Review Team (PSRT) has determined it is safe to do so. Once all of the reactogenicity and 2-week safety data from at least 6 participants have been submitted to the database, the PSRT must assess the accumulated product-specific data as showing no significant safety concerns before proceeding with enrollment of Groups 2, 4 and 5. If the PSRT has determined it is safe to proceed after reviewing data from Groups 1 and 3, the following groups may begin simultaneously:
- Group 2 with no more than 1 participant enrolled per day for the 3 participants
- Group 4 with no more than 1 participant enrolled per day for the 3 participants
- Group 5 with no more than 1 participant enrolled per day for the 3 participants

Once Groups 2, 4 and 5 have been fully enrolled, the study will be placed on a safety hold before proceeding with Part B. Once all of the reactogenicity and 2-week safety data from at least 9 participants have been submitted to the database, the PSRT must assess the accumulated product-specific data as showing no significant safety concerns before proceeding with enrollment of Part B.
Part B enrollments may only proceed if no safety concerns have been identified for any of the product administrations in Part A of the study.
If at any time there is insufficient data to conduct a formal PSRT Safety Review because of participant discontinuations from the study before sufficient data are collected, then additional participants may be enrolled into that group to acquire the requisite data on the required number of participants specified above.
Moreover, the PSRT may recommend additional participants be enrolled into a given treatment group if additional safety evaluations are requested.
Consultation with the HVTN Safety Monitoring Board (SMB), Institutional Review Board (IRB) and Food and Drug Administration (FDA), if needed, as specified by study pause criteria (per Section 9.5.1), will occur if indicated.

1.6 Duration per participant

For participants in Part A (Groups 1-5): 52 weeks of scheduled clinic visits.

~~For participants in Part B (Groups 6 and 7): 100 weeks of scheduled clinic visits.~~

~~For participants in Part B (Group 8): 96 weeks of scheduled clinic visits.~~

On January 13, 2023, a protocol memo was distributed informing the clinical research sites that all vaccinations in HVTN 303 were permanently discontinued. Procedures specified for remaining follow-up visits have been revised. Duration for Part A participants remained unchanged. Duration for Part B participants (for Groups 6 -8) is 56 weeks.

1.7 Estimated total study duration

~~Total study duration is 36 months (includes enrollment, planned safety holds and follow-up).~~

Following cessation of vaccination and reduction of follow-up duration the estimated total study duration is reduced to approximately 18 months.

1.8 Study sites

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

2 Introduction

In the nearly 4 decades since AIDS was first observed and described (1-5), its virologic etiologic agent, HIV-1 (6-8), has continued to be a worldwide threat to public health. In 2018, in the United States alone, an estimated 37,968 new infections occurred, with 1.2 million people living with confirmed infection, and 15,820 deaths among those diagnosed with HIV (9). Global statistics reported in 2019 were even more sobering, with approximately 38 million people living with HIV worldwide, including 1.7 million new HIV cases and 690,000 deaths (9). For individuals who received a diagnosis of HIV-1 during the years spanning 1980 and 1996, median survival was less than a year (10, 11). Advances in therapeutic drug development have transformed the survival prognosis to one of an essentially normal life span for individuals newly diagnosed since 2017 and who have access to effective antiretroviral therapy (ART) (12, 13). Although the rate of new cases of HIV infections is decreasing globally, additional widespread measures are needed to accelerate the reduction of new infections. Development of a protective HIV-1 vaccine thus remains a global health priority to control and eliminate the AIDS pandemic (14).

A central goal of HIV-1 vaccine development remains the elicitation of protective antibodies capable of neutralizing diverse tier 2 (neutralization-resistant) isolates that typify naturally circulating HIV-1 strains. A primary vaccine target has been the HIV-1 envelope glycoprotein (Env), the sole viral component exposed on the virion surface and principal target for neutralizing antibodies. HIV Env is synthesized as a gp160 precursor protein, cleaved to gp120 exterior and gp41 transmembrane subunits by a furin-family protease, and assembled via noncovalent interactions into a trimer of gp120-gp41 heterodimers (15). To infect a target cell, the gp120 subunit binds the primary host CD4 receptor, thereby triggering conformational rearrangements in HIV Env that enable binding to a co-receptor, either CCR5 or CXCR4 chemokine receptor. Co-receptor binding induces further Env-conformational rearrangements, including formation of the pre hairpin intermediate and insertion of the fusion peptide (FP) at the N-terminus of gp41 into the host cell membrane. Subsequent refolding of gp41 into a post fusion six helical bundle leads to fusion of virion and host cell membranes, and entry of HIV-1 into the host cell (16, 17). Extensive glycosylation, sequence variation, and conformational masking of key neutralizing epitopes on the Env allow circulating HIV strains to evade the immune response, and thus poses serious challenges for vaccine development.

2.1 Study rationale

In order to test the effect of vaccination with a well-formed HIV envelope trimer in humans, the Vaccine Research Center (VRC) has previously developed BG505 DS-SOSIP.664 (herein Trimer 4571, VRC-HIVRGP096-00-VP; IND 18602), a soluble, cleaved HIV-1 Env trimer derived from the clade A BG505 Env gene.

The new 201C 433C disulfide mutation (DS) introduced within the gp120 subunit of BG505 DS-SOSIP.664 maintains the Env trimer in a prefusion-closed state that is resistant to CD4-induced conformational change (18). In preclinical testing, Trimer 4571 exhibited the desired target antigenicity with specific recognition by broadly neutralizing antibodies (bnAbs) but little to weak binding by non-neutralizing antibodies, even in the presence of CD4 (18, 19). Immunization with prefusion-stabilized SOSIP trimers in multiple animal models has been shown to elicit neutralizing responses against autologous or closely matched heterologous HIV-1 viruses, although with limited breadth (20-26). Trimer 4571 was tested in the VRC 018 study (NCT03783130), a phase 1 clinical trial in healthy adults. As of June 2020, the study has been completed, no serious adverse events (SAEs) were reported, and study data became available in ClinicalTrials.gov. Overall, the Trimer 4571 data demonstrated a good phase 1 safety profile and immunogenicity in humans, thus indicating it may be useful as a boosting immunogen in HIV-1 vaccination protocols.

One vaccine strategy to induce epitope targeting bnAbs with significant neutralization breadth involves the use of epitope-focused immunogens as priming reagents followed by boosting with stabilized soluble Env trimers, in combination with an adjuvant. Recently, the VRC identified the fusion peptide (FP) as a vulnerable region targeted by neutralizing antibodies (27). The FP is a hydrophobic stretch of 15-20 residues at the N-terminus of gp41 that plays an essential role in the viral entry process. FP embeds in the target cell membrane during the pre hairpin intermediate step, where it anchors the rearranging viral spike and facilitates fusion of the viral and cell membranes. The N-terminal portion of FP is recognized by several broadly neutralizing human antibodies, including N123-VRC34.01 (27), PGT151 (28, 29), and ACS202 (30, 31). These antibodies are derived from natural infection, and analysis of their recognition established the exposed N-terminal half of FP as a site of vulnerability for antibody-mediated neutralization (27).

To evaluate the utility of FP-targeting immunogens to elicit neutralization breadth, the VRC created FP-carrier protein conjugates by covalently linking the N-terminal 6-10 residues corresponding to the most prevalent sequence of FP (FPv1) with an appended cysteine to lysine residues on the keyhole limpet hemocyanin (KLH) carrier protein using a heterobifunctional crosslinker. Adjuvanted FP-carrier conjugate immunogens could induce broad neutralizing FP-directed immune responses in mice capable of neutralizing up to 31% of a cross-clade panel of 208 diverse HIV-1 strains (32). Boosting with Trimer 4571, administered with the lecithin-carbomer based adjuvant Adjuplex, was found to significantly enhance neutralizing titers, especially in guinea pigs and rhesus macaques, with vaccine-elicited rhesus antibodies obtained after FP-carrier priming and stabilized Env-trimer boosting capable of neutralizing up to 59% of the 208-strain panel (33).

In a follow-up study, priming with a cocktail of FP-carrier conjugate and Trimer 4571, prior to boosting with just Trimer 4571, improved autologous potency and elicited earlier cross-clade neutralizing responses in rhesus macaques compared to priming with FP-carrier conjugate alone (34). This finding suggests that priming with an FP-carrier and Trimer 4571 cocktail followed by boosting with HIV-1 Env Trimer alone could serve as an alternate vaccine strategy capable of achieving broad neutralization with potentially fewer immunizations.

Finally, a heterologous trimer boost strategy was recently shown to further improve breadth in guinea pigs. In this study, the animals were given a FP-tetanus toxoid carrier (FP8v1-rTTHC) prime, followed by sequential boosts with Trimer 4571, then Trimer 6931 (a consensus C trimer engineered with a slightly altered FP [see Section 2.9]), and finally boosted with a trimer cocktail (Trimer 4571 + Trimer 6931). The consistency, breadth and potency of neutralization against a panel of representative pseudoviruses was further enhanced with this regimen (34, 35).

Overall, these proof-of-concept studies provide substantial evidence that an epitope-focused vaccine approach using an FP-carrier protein conjugate and prefusion-closed stabilized Env trimers (Trimer 4571 and Trimer 6931) administered with an adjuvant (ie, Adjuplex) in prime-boost regimens can elicit reproducible, neutralizing immune responses against diverse tier-2 primary isolates in animals. This vaccine strategy will be used in the proposed HVTN 303 clinical study.

Overall, the goal of the trial is to elicit tier-2 neutralizing antibodies against FP with breadth. Key immunological questions to be addressed include: 1) Will somatic hypermutation allow focusing on FP during the priming phase?, 2) What is the impact of FP priming on inducing a cross-reactive HIV-1 neutralizing response?, 3) What are the differences in immune responses and types of antibodies elicited by different priming regimens, either priming with FP alone or FP in combination with a stabilized Env trimer?, and 4) Will boosting with diverse trimers and trimer cocktails induce and expand breadth of neutralization responses? This trial continues a project establishing first-in-human data for adjuvanted stabilized Env trimers in combination with the adjuvanted FP vaccine, which has already shown promise in animal models. Data from this trial will be used in design of future antigens and vaccine regimens to increase breadth and potency of FP-directed neutralization as a critical step towards development of a protective HIV-1 vaccine.

2.2 Rationale for evaluation of FP8v1-rTTHC

Initial studies evaluating an FP-focused vaccine approach used FP conjugated to the KLH carrier protein. However, the KLH used as a carrier protein in these earlier studies is a multi-subunit metalloprotein of approximately 8 MDa and is derived from a natural source, posing challenges to standard manufacturing

processes, characterization, and quality control. To identify an FP-based immunogen suitable for clinical development, the VRC evaluated several FP-carrier conjugates made with different carrier proteins used in licensed vaccines, and found a non-toxic, truncated variant of tetanus toxoid, recombinant tetanus toxoid heavy chain fragment C (rTTHC) (36, 37) to be a suitable candidate carrier protein for inducing FP-directed responses (38). The N-terminal eight amino acids of the most prevalent FP sequence (FP8v1: AVGIGAVF) conjugated to rTTHC carrier protein (FP8v1-rTTHC) elicits FP-directed neutralizing responses in multiple animal models when used with the Adjuplex adjuvant. Based on these promising attributes, FP8v1-rTTHC was advanced as a candidate vaccine immunogen for product development and current good manufacturing practice (cGMP) manufacture.

2.3 Rationale for evaluation of FP8v1-rTTHC and Trimer 6931 (ConC-FP8v2 RnS-3mut-2G-SOSIP.664)

Priming with the most prevalent N-terminal eight residues of the fusion peptide (FP8v1) followed by boosting with Trimer 4571 (BG505 DS-SOSIP.664) that contains the matching FP8v1 sequence elicits 20-30% neutralization breadth in sera from vaccine-test animals (32). While the FP-carrier protein priming is important for initiating FP-directed responses (33), Env trimer boosts are critical for maturing responses to achieve broad neutralization (32, 33). Additional boosting with a heterologous clade C HIV-1 Env trimer increased consistency of eliciting broad cross-clade neutralizing responses (33), suggesting a vaccine strategy that uses sequential boosting with a heterologous trimer can enhance cross-clade FP-directed neutralizing responses.

To evaluate the utility of a heterologous HIV-1 Env trimer to boost FP-directed responses, the VRC designed Trimer 6931 (ConC-FP8v2 RnS-3mut-2G-SOSIP.664), a prefusion-closed stabilized trimer with a consensus clade C sequence and a different fusion peptide sequence from that found in FP8v1-rTTHC and Trimer 4571. Trimer 6931 is based on the ConC_Base0 variant (39) and contains additional stabilizing mutations, 302M, 320L, and 329P (3mut), at the trimer apex, two glycine substitutions in gp41 (2G) (40) to further improve stability and antigenicity of the trimer in a prefusion-closed form, as well as the standard SOSIP substitutions (41) and 7 other stabilizing mutations (39). In preclinical studies, Trimer 6931 elicited higher neutralization breadth when used as a heterologous trimer boost in guinea pigs immunized with FP8v1-rTTHC and Trimer 4571 prime-boost regimen, compared to boosting with Trimer 4571 only, as discussed in Section 2.9.

2.4 Rationale for Use of the Adjuplex Adjuvant

Adjuvants improve the elicited immune response to many vaccines (42). Adjuplex is a novel adjuvant based on a purified lecithin and carbomer homopolymer.

Adjuplex has been established as an effective and well tolerated adjuvant for use with different antigens and immunization regimens in animals (43, 44). There is limited human experience with Adjuplex (see Section 2.10.2).

In animal studies, soluble influenza hemagglutinin (HA) protein administered subcutaneously (SC) or intranasally with Adjuplex adjuvant at 1-20% by volume induced robust antigen-specific CD8+ T lymphocytes (CTLs) responses in mice (44). Through histological images of the IM and SC injection sites in the Ovalbumin-Adjuplex model in mice, significant correlation was observed between increasing dose of Adjuplex and the extent and cellularity of the inflammatory infiltrate at the injection site (44).

In another study in mice, soluble HA of influenza A virus adjuvanted with Adjuplex and administered SC has been shown to elicit robust humoral immunity and T-cell responses (43). The optimal dose of Adjuplex for immunogenicity was 1% by volume; HA adjuvanted with 1% of Adjuplex induced high titers of HA-specific IgG with insignificant weight loss in mice. The vaccine administered with the optimal dose of Adjuplex completely protected mice from the lethal influenza virus challenge and was comparatively as effective as the adjuvants monophosphoryl lipid A (MPL) and alum in preventing disease. No signs of local toxicity or intolerance, assessed by swelling or scratching caused by irritation, were noted at any Adjuplex dose, suggesting the Adjuplex to be a potent and well-tolerated adjuvant for subunit vaccines (43).

HIV vaccine candidates adjuvanted with the Adjuplex dose of 20% by volume were found to elicit reproducible, neutralizing immune responses against diverse tier-2 primary isolates in a prime-boost regimen (32). Boosting with Trimer 4571 after FP priming, with Adjuplex adjuvant, was found to significantly enhance neutralizing titers, especially in guinea pigs and rhesus macaques, with vaccine-elicited rhesus antibodies neutralizing up to 59% of the 208-strain panel (33). Adjuplex adjuvant has been shown to enhance immunogenicity of HIV-1 Env trimers compared to alum in non-human primates (45).

Based on VRC pre-clinical data, using the Adjuplex adjuvant elicited a statistically significant higher neutralizing responses compared to the alum and unadjuvanted groups; therefore, Adjuplex was selected for clinical evaluations in HVTN 303 study and further discussed in Section 2.9.

2.5 Rationale for Vaccine Regimen and Dose Selections

The consecutive immunization strategy through the sequential prime-boost regimens allows for the ability to induce potent HIV-1 broadly neutralizing antibodies (bnAb) in animal studies (46). Investigation of different prime-boost regimens may inform on the next generation of HIV-1 vaccines and the immunization regimens capable of eliciting durable, cross-reactive serum neutralizing antibody responses in humans. The pre-clinical studies provide

substantial evidence that an epitope-focused vaccine approach using an FP-carrier protein conjugate (FP8v1-rTTHC) and prefusion-closed stabilized Env trimer(s) (Trimer 4571 and Trimer 6931) administered with an adjuvant (ie, Adjuplex) in a prime-boost regimen can elicit reproducible, neutralizing immune responses against diverse tier-2 primary isolates in animals. In addition, they indicate that a FP-fusion protein + Trimer 4571 prime cocktail, followed by heterologous boosts by each individual trimer and then with a cocktail of the two trimers, has induced the broadest neutralizing response to the FP epitope. These animal studies provide the rationale for Groups 7 and 8 in Part B of this study, which tests the difference in the two priming regimens of FP-conjugate alone (Group 7) or FP-conjugate + Trimer 4571 cocktail (Group 8). Group 6 is an essential control arm, which tests the relative effects of each booster regimen for Groups 7 and 8. The first 4 doses in Group 6 mirror the boosts for Group 7 and the last 3 doses mirror the boost doses in Group 8. This will provide important data on the trimers alone and in cocktail to induce immunity in combination with Adjuplex and importantly, will allow a precise determination the impact of FP and FP + trimer priming. These data can confirm whether the animal study results were predictive, and thus can inform future product development.

Doses were chosen to ensure safety of new products and to maximize immune response. The recombinant tetanus toxoid heavy chain fragment C (rTTHC) has been shown to be safe and effective in multiple vaccines with doses up to at least 200 mcg (a polysaccharide quadrivalent [A, C, W135, Y]). *Neisseria meningitis* tetanus toxoid conjugate vaccine (47), the FP-conjugate (FP8v1-RTTHC), has not been used in humans and will be tested first at 25 mcg (Group 1), the dose used in mice (38) and then at the target dose of 200 mcg (Group 2). Given the proven safety track record for tetanus toxoid conjugate vaccines, an intermediate step dose is not felt to be necessary for safety, and instead the immunological target dose will be tested directly in Group 2. In VRC 018, Trimer 4571 was tested at 100 mcg and 500 mcg in combination with 500 mcg of Alum. Both doses were tolerated well and there was a trend towards improved immune response at the higher dose of immunogen. Consequently, the target doses for each trimer in this study will be 200 mcg, both for improved immunogenicity and to allow a total dose of 400 mcg immunogen when trimers are used in combination with the FP-tetanus toxoid conjugate. As Trimer 6931 has not yet been tested in humans, Group 3 will start with 100 mcg of this immunogen. Groups 4 and 5 will test maximum dosing of each respective trimer at 200 mcg each.

Based on previous VRC experience (48), product injections will be split and completed at 2 injection sites, 1 on each arm. Separating injections into 2 sites would increase germinal center recruitment and therefore could enhance vaccine immunogenicity (unpublished animal data).

2.6 Assessment of Immunogenicity

Vaccinations were stopped on January 13, 2023, resulting in most (33) participants receiving only 1 vaccination and eleven participants in Groups 7 and 8 receiving 2 vaccinations. Specimens to evaluate immunogenicity were collected at baseline and at the specified timepoints after the administration of product until March 13, 2023, when the FDA informed DAIDS that the IND has been placed on Clinical Hold and confirmed that all protocol-related activities must cease except for any participant safety follow-up assessments. The primary immunogenicity time point, after the changes to the vaccination and collection schedule, remains 2 weeks after the last vaccination, however participants only received 1 or 2 vaccinations. Antibody titers will be measured by Electrochemiluminescence (ELICA) using a Meso Scale Discovery (MSD) platform for all participants with one or two vaccinations in Groups 5, 6, 7 and 8.

Additional exploratory assays to assess humoral and cellular immune responses may be performed, including B cell response and neutralization antibody assays.

A specimen collection has been added at approximately 10 months post the

Specimens to evaluate immunogenicity will be collected at baseline and at specified time points throughout the study after the administration of product. HIV-specific humoral immune responses will be assessed by neutralization antibody assays. The primary immunogenicity time point is 2 weeks after the last vaccination.

Trimer 4571- and Trimer 6931-specific antibody titers will be measured by Electrochemiluminescence (ELICA) using a Meso Scale Discovery (MSD) platform. Other exploratory assays to assess humoral and cellular immune responses may be performed with stored samples.

Measurements of antibody, B-cell and T-cell responses may be assessed from stored samples for timepoints throughout the study as exploratory evaluations. This includes a number of high-throughput functional assays and high-throughput biophysical profiling tools to comprehensively characterize the humoral immune response elicited by vaccination regimens.

2.7 Rationale for Leukapheresis

Only participants who received two vaccinations in Part B (Groups 7-8) and who consent to leukapheresis will undergo leukapheresis at 10 months +/- 2 months post–last vaccination in order to collect blood cells for research.

~~Subjects in Groups 6-8 will be encouraged to complete leukapheresis at 2 weeks post- last vaccination to collect blood cells of special interest for research. It is expected that a subset of participants (about half of the participants in Part B, groups 6-8) will agree to undergo leukapheresis. Leukapheresis will be performed 2 weeks post- last vaccination for subjects who receive the full vaccination regimen.~~ The rationale for performing leukapheresis at these timepoints is to assess whether rare-epitope–specific B cells and T cells have been stimulated. While peripheral blood draws of ~500 mL allow isolation of up to 500 million peripheral blood mononuclear cells (PBMCs), these number of cells are often not enough to detect an epitope-specific response in the periphery. Leukapheresis, however, can provide 6-10 billion PBMCs without depleting red blood cells or other blood components and significantly increase the likelihood of detecting antigen-specific B and T cells outside lymph nodes.

2.8 Study Products

Study products are manufactured under cGMP regulations by VRC/National Institute of Allergy and Infectious Diseases (NIAID)/NIH at the VRC Pilot Plant, operated under contract by the Vaccine Clinical Materials Program (VCMP), Leidos Biomedical Research, Inc., Frederick, MD USA.

Manufacturing and preclinical information is included in the Investigator's Brochures (IBs) for each product. Quality assurance lot release testing by the manufacturer and ongoing stability programs verify conformance to product specifications throughout use in clinical trial.

The adjuvant in this study, Adjuplex, is a sterile, pyrogen-free suspension produced by VRC Pilot Plant. Adjuplex comprises highly purified de-oiled soy lecithin and benzene-free carbomer homopolymer formulated in phosphate buffered saline (PBS). Adjuplex adjuvant will be mixed with study products mentioned below at 20% by volume in the pharmacy during product preparation for all vaccinations.

2.8.1 VRC-HIVVCP0108-00-VP (FP8v1-rTTHC or FP conjugate vaccine), Investigational Vaccine

The VRC-HIVRGP0108-00-VP vaccine (FP8v1-rTTHC) is a sterile, aqueous, buffered solution filled into single-dose vials. The peptide-carrier conjugate,

FP8v1-rTTHC, consists of a carrier tetanus toxoid–derived protein plus a linker to chemically attach the fusion peptide (FP).

2.8.2 VRC-HIVRGP096-00-VP (Trimer 4571), Investigational Vaccine

The VRC-HIVRGP096-00-VP vaccine (Trimer 4571) is a sterile, aqueous, buffered solution filled into single-dose vials. Trimer 4571 consists of an HIV-1 envelope trimer variant derived from the clade A HIV-1 strain BG505. It is produced in a Chinese Hamster Ovary (CHO) DG44 cell line.

2.8.3 VRC-HIVRGP0106-00-VP (Trimer 6931), Investigational Vaccine

The VRC-HIVRGP0106-00-VP vaccine (Trimer 6931) is a sterile, aqueous, buffered solution filled into single-dose vials. Trimer 6931 is a recombinant, consensus clade C HIV-1 trimeric envelope glycoprotein. It is produced in a CHO DG44 cell line. The envelope glycoprotein molecule contains the second-most prevalent sequence for the eight N-terminal FP residues (FP8v2) and additional stabilizing mutations (RnS, 3mut, and 2G) to maintain a closed conformation in the presence of CD4, further enhancing the likelihood of cross-clade HIV-1 neutralization when administered as a heterologous trimer boost to FP8v1-rTTHC.

2.9 Preclinical Studies with FP conjugate vaccine, Trimer 4571 and Trimer 6931 and Adjuplex

Preliminary immunogenicity studies in standard vaccine-test animals have demonstrated that boosting with Trimer 4571 significantly enhanced neutralizing titers in animals primed with an FP-carrier conjugate (32), and subsequent boosting with a diverse HIV-1 Env trimer derived from a clade C strain of donor CH505 increased consistency of elicited cross-clade neutralizing responses in guinea pigs (49). To demonstrate FP8v1-rTTHC immunogenicity and to evaluate the utility of Trimer 6931 as a heterologous Env trimer to boost FP-directed immune responses, guinea pigs were immunized using a FP8v1-rTTHC/Trimer 4571 prime-boost regimen and then further boosted with either Trimer 4571 or Trimer 6931, with Adjuplex adjuvant (35). Female Hartley guinea pigs were immunized intramuscularly (IM) with 25 mcg FP8v1-rTTHC at weeks 0, 4 and 8 weeks and immunized with 25 mcg Trimer 4571 at weeks 12 and 16. One group of animals (n = 6 per group) was boosted further with 25 mcg adjuvanted Trimer 4571 at weeks 20, 24 and 36. A second group (n = 6 per group) was boosted with 25 mcg adjuvanted Trimer 6931 at weeks 20 and 24 and given a final immunization of 25 mcg of a trimer cocktail at week 36. At week 38, 2 weeks after the final immunization, the group boosted with Trimer 6931 showed higher serum-neutralization breadth on a panel of 10 heterologous, cross-clade HIV-1 pseudoviruses compared to the group boosted only with HIV-1 Trimer 4571. Overall, this study showed that priming with adjuvanted FP8v1-rTTHC and sequential boosting with Trimer 4571 and Trimer 6931 could induce a broadly

neutralizing response in guinea pigs and demonstrated the utility of Trimer 6931 in boosting FP-directed responses.

Additional preclinical studies were conducted in mice and guinea pigs to evaluate immunogenicity of development-grade FP8v1-rTTHC, Trimer 4571 and Trimer 6931 immunogens, with Adjuplex adjuvant, using different prime-boost regimens. Animals (n = 10 per group) were immunized using the following vaccine regimens: 1) FP8v1-rTTHC prime followed by sequential boosting with Trimer 4571, Trimer 6931 and a trimer cocktail or 2) co administration of FP8v1-rTTHC and Trimer 4571 followed by boosting with Trimer 6931 and a trimer cocktail. Mice were immunized once every 2 weeks and guinea pigs were immunized monthly, except for a 2-month interval used between trimer cocktail boosts. For both mice and guinea pigs, 25 mcg of a single immunogen or 25 mcg of a trimer cocktail or 50 mcg of FP8v1-rTTHC + Trimer 4571 cocktail were administered intramuscularly with Adjuplex adjuvant at a 20% (v/v) dose. The data indicated that development-grade FP8v1-rTTHC, Trimer 4571 and Trimer 6931 are immunogenic in both mice and guinea pigs. Priming with FP8v1-rTTHC conjugate, either alone or in combination with Trimer 4571, followed by heterologous boosting with Trimer 6931, elicited consistent, autologous BG505 neutralizing responses in both mice and guinea pigs. Moreover, both FP-primed and FP + trimer cocktail-primed vaccine regimens with heterologous Env trimer boosts induced cross-clade HIV-1 neutralization in guinea pigs, and boosting with a cocktail of Env trimers expanded the breadth and potency of heterologous neutralizing responses. Additional details for these preclinical immunogenicity studies can be found in the IB.

A repeat-dose toxicity study of Trimer 4571 formulated with alum adjuvant was conducted to evaluate the safety of Trimer 4571 vaccine formulated with adjuvant in New Zealand white rabbits when administered intramuscularly (IM) or subcutaneously (SC) once every 3 weeks for 4 injections (study days 1, 22, 43, and 64). Treatment with the Trimer 4571 vaccine and adjuvant mixture induced appropriate antibody responses in the rabbits. All study injections were well tolerated, and the study rabbits used in this study survived to the scheduled necropsies. Any findings noted did not result in any adverse or limiting toxicity, were considered to be of minimal toxicological significance (eg, noted in only 1 sex, reversible, transient, no alteration in organ function), and were anticipated findings (such as fibrinogen increases and mixed cell infiltration at the injection sites) following administration of immunogenic substances.

A repeat-dose toxicity study to evaluate the safety of the 3 HIV-1 vaccine candidates (FP8v1-r TTHC, HIV-1 Trimer 4571, and HIV-1 Trimer 6931, 200 mcg each) alone or in combination with and without Adjuplex adjuvant (20% v/v) when administered intramuscularly (IM) every 3 weeks for 3 (FP8v1-rTTHC with or without Trimer 4571) or 4 (Trimers 4571 and 6931) injections was conducted in New Zealand white rabbits. Toxicity and reversibility of effects were evaluated after acute and recovery time points. The study was conducted in compliance with

the US Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations (21 CFR Part 58). Treatment with the HIV-1 vaccine candidates was well tolerated, did not result in any animal deaths and induced appropriate antibody responses in the rabbits. The treatments did not result in any treatment-related adverse clinical signs or toxicologically significant or adverse findings.

2.10 Previous Human Experience

There is no human experience with the FP conjugate vaccine (VRC-HIVVCP018-00-VP) or Trimer 6931 (VRC-HIVRGP0106-00-VP) prior to this trial.

2.10.1 Trimer 4571

2.10.1.1 Prevention studies

I. VRC 018

Trimer 4571 was evaluated under protocol VRC 018, "A Phase 1 Dose Escalation, Randomized, Open-Label Clinical Trial to Evaluate Dose, Safety, Tolerability and Immunogenicity of a HIV-1 Vaccine, VRC-HIVRGP096-00-VP, with Alum in Healthy Adults" (NCT03783130). This first-in-human study, conducted at the NIH Clinical Center in Bethesda, MD, was completed in June 2020. The VRC018 study evaluated Trimer 4571 at doses of 100 mcg or 500 mcg, field mixed with 500 mcg of aluminum hydroxide suspension (alum) adjuvant prior to injection, and administered via IM or SC injections at day 0, week 8 and week 20. Sixteen healthy adults, ages 22-48 years old, enrolled in the study; 6 participants received a 100-mcg dose (3 participants by IM and 3 by SC route) of Trimer 4571 and 10 participants received a 500-mcg dose (5 by IM and 5 by SC route).

As of August 2021, final data from this study are available and have been posted on ClinicalTrials.gov (NCT03783130). Overall, Trimer 4571 with alum adjuvant was well-tolerated with no reported SAEs or unexpected reactions, and no study pause criteria were met at any time.

Mild injection site pruritis following second or third product administration was the most frequently recorded unsolicited adverse event (AE), reported for 6/16 (37.5%) participants. All injection site pruritis events were assessed as related to study product, lasted between 1 and 9 days, and resolved with no residual effects.

The only other AE assessed as related to study product was a mild asymptomatic neutropenia recorded for a Group 3 (500 mcg IM) participant 7 days after the second product administration, and which resolved 8 days after onset with no residual effects. Solicited local and systemic reactogenicity symptoms were reported through 7 days post Trimer 4571 product administration. Symptoms of local reactogenicity were reported by 14 of 16 participants (87.6%), with mild pain/tenderness at the injection site being the most frequently reported symptom.

Symptoms of solicited systemic reactogenicity were reported by 11 of 16 participants (68.8%).

Local Reactogenicity

For the 100-mcg recipients, mild pain/tenderness at the injection site was the most frequent local symptom reported by 2 of 3 (66.7%) participants who received the IM or the SC injection, respectively. No other local symptoms were reported by participants who received 100-mcg IM. Among participants who received the 100-mcg SC injection, 2 of 3 (66.7%) participants reported mild to moderate swelling and 2 of 3 (66.7%) participants reported moderate redness. All solicited events in the 100-mcg groups resolved within the 7-day solicited period except for 1 report of pain/tenderness by a 100-mcg IM recipient, which occurred after the first product administration and resolved on day 8.

For the 500-mcg groups, 100% of the participants who received IM or SC injections reported at least 1 local symptom, with mild pain/tenderness at the injection site reported by 10/10 (100%) participants. One of 5 (20.0%) IM recipients reported moderate swelling after the second product administration and moderate redness after the third product administration. One of 5 (20.0%) SC recipients reported moderate swelling and severe redness, and 1 of 5 (20%) SC recipients reported mild redness after the first product administration. The moderate swelling resolved 1 day after the solicited period on day 8. All other events resolved within the solicited period.

Systemic Reactogenicity

Overall, for the 100-mcg groups, 1 of 3 (33.3%) participants who received the IM injection and 2 of 3 (66.7%) participants who received the SC injection reported at least 1 mild systemic symptom. Mild headache was the most frequent symptom, reported by 1 of 3 (33.3%) IM recipients and 2 of 3 (66.7%) SC recipients. One SC recipient reported moderate malaise and moderate nausea after the first product administration, and mild malaise after the second and third product administration. All events resolved within the 7-day solicited period except for 1 report of mild myalgia by a 100-mcg IM recipient, which occurred after the third product administration and resolved 6 days after the solicited period.

For the 500-mcg groups, 5 of 5 (100.0%) IM recipients and 3 of 5 (60.0%) SC recipients reported at least 1 mild systemic symptom. Mild myalgia was the most frequent symptom, reported by 4 of 5 (80.0%) IM recipients and 1 of 5 (20.0%) SC recipients. Mild malaise was reported by 2 of 5 (40.0%) IM and SC recipients, respectively. Mild headache was reported by 1 of 5 (20.0%) IM recipients and 1 of 5 (20.0%) SC recipients. Mild nausea was reported by 1 of 5 (20.0%) SC recipients. All events resolved within the solicited period. There were no reports of fever or severe systemic solicited reactogenicity symptoms.

Immunogenicity:

Immunogenicity of Trimer 4571 was evaluated by measurement of the trimer-specific antibody in serum samples by Electrochemiluminescence (ECLIA) using a Meso Scale Discovery (MSD) platform at baseline and at 2 weeks after the third product administration. Trimer 4571–specific antibody titers were below the negative cut off values in all groups at baseline. All dose groups displayed detectable antibody responses at 2 weeks after regimen completion (week 22) with the geometric mean AUCs of 674 (95% CI 50-9011), 924 (95% CI 175-4880), 2,529 (95% CI 387-16515), and 1,368 (95% CI 551-3397) for the 100-mcg IM, 100-mcg SC, 500-mcg IM, and 500-mcg SC dose groups, respectively.

Trimer 4571 induced antibodies that were detected by antigen (Ag)/antibody (Ab) Combination HIV testing in participants who confirmed to be HIV negative by polymerase chain reaction (PCR). This reaction is referred to as vaccine-induced seropositivity (VISP). The VRC 018 trial used the VITROS 3600 system Ag/Ab detection assay at the NIH Clinical Center for clinical testing. Samples collected from 7 of 16 participants (43.8%) were reactive in the assay, and therefore seropositive, but participants were confirmed as HIV negative by PCR. Of the 7 participants who were seropositive, 1 was a 100-mcg recipient and 6 were 500-mcg recipients. Six of 7 participants with VISP reverted back to non-reactive on later testing. It is unknown whether the remaining 1 participant (500-mcg recipient) reverted back to non-reactive, as the final follow-up visits were conducted by the telephone, but blood samples were not collected due to the coronavirus disease 2019 (COVID-19) public health emergency stay-at-home orders.

II. NIH-19-I-0069 Phase 1 Study (NCT03878121) [Source: Information provided by Vaccine Research Center (VRC)]

Protocol NIH-19-I-0069 is an open-label phase 1 study of recombinant adenovirus serotype 4 (Ad4)-based HIV envelope (Env) vaccines administered intranasally to healthy volunteers. Each study vaccinee (n~100) will receive 1 of 2 Ad4-HIV vaccines at months 0 and 2, followed by a booster vaccination with 500 mcg of the heterologous soluble trimeric protein VRCHIVRGP096-00-VP (HIV-1 Trimer 4571) with alum, administered as an intramuscular (IM) injection at month 6.

The study was initiated in February 2019 and is ongoing.

As of March 31, 2023, 21participants have enrolled in the trial and 15 participants have received HIV-1 Trimer 4571 with alum adjuvant, 500 mcg by IM route at Month 6 of trial participation. Doses of Trimer 4571 were given as a boost 4 months after administration of the second dose of Ad4-Env145NFL or Ad4-Env150KN intranasally (administered at months 0 and 2).

Overall, Trimer 4571 product administrations have been generally well tolerated with 39 reported reactogenicity events after trimer 4571 dosing, 8 of which were

judged to be definitely, possibly or possibly related to Trimer 4571 administration. Solicited local reactions of mild pain at the injection site was reported by 1 of 15 (6.7%) participants. Mild to moderate headache reported by 5 (33.3%) participants and mild to moderate fatigue reported by 3 (20%) participants. No unsolicited events were assessed as related to study product. There were no SAEs reported and no adverse events resulted in the Trimer 4571 product discontinuation in any participant. There have been no SUSARs reported to the FDA and no study pauses due to safety concerns.

III. HVTN 137 (NCT04177355)

HVTN 137 is a Phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 BG505 SOSIP.664 gp140 with TLR agonist and/or alum adjuvants, and specifically, VRC HIV Env Trimer 4571 and TLR agonist, 3M-052-AF, with alum in healthy, HIV-uninfected adults. Part C Group 8, the only group including Trimer 4571, will enroll 10 participants (10:1 vaccine to placebo ratio) to receive Trimer 4571 100 mcg adjuvanted with 5 g 3M-052-AF and 500 g alum administered IM and placebo (N = 1) at Days 0 and 56 with an optional third dose at Day 168.

The study began enrolling on February 5, 2023. As of May 1, 2023, 11 participants have received the first of 3 planned administrations of Trimer 4571 with 3M-052-AF or placebo. Five (5) participants have received the second dose.

Overall product administrations have been generally well-tolerated with reported reactogenicity of mostly mild to moderate in severity. Solicited injection site pain and/or tenderness was reported by 8 of 11 (73%) participants (7 mild, 1 moderate). Mild induration at the injection site was reported by 1 (9%) participant. No participants reported gradable injection site erythema. Solicited systemic reactions were reported by 9 (81.8% participants, including 2 (18%) with mild symptoms, 6 (55%) with moderate symptoms, and 1 (9% with severe symptoms. Systemic events included: malaise/fatigue (n=2 mild, n=5 moderate, n=1 severe); myalgia (n=2 mild, n=4 moderate, n=1 severe); headache (n=2 mild, n=3 moderate, n=1 severe); nausea (n=3 mild, n=2 moderate); chills (n=2 mild, n=1 moderate, n=1 severe); arthralgia (n=2 mild, n=1 severe); and fever (n=3 mild).

Three (3) unsolicited adverse events have been reported by 2 (18.2%) participants. One participant reported 2 events that were determined to be related to study product: mild decreased appetite of 10 days duration and mild brain fog lasting 2 days. Both events resolved without sequelae.

There have been no SAEs and no adverse events resulting in product discontinuation of Trimer 4571.

2.10.1.2 Therapeutic studies

Native-like Envelope Trimer Immunization (NETI) Phase 1 Study (NCT04985760) [Source: information provided by Vaccine Research Center (VRC)]

The NETI protocol is a phase 1; randomized, double-blind, dose escalation clinical trial to assess the safety, tolerability, and immunogenicity of vaccination with Trimer 4571 in adults living with HIV-1 on suppressive ART. Both groups are randomized 3:1 Trimer 4571: vaccine control with alum and administered study product by IM injection as follows:

- Group 1 will receive 100 g of Trimer 4571 with 500 mcg of alum (active, n=6) or volume matched PBS with 500 g alum (control, n= 2) at weeks 0, 8 and 20.

- Group 2 will receive 500 g of Trimer 4571 with 500 mcg of alum (active, n=18) or volume matched PBS with 500 g alum (control, n=6) at weeks 0, 8 and 20.

- Group 2A (n=8) will enroll participants without evidence of precursor broadly neutralizing antibody at screening.

- Group 2B (n=16) will enroll participants with evidence of precursor broadly neutralizing antibody at screening.

The first participant enrolled and received study product on September 30, 2021. As of March 31, 2023, the study is ongoing and 16 participants have received either a 100 mcg dose of Trimer 4571 with alum or vaccine control by IM route. A total of 16 participants have received two doses of study product and 9 have received three doses of study product. Overall product administrations have been generally well tolerated with all reported reactogenicity of grade 1 or grade 2 in severity.

Solicited local reactions were reported by 14 of 16 (87.5%) participants including injection site tenderness (n=11, 68.8%) and injection site pain (n=6, 37.5%). Local symptoms were mild in severity with the exception of 1 participant reporting moderate tenderness. Solicited systemic reactions were reported by 7 (43.8%) participants including fatigue (n=5, 31.3%), arthralgia (n=2, 12.5%), myalgia (n=3, 18.8%), headache (n=2, 12.5%) and 1 (6.3%) reported instance each for chills, malaise, and nausea. One (6.3%) participant reported headache; one (6.3%) reported fatigue; and one (6:3%) reported myalgia after both first and second dose of study product. Of the 40 solicited events reported, 34 (85.0%) were mild and 6 (15.0%) were moderate in severity.

Thirteen (81.3%) participants reported unsolicited adverse events, and 4 events were determined to be possibly or probably related to study product due to

temporal association. One participant experienced new onset eczema, moderate in severity 14 days after first dose of study product, which resolved but recurred 2 days prior to second dose of study product in a localized area after application of local heat. This participant noted mild worsening two days after third dose of study product, which improved with topical steroid application. One subject reporting flushing of the left face, neck and shoulder on the day of first product administration and a tender lymph node in the neck on the day of second study product administration. Both events resolved the same day.

There were 2 SAEs reported: 1 hospitalization due to acute cholecystitis and 1 hospitalization due to COVID-19 pneumonia, both events were assessed as unrelated to study product. A planned interval cholecystectomy for the participant with acute cholecystitis demonstrated incidental finding of gallbladder adenocarcinoma on the resected gallbladder pathology, assessed as unrelated to study product, and subsequently, per investigator discretion, the third dose of the study product was not administered. The participant with COVID-19 pneumonia received a contraindicated medication and per study protocol the third dose of study product was discontinued. There were no related SAEs. There have been no SUSARs reported to the FDA or other regulatory agency and no study pauses due to safety concerns.

2.10.2 Adjuplex

Adjuplex is currently being tested in the following studies:

- A Phase 1 clinical study of a mosaic quadrivalent influenza vaccine (NCT04896086).

- A Phase 1 clinical study of an adenovirus-vectored vaccine against cocaine (NCT02455479). A summary of the cocaine vaccine study objectives and endpoints, a well as study contact information, can be found on the ClinicalTrials.gov website.

- A Phase 1 clinical study (HVTN 303, NCT05470400) with Adjuplex combined with HIV vaccine antigens

2.10.3 Experience in this study (HVTN 303) to date

Local and systemic reactogenicity, adverse events, and transient increases in absolute eosinophil counts

Two participants reported grade 3 fever on day 1 after first vaccination that resolved the same day (Group 7) or the next day (Group 8).

Across Groups 3, 5, 6, 7, and 8, there were 6 participants who reported grade 3 injection site reactions of erythema (4 participants); induration/swelling (1 participant); or both (1 participant) after first or second vaccination, with onset on

days 1, 2, 5, 6, 7 or 8 post-vaccination. A Group 5 participant with grade 3 erythema and grade 3 induration/swelling also reported pain in thumb and knee with possible swelling over his right metacarpal joint; however, at the time of reporting, possible alternative etiologies for these joint symptoms were considered.

One group 8 participant was diagnosed with serum sickness, with onset on day 8 post first vaccination. The participant was prescribed oral corticosteroids, and all symptoms resolved within approximately 2 weeks. The participant was discontinued from study vaccinations.

A second Group 8 participant experienced grade 3 fever one day post first vaccination that lasted 1 day. Two weeks post-vaccination, the participant reported a small area of erythema on the chest that was pruritic, bruising above the right antecubital fossa, right armpit pain/tenderness, and sore throat. All symptoms were resolved after 7 days. One week post 2nd vaccination, the participant reported a nickel-sized area of urticaria/erythema on the right shin that was pruritic and blanched slightly with pressure; the rash resolved after 8 days.

A Group 7 participant reported pruritis and slightly area of redness at/near the injection sites of both arms, with onset two days post second vaccination. The symptoms resolved after 4 days.

In addition, several participants had a transient increase in absolute eosinophil count (AEC) after vaccination. None of the participants with elevated AECs had grade 3 erythema, induration or fever; none had symptoms associated with the AEC increase; none required any treatment or medical care. All elevated values returned to within laboratory reference range.

2.11 Risks and benefits

2.11.1 Risks of the Vaccines:

This is the first study in humans of the investigational vaccines FP8v1-rTTHC and Trimer 6931; therefore, risks are unknown at the time of study start.

The signs and symptoms associated with administration of a similar vaccine, Trimer 4571, are discussed in Section 2.10.1.

Most of the risks noted are based on risks of vaccines in general. Potential side effects resulting from intramuscular injections include stinging, discomfort, redness of skin, or mild bruising at vaccine injection site. As with any injection procedure, infection at the site of injection is a possible risk. Signs of infection at the injection site include severe pain, redness, swelling, warmth or drainage.

Participants may exhibit general signs and symptoms associated with administration of the vaccine injection, including fever, chills, rash, aches and pains, nausea, dizziness and fatigue. These side effects will be monitored, but are generally short term, mild to moderate severity and usually do not require treatment.

There may be side effects from the study products, which may be serious or life threatening, that we do not know about yet.

These vaccines are intended to generate antibodies, which may cause a positive or indeterminate HIV antibody result in standard diagnostic tests that may have a negative employment and social impact. HIV PCR will be used to exclude or confirm HIV infection.

The majority of the human population is immune to tetanus toxoid via vaccination. Since the fragment used in FP8v1-rTTHC (FP conjugate) vaccine is not a complete tetanus toxoid, but rather just the heavy chain it is most likely will not be immunogenic. No risks are expected from the tetanus toxoid fragment.

2.11.2 Risks of Adjuplex

Adjuplex is a novel adjuvant platform and therefore, risks are broadly unknown at the time of study start. As described in Section 2.8, one of Adjuplex components is soy lecithin. Highly purified soy lecithin that is used for Adjuplex preparation does not contain soy protein residues that are linked to induction of an allergic reaction to soy, and therefore an allergic reaction to the Adjuplex is highly unlikely (50, 51).

Based on experience with Adjuplex in animal studies described in Section 2.4, potential inflammatory reactions at injection site may be expected, similar to the injection site reactions observed with other adjuvants.

In a single human phase 1 study described in Section 2.10.2 (NCT02455479), injections of either a vaccine adjuvanted with Adjuplex or Adjuplex alone have been well tolerated, with injection site pain, soreness and tenderness, and occasional fever reported. As of 10 December 2021, there was only one SAE reported in the study, and it was attributed to cocaine drug use, not the vaccine or adjuvant.

Potential inflammatory reactions at the injection site may be expected, similar to the injection site reactions observed with other adjuvants. This can manifest as mild to severe pain, redness, and swelling at the site of administration. When combined with a vaccine, Adjuplex may cause more frequent or severe systemic reactions, such as fever, chills, myalgia, or malaise, compared to formulations containing the vaccine only. Rash, urticaria and transient elevated absolute eosinophil counts and one event of serum sickness have been observed after administration of Adjuplex-adjuvanted vaccines.

There is a limited experience with Adjuplex in humans, there may be other unknown side effects.

2.11.3 Other risks

A skin biopsy is a generally safe procedure. However, complications such as bleeding, pain, bruising, and scarring, and rarely an infection can occur.

2.11.4 Benefits

Study participants will not receive direct health benefit from study participation. Others may benefit from knowledge gained in this study that may aid in the development of an HIV vaccine. The investigational vaccine is not expected to provide protection from HIV infection.

Participants may benefit from more frequent counseling, laboratory tests and physical exams while enrolled in the study. Participants may also experience positive social impacts as a benefit of study participation. When asked, participants say that being in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self-esteem.

3 Objectives and endpoints

On January 13, 2023, a protocol memo was distributed informing the clinical research sites that all vaccinations in HVTN 303 were permanently discontinued. Due to permanent discontinuation of vaccinations, none of the participants received Trimer 6931 in prime-boost regimen. Collection of immunogenicity samples was stopped on March 13, 2023. Sections 2.6 and 3 are the revised version of the original protocol (v1.0) as per the availability of the samples and the content of the current protocol version 2.0.

3.1 Primary objectives and endpoints

Primary objective 1:

To evaluate the safety and tolerability of the following regimens in healthy adults:

- Adjuvanted FP conjugate vaccine administered IM at a dose of 25 or 200 mcg,
- Adjuvanted Trimer 6931, administered IM at a dose of 100 or 200 mcg,
- Adjuvanted Trimer 4571 administered IM at 200 mcg, or
- Prime-boost vaccination regimen of FP conjugate and Trimer 4571.

Primary endpoint 1:

Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and adverse and serious adverse events.

SAEs, medically attended adverse events (MAAEs), adverse events of special interest (AESIs) and AEs leading to early participant withdrawal or permanent discontinuation which will be collected throughout the study and for 12 months following any receipt of study product. Additionally, all adverse events will be collected for 28 days after any receipt of study vaccination. All safety lab related adverse events will be collected throughout duration of study.

Primary objective 2:

To evaluate the ability of FP-conjugate and Trimer 4571 vaccines to elicit FP-specific binding antibodies in Part B participants.

Primary endpoint 2:

Magnitude and response rate of serum antibody binding of FP and envelope trimer antigens as measured by the MSD assay 2 weeks after the last vaccination.

3.2 Exploratory objectives

Exploratory objective 1:

Mapping of FP-specific serum neutralizing activity

Exploratory objective 2:

To evaluate the ability of the vaccine regimen to elicit early FP broad neutralizing antibody memory B-cell lineages.

Exploratory objective 3:

To evaluate the humoral and cellular immune response to vaccination regimens including FP-conjugate vaccine and Trimer 4571 to compare responses between the regimens.

Exploratory objective 4:

To conduct analyses related to furthering the understanding of HIV, immunology, vaccines, and clinical trial conduct. To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed in a subset of participants, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm.

4 Laboratory strategy

The laboratory strategy for this protocol will focus on interrogations of the humoral ~~and cellular immune~~ response as it relates to the ability of FP-conjugate and Trimer 4571 vaccines to elicit FP-specific binding antibodies. ~~the elicitation of broadly neutralizing antibodies. Magnitude, breadth, kinetics and durability of serum neutralizing antibodies will be assessed by using vaccine-matched and non-matched (heterologous) tier 2 HIV-1 Env-pseudotyped viruses in the TZM-bl assay. Serum antibodies will be further interrogated by measuring IgG binding to the vaccine antigens and a specialized set of epitope domain-specific antigens. A role for T-cell help will be investigated by assessing antigen-specific CD4+ T cells by intracellular cytokine staining as measured by flow cytometry.~~ To gain further insights ~~into the requirements for broadly neutralizing antibody induction~~, exploratory studies may be conducted to assess antigen-specific B-cell frequencies and to interrogate B-cell lineages that give rise to FP-specific antibodies in vaccine recipients. Serum neutralizing antibodies may also be assessed in the TZM-bl assay. The detailed laboratory strategy and the technical details are described in the Central Assay Plan and will be updated as new reagents and techniques are incorporated into assay planning. This document will be available on the protocol webpage. Descriptions of the standard HVTN laboratory assays can be found online at https://www.hvtn.org/content/dam/hvtn/scientific-programs/hvtn-laboratory-assay-descriptions.pdf

5 Study design

This is an open-label, dose-escalation study to examine the safety, tolerability, and immunogenicity dose of adjuvanted Fusion Peptide Vaccine alone or in prime-boost regimens with adjuvanted Trimer 4571 ~~and Trimer 6931~~ vaccines in healthy adults. The hypothesis is that the vaccines will be safe, tolerable for human administration and will induce detectable immune response.

5.1 Study population

All inclusion and exclusion criteria must be met for eligibility. Screening procedures to determine eligibility must be performed within 56 days prior to enrollment.

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.

5.1.1 Inclusion criteria

1. Able and willing to complete the informed consent process, including an Assessment of Understanding (AoU): volunteer demonstrates understanding of this study, completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly.

2. 18-50 years old, inclusive, on day of enrollment.

3. Agrees to comply with planned study procedures and be available for clinic follow-up through the last clinic visit.

4. Agrees not to enroll in another study of an investigational agent during participation in the trial, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) investigational agents that may subsequently obtain emergency use authorization (EUA) or undergo licensure by the FDA. If a potential participant is already enrolled in a SARS-CoV-2 clinical trial, prior approvals from the SARS-COV-2 study sponsor and HVTN 303 PSRT are required prior to enrollment in HVTN 303.

5. In good general health without clinically significant medical history.

6. Physical examination and laboratory results without clinically significant findings that would interfere with assessment of safety or reactogenicity.

7. Body Mass Index (BMI) ≤ 40.

8. Assessed as low risk for HIV acquisition as per Appendix K, agrees to discuss HIV infection risks, agrees to risk reduction counseling, and agrees to avoid behavior associated with high risk of HIV exposure through the final study visit. Low risk may include persons stably taking pre-exposure prophylaxis (PrEP) as prescribed for 6 months or longer (see Appendix K for protocol safety review team [PSRT)] consultation requirements).

9. Suitable injection sites in the deltoid muscle of each arm, as assessed by a clinician.

Laboratory Criteria within 56 days prior to enrollment:

10. White blood cells (WBCs) 2,500-12,000/mm^3

11. WBC differential either within institutional normal range or approved by the Investigator of Record (IoR) as "not clinically significant."

12. Platelets = 125,000 – 500,000/mm3

13. Hemoglobin

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

14. Serum creatinine ≤ 1.1 x upper limit of normal (ULN) based on the institutional normal range.

15. Alanine aminotransferase (ALT) ≤1.25 x ULN based on the institutional normal range.

16. Negative for HIV infection by an (US) Food and Drug Administration (FDA)-approved enzyme immunoassay (EIA) or chemiluminescent microparticle immunoassay (CMIA).

17. Negative for anti-Hepatitis C antibodies (anti-HCV) or negative HCV nucleic acid test (NAT) if anti-HCV antibodies are detected.

18. Negative for Hepatitis B surface antigen.

For a volunteer capable of becoming pregnant:

19. Agrees to use effective means of birth control from at least 21 days prior to enrollment through 12 weeks after the last product administration.

20. Negative β-HCG (beta human chorionic gonadotropin) pregnancy test (urine or serum) at screening and prior to each study product administration on the day of study product administration.

5.1.2 Exclusion criteria

1. Active duty and reserve US military personnel

For a volunteer capable of becoming pregnant:

2. Breast-feeding or planning to become pregnant from at least 21 days prior to enrollment through 12 weeks after the last product administration.

Participant has received any of the following:

3. An investigational HIV vaccine (previous placebo recipients are not excluded).

4. 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 ≤ 60 mg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment).

5. Blood products within 60 days prior to enrollment

6. Monoclonal antibodies (mAbs), whether licensed or investigational. Exceptions may be made by the HVTN 303 PSRT on a case-by-case basis

7. Receipt of any of the following:

    - Within 4 weeks prior to enrollment:
        - Any licensed live, attenuated vaccine (except for the Jynneos monkeypox vaccine)
        - Any adenoviral-vectored SARS-CoV-2 vaccine with FDA Emergency Use Authorization (EUA), FDA licensure or World Health Organization (WHO) Emergency Use Listing (EUL)

- ACAM2000 vaccine for Monkeypox
- ACAM2000 received greater than 4 weeks prior to enrollment but vaccination scab is still present

- Within 2 weeks prior to enrollment:
  - Any licensed killed/subunit/inactivated vaccine
  - Any mRNA based or protein SARS-CoV-2 vaccines with FDA EUA, FDA licensure, or WHO EUL
  - Jynneos Monkeypox vaccine for Monkeypox

  Receipt of any SARS-CoV-2 vaccination series should be completed 4 weeks prior to enrollment when possible.

8. Investigational research agents with a half-life of 7 or fewer days within 4 weeks prior to enrollment. If a potential participant has received investigational agents with a half-life greater than 7 days (or unknown half-life) within the past year, PSRT approval is required for enrollment.

9. Current allergen immunotherapy with antigen injections, unless on maintenance schedule.

10. Current anti-TB prophylaxis or therapy.

Participant has any of the following:

11. Serious adverse reactions to vaccines or vaccine components.

12. Hereditary angioedema, acquired angioedema, or idiopathic forms of angioedema.

13. Hypertension that is not well controlled.

    - 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.

14. Asthma is excluded if the participant has ANY of the following:

    - Required either oral or parenteral corticosteroids for an exacerbation two or more times within the past year; OR

    - Needed emergency care, urgent care, hospitalization, or intubation for an acute asthma exacerbation within the past year (eg, would NOT exclude individuals with asthma who meet all other criteria but sought urgent/emergent care solely for asthma medication refills or co-existing conditions unrelated to asthma); OR

    - Uses a short-acting rescue inhaler more than 2 days/week for acute asthma symptoms (ie, not for preventive treatment prior to athletic activity); OR

    - Uses medium-to-high-dose inhaled corticosteroids (greater than 250 mcg fluticasone or therapeutic equivalent per day), whether in single-therapy or dual-therapy inhalers (ie, with a long-acting beta agonist [LABA]); OR

    - Uses more than one medication for maintenance therapy daily. Inclusion of anyone on a stable dose of more than one medication for maintenance therapy daily for greater than two years requires PSRT approval.

15. Autoimmune disease, current or history, including psoriasis.

16. Clinically significant immunodeficiency.

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

18. 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.).

19. Diabetes mellitus type 1 or type 2.

20. Bleeding disorder diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws.

21. Seizure disorder other than: 1) febrile seizures, 2) seizures secondary to alcohol withdrawal more than 3 years ago, or 3) seizures that have not required treatment within the last 3 years.

22. Asplenia or functional asplenia.

23. 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).

24. Any other chronic or clinically significant condition that in the clinical judgement of the investigator would jeopardize the safety or rights of the study participant, including, but not limited to: clinically significant forms of drug or alcohol abuse, serious psychiatric disorders, or cancer that, in the clinical judgement of the site investigator, has a potential for recurrence (excluding basal cell carcinoma).

5.1.3 Leukapheresis Eligibility Criteria

Only participants who received two vaccinations in Part B (Groups 7-8) and who consent to leukapheresis will undergo leukapheresis at 10 months +/- 2 months post–last vaccination in order to collect blood cells for research.

~~Part B (groups 6-8) participants who consent to leukapheresis (see Section 2.7) will be asked to undergo leukapheresis at 2 weeks post- last vaccination in order to collect blood cells of special interest for research.~~ These participants must meet all of the following criteria:

1. Weight ≥ 110 pounds

2. Meeting local site requirements related to these procedures (eg, any specific hemoglobin level requirements by pheresis center)

3. Negative beta-human chorionic gonadotropin (β-HCG) pregnancy test (urine or serum) performed by a study clinician within 72 hours prior to the apheresis procedure

5.2 Participant departure from vaccination schedule or withdrawal

On January 13, 2023, a protocol memo was distributed informing the clinical research sites that all vaccinations in HVTN 303 were permanently discontinued. Hence, the language that are no longer relevant for this amendment is marked with strikethrough.

5.2.1 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:~~

- ~~Intercurrent illness or pre vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction~~

- ~~Treatment with systemic glucocorticoids (eg, prednisone or other glucocorticoid), with the exception that study injection may continue per principal investigator (PI) discretion if the next study injection occurs at least 2 weeks following completion of glucocorticoid treatment~~

- ~~Receipt of any live attenuated vaccines within 4 weeks prior to study vaccine administration~~

- ~~Receipt of any inactivated vaccines within 2 weeks prior to study vaccine administration with exceptions as below:~~

- ~~Timing of study vaccination with respect to SARS-CoV-2 vaccination: if the SARS-CoV-2 vaccination series was not completed prior to enrollment, attempt to time SARS-CoV-2 vaccination no closer than 2 weeks (for protein vaccines and mRNA-based vaccines) or 4 weeks (for adenoviral-vectored) before or after study vaccination.~~

- ~~Timing of study vaccination with respect to Monkeypox vaccination: attempt to separate study vaccination from administration of Monkeypox vaccine prior to study enrollment, or during study based on the instruction below:~~

  - ~~Jynneos vaccine:~~

    - ~~after administration of Jynneos vaccine, a minimum of 2 weeks is required before administration of study vaccine~~

    - ~~after study vaccine administration, a minimum of 2 weeks is required before administration of Jynneos vaccine~~

  - ~~ACAM2000 vaccine: *(Note: in addition to the instructions mentioned below, sites should consult with HVTN 303 PSRT for any participants who may be scheduled to receive ACAM2000 vaccine after enrollment)*~~

    - ~~after administration of ACAM2000 vaccine, a minimum of 4 weeks is required before administration of study vaccine; if vaccination scab is not present~~

    - ~~if ACAM2000 vaccine is received greater than 4 weeks prior to enrollment but vaccination scab is still present, delay study vaccination until the scab is no longer present~~

    - ~~after study vaccine administration, a minimum of 2 weeks is required before administration of ACAM2000 vaccine~~

~~Vaccinations should not be administered outside the visit window period specified in Appendix E and Appendix F.~~

5.2.2 Discontinuation of study vaccine administration

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:

- SAE that is subsequently considered to be related to vaccination
- Pregnancy (regardless of outcome)
- HIV infection
- Grade 3 AE assessed as related to study vaccine, with the following exceptions:
  - Grade 3 subjective reactogenicity and injection site reactions: injection site pain/tenderness and erythema/induration (grade 3 by size only), fatigue, generalized myalgia, generalized arthralgia, chills, headache, nausea (unless intravenous [IV] rehydration required)
  - AEs reviewed by the PSRT and approved for vaccination continuation
- Grade 4 AE assessed as related to study vaccine
- Clinically significant type 1 hypersensitivity associated with study vaccine

For ease of reference and review, the clinically significant type 1 hypersensitivity definition, as per the Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium criteria for anaphylaxis (28), is provided below:

Anaphylaxis is highly likely when any one of the following criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow [PEF], hypoxemia)

   b. Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient [participant] (minutes to several hours):

a. ~~Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)~~

~~b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)~~

~~c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)~~

~~d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)~~

~~3. Reduced BP after exposure to known allergen for that patient [participant] (minutes to several hours). Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline~~

- ~~PI assessment that it is not in the best interest of the participant to continue receiving study vaccine~~
- ~~New diagnosis of or newly disclosed AESI (see Appendix MAppendix M)~~
- ~~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 303 PSRT)~~

~~Participants discontinuing study vaccine for reasons other than HIV infection should be encouraged to participate in follow-up visits and procedures per the protocol. At the discretion of the CRS clinician and the PSRT (for composition of PSRT see Section 9.3), some clinic procedures and sample collections may be modified or discontinued.~~

If a participant becomes HIV-infected during the course of the study, ~~no additional study vaccine will be administered.~~ participants will be encouraged to continue scheduled study visits for up to 12 months following their last study vaccine administration. At post infection follow-up visits, only samples for protocol-specified clinical labs (with the exception of HIV diagnostic testing) will be collected. In addition, some clinic procedures may be modified or discontinued.

5.2.3 Participant departure from vaccine schedule

~~If a participant misses a scheduled vaccination, they are eligible for future vaccinations.~~

5.2.4 Discontinuation of study participation

A participant may be discontinued from protocol participation for the following reasons:

- Participant voluntarily withdraws;
- CRS determines the participant is lost to follow-up;
- The investigational new drug (IND) application Sponsor or regulatory authorities stop the study; or
- PI assessment that it is not in the best interest of the participant to continue participation in the study or that the participant's compliance with the study is not sufficient.

If a participant terminates participation in the study early for any reason, the site principal investigator should consider if the following assessments are appropriate: end-of-study HIV test, complete blood count (CBC) with differential, serum chemistry, physical examination, and if indicated, a pregnancy test (see Appendix A, Appendix B, Appendix C and Appendix D). For participants with HIV infection, please see Section 8.7. If the site principal investigator has questions regarding a termination visit, they should consult with the PSRT.

6 Statistical considerations

On January 13, 2023, a protocol memo was distributed informing the clinical research sites that all vaccinations in HVTN 303 were permanently discontinued. The sample size calculations for safety and immunogenicity were updated based on the actual numbers of participants who were enrolled in the study and the updated objectives in Section 3. The language shown below is the revised version of the original protocol (v1.0) as per the sample size and the design of the current protocol version 2.0.

6.1 Accrual and sample size calculations

Recruitment will target 60 (up to 70) healthy adult participants 18 to 50 years of age. However, due to the early termination, the study enrolled a total of 44 participants (15 in Part A and 29 in Part B). The primary goal of this study is to identify safety concerns that may be associated with the study products.

Since enrollment is concurrent with receiving the first study vaccination, all enrolled participants will provide some safety data. For immunogenicity analyses, the sample calculations are based on the available samples.

6.2 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect SAEs (see Section 9.1) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each vaccine arm of the study (n = 3) in Part A, there is a 90% chance of observing at least 1 event if the true rate of such an event is 53.6% or more; and there is a 90% chance of observing no events if the true rate is 1% or less. For a vaccine arm in Part B (n = 9 or 10), there is a 90% chance of observing at least 1 event if the true rate of such an event is 22.6% or 20.6% or more; and there is a 90% chance of observing no events if the true rate is 1% or less For a combined vaccine arm (Groups 1-5 in Part A and Groups 6-8 in Part B) of the study (n = 44), there is a 90% chance of observing at least 1 event if the true rate of such an event is 5.1% or more; and there is a 90% chance of observing no events if the true rate is 0.2% or less. As a reference, in HVTN vaccine trials from April 2008 through March 2018, 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 3, 9, 10, and 44 are presented in Table 6-1for 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 3, 9, 10, and combined arms of size 44, for different true event rates

True event rate (%)	Pr(0/3)	Pr(1+/3)	Pr(2+/3)	Pr(0/10)	Pr(1+/10)	Pr(2+/10)	Pr(0/9)	Pr(1+/9)	Pr(2+/9)	Pr(0/44)	Pr(1+/44)	Pr(2+/44)
1	97	3	0	90.4	9.6	0.4	91.4	8.6	0.3	64.3	35.7	7.2
4	88.5	11.5	0.5	66.5	33.5	5.8	69.3	30.7	4.8	16.6	83.4	53
5	85.7	14.3	0.7	59.9	40.1	8.6	63	37	7.1	10.5	89.5	65.3
10	72.9	27.1	2.8	34.9	65.1	26.4	38.7	61.3	22.5	1	99	94.3
20	51.2	48.8	10.4	10.7	89.3	62.4	13.4	86.6	56.4	<0.1	>99.9	99.9
30	34.3	65.7	21.6	2.8	97.2	85.1	4	96	80.4	<0.1	>99.9	>99.9
40	21.6	78.4	35.2	0.6	99.4	95.4	1	99	92.9	<0.1	>99.9	>99.9
50	12.5	87.5	50	0.1	99.9	98.9	0.2	99.8	98	<0.1	>99.9	>99.9
60	6.4	93.6	64.8	0	>99.9	99.8	0	>99.9	99.6	<0.1	>99.9	>99.9

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval (CI) for the true rate of an adverse event based on the observed data. Table 6-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method (52). If none of the participants in a group of size of 3 (eg, Groups 1-5 in Part A) or in a group of size of 9 or 10 (eg, the Groups 6-8 in Part B) are experiencing a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 56.1% or 29.9% or 29.2%, respectively. For the combined vaccine arms in Part A and Part B (Groups 1-8, n = 44), the 95% 2-sided upper confidence bound for this rate is 9.8%.

Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 3, 9, 10 or combined arms of size 44

Observed event rate	95% Confidence interval (%)
0/3	[0, 56.1]
1/3	[6.1, 79.2]
2/3	[20.8, 93.9]
0/9	[0, 29.9]
1/9	[2, 43.5]
2/9	[6.3, 54.7]
0/10	[0, 29.2]
1/10	[0.1, 30.5]
2/10	[0.2, 31.9]
0/44	[0.1, 9.8]
1/44	[0.3, 11.4]
2/44	[0.6, 12.9]

6.2.1 Sample size calculations for immunogenicity

The main goals of this trial regarding immunogenicity outcomes involve assessing the ability of FP-conjugate and Trimer 4571 vaccines alone or co-administration to elicit antibody binding FP and envelop trimer antigens and mapping FP-specific serum neutralizing activity induced by the prime vaccines regimens in Groups 5-8 and prime boost vaccines regimens in Groups 7-8. 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% CIs for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-3. Calculations are done using the score test method (52). The sample size of n = 13 (Groups 5-6) and n=9 for each of Groups 7 and 8 will be used to evaluate the immunogenicity post the 1st vaccination induced by the vaccination; the sample sizes of n=4, 5 for Groups 7-8 will be used to evaluate the immunogenicity post the 2nd vaccination.

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, 5, 9, 13)

No. of responses	Observed response rate (%)	95% Confidence interval
2/4	50	[15, 85]
4/4	100	[51, 100]
2/5	40	[11.8, 76.9]
4/5	80	[37.6, 96.4]
3/9	33.3	[12.1, 64.6]
6/9	66.7	[35.4, 87.9]
9/9	100	[70.1, 100]
3/13	23.1	[8.2, 50.3]
6/13	46.2	[23.2, 70.9]
9/13	69.2	[42.4, 87.3]

As shown in Table 6-4, there is limited power for a formal comparison of immunogenicity response rates between Group 7 (n=9) and Group 8 (n=9). 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 exact 2-sided test with a type I error rate of 0.05.

Table 6-4 Power for comparison of response rates between 2 arms (n1 = 9, n2 = 9)

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

The response magnitudes will be compared between the arms among the positive responders. The response magnitudes will be transformed (eg, using log-transformation for response magnitudes) prior to the comparisons if they are not normally distributed. The numbers presented in Table 6-5 are the minimum mean differences (in standard deviations [SDs]) between two arms that can be distinguished with a statistical power of 80% or 90% and a type I error rate of 0.05 given a range of possible true response rates in both arms. The calculations are based on 5,000 simulations with the data being generated from normal distributions with different means and a common standard deviation in two arms and the Wilcoxon rank sum test for comparing the difference between arms.

Table 6-5 Minimum mean difference (in SDs) with 80% or 90% power to distinguish the response levels between Group 7 and Group 8 among positive responders.

Common true response rate (%)	Minimum mean difference in SDs (n1 = 9, n2 = 9)	
	Power = 80%	Power = 90%
50	2.7	3.1
60	2.3	2.6
70	2.0	2.3
80	1.8	2.0
90	1.6	1.9
100	1.5	1.8

6.3 Randomization

In Part A, Groups 1 and 3 will be randomized and will enroll no more than 1 participant per day for 3 participants per group. Contingent on the safety data from Groups 1 and 3, Groups 2, 4, and 5 will be randomized and may enroll simultaneously with no more than 1 participant per day for 3 participants for each group. Contingent on the safety data from Groups 1-5 in Part A, Groups 6-8 in Part B will be randomized and stratified by whether or not participants will be willing to consent to leukapheresis. A maximum of 7 participants per group that do NOT consent to leukapheresis collection will be enrolled. 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 procedures [MOP]).

6.4 Blinding

Participants and site staff will be unblinded to participants' group assignments. Laboratory program staff will be unblinded to whether a sample is from Part A or Part B but will remain blinded to the treatment assignment within Part A or Part B during assay analysis.

6.5 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 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 statistical analysis system (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).

6.5.1 Analysis variables

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

6.5.2 Baseline comparability

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

6.5.3 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.

6.5.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm in Part A and Part B and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition, to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms will be calculated. Kruskal-Wallis tests will be used to test for differences in severity across arms and Wilcoxon rank sum test will be used to test for difference in severity between 2 arms in Part B.

6.5.3.2 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 (in Part A and Part B) 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 Division of AIDS (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.

6.5.3.3 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 (Part A and Part B) 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 time point, as well as changes from baseline for post enrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Section 9.1) will be tabulated by treatment arm for each post vaccination time point. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

6.5.3.4 Reasons for vaccination discontinuation and early study termination

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

6.5.4 Immunogenicity analysis

6.5.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants in Part B will be used according to the initial randomization assignment regardless of how many injections the participants received. Additional analyses may be performed, limited to participants who received all scheduled injections per protocol. Assay results from specimens collected outside of the visit window, or from HIV-infected participants collected post infection, may be 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 post enrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% CI estimates calculated using the score test method (52). Barnard's 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 (54).

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 CIs, for each vaccine arm using maximum likelihood-based methods (55).

For quantitative assay data (eg, the vaccine-induced serum neutralizing antibody [nAb] as measured by the TZM-bl assay, HIV-specific serum IgG binding magnitude, breadth, and avidity to cross-clade panels of gp120 and V1V2 and to V3, CD4i, gp41 and gp41 immunodominant region [IDR] as assessed by the binding antibody multiplex assay [BAMA] or CD4+ T-cell responses to Gag and Env as assessed by ICS), 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.

The difference between arms at a specific timepoint will be tested with a nonparametric Wilcoxon rank sum test if the data are not normally distributed and with a 2-sample t-test if the data appear to be normally distributed. To test for differences among all vaccine arms, first a Kruskal-Wallis rank test or an F-test (depending on the normality assumption) will be used to test for overall differences. Secondly, if the overall test is significant at the 2-sided 0.05 level, then individual tests comparing between vaccine arms will be done. If rank-based tests are used, then the tests will be inverted to construct Hodges-Lehmann point estimates and 2-sided (1-0.05/# of comparisons) × 100% CIs about the differences in location centers of the number of comparisons between vaccine arms. If rank-based tests are used, then the tests will be inverted to construct Hodges-Lehmann point estimates and 2-sided (1-0.05/# of comparisons) × 100% CIs about the differences in location centers of the number of comparisons between vaccine arms. When all pair-wise comparisons between the multiple vaccine arms are of interest, the Tukey procedure (56) will be used. If only specific comparisons

between pairs of the multiple vaccine arms are of interest, the Holm-Bonferroni procedure will be used. An appropriate data transformation (eg, log10 transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance). Significance of the differences between pairs will be evaluated using 2 procedures, first based on whether the simultaneous 95% CIs exclude zero and secondly, based on whether the nominal (unadjusted) 95% CIs exclude zero.

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, nAb, BAMA, ICS). If treatment arm differences for these assays are best summarized by a mixture model, then either Lachenbruch's test statistic (57) or an alternative 2-part test (58) (as defined in the SAP) will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal response distributions. Lachenbruch's test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

More sophisticated analyses employing repeated measures methodology (for example, linear mixed models or marginal mean models fit by generalized estimating equations [GEE]) may be utilized to incorporate immune responses over several timepoints and to test for differences over time. However, inference from such analyses would be limited by the small sample size of this study. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05$.

Based upon previous HVTN trials, missing 15% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed PBMCs. To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data are missing for an endpoint (at least 1 value missing from more than 20% of participants), then using the methods

that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For 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 (59) will be used because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE (60) 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.

6.5.4.2 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, nAb, BAMA, 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; for a larger number of readouts, a heatmap showing the degree of correlation between any two pairs may be used. 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 (61). 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 [PFS], Env-specific CD8+ T cell total magnitude) from the origin to the point defined by its first 2 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 2 arrows conveys information about the correlation of the 2

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.

6.5.4.3 Primary analyses of neutralization magnitude-breadth curves

The area-under-the-magnitude-breadth curve (AUC-MB) to non matched (heterologous) tier 2 HIV-1 Env-pseudotyped viruses in the TZM-bl assay (62) will be computed for each participant with evaluable neutralization data, as described in Huang et al, 2009 (63). AUC-MB to a vaccine-matched panel may also be computed when the panel consists of at least 3 isolates. Dunnett's procedure will be applied with 2-sided alpha = 0.05 to determine which of the 3 vaccine groups (Groups 6-8 in Part B) have a significantly higher mean AUC-MB than that of the pooled baseline samples from Groups 6-8, as described in Liu et al, 1997 (64) (see their formula [1.1]). This procedure will be applied to construct 95% CIs about the 3 differences in mean AUC-MB for each vaccine regimen (Groups 6-8) versus the pooled baseline samples from Groups 6-8, which simultaneously have at least 95% coverage probability.

Select the best vaccine regimen among those passing the tier-2 screen

The vaccine regimens that passed the tier-2 screen will be ranked by the estimated mean of the AUC-MB curves. The vaccine regimen with the highest estimated mean will be selected as the best regimen.

6.5.4.4 Secondary analyses of neutralization magnitude-breadth curves

Superiority comparisons of vaccine regimens passing the tier-2 screen

For the set of vaccine regimens that passed the tier-2 screen, an F-test will be performed for whether any of the mean AUC-MBs differ. If this test is not significant (p-value > 0.05), then the conclusion will be that there were no significant differences in mean AUC-MBs among the vaccine regimens that advanced. If the F-test is significant, then simultaneous 95% CIs about the mean-differences in AUC-MBs will be reported. These CIs are computed as the estimated mean-difference plus or minus $t_{N-m,025}$ multiplied by the square-root of S^2 $(1/n_i + 1/n_j)$, where $t_{N-m,025}$ is the 97.5th percentile of a t-distribution with N – m degrees of freedom, where N is the total number of vaccine recipients evaluated (summing over the advanced vaccine regimens) and m is the number of advanced vaccine regimens. In addition, S^2 is an estimate of the common sample variance of the AUC-MB, whereas n_i and n_j are the sample sizes of evaluable participants

for vaccine regimens i and j being compared. Following Fisher's least significant difference procedure, the pairs of vaccine regimens with this CI, excluding zero, are deemed to have a significant difference. Nominal (unadjusted) 95% CIs about pairs of vaccine arms will also be reported.

Omnibus comparison of magnitude-breadth distributions

The analyses of magnitude-breadth described above are based on the endpoint area-under-the-curve, which is interpreted as the average log10 half-maximal inhibitory concentration (IC50) to the set of isolates in the test panel. Use of this endpoint is maximally statistically powerful if 1 vaccine arm has greater magnitude and breadth than the comparator vaccine arm, but may miss an effect wherein 1 vaccine arm has greater magnitude and the comparator vaccine arm has greater breadth. Therefore, a secondary analysis may compare the distribution of magnitude-breadth curves among vaccine arms using the test statistic $\max|B_d^G|$ from Huang, et al (63), which is designed to detect general differences in magnitude-breadth curve distributions.

Selecting the best vaccine regimen among those passing the tier-2 screen

For each vaccine regimen that passes the tier-2 screen, the best vaccine regimen will be deemed as that with the greatest value of the $\max|B_d^G|$ test statistic comparing its distribution of magnitude-breadth curves versus the pooled placebo group.

Superiority comparisons of vaccine regimens passing the tier-2 screen

Similarly, the $\max|B_d^G|$ test statistic will be used to compare the distribution of magnitude-breadth curves between each pair of advanced vaccine regimens. The Holm-Bonferroni procedure will be applied to determine the pairs of regimens with significant differences in distribution controlling the family-wise false positive error rate at no more than 0.05. Nominal (unadjusted) 95% CIs about pairs of vaccine arms will also be reported.

6.5.4.5 Analysis of binding antibody IgG/IgA responses measured by BAMA

The analysis of IgG, IgG3, and IgA binding antibody response to gp120, V1V2 and other antigens (V3, CD4i, gp41, and gp41 IDR) as measured by the BAMA assay will be evaluated and compared as described under the general approach. Scores measured the cross-reactivity among the gp120 and V1V2 will be derived. The details of the score derivations will be described in SAP. The derived scores will be evaluated and compared as described under the general approach.

6.5.4.6 Analysis of CD4+ and CD8+ T-cell response as measured by the ICS assay

The analysis of CD4+ and CD8+ T-cell response rates as measured by the ICS assay will be evaluated and compared as described under the general approach. For each T-cell subset, the positivity call for each peptide pool will include a multiple-comparison adjustment for the number of peptide pools used in the assay. In general, the Mixture Models for Single-cell Assays (MIMOSA) statistical framework (65) and/or the Fisher's exact test-based positivity criteria will be used. Details of the positivity criteria will be discussed in the SAP. The magnitude of marginal response will be analyzed as described for quantitative data in the general approach section. For each T-cell subset, graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint. When 3 or more cytokines are being measured by the ICS assay, the polyfunctionality of ICS responses may also be analyzed as an exploratory endpoint. Besides descriptive plots of the magnitude of polyfunctional responses, the COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets) statistical framework (66) may also be used to perform joint modelling of multiple T-cell subsets of different cytokine combinations. For example, the functionality score (FS) and the polyfunctionality score (PFS) may be used to summarize the multi parameter ICS responses.

6.5.5 Analyses prior to end of scheduled follow-up visits

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

6.5.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months for review by the safety monitoring board (SMB). During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 303 PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.5.5.2 Immunogenicity

An unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory

immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after at least 1 of the primary immunogenicity endpoints of the same class (humoral, cell-mediated, innate or mucosal) or, if no primary endpoint of the same class, at least 1 of the primary immunogenicity endpoints reaches the aforementioned threshold. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key HVTN members and investigators. Distribution of reports will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the scheduled follow-up visits.

7 Study product preparation, storage, and administration

On January 13, 2023, a protocol memo was distributed informing the clinical research sites that all vaccinations in HVTN 303 were permanently discontinued. The vaccination that did not take place due to this has been marked with ~~strikethrough~~.

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 1-1. See the Investigator's Brochures (IBs) for further information about study products.

7.1 Vaccine Regimen

The schedule of study product administration is shown in Section 1.5 and additional information is given below.

Part A

Group 1

Treatment 1 (T1):

25 mcg FP Conjugate Vaccine (FP8v1-rTTHC; VRC-HIVVCP0108-00-VP) + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Week 0.

Group 2

Treatment 2 (T2):

200 mcg FP conjugate vaccine (FP8v1-rTTHC; VRC-HIVVCP0108-00-VP) + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Week 0.

Group 3

Treatment 3 (T3):

100 mcg Trimer 6931 (HIV-1 Trimer 6931 Vaccine; VRC-HIVRGP0106-00-VP) + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Week 0.

Group 4

Treatment 4 (T4):

200 mcg Trimer 6931 (HIV-1 Trimer 6931 Vaccine; VRC-HIVRGP0106-00-VP) + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL), intramuscularly at Week 0.

Group 5

Treatment 5 (T5):

200 mcg Trimer 4571 (HIV-1 Trimer 4571 Vaccine; VRC-HIVRGP096-00-VP) + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Week 0.

Part B

Group 6

Treatment 6 (T6):

200 mcg Trimer 4571 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Weeks ~~and 12.~~ 0

~~200 mcg Trimer 6931 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Week 24.~~

~~100 mcg Trimer 4571 + 100 mcg Trimer 6931 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), at Weeks 36 and 48.~~

Group 7

Treatment 7 (T7):

200 mcg FP conjugate vaccine + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), at Weeks 0 and 4 ~~and 8~~.

~~200 mcg Trimer 4571 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Weeks 12 and 24.~~

~~200 mcg Trimer 6931 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Week 36.~~

~~100 mcg Trimer 4571 + 100 mcg Trimer 6931 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), at Week 48.~~

Group 8

Treatment 8 (T8):

200 mcg FP conjugate vaccine + 200 mcg Trimer 4571 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), at Weeks 0 and 4 ~~and 8~~.

~~200 mcg Trimer 6931 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Week 20.~~

~~100 mcg Trimer 4571 + 100 mcg Trimer 6931 + 20% dose/volume (d/v) Adjuplex, to be administered as 0.8 mL, divided into 2 syringes (0.4 mL each), intramuscularly at Weeks 32 and 44.~~

7.2 Study Product Formulation and Storage

This study includes 3 investigational vaccines, a diluent, and 1 adjuvant as follows:

7.2.1 FP Conjugate Vaccine Labeled as FP8v1-rTTHC Vaccine (VRC-HIVVCP0108-00-VP)

FP Conjugate Vaccine will be provided as 3-mL single-use glass vials with a 0.7-mL fill volume, at a concentration of 1 mg/mL. After being thawed, each vial contains a clear, colorless, preservative-free sterile solution; some white or translucent particles may be present.

FP Conjugate Vaccine must be stored until use at -35°C to -15°C. Unopened vials may be stored cumulatively for up to 48 hours at 2ºC to 8ºC and for up to 24 hours at 15°C to 27°C post thaw. Vials should not be refrozen after being thawed.

7.2.2 Trimer 4571 Labeled as HIV Trimer 4571 Vaccine (VRC-HIVRGP096-00-VP)

Trimer 4571 will be provided as 3-mL single-use glass vials with a 1.2-mL fill volume, at a concentration of 500 mcg/mL. After being thawed, each vial contains a clear, colorless, preservative-free, sterile solution; some small white or translucent particles may be present.

Trimer 4571 must be stored until use at -35°C to -15°C. Unopened vials may be stored cumulatively for up to 48 hours at 2°C to 8°C and up to 24 hours at 15°C to 27°C post-thaw. Vials should not be refrozen after being thawed.

7.2.3 Trimer 6931 Labeled as HIV Trimer 6931 Vaccine (VRC-HIVRGP0106-00-VP)

Trimer 6931 will be provided as 3-mL single-use glass vials with a 1.2-mL fill volume, at a concentration of 1 mg/mL. After being thawed, each vial contains a clear, colorless, preservative-free, sterile solution; some small white or translucent particles may be present.

Trimer 6931 must be stored until use at -35°C to -15°C. Unopened vials may be stored cumulatively for up to 48 hours at 2°C to 8°C and up to 24 hours at 15°C to 27°C post-thaw. Vials should not be refrozen after being thawed.

7.2.4 Adjuplex Adjuvant (VRC-GENADJ0110-AP-NV)

Adjuplex adjuvant will be provided as 3-mL single-use glass vials with a fill volume of 0.7 mL. Each vial contains an off-white, semi-opaque (not transparent), preservative-free, sterile liquid with no visible particles and is homogenous after a gentle shake.

Adjuplex will be mixed with study products in the pharmacy during preparation prior to vaccination to maintain a 20% (v/v) dose of Adjuplex.

Adjuplex adjuvant vials must be stored until use at 2°C to 8°C. DO NOT FREEZE. Unopened vials may be stored for up to 8 hours at 15°C to 27°C.

7.2.5 PBS Diluent Labeled as Phosphate Buffered Saline pH 7.2 (VRC-PBSPLA043-00-VP)

PBS diluent will be provided as 3-mL single-use glass vials with a fill volume of 1.5 mL. After being thawed, each vial contains a clear, colorless, preservative-free, sterile buffered solution.

PBS diluent must be stored until use at -35°C to -15°C. Unopened vials may be stored cumulatively for up to 48 hours at 2°C to 8°C and up to 24 hours at 15°C to 27°Cpost-thaw. Vials should not be refrozen after being thawed.

7.3 Preparation of Study Products

Prior to preparation, a new prescription will be sent to the pharmacy and must contain the participant's PTID, group number assignment, and week number for the visit.

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

Any unused portion of study product cannot be used for another participant. Any 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.

Refer to the HVTN 303 *Study Specific Procedures* (SSP) for further details on study product preparation.

7.3.1 FP Conjugate Vaccine (25 mcg)

The pharmacy will need to prepare a 25-mcg dose of FP Conjugate with adjuvant for the following participants at the listed study weeks:

- Group 1; Week 0

Preparation of 25-mcg FP Conjugate Vaccine with Adjuvant:
When preparing doses, glass or polypropylene containers are preferred for compounding; avoid polystyrene and polytetrafluoroethylene (PTFE) materials. Pipettors with plastic disposable tips may be used in place of syringes for compounding.

1. Remove 1 vial of FP8v1-rTTHC Vaccine (VRC-HIVVCP0108-00-VP) and 2 vials of Phosphate Buffered Saline (PBS; VRC-PBSPLA043-00-VP) from frozen storage. Thaw and equilibrate vials at ambient temperature (15°C to 27°C) for a minimum of 30 minutes. **Vials should NOT be moved directly from the freezer to a refrigerator to thaw.**

2. Remove 1 vial of Adjuplex adjuvant (VRC-GENADJ0110-AP-NV) from 2°C to 8°C storage and equilibrate vial at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.

3. Gently swirl the equilibrated FP8v1-rTTHC and PBS vials until the contents look homogenous. **DO NOT SHAKE THE VIALS.** If some white to translucent particles are observed, vials may be used for the preparation of the IM administration.

4. Gently swirl the equilibrated vial of Adjuplex until the contents look homogenous. The Adjuplex adjuvant emulsion will retain some opacity.

5. Transfer 2.00 mL of PBS into a new empty sterile container using a 3.0 mL syringe and a large-bore needle (eg, 18G, 16G).

6. Transfer an additional 0.46 mL of PBS into the same sterile container using a 1.0-mL syringe and a new large-bore needle (eg, 18G, 16G).

7. Transfer 0.1 mL of FP8v1-rTTHC Vaccine into the same sterile conatiner using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

8. Transfer 0.64 mL Adjuplex into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

9. Close and invert the container gently 10 times to mix the compounded dose.

10. Withdraw 0.4 mL of the compounded dose twice into 2 separate new 1-mL syringes using two new large-bore needles (eg, 18G, 16G). Each syringe will be administered IM to one of the deltoids for a total dose volume of 0.8 mL.

11. Remove the needles used in Step 10 to withdraw compounded vaccine and attach appropriately sized needles for administration to the prepared syringes before dispensing.

12. Place a participant-specific label on each prepared syringe for administration.

NOTE: After preparation into a syringe for administration, the adjuvanted FP8v1-rTTHC vaccine may be stored at 2°C to 8°C for up to 8 hours and/or at 15°C to 27°C for a maximum of 4 hours, including administration time. The prepared study product may not be stored in direct sunlight.

7.3.2 FP Conjugate Vaccine (200 mcg)

The pharmacy will need to prepare a 200-mcg dose of FP Conjugate with adjuvant for the following participants at the listed study weeks:

- Group 2; Week 0
- Group 7; Weeks 0, 4 ~~and 8~~

Preparation of 200-mcg FP Conjugate Vaccine with Adjuvant:
When preparing doses, glass or polypropylene containers are preferred for compounding; avoid polystyrene and polytetrafluoroethylene (PTFE) materials. Pipettors with plastic disposable tips may be used in place of syringes for compounding

1. Remove 1 vial of FP8v1-rTTHC Vaccine (VRC-HIVVCP0108-00-VP) and 1 vial of Phosphate Buffered Saline (PBS; VRC-PBSPLA043-00-VP) from frozen storage. Thaw and equilibrate vials at ambient temperature (15°C to 27°C) for a

minimum of 30 minutes. **Vials should NOT be moved directly from the freezer to a refrigerator to thaw.**

2. Remove 1 vial of Adjuplex adjuvant (VRC-GENADJ0110-AP-NV) from 2°C to 8°C storage and equilibrate vial at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.

3. Gently swirl the equilibrated FP8v1-rTTHC and PBS vials until the contents look homogenous. **DO NOT SHAKE THE VIALS.** If some white to translucent particles are observed, vials may be used for the preparation of the IM administration.

4. Gently swirl the equilibrated vial of Adjuplex until the contents look homogenous. The Adjuplex adjuvant emulsion will retain some opacity.

5. Transfer 0.66 mL of PBS into a new sterile container using a 1-mL syringe and a large-bore needle (eg, 18G, 16G).

6. Transfer 0.3 mL of FP8v1-rTTHC Vaccine into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

7. Transfer 0.24 mL of adjuvant into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

8. Close and invert the container gently 10 times to mix the compounded dose.

9. Withdraw 0.4 mL of the compounded dose twice into 2 separate 1-mL syringes using 2 new large-bore needles (eg, 18G, 16G). Each syringe will be administered IM to one of the deltoids for a total dose volume of 0.8 mL

10. Remove the needles used in Step 9 to withdraw compounded vaccine and attach appropriately sized needles for administration to the prepared syringes before dispensing.

11. Place a participant-specific label on each prepared syringe for administration.

NOTE: after preparation into a syringe for administration, the adjuvanted FP8v1-rTTHC vaccine may be stored at 2°C to 8°C for up to 8 hours and/or at 15°C to 27°C for a maximum of 4 hours, including administration time. The prepared study product may not be stored in direct sunlight.

7.3.3 Trimer 6931 (100 mcg)

The pharmacy will need to prepare a 100-mcg dose of Trimer 6931 with adjuvant for the following participants at the listed study weeks:

- Group 3; Week 0

Preparation of 100-mcg Trimer 6931 Vaccine with Adjuvant:
When preparing doses, glass or polypropylene containers are preferred for compounding; avoid polystyrene and polytetrafluoroethylene (PTFE) materials. Pipettors with plastic disposable tips may be used in place of syringes for compounding

1. Remove 1 vial of HIV-1 Trimer 6931 Vaccine (VRC-HIVRGP0106-00-VP) and 1 vial of Phosphate Buffered Saline (PBS; VRC-PBSPLA043-00-VP) from frozen storage. Thaw and equilibrate vials at ambient temperature (15°C to 27°C) for a minimum of 30 minutes. **Vials should NOT be moved directly from the freezer to a refrigerator to thaw.**

2. Remove 1 vial of Adjuplex adjuvant (VRC-GENADJ0110-AP-NV) from 2°C to 8°C storage and equilibrate vial at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.

3. Gently swirl the equilibrated HIV-1 Trimer 6931 and PBS vials until the contents look homogenous. **DO NOT SHAKE THE VIALS.** If some white to translucent particles are observed, vials may be used for the preparation of the IM administration.

4. Gently swirl the equilibrated vial of Adjuplex until the contents look homogenous. The Adjuplex adjuvant emulsion will retain some opacity.

5. Transfer 0.81 mL of PBS into a new sterile container using 1-mL syringe and a large-bore needle (eg, 18G, 16G).

6. Transfer 0.15 mL of HIV-1 Trimer 6931 into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

7. Transfer 0.24 mL of Adjuplex into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

8. Close and invert the container gently 10 times to mix the compounded dose.

9. Withdraw 0.4 mL of the compounded dose twice into 2 separate 1-mL syringes using 2 new large-bore needles (eg, 18G, 16G). Each syringe will be administered IM to one of the deltoids for a total dose volume of 0.8 mL.

10. Remove the needles used in Step 9 to withdraw compounded vaccine and attach appropriately sized needles for administration to the prepared syringes before dispensing.

11. Place a participant-specific label on each prepared syringe for administration.

NOTE: after preparation into a syringe for administration, the adjuvanted Trimer 6931 vaccine may be stored at 2°C to 8°C for up to 8 hours and/or at 15°C to 27°C

for a maximum of 4 hours, including administration time. The prepared study product may not be stored in direct sunlight.

7.3.4 Trimer 6931 (200 mcg)

The pharmacy will need to prepare a 200-mcg dose of Trimer 6931 with adjuvant for the following participants at the listed study weeks:

- Group 4; Week 0
- ~~Group 6; Week 24~~
- ~~Group 7; Week 36~~
- ~~Group 8; Week 20~~

Preparation of 200-mcg Trimer 6931 Vaccine with Adjuvant:

When preparing doses, glass or polypropylene containers are preferred for compounding; avoid polystyrene and polytetrafluoroethylene (PTFE) materials. Pipettors with plastic disposable tips may be used in place of syringes for compounding.

1. Remove 1 vial of HIV-1 Trimer 6931 Vaccine (VRC-HIVRGP0106-00-VP) and 1 vial of Phosphate Buffered Saline (PBS; VRC-PBSPLA043-00-VP) from frozen storage. Thaw and equilibrate vials at ambient temperature (15°C to 27°C) for a minimum of 30 minutes. **Vials should NOT be moved directly from the freezer to a refrigerator to thaw.**

2. Remove 1 vial of Adjuplex adjuvant (VRC-GENADJ0110-AP-NV) from 2°C to 8°C storage and equilibrate vial at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.

3. Gently swirl the equilibrated HIV-1 Trimer 6931 and PBS vials until the contents look homogenous. **DO NOT SHAKE THE VIALS.** If some white to translucent particles are observed, vials may be used for the preparation of the IM administration.

4. Gently swirl the equilibrated vial of Adjuplex until the contents look homogenous. The Adjuplex adjuvant emulsion will retain some opacity.

5. Transfer 0.66 mL of PBS into a new sterile container using a 1-mL syringe and a large-bore needle (eg, 18G, 16G).

6. Transfer 0.3 mL of HIV-1 Trimer 6931 into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

7. Transfer 0.24 mL of Adjuplex into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

8. Close and invert the container gently 10 times to mix the compounded dose.

9. Withdraw 0.4 mL of the compounded mixture twice into 2 separate 1-mL syringes using 2 new needles. Each syringe will be administered IM to one of the deltoids for a total dose volume of 0.8 mL.

10. Remove the needles used in Step 9 to withdraw compounded vaccine and attach appropriately sized needles for administration to the prepared syringes before dispensing.

11. Place a participant-specific label on each prepared syringe for administration.

NOTE: after preparation into a syringe for administration, the adjuvanted Trimer 6931 vaccine may be stored at 2°C to 8°C for up to 8 hours and/or at 15°C to 27°C for a maximum of 4 hours, including administration time. The prepared study product may not be stored in direct sunlight.

7.3.5 Trimer 4571 (200 mcg)

The pharmacy will need to prepare a 200-mcg dose of Trimer 4571 with adjuvant for the following participants at the listed study weeks:

- Group 5; Week 0
- Group 6; Weeks 0 ~~and 12~~
- ~~Group 7; Weeks 12 and 24~~

Preparation of 200-mcg Trimer 4571 Vaccine with Adjuvant:

When preparing doses, glass or polypropylene containers are preferred for compounding; avoid polystyrene and polytetrafluoroethylene (PTFE) materials. Pipettors with plastic disposable tips may be used in place of syringes for compounding.

1. Remove 1 vial of HIV-1 Trimer 4571 Vaccine (VRC-HIVRGP096-00-VP) and 1 vial of Phosphate Buffered Saline (PBS; VRC-PBSPLA043-00-VP) from frozen storage. Thaw and equilibrate vials at ambient temperature (15°C to 27°C) for a minimum of 30 minutes. **Vials should NOT be moved directly from the freezer to a refrigerator to thaw.**

2. Remove 1 vial of Adjuplex adjuvant (VRC-GENADJ0110-AP-NV) from 2°C to 8°C storage and equilibrate vial at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.

3. Gently swirl the equilibrated HIV-1 Trimer 4571 and PBS vials until the contents look homogenous. **DO NOT SHAKE THE VIALS.** If some white to translucent particles are observed, vials may be used for the preparation of the IM administration.

4. Gently swirl the equilibrated vial of Adjuplex until the contents look homogenous. The Adjuplex adjuvant emulsion will retain some opacity.

5. Transfer 0.36 mL of PBS into a new sterile container using a 1-mL syringe and a large-bore needle (eg, 18G, 16G).

6. Transfer 0.6 mL of HIV-1 Trimer 4571 into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

7. Transfer 0.24 mL of Adjuplex into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

8. Close and invert the container gently 10 times to mix the compounded dose.

9. Withdraw 0.4 mL of the compounded dose twice into 2 separate 1-mL syringes using 2 new large-bore needles (eg, 18G, 16G). Each syringe will be administered IM to one of the deltoids for a total dose volume of 0.8 mL.

10. Remove the needles used in Step 9 to withdraw compounded vaccine and attach appropriately sized needles for administration to the prepared syringes before dispensing.

11. Place a participant-specific syringe on each prepared syringe for administration.

NOTE: after preparation into a syringe for administration, the adjuvanted Trimer 4571 vaccine may be stored at 2°C to 8°C for up to 8 hours and/or at 15°C to 27°C for a maximum of 4 hours, including administration time. The prepared study product may not be stored in direct sunlight

7.3.6 Trimer 4571 (100 mcg) + Timer 6931 (100 mcg):

~~The pharmacy will need to prepare a dose containing 100 mcg Trimer 4571 + 100 mcg Trimer 6931 + adjuvant for the following participants at the listed study weeks:~~

- ~~Group 6; Weeks 36 and 48~~
- ~~Group 7; Week 48~~
- ~~Group 8; Weeks 32 and 44~~

~~Preparation of 100-mcg Trimer 4571 Vaccine + 100-mcg Trimer 6931 with Adjuvant:~~

~~When preparing doses, glass or polypropylene containers are preferred for compounding; avoid polystyrene and polytetrafluoroethylene (PTFE) materials. Pipettors with plastic disposable tips may be used in place of syringes for compounding.~~

~~1. Remove 1 vial of HIV-1 Trimer 4571 Vaccine (VRC-HIVRGP096-00-VP), 1 vial of HIV-1 Trimer 6931 Vaccine (VRC-HIVRGP0106-00-VP) and 1 vial of Phosphate Buffered Saline (PBS; VRC-PBSPLA043-00-VP) from frozen storage. Thaw and equilibrate vials at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.~~ **~~Vials should NOT be moved directly from the freezer to a refrigerator to thaw.~~**

~~2. Remove 1 vial of Adjuplex adjuvant (VRC-GENADJ0110-AP-NV) from 2°C to 8°C storage and equilibrate vial at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.~~

~~3. Gently swirl the equilibrated HIV-1 Trimer 4571, HIV-1 Trimer 6931 and PBS vials until the contents look homogenous.~~ **~~DO NOT SHAKE THE VIALS.~~** ~~If some white to translucent particles are observed, vials may be used for the preparation of the IM administration.~~

~~4. Gently swirl the equilibrated vial of Adjuplex until the contents look homogenous. The Adjuplex adjuvant emulsion will retain some opacity.~~

~~5. Transfer 0.51 mL of PBS into a new sterile container using 1-mL syringe and a large-bore needle (eg, 18G, 16G).~~

~~6. Transfer 0.3 mL of HIV-1 Trimer 4571 into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).~~

~~7. Transfer 0.15 mL of HIV-1 Trimer 6931 into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).~~

~~8. Transfer 0.24 mL of Aduplex into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).~~

~~9. Close and invert the container gently 10 times to mix the compounded dose.~~

~~10. Withdraw 0.4 mL of the compounded dose twice into 2 separate 1-mL syringes using 2 new large-bore needles (eg, 18G, 16G). Each syringe will be administered IM to one of the deltoids for a total dose volume of 0.8 mL.~~

~~11. Remove the needles used in Step 10 to withdraw compounded vaccine and attach appropriately sized needles for administration on the prepared syringes before dispensing.~~

~~12. Place a participant-specific label on each prepared syringe for administration.~~

~~NOTE: after preparation into a syringe for administration, the adjuvanted Trimer 4571 + Trimer 6931 vaccine may be stored at 2°C to 8°C for up to 8 hours and/or at 15°C to 27°C for a maximum of 4 hours, including administration time. The prepared study product may not be stored in direct sunlight.~~

7.3.7 FP Conjugate Vaccine (200 mcg) + Timer 4571 (200 mcg):

The pharmacy will need to prepare a dose containing 200 mcg FP Conjugate Vaccine + 200 mcg Trimer 4571 + adjuvant for the following participants at the listed study weeks:

- Group 8; Weeks 0, 4 ~~and 8~~

Preparation of 200-mcg FP Conjugate Vaccine + 200-mcg Trimer 4571 with Adjuvant:

When preparing doses, glass or polypropylene containers are preferred for compounding; avoid polystyrene and polytetrafluoroethylene (PTFE) materials. Pipettors with plastic disposable tips may be used in place of syringes for compounding.

1. Remove 1 vial of FP8v1-rTTHC Vaccine (VRC-HIVVCP0108-00-VP), 1 vial of HIV-1 Trimer 4571 Vaccine (VRC-HIVRGP096-00-VP) and 1 vial of Phosphate Buffered Saline (PBS; VRC-PBSPLA043-00-VP) from frozen storage. Thaw and equilibrate vials at ambient temperature (15°C to 27°C) for a minimum of 30 minutes. **Vials should NOT be moved directly from the freezer to a refrigerator to thaw.**

2. Remove 1 vial of Adjuplex adjuvant (VRC-GENADJ0110-AP-NV) from 2°C to 8°C storage and equilibrate vial at ambient temperature (15°C to 27°C) for a minimum of 30 minutes.

3. Gently swirl the equilibrated FP Conjugate Vaccine, HIV-1 Trimer 4571 and PBS vials until the contents look homogenous. **DO NOT SHAKE THE VIALS.** If some white to translucent particles are observed, vials may be used for the preparation of the IM administration.

4. Gently swirl the equilibrated vial of Adjuplex until the contents look homogenous. The Adjuplex adjuvant emulsion will retain some opacity.

5. Transfer 0.06 mL of PBS into a new sterile container using a new 1-mL syringe and a large-bore needle (eg, 18G, 16G).

6. Transfer 0.3 mL of FP Conjugate Vaccine into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

7. Transfer 0.6 mL of HIV-1 Trimer 4571 into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

8. Transfer 0.24 mL of Adluplex into the same sterile container using a new 1-mL syringe and a new large-bore needle (eg, 18G, 16G).

9. Close and invert the container gently 10 times to mix the compounded dose.

10. Withdraw 0.4 mL of the compounded dose twice into 2 separate 1-mL syringes using 2 new large-bore needles (eg, 18G, 16G). Each syringe will be administered IM to one of the deltoids for a total dose volume of 0.8 mL.

11. Remove the needles used in Step 10 to withdraw compounded vaccine and attach appropriately sized needles on the prepared syringe before dispensing.

12. Place a participant-specific label on each prepared syringe for administration.

NOTE: after preparation into a syringe for administration, the adjuvanted FP Conjugate + Trimer 4571 vaccine may be stored at 2°C to 8°C for up to 8 hours and/or at 15°C to 27°C for a maximum of 4 hours, including administration time. The prepared study product may not be stored in direct sunlight.

7.4 Labeling of Study Products

Label the prepared study product in a syringe as follows:

- Participant identifier(s)
- Study product name
- Total dose or dosages
- Volume in mL
- Route of administration (IM)
- Instructions to “Invert syringe 5 times prior to administration”
- Beyond-use date and time

- Any additional information required by jurisdiction

7.5 Study Product Administration

The prepared dose will be administered as two IM injections in a deltoid muscle, 0.4 mL in volume for each arm. The prepared syringe must be inverted 5 times prior to administration to the participant.

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

7.6 Acquisition of study products

FP Conjugate Vaccine, Trimer 4571, Trimer 6931, PBS diluent and Adjuplex adjuvant are provided by the VRC, NIAID, DAIDS. Once a 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.

7.7 Study Product Accountability

The 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.

7.8 Final Disposition of study product

For US clinical research sites, all unused study products must be returned to the clinical research products management center (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.

8 Clinical procedures

8.1 Screening

Screening for eligibility will be performed after informed consent has been obtained and properly documented before enrollment. Screening evaluations and sample collection include medical history review, physical exam, and any clinical laboratory tests as detailed in the Schedule of procedures (Appendix A, Appendix B, Appendix C and Appendix D) needed to confirm eligibility. Persons assigned female sex at birth who are of reproductive potential will be given a pregnancy test. Additional assessments of health may be conducted at screening based on clinical judgment.

If screening evaluations suggest a current concerning health condition or infection, then appropriate laboratory tests may be conducted to evaluate these conditions. Additional assessments of health may be conducted at screening based on clinical judgment. Screening evaluations for specific eligibility criteria (Section 5) must be completed within the time interval specified prior to enrollment for the given parameter and may be repeated, as needed, to confirm eligibility.

An Assessment of Understanding (AoU) will be completed prior to enrollment. Records will be kept documenting the reasons that screened participants did not enroll or were ineligible.

8.2 Definition of Study Day and Study Visit

Study Day 0 is defined as the day of the first vaccination. A study visit may be conducted remotely, such as via phone, text message, email, or other electronic means, in lieu of, or in combination with, in-person visits. As long as they are completed within the visit window (see Appendix E and Appendix F), procedures for a study visit can be completed over multiple days. The procedures for documenting remote visits and out of window visits are described in the HVTN 303 SSP.

8.3 Reactogenicity Assessments

Pre-Vaccine Administration: Medical history and evaluations including vital signs and planned injection site evaluation are performed prior to each vaccine administration.

Post-Vaccine Administration Follow-Up in Clinic: Following each vaccine administration, participants will be observed for a minimum of 30 minutes post injection, vital signs will be recorded, the injection site will be inspected for

evidence of local reaction, and any evidence of systemic symptoms will be assessed. Participants will be contacted on the day after each study injection as indicated on the Schedule of Procedures (Appendix A, Appendix B, Appendix C and Appendix D).

Post-Clinic Follow-Up: Participants are asked to record symptoms on a daily basis using an electronic participant diary. Signs and symptoms considered to represent reactogenicity from the vaccine include systemic events of increased body temperature, fatigue, generalized myalgia, generalized arthralgia, headache, chills, nausea, and local events at the injection site, including pain/tenderness, induration, erythema, and bruising. Participant diaries will be reviewed by a clinician and reconciled for accuracy and completeness.

Participants will be given a thermometer for oral temperature measurement, a ruler, and provided access to the electronic diary. Participants will be encouraged to use the preferred electronic diary but will have the option to use a paper diary. The paper diary, if used, will be transcribed into the study database and stored in the participant file for monitoring purposes. The participant will use the diary to record daily their highest temperature as well as local and systemic signs and symptoms for 10 full days following each study vaccination. Participants will be provided training on diary completion, proper thermometer usage, and the use of the measuring device to measure any injection site induration and/or erythema.

In LOA #3 for protocol version 1.0 (distributed to the sites on Dec 14, 2022) the reactogenicity assessment period was increased from 7 days to 10 days. However, as vaccinations were permanently discontinued on January 13, 2023, the change in post-vaccine reactogenicity assessment period was no longer applicable and was not implemented. The reactogenicity assessment period of the previously vaccinated participants remained 7 days following vaccine administration.

Remote or in-person contact between the participant and the site staff should take place on the day after each study injection. The reactogenicity assessment period is 10 days following vaccine administration. Clinicians will follow and collect resolution information for any reactogenicity signs and symptoms that have not resolved within 10 days.

Any post vaccination reaction grade 2 or higher will be assessed by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved. Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

Given the first-in-human use of the various adjuvanted study products alone or in prime-boost regimens, the HVTN 303 PSRT may recommend that skin biopsies be obtained if unusual skin reactions following vaccinations are reported. Sites are

encouraged to have a process in place for this optional study procedure. Refer to the HVTN 303 SSP for further details.

Clinicians or site staff may photograph the skin reactions to document unusual skin findings.

8.4 Leukapheresis for Part B participants

Only participants who received two vaccinations in Part B (Groups 7-8) and who consent to leukapheresis will undergo leukapheresis at 10 months +/- 2 months post–last vaccination in order to collect blood cells for research.

~~Part B (groups 6-8) participants who consented to leukapheresis (see Sections 2.7 and 5.1.3) will undergo leukapheresis at 2 weeks post- last vaccination in order to collect blood cells of special interest for research.~~ This procedure requires participants meeting additional eligibility criteria (see Section 5.1.3). Collection of PBMC via leukapheresis will be performed in accordance with the standard practices of the participating apheresis center. Ideally, in this procedure, approximately 1 to 4 liters of blood will be processed over 1 to 2 hours using peripheral veins for venous access. The blood will be anti-coagulated in accordance with standard practice of the apheresis center.

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

8.5 Visit windows and missed visits

The schedule of visits and evaluations performed at each visit are shown in Appendix A, Appendix B, Appendix C and Appendix D. Visit windows are shown in Appendix E and Appendix F. The procedures for documenting missed visits and out of window visits are described in the HVTN 303 SSP. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

8.6 Monitoring for COVID-19 symptoms

Participants will be monitored for symptoms of COVID-19 at each study visit (see Appendix A, Appendix B, Appendix C and Appendix D).

8.7 Monitoring for HIV infection

Study participants will be tested for HIV infection periodically throughout the study, as indicated in the schedules included in Appendix A, Appendix B, Appendix C and Appendix D. The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see HVTN Laboratory Center MOP), which is able to distinguish vaccine-induced antibody responses from actual HIV infections. Participants will be promptly informed and counseled if they become HIV-infected during the study and will be referred for treatment (see Section 5.2.2).

Study participants will receive regularly scheduled counseling regarding avoidance of HIV infection in accordance with the most recent Centers for Disease Control and Prevention HIV counseling guidelines.

Although the study vaccine will not cause HIV infection, it may induce antibodies detectable by standard HIV infection screening techniques. This is referred to as VISP. The following steps will be taken to protect participants from adverse consequences associated with VISP:

- Participants will be counseled to avoid HIV antibody testing outside of the HVTN CRS during study participation.

- Participants can receive HIV diagnostic testing from the CRS following their last scheduled visit until they are told they do not have VISP.

- Participants with VISP will be periodically offered free-of-charge poststudy HIV diagnostic testing (per the HVTN poststudy HIV testing algorithm) as medically/socially indicated (approximately every 6 months) unless or until HIV antibody testing is no longer standard in clinical settings.

- Unless participants request that their names be removed, the names of all participants in HVTN studies are entered into a secure VISP registry in order to verify that an individual received an HIV vaccine (and therefore has the potential for VISP) and to qualify former participants for post study HIV testing to distinguish between VISP and HIV infection. Information in the VISP registry is not used for research.

8.8 Early termination visit

If a participant terminates participation in the study early for any reason, the site PI should consider if the following assessments are appropriate: end-of-study HIV test, CBC with differential, serum chemistry, physical examination, social impact assessment and, if indicated, a pregnancy test (see Appendix A, Appendix B, Appendix C and Appendix D). For participants with HIV infection, please see

Section 5.2.2. If the site PI has questions regarding a termination visit, they should consult with the PSRT.

9 Safety and adverse events

9.1 Adverse events

Unsolicited AEs will be collected over a period of 28 days after each vaccination. All collected AEs are captured in the clinical database on the appropriate case report form (CRF). Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting (see Section 9.2.1), (2) if the AE meets the criteria for a safety pause/prompt AE review (see Section 9.5), (3) if the AE meets the criteria for an MAAE, and (4) if the AE is a potential immune-mediated medical condition (PIMMC) or an AESI. A sample list of AESIs and PIMMCs is provided in Appendix M.

In addition, a limited set of AEs will be collected and reported for 12 months following any vaccine administration.

- SAEs/EAEs,
- AESIs,
- MAAEs (defined as any AEs leading to an unscheduled visit to a healthcare professional),
- Safety lab-related AEs,
- AEs leading to early participant withdrawal or early discontinuation of study vaccine(s) administration.

AEs will be graded according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 (https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables), with the following exceptions:

- 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 303 SSP).
- 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.
- Do not grade creatinine clearance or estimated glomerular filtration rate (eGFR) based on the change from the baseline parameter. Do not grade on the basis of eGFR if there is clinical concern for kidney injury.

- 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)

- For severity grading of the solicited bruising parameter at the product administration site, the definitions based on size of the largest diameter and listed for the "Injection Site Erythema or Redness" will be used. The severity grade definition for "Bruising" provided under the Dermatologic Clinical Conditions will be used only for unsolicited AEs involving bruising at other body locations.

9.2 Serious adverse events

The term "Serious Adverse Event" (SAE) is defined in 21 CFR 312.32 as follows:

"An adverse event or suspected adverse reaction is considered serious if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- congenital anomaly/birth defect,
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or

at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse."

"Life-threatening" refers to an AE that, at occurrence, represents an immediate risk of death to the participant. Similarly, a hospital admission for an elective procedure is not considered an SAE.

9.2.1 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 Expedited Adverse Event [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 internet-based DAIDS Adverse Experience Reporting System (DAERS) must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs may be submitted using 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 NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

The SAE Reporting Category will be used throughout the study. All SAEs observed in this clinical trial (including PIMMCs) that are unexpected, and judged to be related to the study products, will be reported by the NIAID/DAIDS to the FDA in accordance with 21 CFR 312.32 (IND Safety Reports). After completion of the study, the suspected unexpected serious adverse reaction (SUSAR) reporting category will be used if clinical staff becomes aware of an event on a passive basis.

The study products for which expedited reporting are required are:

- VRC-HIVVCP0108-00-VP (FP8v1-rTTHC; FP conjugate vaccine) with Adjuplex
- VRC-HIVRGP096-00-VP (Trimer 4571) with Adjuplex
- VRC-HIVRGP0106-00-VP (Trimer 6931) with Adjuplex

There is no placebo product administered in this protocol.

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).

9.3 Safety monitoring

9.3.1 Protocol Safety Review Team (PSRT)

The PSRT comprises the Study Chairs, HVTN Protocol Team Leader, DAIDS Medical Officer, HVTN Clinical Safety Specialist (CSS), and CRS Clinic Coordinator. The Protocol Team clinic coordinator, clinical data manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 303 PSRT meetings. The clinician members of HVTN 303 PSRT are responsible for decisions related to participant safety. The PSRT will review study safety information on a weekly basis through 4 weeks after the last participant receives the final study injection. Less frequent safety reviews will be conducted at the discretion of the PSRT.

9.3.2 HVTN Safety Monitoring Board (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 (including cumulative reactogenicity events, AEs, laboratory safety data, and individual SAE reports) approximately every 4 months. The SMB conducts additional special reviews at the request of the HVTN 303 PSRT.

Study sites will receive SMB summary minutes and are responsible for forwarding them to their Institutional Review Board (IRB)/Ethics Committee (EC) and any applicable Regulatory Entity (RE).

9.4 Safety reviews

There are 2 planned safety holds in this study: the first to determine dose escalations between groups 1 and 2 and between groups 3 and 4, and the second to review safety data for the higher-dose groups prior to proceeding to Part B. The PSRT must assess the accumulated product-specific data as showing no significant safety concerns before proceeding with activation of additional groups.

9.4.1 Safety considerations for Part A: Initial Safety reviews

Part A of the study may begin with direct enrollment of participants into the following groups simultaneously:

- Group 1 with no more than 1 participant enrolled per day for the 3 participants
- Group 3 with no more than 1 participant enrolled per day for the 3 participants

After Groups 1 and 3 have been fully enrolled, the study will be placed on a safety hold. No additional enrollments will proceed until the PSRT has determined it is safe to do so. Once all of the reactogenicity and 2-week safety data from at least 6 participants have been submitted to the database, the PSRT must assess the accumulated product-specific data as showing no significant safety concerns before proceeding with enrollment of Groups 2, 4 and 5.

If the PSRT has determined it is safe to proceed after reviewing data from Groups 1 and 3, the following groups may begin simultaneously:

- Group 2 with no more than 1 participant enrolled per day for the 3 participants
- Group 4 with no more than 1 participant enrolled per day for the 3 participants
- Group 5 with no more than 1 participant enrolled per day for the 3 participants

Once Groups 2, 4 and 5 have been fully enrolled, the study will be placed on a safety hold before proceeding with Part B. Once all of the reactogenicity and 2-week safety data from at least 9 participants has been submitted to the database, the PSRT must assess the accumulated product-specific data as showing no significant safety concerns before proceeding with enrollment of Part B. Please see Table 9-1 for more details.

9.4.2 Safety evaluations for opening enrollment in Part B

Once all of the cumulative safety data from all Part A participants have been reviewed and the PSRT determines there are no safety concerns, Part B may proceed with enrollment into Groups 6, 7, and 8 simultaneously.

Table 9-1 Summary of planned safety reviews

Planned Safety Review	Timepoint/Data Reviewed	Actions
Planned Safety Hold #1: Groups 1 and 3 Enrollment is on hold	Reactogenicity and safety data from the "Week 2" post vaccination visit will be reviewed for the following: • At least 3 recipients of the FP conjugate vaccine (with Adjuplex) at a dose of 25 mcg (Group 1) • At least 3 recipients of the Trimer 6931 (with Adjuplex) at a dose of 100 mcg (Group 3)	An interim safety review by the PSRT will determine: • If the FP conjugate vaccine plus Adjuplex (Group 1) is safe for further evaluation at a dose of 200 mcg (Group 2) • If the Trimer 6931 plus Adjuplex (Group 3) is safe for further evaluation at a dose of 200 mcg (Group 4) If the PSRT determines it is safe to proceed, enrollments into Groups 2, 4 and 5 may begin with no more than 1 participant enrolled per day for the 3 participants in each of these groups
Planned Safety Hold #2: Groups 2, 4 and 5 Enrollment is on hold	Reactogenicity and safety data from the "Week 2" post vaccination visit will be reviewed for the following: 1. At least 3 recipients of FP conjugate vaccine plus Adjuplex at a dose of 200 mcg (Group 2) 2. At least 3 recipients of Trimer 6931 plus Adjuplex at a dose of 200 mcg (Group 4) 3. At least 3 recipients Trimer 4571 plus Adjuplex at a dose of 200 mcg (Group 5)	An interim safety review by the PSRT will determine if it is safe to proceed with all groups in Part B. If the PSRT determines it is safe to proceed, enrollments into Groups 6, 7, and 8 may begin and is unrestricted to the number of participants enrolled per day.

9.5 Safety pause and prompt PSRT AE review

The PSRT (see Section 9.3) will closely monitor participant safety. The trial can be paused at any time for any reason by the PSRT. When a trial is placed on safety pause, all enrollment and vaccination will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 303 PSRT AE review are summarized in Table 9-2.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 303 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 5.2.3.

Table 9-2 Pause rules

Event and relationship to study vaccine	Severity Grade	HVTN Site Actions	HVTN Core action
SAE, **related**	5 or 4	**Phone** 24/7 Safety Phone immediately **Email** vtn.clin.safety.spec@hvtn.org **Submit** CRFs immediately	Immediate pause
SAE, **related**	3, 2, or 1	**Email** clinical safety specialist **Submit** CRFs immediately	Prompt PSRT AE review
AE, **related (see grade 3 exceptions)**	4 or 3	**Email** clinical safety specialist **Submit** CRFs immediately	Prompt PSRT AE review

Exceptions to the related Grade 3 AEs (for Grade 3 subjective reactogenicity events):

- injection site pain/tenderness
- fatigue
- generalized myalgia
- generalized arthralgia
- chills
- headache
- nausea (unless IV rehydration required)

Unrelated Participant Death: Sites will call the CSS office phone upon learning of any unrelated participant deaths. The site will also email the CSS and immediately submit CRFs. The PSRT will then be immediately notified.

If the site needs to contact the CSS, refer to phone numbers and email addresses found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn300).

9.5.1 Plan for review of pause rules

For all safety pauses, HVTN Core notifies the HVTN 303 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 303 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 303 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 303 PSRT notification or prompt HVTN 303 PSRT AE review is triggered, HVTN Core notifies the HVTN 303 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 303 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.

9.6 Total blood volume

Required blood volumes per visit are shown in Appendix A, Appendix B, Appendix C and Appendix D. 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, per American Red Cross guidelines for blood donation (https://www.redcrossblood.org/donate-blood/how-to-donate/eligibility-requirements.html).

The preferred laboratory specimen tube types for research samples are shown in Appendix A, Appendix B, Appendix C and Appendix D. Alternate tube types may be used under certain circumstances (eg, ACD tube shortages) upon approval of the HVTN Laboratory Center. Refer to the HVTN 303 Specimen Collection SSP for more information.

9.7 Study termination

This study may be terminated early by the determination of the HVTN 303 PSRT, the NIH, the United States Department of Health and Human Services Office for

Human Research Protections (OHRP), the FDA, or study product developers. 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.

9.8 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 for persons who are confirmed pregnant, pregnancy testing is not required unless clinically indicated. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome. Pregnancies and pregnancy outcomes will be reported as described in the HVTN 303 SSP. If the participant is no longer pregnant, refer to Section 5.2.2.

10 Protocol conduct and informed consent

10.1 Protocol conduct

This research study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) (the international council for harmonization of technical requirements for pharmaceuticals for human use (ICH) E6 [R2]), HVTN and DAIDS policies and procedures as specified in the *HVTN Manual of Operations* and DAIDS Clinical Research Policies and Standard Procedures Documents, and all applicable regulatory requirements. These policies and procedures include protocol monitoring (on-site and remote) and compliance. DAIDS and HVTN policies and procedures are available for review by any IRB/EC/RE upon request. 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 303 *Study Specific Procedures*.

HVTN scientists and operational staff are committed to substantive community input into the planning, conduct, and follow-up of its research, ensuring that locally appropriate cultural and linguistic needs of study populations are met. Community Advisory Boards (CAB) are required by DAIDS and are supported at all HVTN research sites to ensure community input in accordance with Good Participatory Practices (GPP) and all local and national guidelines.

10.2 Informed consent

The sample informed consent forms (SICFs) describe the investigational vaccines and all aspects involved in study participation. Documentation of appropriate informed consent must be in place prior to conducting study procedures with participants. Periodic assessment of participants' continued understanding of key study concepts and informed consent must also be documented. Study sites are strongly encouraged to have their local CABs review their site-specific consent forms. This review should include, but should not be limited to, issues of cultural competence, local language considerations, and the SICF's level of comprehensibility.

If any new information is learned that might affect the participants' decisions to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign revised informed consent forms.

An HVTN CRS may employ recruitment efforts prior to the participant consenting. For example, some HVTN CRSs use a telephone script to prescreen people before they come to the clinic for a full screening visit. Participants must sign a screening or protocol-specific consent before any procedures are performed to determine eligibility. HVTN CRSs must submit recruitment and prescreening

materials to their IRB/EC and any applicable RE for human participants protection review and approval.

10.2.1 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. Sites should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

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.

11 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.

Only genetic testing that is in accordance with the language in the sample informed consent forms (see Appendix G, Appendix H, Appendix I and Appendix J) may be performed on samples.

11.1 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 destruction or a time limit for storage is required by IRB/EC, or RE.

Other use of specimens is defined as studies not covered by the protocol or the informed consent form for the main study (see Appendix G, Appendix H, Appendix I and Appendix J).

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

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~~Appendix A Schedule of procedures for Part A (Groups 1-5)~~

See updated procedure for Part A (Groups 1-5) post termination of vaccination in Appendix B.

Schedule of Procedures Part A: Groups 1-5													
Visit Number		**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Study Week			**0**	1	2	4	8	12	16	24	32	40	52
Study Day		**-56 to 0**	**0**	7	14	28	56	84	112	168	224	280	364
Procedure			**Vac**										
Study Procedures	**Tube**	**Screen[1]**											
Assessment of Understanding		√											
Informed Consent		√											
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5]			√										
Reactogenicity Diary[6]			√										
Follow-Up Contact[7]			√										
Adverse Events (AEs)			√	√	√	√							
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√		√			√					
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire							√			√			√
HIV Risk Reduction Counseling[9]		√	√		√			√		√		√	√
Clinical Labs													
Pregnancy Test (urine or serum)[8,10]		√	√					√					
CBC/Differential[10]	EDTA	3	3	3	3	3	0	0	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	0	0	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	10	0	10	0	10	20
Research Samples[12, 13]													
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
PBMC	ACD	0	85	17	42.5	0	0	17	0	17	0	0	17
Daily Volume (mL)		**17.0**	**100.5**	**32.5**	**58.0**	**15.5**	**8.5**	**35.5**	**8.5**	**35.5**	**8.5**	**18.5**	**45.5**
56-day total Cumulative Volume (mL)		**17.0**	**117.5**	**150.0**	**208.0**	**223.5**	**232.0**	**59.5**	**52.5**	**44.0**	**44.0**	**27.0**	**45.5**

~~Green shaded columns represent vaccination visits.~~

~~1 Screening evaluations at Visit 01 are performed no more than 56 days before Day 0.~~

~~2 A complete physical exam is performed at screening and last clinic visit, to include 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. At other visits, a targeted physical exam will be performed as needed, based on participant report or indications of illness.~~

~~3 Medical history: A complete history is performed during screening. At enrollment and subsequently, an interim medical history may be performed.~~

~~4 Concomitant medications, including prescription and non-prescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots are recorded during screening, at enrolment, and at each subsequent clinic visit.~~

~~5 Vaccination (in clinic assessments): At least 30 minutes after each study injection and prior to clinic discharge, participants will have vital signs taken and the injection site will be assessed, and systemic symptoms will be assessed.~~

~~6 Reactogenicity diary: Participants will complete a 10-day diary as noted in Section 8.3. If any solicited reactogenicity is unresolved by Day 10, it will continue to be reviewed with the participant at each visit until resolved.~~

~~7 Follow-Up Contact: Participants will be contacted the day after vaccination which might not necessarily be within the visit window for vaccination.~~

~~8 Contraception status assessment and pregnancy test are required only for participants who were assigned female sex at birth and are capable of becoming pregnant. Pregnancy test may be performed on urine or blood specimens. Persons who are NOT capable of becoming pregnant due to total hysterectomy or bilateral oophorectomy (verified by medical records), or menopause (no menses for ≥ 1 year) are not required to undergo pregnancy testing. For persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.~~

~~9 HIV risk reduction and post-test counseling, including potential for VISP will be conducted as indicated above and at other visits if deemed clinically appropriate. A wallet card and letter regarding VISP will be provided to volunteers as noted in Section 8.6.~~

~~10 Local labs may assign appropriate alternative tube types for locally performed tests.~~

~~11 HIV screening test: see Section 5.1.1.~~

~~12 Research Samples: Blood draw volumes for each tube type shown.~~

~~13 Skin biopsies will be obtained if unusual skin reactions following vaccinations are reported (see Section 8.3)~~

Appendix B Follow-up schedule of procedures for Part A (Groups 1-5) post termination of vaccinations

Schedule of Procedures Part A: Groups 1-5													
Visit Number		**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Study Week			**0**	1	2	4	8	12	16	24	32	40	52
Study Day		**-56 to 0**	**0**	7	14	28	56	84	112	168	224	280	364
Procedure			**Vac**										
Study Procedures	**Tube**	**Screen[1]**											
Assessment of Understanding		√											
Informed Consent		√											
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5]			√										
Reactogenicity Diary[6]			√										
Follow-Up Contact[7]			√										
Adverse Events (AEs)			√	√	√	√							
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√		√			√					
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire							√			√			√
HIV Risk Reduction Counseling[9]		√	√		√			√		√		√	√
Clinical Labs													
Pregnancy Test (urine or serum)[8,10]		√	√					√					
CBC/Differential[10]	EDTA	3	3	3	3	3	0	0	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	0	0	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	10	0	10	0	10	20
Research Samples[12, 13]													
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	0	0	0
PBMC	ACD	0	85	17	42.5	0	0	17	0	17	0	0	0
Daily Volume (mL)		**17.0**	**100.5**	**32.5**	**58.0**	**15.5**	**8.5**	**35.5**	**8.5**	**35.5**	**0.0**	**10.0**	**20.0**
56-day total Cumulative Volume (mL)		**17.0**	**117.5**	**150.0**	**208.0**	**223.5**	**232.0**	**59.5**	**52.5**	**44.0**	**35.5**	**10.0**	**20.0**

Green shaded columns represent vaccination visits.

1 Screening evaluations at Visit 01 are performed no more than 56 days before Day 0.

2 A complete physical exam is performed at screening and last clinic visit, to include 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. At other visits, a targeted physical exam will be performed as needed, based on participant report or indications of illness.

3 Medical history: A complete history is performed during screening. At enrollment and subsequently, an interim medical history may be performed.

4 Concomitant medications, including prescription and non-prescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots are recorded during screening, at enrolment, and at each subsequent clinic visit.

5 Vaccination (in clinic assessments): At least 30 minutes after each study injection and prior to clinic discharge, participants will have vital signs taken and the injection site will be assessed, and systemic symptoms will be assessed.

6 Reactogenicity diary: Participants will complete a 7-day diary as noted in Section 8.3. If any solicited reactogenicity is unresolved by Day 10, it will continue to be reviewed with the participant at each visit until resolved.

7 Follow-Up Contact: Participants will be contacted the day after vaccination which might not necessarily be within the visit window for vaccination.

8 Contraception status assessment and pregnancy test are required only for participants who were assigned female sex at birth and are capable of becoming pregnant. Pregnancy test may be performed on urine or blood specimens. Persons who are NOT capable of becoming pregnant due to total hysterectomy or bilateral oophorectomy (verified by medical records), or menopause (no menses for ≥ 1 year) are not required to undergo pregnancy testing. For persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

9 HIV risk reduction and post-test counseling, including potential for VISP will be conducted as indicated above and at other visits if deemed clinically appropriate. A wallet card and letter regarding VISP will be provided to volunteers as noted in Section 8.6.

10 Local labs may assign appropriate alternative tube types for locally performed tests.

11 HIV screening test: see Section 5.1.1.

12 Research Samples: Blood draw volumes for each tube type shown.

13 Skin biopsies will be obtained if unusual skin reactions following vaccinations are reported (see Section 8.3)

~~Appendix C Schedules of procedures for Part B (Tables for Group 6, Group 7, and Group 8)~~

See updated schedule of procedures for Part B (Tables for Groups 6-8) post termination of vaccination in Appendix D

~~Part B, Group 6~~

Schedule of Procedures Part B: Group 6^																			
Visit Number		**100**	**101**	**102**	**103**	**110**	**111**	**112**	**116**	**117**	**118**	**122**	**123**	**124**	**129**	**130**	**131**	**133**	**135**	**137**
Study Week			0	1	2	12	13	14	24	25	26	36	37	38	48	49	50	60	72	100
Study Day		-56 to 0	0	7	14	84	91	98	168	175	182	252	259	266	336	343	350	420	504	700
Procedure			Vac 1			Vac 2			Vac 3			Vac 4			Vac 5					
Study Procedures	**Tube**	**Screen[1]**																		
Assessment of Understanding		√																		
Informed Consent		√																		
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5, 17]			√			√			√			√			√					
Reactogenicity Diary[6]			√			√			√			√			√					
Follow-Up Contact[7]			√			√			√			√			√					
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√			√			√			√			√			√		
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire						√			√			√			√			√		√
HIV Risk Reduction Counseling[9]		√			√			√			√		√			√		√		√
Clinical Labs																				
Pregnancy Test (urine or serum)[8,10]		√	√			√			√			√			√		[√][14]			
CBC/Differential[10]	EDTA	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	10	0	0	10	0	0	10	0	0	10	10	10	20
Research Samples[12, 15, 16]																				
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	25.5	8.5	8.5	8.5
PBMC	ACD	0	85	17	42.5	17	17	42.5	17	17	42.5	17	17	42.5	85	17	119[13]	17	102	17
Leukapheresis[14]																	√			
Daily Volume (mL)		**17**	**100.5**	**32.5**	**58**	**32.5**	**32.5**	**68**	**32.5**	**32.5**	**68**	**32.5**	**32.5**	**61**	**93.5**	**25.5**	**154.5**	**35.5**	**120.5**	**45.5**
56-day total Cumulative Volume (mL)		**17**	**117.5**	**150**	**208**	**32.5**	**65**	**133**	**32.5**	**65**	**133**	**32.5**	**65**	**126**	**93.5**	**119**	**273.5**	**35.5**	**120.5**	**45.5**

~~Part B, Group 7~~

Schedule of Procedures Part B: Group 7^																									
Visit Number		**100**	**101**	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**	**110**	**111**	**112**	**116**	**117**	**118**	**122**	**123**	**124**	**129**	**130**	**131**	**133**	**135**	**137**
Study Week			0	1	2	4	5	6	8	9	10	12	13	14	24	25	26	36	37	38	48	49	50	60	72	100
Study Day		**-56 to 0**	0	7	14	28	35	42	56	63	70	84	91	98	168	175	182	252	259	266	336	343	350	420	504	700
Procedure			Vac 1			Vac 2			Vac 3			Vac 4			Vac 5			Vac 6			Vac 7					
Study Procedures	**Tube**	**Screen[1]**																								
Assessment of Understanding		√																								
Informed Consent		√																								
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5,17]			√			√			√			√			√			√			√					
Reactogenicity Diary[6]			√			√			√			√			√			√			√					
Follow-Up Contact[7]			√			√			√			√			√			√			√					
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√			√			√			√			√			√			√			√		
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire									√			√			√			√			√			√		√
HIV Risk Reduction Counseling[9]		√			√			√			√			√			√			√		√		√		√
Clinical Labs																										
Pregnancy Test (urine or serum)[8,10]		√	√			√			√			√			√			√			√		[√][14]			
CBC/Differential[10]	EDTA	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	10	0	0	10	0	0	10	10	10	20
Research Samples[12, 15, 16]																										
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	25.5	8.5	8.5	8.5
PBMC	ACD	0	85	17	42.5	17	17	42.5	17	17	42.5	17	17	42.5	17	17	42.5	17	17	42.5	17	17	119[13]	17	102	17
Leukapheresis[14]																							√			
Daily Volume (mL)		**17**	**100.5**	**32.5**	**58**	**32.5**	**32.5**	**58**	**32.5**	**32.5**	**58**	**32.5**	**32.5**	**68**	**32.5**	**32.5**	**68**	**32.5**	**32.5**	**61**	**25.5**	**25.5**	**154.5**	**35.5**	**120.5**	**45.5**
56-day total Cumulative Volume (mL)		**17**	**117.5**	**150**	**208**	**240.5**	**273**	**331**	**363.5**	**278.5**	**304**	**278.5**	**278.5**	**314**	**32.5**	**65**	**133**	**32.5**	**65**	**126**	**25.5**	**51**	**205.5**	**35.5**	**120.5**	**45.5**

~~Part B, Group 8~~

Schedule of Procedures Part B: Group 8^																								
Visit Number		**100**	**101**	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**	**113**	**114**	**115**	**119**	**120**	**121**	**122**	**125**	**126**	**127**	**128**	**132**	**134**	**136**
Study Week			0	1	2	4	5	6	8	9	10	20	21	22	32	33	34	36	40	44	45	46	56	68	96
Study Day		**-56 to 0**	0	7	14	28	35	42	56	63	70	140	147	154	224	231	238	252	280	308	315	322	392	476	672
Procedure			Vac 1			Vac 2			Vac 3			Vac 4			Vac 5					Vac 6					
Study Procedures	**Tube**	**Screen[1]**																							
Assessment of Understanding		√																							
Informed Consent		√																							
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5,17]			√			√			√			√			√					√					
Reactogenicity Diary[6]			√			√			√			√			√					√					
Follow-Up Contact[7]			√			√			√			√			√					√					
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√			
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√			√			√			√			√		√			√			√		
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire									√			√			√					√					√
HIV Risk Reduction Counseling[9]		√			√			√			√			√				√		√		√		√
Clinical Labs																									
Pregnancy Test (urine or serum)[8,10]		√	√			√			√			√			√					√		[√][14]			
CBC/Differential[10]	EDTA	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	0	0	0	10	0	0	10	0	0	10	0	0	0	0	10	0	10	20
Research Samples[12, 15,16]																									
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	25.5	8.5	8.5	8.5
PBMC	ACD	0	85	17	42.5	17	17	42.5	17	17	42.5	17	17	42.5	17	17	42.5	17	17	17	17	119[13]	17	102	17
Leukapheresis[14]																						√			
Daily Volume (mL)		**17**	**100.5**	**32.5**	**58**	**32.5**	**32.5**	**58**	**32.5**	**32.5**	**68**	**32.5**	**32.5**	**68**	**32.5**	**32.5**	**68**	**32.5**	**25.5**	**25.5**	**25.5**	**154.5**	**25.5**	**120.5**	**45.5**
56-day total Cumulative Volume (mL)		**17**	**117.5**	**150**	**208**	**240.5**	**273**	**331**	**363.5**	**278.5**	**314**	**32.5**	**65**	**133**	**32.5**	**65**	**133**	**165.5**	**191**	**83.5**	**76.5**	**231**	**25.5**	**120.5**	**45.5**

~~Green shaded columns represent vaccination visits~~

~~^If a participant discontinues study injections, the schedule of evaluations should be followed for Visits 28-44, starting from a visit for the last completed injection.~~

~~[1] Screening evaluations at Visit 100 are performed no more than 56 days before Day 0.~~

~~[2] A complete physical exam is performed at screening and last clinic visit, to include 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. At other visits, a targeted physical exam will be performed as needed, based on participant report or indications of illness.~~

~~[3] Medical history: A complete history is performed during screening. At enrollment and subsequently, an interim medical history may be performed.~~

~~[4] Concomitant medications, including prescription and non-prescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots are recorded during screening, at enrolment, and at each subsequent clinic visit.~~

~~[5] Vaccination (in clinic assessments): At least 30 minutes after each study injection and prior to clinic discharge, participants will have vital signs taken and the injection site will be assessed, and systemic symptoms will be assessed.~~

~~[6] Reactogenicity diary: Participants will complete a 10-day diary as noted in Section 8.3. If any solicited reactogenicity is unresolved by Day 7, it will continue to be reviewed with the participant at each visit until resolved.~~

~~[7] Follow-Up Contact: Participants will be contacted by telephone the day after each vaccination which might not necessarily be within the visit window for vaccination.~~

~~[8] Contraception status assessment and pregnancy test are required only for participants who were assigned female sex at birth and are capable of becoming pregnant. Pregnancy test may be performed on urine or blood specimens. Persons who are NOT capable of becoming pregnant due to total hysterectomy or bilateral oophorectomy (verified by medical records), or menopause (no menses for ≥ 1 year) are not required to undergo pregnancy testing. For persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.~~

~~[9] HIV risk reduction and post-test counseling, including potential for VISP will be conducted as indicated above and at other visits if deemed clinically appropriate. A wallet card and letter regarding VISP will be provided to volunteers as noted in Section 8.6.~~

~~[10] Local labs may assign appropriate alternative tube types for locally performed tests.~~

~~[11] HIV screening test: see Section 5.1.1.~~

~~[12] Research Samples: Blood draw volumes for each tube type shown.~~

~~[13] PBMC for assays & storage: To be collected only from participants who will not undergo leukapheresis.~~

~~[14] Leukapheresis: Will only be collected from participants who agreed to provide this sample type and who have met leukapheresis eligibility criteria (see Section 8.4). A pregnancy test must be performed and confirmed negative within 72 hours prior to leukapheresis (see also: footnote 8). For participants who decide not to provide leukapheresis samples, 119 mL of ACD blood will be collected instead of leukapheresis.~~

~~[15] Skin biopsies will be obtained if unusual skin reactions following vaccinations are reported (see Section 8.3)~~

~~[16] Collection of research samples has been discontinued since March 13, 2023~~

~~[17] Vaccinations have been permanently discontinued since January 13, 2023~~

Appendix D Follow-up schedule of procedures for Part B (Tables for Group 6, Group 7, and Group 8) post termination of vaccinations

Part B, Group 6

Schedule of Procedures Part B: Group 6^																		
Visit Number		**100**	**101**	**102**	**103**	**110**	**111**	**112**	**116**	**117**	**118**	**122**	**123**	**124**	**129**	**130**	**131**	**132**
Study Week			0	1	2	12	13	14	24	25	26	36	37	38	48	49	50	56
Study Day		-56 to 0	0	7	14	84	91	98	168	175	182	252	259	266	336	343	350	392
Procedure			Vac 1															
Study Procedures	**Tube**	**Screen[1]**																
Assessment of Understanding		√																
Informed Consent		√																
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5,17]			√															
Reactogenicity Diary[6]			√															
Follow-Up Contact[7]			√															
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√			√			√			√			√			
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire						√			√			√			√			√
HIV Risk Reduction Counseling[9]		√			√			√			√		√			√		√
Clinical Labs																		
Pregnancy Test (urine or serum)[8,10]		√	√															
CBC/Differential[10]	EDTA	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	10	0	0	10	0	0	10	0	0	10	20
Research Samples[12, 15,16]																		
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	0	0	0	0	0	0	0
PBMC	ACD	0	85	17	42.5	17	17	42.5	17	17	42.5	0	0	0	0	0	0	0
Daily Volume (mL)		**17**	**100.5**	**32.5**	**58**	**32.5**	**32.5**	**68**	**32.5**	**32.5**	**68**	**7**	**7**	**10**	**0**	**0**	**10**	**20**
56-day total Cumulative Volume (mL)		**17**	**117.5**	**150**	**208**	**32.5**	**65**	**133**	**32.5**	**65**	**133**	**7**	**14**	**24**	**0**	**0**	**10**	**30**

Part B, Group 7

Schedule of Procedures Part B: Group 7^																							
Visit Number		**100**	**101**	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**	**110**	**111**	**112**	**116**	**117**	**118**	**122**	**123**	**124**	**129**	**130**	**131**	**132**
Study Week			0	1	2	4	5	6	8	9	10	12	13	14	24	25	26	36	37	38	48	49	50	56
Study Day		**-56 to 0**	0	7	14	28	35	42	56	63	70	84	91	98	168	175	182	252	259	266	336	343	350	392
Procedure			Vac 1			Vac 2																		
Study Procedures	**Tube**	**Screen[1]**																						
Assessment of Understanding		√																						
Informed Consent		√																						
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5,17]			√			√																		
Reactogenicity Diary[6]			√			√																		
Follow-Up Contact[7]			√			√																		
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√			√			√			√			√			√			√			
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire									√			√			√			√			√			√
HIV Risk Reduction Counseling[9]		√			√			√			√			√			√			√		√		√
Clinical Labs																								
Pregnancy Test (urine or serum)[8,10]		√	√			√																[√][14]		
CBC/Differential[10]	EDTA	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	10	0	0	10	0	0	10	20
Research Samples[12, 15,16]																								
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	0	0	0	0	0	0	0	0	0	0
PBMC	ACD	0	85	17	42.5	17	17	42.5	17	17	42.5	17	17	42.5	0	0	0	0	0	0	0	204[13]	0	0
Leukapheresis[14]																						√		
Daily Volume (mL)		**17**	**100.5**	**32.5**	**58**	**32.5**	**32.5**	**58**	**32.5**	**32.5**	**58**	**32.5**	**32.5**	**68**	**7**	**7**	**17**	**7**	**7**	**10**	**0**	**204**	**10**	**20**
56-day total Cumulative Volume (mL)		**17**	**117.5**	**150**	**208**	**240.5**	**273**	**331**	**363.5**	**278.5**	**304**	**278.5**	**278.5**	**314**	**7**	**14**	**31**	**7**	**14**	**24**	**0**	**204**	**214**	**234**

Part B, Group 8

Schedule of Procedures Part B: Group 8^																						
Visit Number		**100**	**101**	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**	**113**	**114**	**115**	**119**	**120**	**121**	**122**	**125**	**126**	**127**	**128**	**132**
Study Week			0	1	2	4	5	6	8	9	10	20	21	22	32	33	34	36	40	44	45	46	56
Study Day		**-56 to 0**	0	7	14	28	35	42	56	63	70	140	147	154	224	231	238	252	280	308	315	322	392
Procedure			Vac 1			Vac 2																	
Study Procedures	**Tube**	**Screen[1]**																					
Assessment of Understanding		√																					
Informed Consent		√																					
Physical Exam[2]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Medical History[3]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Concomitant Medications[4]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[5,17]			√			√																	
Reactogenicity Diary[6]			√			√																	
Follow-Up Contact[7]			√			√																	
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
COVID-19 symptom check			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[8]		√	√			√			√			√			√		√			√			
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Social impact questionaire									√			√			√					√			√
HIV Risk Reduction Counseling[9]		√			√			√			√			√					√		√		√
Clinical Labs																							
Pregnancy Test (urine or serum)[8,10]		√	√			√																[√][14]	
CBC/Differential[10]	EDTA	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0
ALT/Creatinine[10]	SST	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	0	0	0
HIV screening test[10,11]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HBsAg/anti-HCV[10]	SST	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV diagnostic test	EDTA	0	0	0	0	0	0	0	0	0	10	0	0	10	0	0	10	0	0	0	0	10	20
Research Samples[12, 15,16]																							
Serum	SST	0	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	0	0	0	0	0	0	0	0	0	0	0	0
PBMC	ACD	0	85	17	42.5	17	17	42.5	17	17	42.5	0	0	0	0	0	0	0	0	0	0	204[13]	0
Leukapheresis[14]																						√	
Daily Volume (mL)		**17**	**100.5**	**32.5**	**58**	**32.5**	**32.5**	**58**	**32.5**	**32.5**	**68**	**7**	**7**	**17**	**7**	**7**	**17**	**7**	**0**	**0**	**0**	**214**	**20**
56-day total Cumulative Volume (mL)		**17**	**117.5**	**150**	**208**	**240.5**	**273**	**331**	**363.5**	**278.5**	**314**	**7**	**14**	**31**	**7**	**14**	**31**	**38**	**38**	**7**	**0**	**214**	**20**

Green shaded columns represent vaccination visits

Yellow shaded columns represent visits without any sample collection. If these visits have already happened, please collect data in the CRFs. Otherwise, please skip these visits.

1 Screening evaluations at Visit 100 are performed no more than 56 days before Day 0.

2 A complete physical exam is performed at screening and last clinic visit, to include 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. At other visits, a targeted physical exam will be performed as needed, based on participant report or indications of illness.

3 Medical history: A complete history is performed during screening. At enrollment and subsequently, an interim medical history may be performed.

4 Concomitant medications, including prescription and non-prescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots are recorded during screening, at enrolment, and at each subsequent clinic visit.

5 Vaccination (in clinic assessments): At least 30 minutes after each study injection and prior to clinic discharge, participants will have vital signs taken and the injection site will be assessed, and systemic symptoms will be assessed.

6 Reactogenicity diary: Participants will complete a 7-day diary as noted in Section 8.3. If any solicited reactogenicity is unresolved by Day 10, it will continue to be reviewed with the participant at each visit until resolved.

7 Follow-Up Contact: Participants will be contacted by telephone the day after each vaccination which might not necessarily be within the visit window for vaccination.

8 Contraception status assessment and pregnancy test are required only for participants who were assigned female sex at birth and are capable of becoming pregnant. Pregnancy test may be performed on urine or blood specimens. Persons who are NOT capable of becoming pregnant due to total hysterectomy or bilateral oophorectomy (verified by medical records), or menopause (no menses for ≥ 1 year) are not required to undergo pregnancy testing. For persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

9 HIV risk reduction and post-test counseling, including potential for VISP will be conducted as indicated above and at other visits if deemed clinically appropriate. A wallet card and letter regarding VISP will be provided to volunteers as noted in Section 8.6.

10 Local labs may assign appropriate alternative tube types for locally performed tests.

11 HIV screening test: see Section 5.1.1.

12 Research Samples: Blood draw volumes for each tube type shown.

13 PBMC for assays & storage: To be collected only from participants who received two vaccinations and who will not undergo leukapheresis. There is a wide visit window for this collection (see Appendix F).

14 Leukapheresis: Will only be collected from participants who received two vaccinations and have agreed to provide this sample type and who have met leukapheresis eligibility criteria (see Section 8.4). A pregnancy test must be performed and confirmed negative within 72 hours prior to leukapheresis (see also: footnote 8). For participants who received two vaccinations and decide not to provide leukapheresis samples, 204 mL of ACD blood will be collected instead of leukapheresis. There is a wide visit window for this collection (see Appendix F).

15 Skin biopsies will be obtained if unusual skin reactions following vaccinations are reported (see Section 8.3)

16 Collection of research samples has been discontinued since March 13, 2023

17 Vaccinations have been permanently discontinued since January 13, 2023

~~Appendix E Visit windows~~

See updated visit windows post termination of vaccination in Appendix F

Visit windows for Part A (Groups 1-5)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
01	Screening	-56	-	0	-	+0
02	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
03	1-week post-vaccination 1	-2	-1	7	+1	+3
04	2 weeks post-vaccination 1 **Primary Immunogenicity**	-4	-2	14	+2	+7
05	Follow-up	-7	-2	28	+2	+7
06	Follow-up	-7	-3	56	+3	+7
07	Follow-up	-7	-3	84	+3	+7
08	Follow-up	-14	-3	112	+3	+14
09	Follow-up	-14	-7	168	+7	+14
10	Follow-up	-14	-7	224	+7	+14
11	Follow-up	-14	-7	280	+7	+14
12	Final Visit	-21	-14	364	+14	+21

* All target dates are relative to Day 0.

Visit windows for Part B (Group 6)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
100	Screening	-56	-	0	-	+0
101	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
102	1-week post-vaccination 1	-2	-1	7	+1	+3
103	2 weeks post-vaccination 1	-4	-2	14	+2	+14
110	**Vaccination 2**	**-14**	**-3**	**84**	**+3**	**+14**
111	1-week post-vaccination 2	-2	-1	91	+1	+3
112	2 weeks post-vaccination 2	-4	-2	98	+2	+14
116	**Vaccination 3**	**-21**	**-3**	**168**	**+3**	**+21**
117	1-week post-vaccination 3	-2	-1	175	+1	+3
118	2 weeks post-vaccination 3	-4	-2	182	+2	+14
122	**Vaccination 4**	**-21**	**-3**	**252**	**+3**	**+21**
123	1-week post-vaccination 4	-2	-1	259	+1	+3
124	2 weeks post-vaccination 4	-4	-2	266	+2	+14
129	**Vaccination 5**	**-21**	**-3**	**336**	**+3**	**+21**
130	1-week post-vaccination 5	-2	-1	343	+1	+3
131	2 weeks post-vaccination 5 **Primary Immunogenicity** Leukapheresis	-4	-2	350	+2	+14
133	Follow-up	-28	-3	420	+3	+28
135	Follow-up	-28	-3	504	+3	+28
137	Final Visit	-28	-7	700	+7	+28

*Target dates are relative to Day 0 (Enrollment), with the exception of post-vaccination visits, which are relative to the prior vaccination visit.

Visit windows for Part B (Group 7)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
100	Screening	-56	-	0	-	+0
101	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
102	1-week post-vaccination 1	-2	-1	7	+1	+3
103	2 weeks post-vaccination 1	-4	-2	14	+2	+7
104	**Vaccination 2**	**-6**	**-3**	**28**	**+3**	**+7**
105	1-week post-vaccination 2	-2	-1	35	+1	+3
106	2 weeks post-vaccination 2	-	-2	42	+2	-
107	**Vaccination 3**	**-6**	**-3**	**56**	**+3**	**+7**
108	1-week post-vaccination 3	-2	-1	63	+1	+3
109	2 weeks post-vaccination 3	-	-2	70	+2	-
110	**Vaccination 4**	**-6**	**-3**	**84**	**+3**	**+14**
111	1-week post-vaccination 4	-2	-1	91	+1	+3
112	2 weeks post-vaccination 4	-4	-2	98	+2	+14
116	**Vaccination 5**	**-21**	**-3**	**168**	**+3**	**+21**
117	1-week post-vaccination 5	-2	-1	175	+1	+3
118	2 weeks post-vaccination 5	-4	-2	182	+2	+14
122	**Vaccination 6**	**-21**	**-3**	**252**	**+3**	**+21**
123	1-week post-vaccination 6	-2	-1	259	+1	+3
124	2 weeks post-vaccination 6	-4	-2	266	+2	+14
129	**Vaccination 7**	**-21**	**-3**	**336**	**+3**	**+21**
130	1-week post-vaccination 7	-2	-1	343	+1	+3
131	2 weeks post-vaccination 7 **Primary Immunogenicity** Leukapheresis	-4	-2	350	+2	+14
133	Follow-up	-28	-3	420	+3	+28
135	Follow-up	-28	-7	504	+7	+28
137	Final Visit	-28	-7	700	+7	+28

*Target dates are relative to Day 0 (Enrollment), with the exception of post-vaccination visits, which are relative to the prior vaccination visit.

Visit windows for Part B (Group 8)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
100	Screening	-56	-	0	-	+0
101	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
102	1-week post-vaccination 1	-2	-1	7	+1	+3
103	2 weeks post-vaccination 1	-4	-2	14	+2	+7
104	**Vaccination 2**	**-6**	**-3**	**28**	**+3**	**+7**
105	1-week post-vaccination 2	-2	-1	35	+1	+3
106	2 weeks post-vaccination 2	-	-2	42	+2	-
107	**Vaccination 3**	**-6**	**-3**	**56**	**+3**	**+7**
108	1-week post-vaccination 3	-2	-1	63	+1	+3
109	2 weeks post-vaccination 3	-4	-2	70	+2	+14
113	**Vaccination 4**	**-14**	**-3**	**140**	**+3**	**+14**
114	1-week post-vaccination 4	-2	-1	147	+1	+3
115	2 weeks post-vaccination 4	-4	-2	154	+2	+14
119	**Vaccination 5**	**-21**	**-3**	**224**	**+3**	**+21**
120	1-week post-vaccination 5	-2	-1	231	+1	+3
121	2 weeks post-vaccination 5	-4	-2	238	+2	+14
122	4 weeks post-vaccination 5	-7	-2	252	+2	+7
125	8 weeks post-vaccination 5	-7	-3	280	+3	+7
126	**Vaccination 6**	**-21**	**-3**	**308**	**+3**	**+21**
127	1-week post-vaccination 6	-2	-1	315	+1	+3
128	2 weeks post-vaccination 6 **Primary Immunogenicity** Leukapheresis	**-4**	**-2**	**322**	**+2**	**+14**
132	Follow-up	-28	-4	392	+4	+28
134	Follow-up	-28	-7	476	+7	+28
136	Final Visit	-28	-7	672	+7	+28

*Target dates are relative to Day 0 (Enrollment), with the exception of post-vaccination visits, which are relative to the prior vaccination visit.

Appendix F Follow up visit windows post termination of vaccination

Visit windows for Part A (Groups 1-5)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
01	Screening	-56	-	0	-	+0
02	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
03	1-week post-vaccination 1	-2	-1	7	+1	+3
04	2 weeks post-vaccination 1 **Primary Immunogenicity**	-4	-2	14	+2	+7
05	Follow-up	-7	-2	28	+2	+7
06	Follow-up	-7	-3	56	+3	+7
07	Follow-up	-7	-3	84	+3	+7
08	Follow-up	-14	-3	112	+3	+14
09	Follow-up	-14	-7	168	+7	+14
10	Follow-up	-14	-7	224	+7	+14
11	Follow-up	-14	-7	280	+7	+14
12	Final Visit	-21	-14	364	+14	+21

* All target dates are relative to Day 0.

Visit windows for Part B (Group 6)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
100	Screening	-56	-	0	-	+0
101	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
102	1-week post-vaccination 1	-2	-1	7	+1	+3
103	2 weeks post-vaccination 1	-4	-2	14	+2	+14
110	Follow-up visit	-14	-3	84	+3	+14
111	Follow-up visit	-2	-1	91	+1	+3
112	Follow-up visit	-4	-2	98	+2	+14
116	Follow-up visit	-21	-3	168	+3	+21
117	Follow-up visit	-2	-1	175	+1	+3
118	Follow-up visit	-4	-2	182	+2	+14
122	Follow-up visit	-21	-3	252	+3	+21
123	Follow-up visit	-2	-1	259	+1	+3
124	Follow-up visit	-4	-2	266	+2	+14
129	Follow-up visit	-21	-3	336	+3	+21
130	Follow-up visit	-2	-1	343	+1	+3
131	Follow-up visit	-4	-2	350	+2	+14
132	Follow-up visit	-28	-3	392	+3	+28

*Target dates are relative to Day 0 (Enrollment), with the exception of post-vaccination visits, which are relative to the prior vaccination visit.

Visit windows for Part B (Group 7)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
100	Screening	-56	-	0	-	+0
101	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
102	1-week post-vaccination 1	-2	-1	7	+1	+3
103	2 weeks post-vaccination 1	-4	-2	14	+2	+7
104	**Vaccination 2**	**-6**	**-3**	**28**	**+3**	**+7**
105	1-week post-vaccination 2	-2	-1	35	+1	+3
106	2 weeks post-vaccination 2	-	-2	42	+2	-
107	Follow-up visit	-6	-3	56	+3	+7
108	Follow-up visit	-2	-1	63	+1	+3
109	Follow-up visit	-	-2	70	+2	-
110	Follow-up visit	-6	-3	84	+3	+14
111	Follow-up visit	-2	-1	91	+1	+3
112	Follow-up visit	-4	-2	98	+2	+14
116	Follow-up visit	-21	-3	168	+3	+21
117	Follow-up visit	-2	-1	175	+1	+3
118	Follow-up visit	-4	-2	182	+2	+14
122	Follow-up visit	-21	-3	252	+3	+21
123	Follow-up visit	-2	-1	259	+1	+3
124	Follow-up visit	-4	-2	266	+2	+14
129	Follow-up visit	-21	-3	336	+3	+21
	Leukapheresis	Leukapheresis (or large blood draw) must be completed at only one of the following visits (130, 131, or 132) but NOT at all 3 visits for the subset of participants who received two vaccinations (see Section 8.4).				
130	Follow-up visit	-2	-1	343	+1	+3
131	Follow-up visit	-4	-2	350	+2	+14
132	Follow-up visit	-28	-3	392	+3	+28

*Target dates are relative to Day 0 (Enrollment), with the exception of post-vaccination visits, which are relative to the prior vaccination.

Visit windows for Part B (Group 8)						
Visit Number	**Visit Type**	**Lower Allowable Window (-)**	**Lower Target Window (-)**	**Target Day***	**Upper Target Window (+)**	**Upper Allowable Window (+)**
100	Screening	-56	-	0	-	+0
101	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
102	1-week post-vaccination 1	-2	-1	7	+1	+3
103	2 weeks post-vaccination 1	-4	-2	14	+2	+7
104	**Vaccination 2**	**-6**	**-3**	**28**	**+3**	**+7**
105	1-week post-vaccination 2	-2	-1	35	+1	+3
106	2 weeks post-vaccination 2	-	-2	42	+2	-
107	Follow-up visit	-6	-3	56	+3	+7
108	Follow-up visit	-2	-1	63	+1	+3
109	Follow-up visit	-4	-2	70	+2	+14
113	Follow-up visit	-14	-3	140	+3	+14
114	Follow-up visit	-2	-1	147	+1	+3
115	Follow-up visit	-4	-2	154	+2	+14
119	Follow-up visit	-21	-3	224	+3	+21
120	Follow-up visit	-2	-1	231	+1	+3
121	Follow-up visit	-4	-2	238	+2	+14
122	Follow-up visit	-7	-2	252	+2	+7
125	Follow-up visit	-7	-3	280	+3	+7
126	Follow-up visit	-21	-3	308	+3	+21
127	Follow-up visit	-2	-1	315	+1	+3
	Leukapheresis	Leukapheresis (or large blood draw) must be completed at only one of these 2 visits (128 or 132) but NOT both visits for the subset of participants who received two vaccinations (see Section 8.4).				
128	Follow-up visit	-4	-2	322	+2	+14
132	Follow-up visit	-28	-4	392	+4	+28

*Target dates are relative to Day 0 (Enrollment), with the exception of post-vaccination visits, which are relative to the prior vaccination visit.

Appendix G Sample informed consent form for Part A (Groups 1-5)

Title: A Phase 1, Open-Label Clinical Trial to Evaluate Safety, Tolerability, and Immunogenicity of Adjuvanted HIV-1 Fusion Peptide Conjugate Vaccine (VRC-HIVVCP0108-00-VP) Alone or in Prime-Boost Regimens with Adjuvanted HIV-1 Envelope Trimer 4571 (VRC-HIVRGP096-00-VP) and HIV-1 Trimer 6931 (VRC-HIVRGP0106-00-VP) Vaccines in Healthy Adults

HVTN protocol number: HVTN 303

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.

Key information

- Being in this research study is voluntary. It is your choice.
- You are being asked to take part in this study because you are age 18-50, HIV negative and healthy.
- The purpose of the study is to see if the study vaccines are safe to give to people and do not make people too uncomfortable.
- Another purpose of the study is to see how a person's immune system responds to the study vaccines. (Your immune system protects you from infections and disease.)
- You will be in this study for up to 12 months of clinic visits.
- Procedures will include blood draws and injections of study vaccine. There is also an optional procedure of a skin biopsy that we may ask you to have done. We will tell you more about this procedure later in this consent form.
- There are risks from participating.
  - The vaccine you receive has not been given to people before or has only been given to a small number of people. We do not know all the risks of the vaccines in this study. A few known risks are pain and itching at the injection site, muscle pain, swelling, skin redness, soreness, tenderness, headache, nausea, fatigue and fever.
  - Taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding. A skin biopsy can cause bleeding, pain, bruising, and scarring, and rarely an infection.

- We will tell you more information about risks later in this consent form.

- We do not expect the study vaccines to benefit you in any way.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test 3 HIV vaccines. HIV is the virus that causes AIDS. The study vaccines are made from two parts: a protein and an adjuvant. Adjuvants are substances that help the immune system respond better (Your immune system protects you from disease). In this study we will test 3 different proteins with 1 adjuvant.

Up to 70 people will take part in this study at multiple sites in the United States. 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.

The study is divided into 2 parts, Part A and Part B. In Part A, participants will get 1 of the 3 study vaccines. Up to 20 people will be in Part A. If there are no safety concerns in Part A, we will do Part B. Up to 50 people will be in Part B. Part B will test the study vaccines in different combinations. You are being invited to join Part A of the study.

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

   - Are the study vaccines safe to give to people?

   - Are people able to take the vaccines without becoming too uncomfortable?

   - How do people's immune systems respond to the study vaccines? (Your immune system protects you from infections and disease.)

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

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

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

3. **These study vaccines are experimental.**

   The study vaccines include two parts. One part is a lab-made piece of protein that looks like a protein found on the outside of HIV. The second part is an adjuvant called Adjuplex. (An adjuvant is something that helps the immune system respond better.) From here on, we will call it "the adjuvant".

We are testing 3 different proteins in this study and each of these will be mixed with the adjuvant. We will use the term “vaccine” or “study vaccine” to refer to the combination of a protein and the adjuvant. The study vaccines are experimental HIV vaccines. That means we do not know if they will be safe to use in people, or if they will work to prevent HIV infection. These study vaccines are used only in research studies.

The 3 proteins being tested are called Fusion Peptide (FP conjugate), Trimer 4571 and Trimer 6931. They are all lab-made small pieces of proteins that look like parts of proteins found in the HIV virus. The immune system will be able to see the pieces that look like parts of HIV and learn how to recognize them. Researchers hope that the immune system will respond by making antibodies that could fight HIV. (The body makes antibodies to fight germs after it is exposed to them. The antibodies attack germs and keep them from infecting the body’s cells.) Each of the 3 proteins in the study is made slightly differently to see how the immune system will respond to these different structures. The proteins were developed by the Vaccine Research Center (VRC) at the US National Institutes of Health.

The FP conjugate protein and the Trimer 6931 protein have not been given to people before. The Trimer 4571 protein mixed with a different adjuvant has been given to a small number of people in several other studies.

The Adjuplex adjuvant is being provided for this study by the VRC. The adjuvant is made of a fatty substance called lecithin and a suspending agent called carbomer mixed together in a saline solution. It has been given to 20 people in a different study.

These combinations of protein and adjuvant have been tested in mice, guinea pigs and rabbits. The adjuvant has also been tested in studies using mice and rabbits. In these studies, animals that got these products had symptoms consistent with the general risks of other vaccines (as described below). Even if something looks like it is safe or works in animals, it may not be true for people.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, dizziness, and feeling tired. Vaccines can also cause stinging, discomfort, redness, mild bruising or an infection where you got the injection. Signs of infection at the injection site include severe pain, redness, swelling, warmth or drainage. Rarely, people have side effects that limit their normal activities or make them go to the doctor.

Risks of the study vaccines:

FP conjugate vaccine and Trimer 6931 vaccine

The FP conjugate vaccine and the Trimer 6931 vaccine have not been given to people before so we do not know what the risks may be. However, we expect they

will be similar to the general risks of vaccines described above. There may be other side effects, even serious or life-threatening ones, which we do not know about yet.

Trimer 4571 vaccine

This vaccine mixed with a different adjuvant was given to people for the first time in a study called VRC 018 that ended in June 2020. The 16 participants in it each got 3 injections at doses higher and lower than the dose we will give in this study. The injections did not cause any serious health problems. 14 people had mild pain where they got the injection and 6 people had muscle pain, which went away within 7 days. 6 people had mild itching where they got the injection that lasted between 1 and 9 days. One person had swelling after their second injection and skin redness after their third. Both of these went away within 7 days. Another person had moderate swelling and severe skin redness where they got the injection. Both of these disappeared at 8 days after the injection. 4 people had mild headaches and 3 people felt nauseous after injection. These symptoms went away within 7 days. One person had a decrease in neutrophils which is a type of white blood cell. This went away after 8 days.

As of January 2022, there are 2 other studies giving the Trimer 4571 vaccine to people. NIH-19-I-0069 will enroll 200 participants and give them a different study vaccine followed by a booster injection of a higher dose of the Trimer 4571 vaccine than we will give in this study with a different adjuvant 6 months later. As of January 5, 2022, 12 participants have gotten their booster injection. The injections have not caused serious health problems. The common side effects are mild pain at the injection site, mild headache and mild fatigue. The other study, called NETI, will give participants with HIV infection multiple injections of either placebo or lower and higher doses of Trimer 4571 than we will use in this study with a different adjuvant. As of January 1, 2022, 5 participants have received a total of 7 injections. The common side effects are mild to moderate injection site pain and tenderness, fatigue, joint stiffness and headache.

Because this protein has only been given to a small number of people, we do not know all the side effects. There may be side effects, which may be serious or life threatening, that we do not know about yet.

Risks of the Adjuplex adjuvant:

The 20 people who got a different vaccine with this adjuvant in a different study have not had serious health problems from it. Some people have had injection site pain, soreness, tenderness and occasional fever after getting this adjuvant. Because this adjuvant with a different vaccine has only been given to a small number of people, we do not know all the side effects.

These are the side effects we know about. There may be others that we don’t know about. We will tell you if we learn about new side effects that could affect your willingness to stay in the study.

Joining the study

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

   Take your time. Talk to people you trust. If you decide not to join this study or if you leave after you have joined, your other care at this clinic and the benefits or rights you would normally have will not be affected.

   You cannot be in this study while you are in another study where you get a study product. If you do not join this study, you may be able to join another study.

   If you join this study, you may not be allowed to join other HIV vaccine or HIV prevention trials now or in the future.

   During the study, you should not donate blood or tissue.

   Site: Remove item 5 if you use a separate screening consent that covers these procedures.

5. **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 run blood tests. These tell us about the health of your kidneys and liver. We will ask you about medicines you are taking, including HIV pre-exposure prophylaxis (PrEP). We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy.

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

   Site: adapt the following section per the care available at your site

6. **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 help you get care elsewhere. For health problems unrelated to the study, we will not pay for care.

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

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

   You should not become pregnant during the study because we do not know how the study vaccines could affect a developing baby. You must agree to use effective birth control from at least 3 weeks before your study injection through 12 weeks later. 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 join the study, here is what will happen:

8. **You will come to the clinic for scheduled visits about [#] times over 12 months.**

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

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

   You may have to come for more visits if you have a lab or health issue. We may contact you after the study ends (for example, to tell you about the study results). We may also contact you about other studies you may want to join.

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

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

   There is also compensation for the optional skin biopsy study procedure we may ask you to have done. We will tell you more about the procedure below. If you have the skin biopsy procedure, we will give you *[Site: Insert amount]*.

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

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

10. We will give you one of the study vaccines.

When you join the study, you will get one of the study vaccines, split into 2 injections. We will give the injections with a needle and syringe into the muscle of both upper arms. If there is a reason we cannot give an injection into one of your arms, then we will give both injections into the other arm.

You will be in 1 of 5 groups. Which group you are in will depend on when you join the study. The table below shows the groups and what study vaccine and dose each will get. Groups 1-4 will get the 2 study vaccines that have not been given to people before. These are the FP conjugate vaccine and the Trimer 6931 vaccine. We will start by giving a low dose of each of these vaccines. Group 1 will get a low dose of the FP conjugate vaccine and Group 3 will get a low dose of the Trimer 6931 vaccine.

Because this is the first time these study vaccines are being given to people, we want to be very careful. Only 1 participant in each group will get the study vaccine each day. Once 3 people in Group 1 and 3 people in Group 3 have gotten the study vaccine, we will pause the study for a review by a team that is watching the study closely to ensure it is safe for participants. They will look at how these 6 participants responded to their study vaccines and decide if it is safe to give a higher dose of these 2 study vaccines. If this team allows us to continue with the study, we will then begin enrolling Groups 2, 4 and 5.

Group 2 will get a higher dose of the FP conjugate vaccine and Group 4 will get a higher dose of the Trimer 6931 vaccine. Group 5 will get the study vaccine that has been given to people before, the Trimer 4571 vaccine. In these groups, just like with Groups 1 and 3, only 1 participant in each group will get the study vaccine each day.

Once 3 people in each group (Group 2, Group 4 and Group 5) have gotten the study vaccine, we will again pause the study for a review by the same team watching the safety of participants. They will look at how these 9 participants responded to their study vaccines and decide if it is safe to start Part B of the study, which will give multiple injections of the study vaccines to participants. It is possible they may ask us at any time to enroll up to 5 more participants in Part A to provide more information to help them with their decision.

Group	Number of participants	Study vaccine and dose
1	3	25 mcg FP conjugate vaccine
2	3	200 mcg FP conjugate vaccine
3	3	100 mcg Trimer 6931 vaccine
4	3	200 mcg Trimer 6931 vaccine
5	3	200 mcg Trimer 4571 vaccine

You will have to wait in the clinic for at least 30 minutes after your injections to see if there are any reactions or health concerns. Then for that night and for 10 more days, you will use an electronic participant diary to record how you are feeling, including your highest daily temperature. We will give you a thermometer to help you do this. We will also give you a ruler to measure any swelling at the injection site. If you are unable or unwilling to use the electronic participant diary, please talk with us about other options that may be available. We will contact you the day after your injection to ask how you are doing. Contact the clinic staff if you have any issues or concerns after getting an injection.

11. In addition to giving you a study vaccine, we will do the procedures shown in the following table.

			Time after first injection visit									
Procedure	**Screening visit(s)**	**Injection visit**	**1 week**	**2 weeks**	**4 weeks**	**8 weeks**	**12 weeks**	**16 weeks**	**24 weeks**	**32 weeks**	**40 weeks**	**52 weeks**
Injection		√										
Medical history	√											
COVID-19 symptom check		√	√	√	√	√	√	√	√	√	√	√
Complete physical	√											√
Brief physical		√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√
Pregnancy test*	√	√					√					
HIV testing	√						√		√		√	√
Risk reduction counseling	√	√		√			√		√		√	√
Interview/ questionnaire	√	√	√	√	√	√	√	√	√	√	√	√

* For persons who were assigned female sex at birth and who are capable of becoming pregnant. Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) or are in menopause, do not have to have pregnancy tests.

When we take blood, the amount will depend on the lab tests we need to do at a certain visit. It will be some amount between 8.5 mL and 101 mL (about ½

tablespoon to 7 tablespoons). Your body will make new blood to replace the blood we take out.

When we do tests, we will review the results with you at your next visit, or sooner if necessary. We will tell you about any results that are important to your health.

12. If you have an unusual reaction on your skin due to a study injection, we may want to photograph it.

Taking photographs may help researchers learn more about reactions to the study products. We will not take photos of your face. Your name and other identifying information will not be included on the photos. The photos will only be used to research the safety of the study products.

13. If you have an unusual reaction on your skin to a study injection, we may want to do a skin biopsy.

A skin biopsy collects a small sample of skin cells so they can be examined in a lab. This may help researchers better understand your skin reaction and learn more about the study products.

We will ask you at the end of this consent form if you agree to the skin biopsy procedure. You can change your mind at any time. It is possible that you may not be asked to have a skin biopsy, even if you agree to it.

If we ask you to have a skin biopsy, this procedure will be done at [site: insert location].

Sites referring out to another facility for the skin biopsy:

- *must obtain copies of the informed consent form and any other educational materials that are available from the facility where it will be done so that they can be reviewed with potential participants during the study informed consent process.*

- *include these sentences:* There will be another consent form for you to review and sign at the facility. As part of the consent process, you will be able to discuss the procedure in more detail with the clinician who will do the procedure. The discussion will include the location, size, depth and risks of the skin biopsy.

Sites doing the skin biopsy in the clinic and that have their own separate ICF for the procedure:

- *must review it and any other educational materials they have about it with the potential participant during the study informed consent process.*

- *Include these sentences:* We will give you a separate consent form to review and sign. As part of the consent process, you will be able to discuss the procedure in more detail with the clinician who will do the procedure. The discussion will include the location, size, depth and risks of the skin biopsy.

Sites doing the skin biopsy in the clinic and that do not have their own separate ICF for the procedure must modify the general information below about skin biopsies to provide the details of the procedure & its risks at their clinic.

There are several different tools that can be used to do a skin biopsy. The clinician doing the procedure will examine the reaction on your skin to determine which one to use. They will tell you how the tool works and the general size of the sample they will take from your skin. They will apply a cleaning solution to your skin in the area where the biopsy will be done and numb the area with an injection of a local anesthetic. Once the area is numb, the clinician will take a small sample of skin. This procedure may require a few stitches to close the wound. A skin biopsy may cause a scar. This scar will likely fade over time. A skin biopsy typically takes about 15 minutes total, including the preparation time, dressing the wound, and instructions for at-home care.

14. We will counsel you about protecting yourself from HIV.

We will ask you 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 body, including your immune system, responds to the study vaccine.

We will send your samples (without your name or other identifying information) to labs in the United States approved by the HVTN for this study. In rare cases, some of your samples may be sent to labs 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. This genetic testing will involve only 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.

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 how the virus is impacted by the study vaccine.

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.

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, the HVTN will destroy all extra samples that it has. Your decision will not affect your being in this study or have any negative consequences here.

Where are the samples stored? Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

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

rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

What information is shared with HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with the HVTN, other researchers, or with anyone else who does not need to know your name. Your name will not be part of the information. However, some information that the HVTN shares may be personal, such as your race, ethnicity, sex, health information from the study, and HIV status. The HVTN 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.

Site: Check HIPAA authorization for conflicts with this section.

All of your samples and most of your study records will be labeled with a code number. Samples and study records are kept in secure locations. When you provide information in the electronic participant diary after the injection visits, that information only has your code number. Your information goes directly from the electronic participant diary into your study record.

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 vaccines(s) you received

No HIV test results are 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 can see your study records. Your records may also be reviewed by groups that 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 (FDA),
- Any regulatory agency that reviews clinical trials,
- *US sites may, but are not required to include:* [Insert name of local IRB/EC] ,
- Advarra Institutional Review Board (IRB)
- *Site:* [Insert name of local and/or national regulatory authority as appropriate]

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

All reviewers will keep your records private.

We cannot guarantee absolute privacy. If you 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]

Site: Include the following boxed text. You can remove the box around the text.

To help protect your privacy, we have a Certificate of Confidentiality from the US government. 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. However, we cannot withhold information from the US government because it funds this research. You can still give 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 Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

18. If you become pregnant or get HIV during the study, we will encourage you to stay in the study until your final scheduled clinic visit if you choose.

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

study, we may take fewer samples from you. If you get HIV, we will help you get care and support. 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 your HIV care. If you stay in the study, we will take fewer samples from you.

19. We may take you out of the study at any time.

We may take you out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you, or
- the study is stopped for any reason.

Other Risks

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

In addition to the risks of the study vaccines and the procedures that were described above, this section describes the other risks we know about. There may be other risks, even serious ones. We will tell you if we learn anything new that may affect your decision 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, stinging, swelling, itching, a sore, bleeding, and rarely infection where you got the injection. Taking blood can cause a low blood cell count (anemia), making you feel tired.

Risks of skin biopsy:

A skin biopsy can cause bleeding, pain, bruising, and scarring, and rarely, an infection. The injection of a local anesthetic can cause brief stinging or burning in the area of the injection.

Personal problems/discrimination/testing HIV antibody positive:

Some people report personal problems or discrimination because they joined an HIV vaccine study. Family or friends may worry, get upset, or assume that you have HIV. Rarely, someone has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

Most vaccines cause the body to make antibodies to prevent infection. Your body may make antibodies to HIV because you received an HIV study vaccine. Those

antibodies could cause you to test positive on some types of HIV 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 is likely to say you have HIV, even if you don't. For this reason, you should get HIV tests only at this clinic. Our tests can tell the difference between true HIV infection and a positive result caused by the study vaccine. If you have VISP, we can arrange free HIV testing for as long as you need it.

It is unlikely, but you could test antibody negative at the end of the study and then test positive sometime later, even though you don't have HIV.

Site: Modify the following paragraph if applicable. If someone believes you have HIV, you could face discrimination and other problems. In some countries, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If you have VISP, 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 VISP, and how you can avoid some of these problems.

If you become pregnant during or after the study and have VISP, the antibodies might be passed to your baby. We know that this happens with some other vaccines. The antibodies are not a danger to the baby and they go away, usually in about 6 months.

You should 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, 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 tests that can distinguish true HIV infection from VISP. 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 questions about HIV risks, such as having sex and using drugs. Also, waiting for HIV test results could make you feel anxious. You could feel worried if 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:

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 happens, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information.

Risks of genetic testing:

It is possible that genetic tests could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, genetic test results are not part of your study record, so it is almost impossible for anyone to connect them to you personally.

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

Unknown risks:

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

We do not know if getting this study vaccines will affect how you respond to any future approved HIV vaccine. Currently, no HIV vaccine has been approved for use.

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

Benefits

21. The study may not benefit you.

We do not expect the study 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 might detect health problems you don't yet know about.

When asked, most study participants say that participating in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self-esteem.

This study may help in the search for a vaccine to prevent HIV. However, if the study vaccines later become approved for sale, 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.

We list these in the Bill of Rights and Responsibilities (BRR) for HIV Research. 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. This will not affect your care at this clinic and your legal rights, but it is important for you to let us know.

Previously collected information about you will remain in the study records and will be included in the analysis of results. Your information cannot be removed from the study records.

We will ask you to come to the clinic one last time for a physical exam. We may ask to take some blood samples from you and ask you to answer some questions. If you were assigned female sex at birth, we may test you for pregnancy. Whether you come for this last visit is up to you.

Injuries

Sites: Approval from HVTN Regulatory Affairs (at vtn.core.reg@fredhutch.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 care that we can give here. For care that we cannot provide, we will explain how we will help you get care elsewhere.

If you become sick or injured in this study, the HVTN has a process to decide if this is related to the study vaccines and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

The HVTN has limited funds to pay medical costs that it determines are reasonable. If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Questions

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

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

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

An institutional review board (IRB) is an independent committee established to help protect the rights of research subjects. If you have any questions about your rights as a research participant, and/or concerns or complaints regarding this research study, contact:

- By mail:

  Study Subject Adviser

  Advarra IRB

  6100 Merriweather Dr, Suite 600

  Columbia, MD 21044

- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: #######.

US sites may include, but are not required to: You may also contact the [name of local IRB/EC] at [insert contact information].

Your permissions and signature

26. In Section 13 of this form, we told you about the skin biopsy procedure we may ask you to have done. If you agree to the skin biopsy, please write your initials or make your mark in the box below. If you do not agree to the skin biopsy, leave the box blank. You can change your mind after signing this form.

☐ I agree to the skin biopsy.

27. In Section 16 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used. You can change your mind after signing this form.

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

OR

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

OR

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

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

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

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

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

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

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

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

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

Appendix H Sample addendum to informed consent form for Part A (Groups 1-5) post termination of vaccinations

Title: A Phase 1, Open-Label Clinical Trial to Evaluate Safety, Tolerability, and Immunogenicity of Adjuvanted HIV-1 Fusion Peptide Conjugate Vaccine (VRC-HIVVCP0108-00-VP) Alone or in Prime-Boost Regimens with Adjuvanted HIV-1 Envelope Trimer 4571 (VRC-HIVRGP096-00-VP) and HIV-1 Trimer 6931 (VRC-HIVRGP0106-00-VP) Vaccines in Healthy Adults

HVTN protocol number: HVTN 303

Site: [Insert site name]

Key information

- Being in this research study is voluntary. It is your choice.
- Study injections have been stopped for all participants.
- If you stay in this study, you will be in it until one year after you completed your study injection visit.
- Procedures will still include blood draws.
- There are risks from participating.
  - Taking blood can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding.

1. Review of study information

This study started in August 2022 and planned to enroll 60 participants. All study injections were stopped on January 13, 2023 because of reactions in some participants. There were 44 participants in the study at that time and we have not enrolled any more. Part A is fully enrolled with 15 participants, all of whom completed their 1 planned study injection visit. Part B enrolled 29 participants across Groups 6-8. Group 6 participants were planned to have only 1 study injection visit. There are 10 participants enrolled in Group 6 and all completed their 1 study injection visit. Groups 7 and 8 were planned to have 3 study injection visits. No participant completed 3 study injection visits. There are 10 participants enrolled in Group 7 and 5 of them completed their second study injection visit. There are 9 participants in Group 8 and 6 of them completed their second study injection visit.

We sent you a letter to tell you that study injections had been stopped and to give you more information about the reactions participants experienced. We also asked

you to continue to come for scheduled clinic visits and blood draws so we could monitor your safety. The letter noted that study leadership and experts monitoring participant safety in this study would be discussing changes to the study to help determine the cause of the reactions.

In a more recent, second letter, we told you that the symptoms experienced by all participants who had reactions went away and these participants have not had any more problems. We noted that 7 months of safety monitoring of all participants showed no problems in any participants and so we were stopping the blood draws for safety.

In order to help determine the cause of the reactions, changes are being made to this study. We will tell you about these below. We will also ask you if you agree to stay in the study.

2. You are free to continue or leave the study.

If you choose to leave, your other care at this clinic and the benefits or rights you would normally have will not be affected. We will ask you to come to the clinic one last time for a physical exam. We may ask to take some blood samples from you and ask you to answer some questions. If you were assigned female sex at birth, we may test you for pregnancy. Whether you come for this last visit is up to you.

3. If you stay in the study, this will change:

In the consent form you signed when you joined the study, we said that if you had an unusual reaction on your skin after a study injection, we may want to do a skin biopsy. We did do a skin biopsy on 2 Part B participants who had unusual reactions after their study injections. We will not do any more skin biopsies in this study.

4. Many things described in the informed consent form you signed when you joined the study remain the same.

These include:

- The potential risks and benefits of being in the study;
- Your rights and responsibilities in the study;
- How your samples will be used;
- What we will do if we find you have a health problem;
- How we will protect your private information and who can access your study records;

- Reasons we might take you out of the study;
- What will happen if you get sick or injured during the study.
- As before, there is no cost to you for being in the study. We will give you [Site: Insert compensation] for each study visit you complete.
- In the consent form you signed when you joined the study, we told you about possible use of your samples and information in other studies. At the end of that consent form, you chose whether the HVTN could use your extra samples and information in other studies. The HVTN will continue to honor the choice you made in that consent form. You can change your mind if you want. Your decision will not affect your being in this study or have any negative consequences here.

The schedule of visits will stay the same. The procedures we do at each visit will also remain the same, except that we will not take blood from you at the visit scheduled 32 weeks after your first injection. You will still be in the study for 1 year after you completed your study injection visit.

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].

An institutional review board (IRB) is an independent committee established to help protect the rights of research subjects. If you have any questions about your rights as a research participant, and/or concerns or complaints regarding this research study, contact:

- By mail:

  Study Subject Adviser

  Advarra IRB

  6100 Merriweather Dr, Suite 600

  Columbia, MD 21044
- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: #######.

US sites may include, but are not required to: You may also contact the [name of local IRB/EC] at [insert contact information].

5. **If you agree to continue in 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 stay in it. You understand what the possible risks and benefits are.
   - You have had your questions answered and know that you can ask more.
   - You agree to stay in this study.

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

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

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

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

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

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

Appendix I Sample informed consent form for Part B (Groups 6-8)

Title: A Phase 1, Open-Label Clinical Trial to Evaluate Safety, Tolerability, and Immunogenicity of Adjuvanted HIV-1 Fusion Peptide Conjugate Vaccine (VRC-HIVVCP0108-00-VP) Alone or in Prime-Boost Regimens with Adjuvanted HIV-1 Envelope Trimer 4571 (VRC-HIVRGP096-00-VP) and HIV-1 Trimer 6931 (VRC-HIVRGP0106-00-VP) Vaccines in Healthy Adults

HVTN protocol number: HVTN 303

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.

Key information

- Being in this research study is voluntary. It is your choice.
- You are being asked to take part in this study because you are age 18-50, HIV negative and healthy.
- The purpose of the study is to see if the study vaccines are safe to give to people and do not make people too uncomfortable.
- Another purpose of the study is to see how a person's immune system responds to the study vaccines. (Your immune system protects you from infections and disease.)
- You will be in this study for up to 25 months of clinic visits.
- Procedures will include blood draws and injections of study vaccine. If you agree, we may collect white blood cells by a procedure called leukapheresis. There is also an optional procedure of a skin biopsy that we may ask you to have done. We will tell you more about these procedures later in this consent form.
- There are risks from participating.
    - The vaccines you receive have only been given to a small number of people. We do not know all the risks of the vaccines in this study. A few known risks are pain and itching at the injection site, muscle pain, swelling, skin redness, soreness, tenderness, headache, nausea, fatigue and fever.

  - Taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding.
  - Leukapheresis can cause pain, bruising, and rarely an infection. A skin biopsy can cause bleeding, pain, bruising, and scarring, and rarely an infection.
  - We will tell you more information about risks later in this consent form.

- We do not expect the study vaccines to benefit you in any way.

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test 3 HIV vaccines. HIV is the virus that causes AIDS. The study vaccines are made from two parts: a protein and an adjuvant. Adjuvants are substances that help the immune system respond better. (Your immune system protects you from disease.) In this study we will test 3 different proteins with 1 adjuvant.

Up to 70 people will take part in this study at multiple sites in the United States. 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.

The study is divided into 2 parts, Part A and Part B. Up to 20 people are currently in Part A. In Part A, participants got just 1 of the 3 study vaccines. Because there have been no safety concerns to date in Part A, we are now starting Part B. It will test the 3 study vaccines in different combinations. Up to 50 people will be in Part B. You are being invited to join Part B of the study.

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

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

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

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

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

3. These study vaccines are experimental.

The study vaccines include two parts. One part is a lab-made piece of protein that looks like a protein found on the outside of HIV. The second part is an adjuvant called Adjuplex. (An adjuvant is something that helps the immune system respond better.) From here on, we will call it "the adjuvant".

We are testing 3 different proteins in this study and each of these will be mixed with the adjuvant. We will use the term "vaccine" or "study vaccine" to refer to the combination of a protein and the adjuvant. The study vaccines are experimental HIV vaccines. That means we do not know if they will be safe to use in people, or if they will work to prevent HIV infection. These study vaccines are used only in research studies.

The 3 proteins being tested are called Fusion Peptide (FP conjugate), Trimer 4571 and Trimer 6931. They are all lab-made small pieces of proteins that look like parts of proteins found in the HIV virus. The immune system will be able to see the pieces that look like parts of HIV and learn how to recognize them. Researchers hope that the immune system will respond by making antibodies that could fight HIV. (The body makes antibodies to fight germs after it is exposed to them. The antibodies attack germs and keep them from infecting the body's cells.) Each of the 3 proteins in the study is made slightly differently to see how the immune system will respond to these different structures. The proteins were developed by the Vaccine Research Center (VRC) at the US National Institutes of Health.

The FP conjugate protein and the Trimer 6931 protein were given to people for the first time in Part A of this study. The Trimer 4571 protein mixed with a different adjuvant has been given to a small number of people in several other studies. The Trimer 4571 protein was also given to people in Part A of this study.

The Adjuplex adjuvant is being provided for this study by the VRC. The adjuvant is made of a fatty substance called lecithin and a suspending agent called carbomer mixed together in a saline solution. It has been given to 20 people in a different study and to the participants in Part A of this study

There have been no safety concerns to date in the participants who got these study vaccines in Part A of this study so we will now give them to participants in Part B.

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, dizziness, and feeling tired. Vaccines can also cause stinging, discomfort, redness, mild bruising or an infection where you got the injection. Signs of infection at the injection site include severe pain, redness, swelling, warmth or drainage. Rarely, people have side effects that limit their normal activities or make them go to the doctor.

Risks of the study vaccines:

FP conjugate vaccine and Trimer 6931 vaccine

The FP conjugate vaccine and the Trimer 6931 vaccine were given to a small number of people for the first time in Part A of this study so we do not know what all the risks may be. However, we expect they will be similar to the general risks of vaccines described above. There may be other side effects, even serious or life-threatening ones, which we do not know about yet.

Trimer 4571 vaccine

This vaccine mixed with a different adjuvant was given to people for the first time in a study called VRC 018 that ended in June 2020. The 16 participants in iteach got 3 injections at doses higher and lower than the dose we will give in this study. The injections did not cause any serious health problems. 14 people had mild pain where they got the injection and 6 people had muscle pain, which went away within 7 days. 6 people had mild itching where they got the injection that lasted between 1 and 9 days. One person had swelling after their second injection and skin redness after their third. Both of these went away within 7 days. Another person had moderate swelling and severe skin redness where they got the injection. Both of these disappeared at 8 days after the injection. 4 people had mild headaches and 3 people felt nauseous after injection. These symptoms went away within 7 days. One person had a decrease in neutrophils which is a type of white blood cell. This went away after 8 days.

As of January 2022, there are 2 other studies giving the Trimer 4571 vaccine to people. NIH-19-I-0069 will enroll 200 participants and give them a different study vaccine followed by a booster injection of a higher dose of the Trimer 4571 vaccine than we will give in this study with a different adjuvant 6 months later. As of January 5, 2022, 12 participants have gotten their booster injection. The injections have not caused serious health problems. The common side effects are mild pain at the injection site, mild headache and mild fatigue. The other study, called NETI, will give participants with HIV infection multiple injections of either placebo or lower and higher doses of Trimer 4571 than we will use in this study with a different adjuvant. As of January 1, 2022, 5 participants have received a total of 7 injections. The common side effects are mild to moderate injection site pain and tenderness, fatigue, joint stiffness and headache.

The same Trimer 4571 protein vaccine we will give to participants in Part B was given to a small number of people for the first time in Part A so, we do not know all the side effects. There may be serious or life-threatening side effects that we do not know about yet.

Risks of the Adjuplex adjuvant:

The 20 people who got a different vaccine with this adjuvant in a different study have not had serious health problems from it. Some people have had injection site

pain, soreness, tenderness and occasional fever after getting this adjuvant. Because this adjuvant with a different vaccine has only been given to a small number of people, we do not know all the side effects.

These are the side effects we know about. There may be others that we don't know about. We will tell you if we learn about new side effects that could affect your willingness to stay in the study.

Joining the study

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

   Take your time. Talk to people you trust. If you decide not to join this study or if you leave after you have joined, your other care at this clinic and the benefits or rights you would normally have will not be affected.

   You cannot be in this study while you are in another study where you get a study product. If you do not join this study, you may be able to join another study.

   If you join this study, you may not be allowed to join other HIV vaccine or HIV prevention trials now or in the future.

   During the study, you should not donate blood or tissue.

   Site: Remove item 5 if you use a separate screening consent that covers these procedures.

5. **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 tests. These tell us about the health of your kidneys and liver. We will ask you about medicines you are taking, including HIV pre-exposure prophylaxis (PrEP). We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy.

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

Site: adapt the following section per the care available at your site

6. **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 help you get care elsewhere. For health problems unrelated to the study, we will not pay for care.

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

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

   You should not become pregnant during the study because we do not know how the study vaccines could affect a developing baby. You must agree to use effective birth control from at least 3 weeks before your first study injections through 12 weeks after your last study injections. 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 join the study, here is what will happen:

8. **You will come to the clinic for scheduled visits over about 25 months.**

   The number of visits you will have depends on which group you are assigned to when you join the study. It will be between 18 and 24. We will tell you more about the groups below.

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

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

   You may have to come for more visits if you have a lab or health issue. We may contact you after the study ends (for example, to tell you about the study results). We may also contact you about other studies you may want to join.

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

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

There is also compensation for the leukapheresis study procedure that we will do on about half of all study participants and for the skin biopsy study procedure we may ask you to have done. We will tell you more about these procedures below. If you have the leukapheresis procedure, we will give you *[Site: Insert text]*. If you have the skin biopsy procedure, we will give you *[Site: Insert text]*.

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

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

10. We will give you the study vaccines on a schedule.

You will be in 1 of 3 groups shown in the table below. Which group you are in will depend on when you join the study. Each group will get different combinations of the study vaccines. The table below shows which study vaccines each group will get and at what timepoint. When we give you the study vaccines, it will always be in 2 injections. When we give you 2 study vaccines at the same time, the pharmacist will mix them together and then split the mixture into 2 syringes. We will give the injections with a needle and syringe into the muscle of both upper arms. If there is a reason we cannot give an injection into one of your arms, then we will give both injections into the other arm.

Group 6 will get injections 5 times. They will get the Trimer 4571 vaccine 2 times, the Trimer 6931 vaccine once, then a combination of the Trimer 4571 and the Trimer 6931 vaccines 2 times. Group 7 will get injections 7 times. They will get the FP conjugate vaccine 3 times, then the Trimer 4571 vaccine 2 times, then the Trimer 6931 vaccine 1 time, then a combination of the Trimer 4571 and the Trimer 6931 vaccines 1 time. Group 8 will get injections 6 times. They will get a combination of the FP conjugate vaccine and the Trimer 4571 vaccine 3 times, then the Trimer 6931 vaccine once, then a combination of the Trimer 4571 and the Trimer 6931 vaccines 2 times.

We plan to enroll 15 participants in each group, for a total of 45. We may need to enroll up to 5 more participants if we need more information on how participants are responding to the study vaccines.

You will have to wait in the clinic for at least 30 minutes after your injections to see if there are any problems. Then for that night and for 10 more days, you will use an electronic participant diary to record how you are feeling, including your highest daily temperature. We will give you a thermometer to help you do this.

We will also give you a ruler to measure any swelling at the injection site. If you are unable or unwilling to use the electronic participant diary, please talk with us about other options that may be available. We will contact you the day after your injections to ask how you are doing. Contact the clinic staff if you have any issues or concerns after getting injections.

			Time after first injection visit								
Group	**Number of participants**	**First injection visit**	**4 weeks**	**8 weeks**	**12 weeks**	**20 weeks**	**24 weeks**	**32 weeks**	**36 weeks**	**44 weeks**	**48 weeks**
6	15	200 mcg Trimer 4571 vaccine			200 mcg Trimer 4571 vaccine		200 mcg Trimer 6931 vaccine		100 mcg Trimer 4571 vaccine + 100 mcg Trimer 6931 vaccine		100 mcg Trimer 4571 vaccine + 100 mcg Trimer 6931 vaccine
7	15	200 mcg FP conjugate vaccine	200 mcg FP conjugate vaccine	200 mcg FP conjugate vaccine	200 mcg Trimer 4571 vaccine		200 mcg Trimer 4571 vaccine		200 mcg Trimer 6931 vaccine		100 mcg Trimer 4571 vaccine + 100 mcg Trimer 6931 vaccine
8	15	200 mcg FP conjugate vaccine + 200 mcg Trimer 4571 vaccine	200 mcg FP conjugate vaccine + 200 mcg Trimer 4571 vaccine	200 mcg FP conjugate vaccine + 200 mcg Trimer 4571 vaccine		200 mcg Trimer 6931 vaccine		100 mcg Trimer 4571 vaccine + 100 mcg Trimer 6931 vaccine		100 mcg Trimer 4571 vaccine + 100 mcg Trimer 6931 vaccine	

11. In addition to giving you the study vaccines, we will do the procedures shown in the following tables.

Group 6																			
			Time after first injection visit																
Procedure	**Screening visit(s)**	**First injection visit**	**1 week**	**2 weeks**	**12 weeks**	**13 weeks**	**14 weeks**	**24 weeks**	**25 weeks**	**26 weeks**	**36 weeks**	**37 weeks**	**38 weeks**	**48 weeks**	**49 weeks**	**50 weeks**	**60 weeks**	**72 weeks**	**100 weeks**
Injection		√			√			√			√			√					
Medical history	√																		
COVID-19 symptom check		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Complete physical	√																		√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Leukapheresis or larger blood draw*																√			
Pregnancy test**	√	√			√			√			√			√		√**			
HIV testing	√						√			√			√			√	√	√	√
Risk reduction counseling	√			√			√			√		√			√		√		√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:

* Leukapheresis will only be collected from participants who agreed to provide this sample type and are eligible. For participants who do not provide leukapheresis samples, a larger blood draw will be done instead.

** For persons who were assigned female sex at birth and who are capable of becoming pregnant. A negative pregnancy test is required within 72 hours before leukapheresis.

Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) or are in menopause, do not have to have pregnancy tests.

Group 7																									
			Time after first injection visit																						
Procedure	**Screening visit(s)**	**First injection visit**	**1 week**	**2 weeks**	**4 weeks**	**5 weeks**	**6 weeks**	**8 weeks**	**9 weeks**	**10 weeks**	**12 weeks**	**13 weeks**	**14 weeks**	**24 weeks**	**25 weeks**	**26 weeks**	**36 weeks**	**37 weeks**	**38 weeks**	**48 weeks**	**49 weeks**	**50 weeks**	**60 weeks**	**72 weeks**	**100 weeks**
Injection		√			√			√			√			√			√			√					
Medical history	√																								
COVID-19 symptom check		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Complete physical	√																								√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Leukapheresis or larger blood draw*																						√			
Pregnancy test**	√	√			√			√			√			√			√			√		√**			
HIV testing	√												√			√			√			√	√	√	√
Risk reduction counseling	√			√			√			√			√			√			√		√		√		√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:

* Leukapheresis will only be collected from participants who agreed to provide this sample type and are eligible. For participants who do not provide leukapheresis samples, a larger blood draw will be done instead.

** For persons who were assigned female sex at birth and who are capable of becoming pregnant. A negative pregnancy test is required within 72 hours before leukapheresis. Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) or are in menopause, do not have to have pregnancy tests.

Group 8																							
			Time after first injection visit																				
Procedure	**Screening visit(s)**	**First injection visit**	**1 week**	**2 weeks**	**4 weeks**	**5 weeks**	**6 weeks**	**8 weeks**	**9 weeks**	**10 weeks**	**20 weeks**	**21 weeks**	**22 weeks**	**32 weeks**	**33 weeks**	**34 weeks**	**36 weeks**	**40 weeks**	**44 weeks**	**45 weeks**	**46 weeks**	**56 weeks**	**68 weeks**	**96 weeks**
Injection		√			√			√			√			√					√					
Medical history	√																							
COVID-19 symptom check		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Complete physical	√																							√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Leukapheresis or larger blood draw*																					√			
Pregnancy test**	√	√			√			√			√			√					√		√**			
HIV testing	√									√			√			√					√		√	√
Risk reduction counseling	√			√			√			√			√					√		√		√		√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:

* Leukapheresis will only be collected from participants who agreed to provide this sample type and are eligible. For participants who do not provide leukapheresis samples, a larger blood draw will be done instead.

** For persons who were assigned female sex at birth and who are capable of becoming pregnant. A negative pregnancy test is required within 72 hours before leukapheresis.

Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) or are in menopause, do not have to have pregnancy tests.

When we take blood, the amount will depend on the lab tests we need to do at a certain visit. It will be some amount between 12 mL and 182 mL (about 1 tablespoon to 12 tablespoons). Your body will make new blood to replace the blood we take out.

When we do tests, we will review the results with you at your next visit, or sooner if necessary. We will tell you about any results that are important to your health.

12. **If you have an unusual reaction on your skin due to a study injection, we may want to photograph it.**

    Taking photographs may help researchers learn more about reactions to the study products. We will not take photos of your face. Your name and other identifying information will not be included on the photos. The photos will only be used to research the safety of the study products.

13. **If you have an unusual reaction on your skin to a study injection, we may want to do a skin biopsy.**

    A skin biopsy collects a small sample of skin cells so they can be examined in a lab. This may help researchers better understand your skin reaction and learn more about the study products.

    We will ask you at the end of this consent form if you agree to the skin biopsy procedure. You can change your mind at any time. It is possible that you may not be asked to have a skin biopsy, even if you agree to it.

    If we ask you to have a skin biopsy, this procedure will be done at [site: insert location].

    Sites referring out to another facility for the skin biopsy:

    - *must obtain copies of the informed consent form and any other educational materials that are available from the facility where it will be done so that they can be reviewed with potential participants during the study informed consent process.*

    - *include these sentences:* There will be another consent form for you to review and sign at the facility. As part of the consent process, you will be able to discuss the procedure in more detail with the clinician who will do the procedure. The discussion will include the location, size, depth and risks of the skin biopsy.

    Sites doing the skin biopsy in the clinic and that have their own separate ICF for the procedure:

- *must review it and any other educational materials they have about it with the potential participant during the study informed consent process.*

- *Include these sentences:* We will give you a separate consent form to review and sign. As part of the consent process, you will be able to discuss the procedure in more detail with the clinician who will do the procedure. The discussion will include the location, size, depth and risks of the skin biopsy.

Sites doing the skin biopsy in the clinic and that do not have their own separate ICF for the procedure must modify the general information below about skin biopsies to provide the details of the procedure & its risks at their clinic.

There are several different tools that can be used to do a skin biopsy. The clinician doing the procedure will examine the reaction on your skin to determine which one to use. They will tell you how the tool works and the general size of the sample they will take from your skin. They will apply a cleaning solution to your skin in the area where the biopsy will be done and numb the area with an injection of a local anesthetic. Once the area is numb, the clinician will take a small sample of skin. This procedure may require a few stitches to close the wound. A skin biopsy may cause a scar. This scar will likely fade over time. A skin biopsy typically takes about 15 minutes total, including the preparation time, dressing the wound, and instructions for at-home care.

14. We would like to collect white blood cells from about half of the participants in Part B using a procedure called leukapheresis.

The leukapheresis procedure collects more white blood cells than we can collect in an ordinary blood draw. During leukapheresis, the white blood cells are removed from the blood and the rest of the components of blood are put back into the body. Blood is made up of red cells that carry oxygen, white cells that fight infection, platelets that help form clots, and plasma, which is the fluid left over when all the cells are removed. Researchers will use white blood cell samples to look more deeply at immune responses. They will try to detect any rare cells that respond to the study vaccines, and whether these ways of making vaccines can stimulate the right kinds of cell responses. This information will guide the ways that vaccines are designed in the future. To get the number of white blood cells we need for this research, this procedure should take between 1-2 hours.

We would like to collect these samples from about half of the participants in Groups 6, 7 and 8. If more than half of participants agree to the procedure, we will collect samples from all who agree. We will ask you at the end of this consent form if you agree to the leukapheresis procedure. You can change your mind at any time. It is possible that you may not be eligible to do leukapheresis, even if you agree to the procedure. We can discuss the eligibility criteria with you now if you would like.

The leukapheresis procedure will be done about 2 weeks after your last injection visit. Participants who do not have the leukapheresis procedure will instead have a

larger blood draw of around 182 mL (about 12 tablespoons) 2 weeks after their last injection visit. Before this point in the study, the largest blood draw was around 100 mL (about 7 tablespoons), with most of them being well below that. To compare, people who donate blood in the US usually donate 500 mL (1 pint or 32 tablespoons) at a time.

Leukapheresis may not be available at every clinic participating in the study. Some clinics may send their participants to a different facility in the area to have the leukapheresis procedure done.

Site: Sites must obtain copies of the informed consent form and any other educational materials that are available from the facilities where leukapheresis will be performed, so that they can be reviewed with potential participants during the study informed consent process.

The leukapheresis procedure will be done at *[site: insert location]*. Your eligibility to have this procedure will be decided by the staff at our clinic and at the facility before you have the procedure. There will be another consent form for you to review and sign at the facility. It will provide additional details about the procedure and any risks involved.

For the procedure, a clinician will insert a sterile needle into a vein in each of your arms. The needles are attached to tubes. Your blood will go out of your body through one tube and into a machine that separates the blood and takes out the white blood cells. After the white blood cells are taken out, the rest of the blood will go back into your body through the tube going into your other arm. Sometimes the fluid lost during the procedure is replaced by a sterile salt water solution, or a solution containing a protein called albumin. This protein is normally found in human blood. An anticoagulant may be added to your blood during the procedure. Anticoagulants prevent blood from clotting.

If you notice any symptoms during leukapheresis, please let the nurse know immediately. Usually the symptoms can be reversed quickly by adding fluid or by slowing down the procedure. If there are any problems, the staff will use the appropriate medical procedures to treat you. It is normal to feel tired for up to 24 hours after having leukapheresis.

15. We will counsel you about protecting yourself from HIV.

We will ask you 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.

16. The HVTN will test your samples to see how your body, including your immune system, responds to the study vaccines.

We will send your samples (without your name or other identifying information) to labs in the United States approved by the HVTN for this study. In rare cases,

some of your samples may be sent to labs 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. This genetic testing will involve only 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.

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 how the virus is impacted by the study vaccine.

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.

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

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

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

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

What information is shared with HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with the HVTN, other researchers, or with anyone else who does not need to know your name. Your name will not be part of the information. However, some information that the HVTN shares may be personal, such as your race, ethnicity, sex, health information from the study, and HIV status. The HVTN 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.

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

Site: Check HIPAA authorization for conflicts with this section.

All of your samples and most of your study records will be labeled with a code number. Samples and study records are kept in secure locations. When you provide information in the electronic participant diary after the injection visits, that information only has your code number. Your information goes directly from the electronic participant diary into your study record.

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 vaccines(s) you received

No HIV test results are 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 can see your study records. Your records may also be reviewed by groups that 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 (FDA),
- Any regulatory agency that reviews clinical trials,
- *US sites may, but are not required to include:* [Insert name of local IRB/EC] ,
- Advarra Institutional Review Board (IRB)
- *Site:* [Insert name of local and/or national regulatory authority as appropriate]
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will keep your records private.

We cannot guarantee absolute privacy. If you 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]

Site: Include the following boxed text. You can remove the box around the text.

To help protect your privacy, we have a Certificate of Confidentiality from the US government. 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. However, we cannot withhold information from the US government because it funds this research. You can still give 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 Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

19. We may stop your injections 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 until your final scheduled clinic visit if you choose. If you stay in the study, we may take fewer samples from you. If you leave the study while you are 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 help you get care and support. 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 your HIV care. We will encourage you to stay in the study until your final scheduled clinic visit if you choose. If you stay in the study, we will take fewer samples from you.

We will stop your injections if you enroll in a different research study where you get another study product. We may stop your injections if we think that giving you more of them is not in your best interest.

20. We may take you out of the study at any time.

We may take you out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you, or
- the study is stopped for any reason.

Other Risks

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

In addition to the risks of the study vaccines and the procedures that were described above, this section describes the other risks we know about. There may

be other risks, even serious ones. We will tell you if we learn anything new that may affect your decision to stay in the study.

Risks of skin biopsy:

A skin biopsy can cause bleeding, pain, bruising, and scarring, and rarely, an infection. The injection of a local anesthetic can cause brief stinging or burning in the area of the injection.

Risks of leukapheresis:

Generally, the risks of leukapheresis include pain, bruising and rarely, infection. Rarely, albumin can cause an allergic reaction. If the leukapheresis procedure has to be stopped, it could result in the loss of up to 1 cup of blood. Your body makes new blood within 2 weeks.

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, stinging, swelling, itching, a sore, bleeding, and rarely 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:

Some people report personal problems or discrimination because they joined an HIV vaccine study. Family or friends may worry, get upset, or assume that you have HIV. Rarely, someone has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

Most vaccines cause the body to make antibodies to prevent infection. Your body may make antibodies to HIV because you received an HIV study vaccine. Those antibodies could cause you to test positive on some types of HIV 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 is likely to say you have HIV, even if you don't. For this reason, you should get HIV tests only at this clinic. Our tests can tell the difference between true HIV infection and a positive result caused by the study vaccine. If you have VISP, we can arrange free HIV testing for as long as you need it.

It is unlikely, but you could test antibody negative at the end of the study and then test positive sometime later, even though you don't have HIV.

Site: Modify the following paragraph if applicable. If someone believes you have HIV, you could face discrimination and other problems. In some countries, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If you have VISP, 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 VISP, and how you can avoid some of these problems.

If you become pregnant during or after the study and have VISP, the antibodies might be passed to your baby. We know that this happens with some other vaccines. The antibodies are not a danger to the baby and they go away, usually in about 6 months.

You should 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, 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 tests that can distinguish true HIV infection from VISP. 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 questions about HIV risks, such as having sex and using drugs. Also, waiting for HIV test results could make you feel anxious. You could feel worried if 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:

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 happens, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information.

Risks of genetic testing:

It is possible that genetic tests could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, genetic test results are not part of your study record, so it is almost impossible for anyone to connect them to you personally.

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

Unknown risks:

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

We do not know if getting this study vaccines will affect how you respond to any future approved HIV vaccine. Currently, no HIV vaccine has been approved for use.

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

Benefits

22. The study may not benefit you.

We do not expect the study vaccines to benefit you in any way. However, being in the study might still help you in some ways. The counseling that you get as part of the study may help you avoid getting HIV. The lab tests and physical exams might detect health problems you don't yet know about.

When asked, most study participants say that participating in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self-esteem.

This study may help in the search for a vaccine to prevent HIV. However, if the study vaccines later become approved for sale, there are no plans to share any money with you.

Your rights and responsibilities

23. If you join the study, you have rights and responsibilities.

We list these in the Bill of Rights and Responsibilities (BRR) for HIV Research. We will give you a copy of it.

Leaving the study

24. Tell us if you decide to leave the study.

You are free to leave the study at any time and for any reason. This will not affect your care at this clinic and your legal rights, but it is important for you to let us know.

Previously collected information about you will remain in the study records and will be included in the analysis of results. Your information cannot be removed from the study records.

We will ask you to come to the clinic one last time for a physical exam., We may ask to take some blood samples from you and ask you to answer some questions. If you were assigned female sex at birth, we may test you for pregnancy. Whether you come for this last visit is up to you.

Injuries

Sites: Approval from HVTN Regulatory Affairs (at vtn.core.reg@fredhutch.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.

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

If you become sick or injured in this study, the HVTN has a process to decide if this is related to the study vaccines and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

The HVTN has limited funds to pay medical costs that it determines are reasonable. If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Questions

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

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

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

An institutional review board (IRB) is an independent committee established to help protect the rights of research subjects. If you have any questions about your rights as a research participant, and/or concerns or complaints regarding this research study, contact:

- By mail:

  Study Subject Adviser

  Advarra IRB

  6100 Merriweather Dr, Suite 600

  Columbia, MD 21044

- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: #######.

US sites may include, but are not required to: You may also contact the [name of local IRB/EC] at [insert contact information].

Your permissions and signature

27. In Section 13 of this form, we told you about the skin biopsy procedure we may ask you to have done. If you agree to the skin biopsy, please write your initials or make your mark in the box below. If you do not agree to the skin biopsy, leave the box blank. You can change your mind after signing this form.

☐ I agree to the skin biopsy.

28. In Section 14 of this form, we told you about the procedure of leukapheresis. If you agree to leukapheresis, please write your initials or make your mark in the box below. If you do not agree to leukapheresis, leave the box blank. You can change your mind after signing this form.

☐ I agree to leukapheresis.

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

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

OR

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

OR

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

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

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

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

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

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

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

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

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

Appendix J Sample addendum to informed consent form for Part B (Groups 6-8) post termination of vaccinations

Title: A Phase 1, Open-Label Clinical Trial to Evaluate Safety, Tolerability, and Immunogenicity of Adjuvanted HIV-1 Fusion Peptide Conjugate Vaccine (VRC-HIVVCP0108-00-VP) Alone or in Prime-Boost Regimens with Adjuvanted HIV-1 Envelope Trimer 4571 (VRC-HIVRGP096-00-VP) and HIV-1 Trimer 6931 (VRC-HIVRGP0106-00-VP) Vaccines in Healthy Adults

HVTN protocol number: HVTN 303

Site: [Insert site name]

Key information

- Being in this research study is voluntary. It is your choice.
- Study injections have been stopped for all participants. We will not give any more injections.
- If you stay in this study, you will be in it until about one year after your last study injection.
- Procedures will still include blood draws. If you completed 2 injection visits earlier in this study and if you agree, we may collect white blood cells by a procedure called leukapheresis. We will tell you more about this procedure later in this consent form.
- There are risks from participating.
  - Taking blood can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding.
  - Leukapheresis can cause pain, bruising, and rarely an infection.

1. Review of study information

This study started in August 2022 and planned to enroll 60 participants. All study injections were stopped on January 13, 2023 because of reactions in some participants. There were 44 participants in the study at that time and we have not enrolled any more. Part A is fully enrolled with 15 participants, all of whom completed their 1 planned study injection visit. Part B enrolled 29 participants across Groups 6-8. Group 6 participants were planned to have only 1 study injection visit. There are 10 participants enrolled in Group 6 and all completed their 1 study injection visit. Groups 7 and 8 were planned to have 3 study injection visits. No participant completed 3 study injection visits. There are 10 participants enrolled in Group 7 and 5 of them completed their second study

injection visit. There are 9 participants in Group 8 and 6 of them completed their second study injection visit.

We sent you a letter to tell you that study injections had been stopped and to give you more information about the reactions participants experienced. We also asked you to continue to come for scheduled clinic visits and blood draws so we could monitor your safety. The letter noted that study leadership and experts monitoring participant safety in this study would be discussing changes to the study to help determine the cause of the reactions.

In a more recent, second letter, we told you that the symptoms experienced by all participants who had reactions went away and these participants have not had any more problems. We noted that 7 months of safety monitoring of all participants showed no problems in any participants and so we were stopping the future blood draws for safety.

In order to help determine the cause of the reactions, changes are being made to this study. We will tell you about these below. We will also ask you if you agree to stay in the study.

2. You are free to continue or leave the study.

If you choose to leave, your other care at this clinic and the benefits or rights you would normally have will not be affected. We will ask you to come to the clinic one last time for a physical exam., We may ask to take some blood samples from you and ask you to answer some questions. If you were assigned female sex at birth, we may test you for pregnancy. Whether you come for this last visit is up to you.

3. If you stay in the study, these things will change.

You will be in it until one year after you completed your last study injection visit.

The schedule of visits and procedures below shows the injections and visits you have already completed and some changes to future visits.

Group 6																	
			Time after first injection visit														
Procedure	**Screening visit(s)**	**First injection visit**	**1 week**	**2 weeks**	**12 weeks**	**13 weeks**	**14 weeks**	**24 weeks**	**25 weeks**	**26 weeks**	**36 weeks**	**37 weeks**	**38 weeks**	**48 weeks**	**49 weeks**	**50 weeks**	**56 weeks**
Injection		√															
Medical history	√																
COVID-19 symptom check		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Complete physical	√																√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√			√	√
Pregnancy test**	√	√															
HIV testing	√						√			√			√			√	√
Risk reduction counseling	√			√			√			√		√			√		√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:

** For persons who were assigned female sex at birth and who are capable of becoming pregnant.

Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) or are in menopause, do not have to have pregnancy tests.

Group 7																							
			Time after first injection visit																				
Procedure	**Screening visit(s)**	**First injection visit**	**1 week**	**2 weeks**	**4 weeks**	**5 weeks**	**6 weeks**	**8 weeks**	**9 weeks**	**10 weeks**	**12 weeks**	**13 weeks**	**14 weeks**	**24 weeks**	**25 weeks**	**26 weeks**	**36 weeks**	**37 weeks**	**38 weeks**	**48 weeks**	**49 weeks**	**50 weeks**	**56 weeks**
Injection		√			√																		
Medical history	√																						
COVID-19 symptom check		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Complete physical	√																						√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√
Leukapheresis or larger blood draw*																					√		
Pregnancy test**	√	√			√																√**		
HIV testing	√												√			√			√			√	√
Risk reduction counseling	√			√			√			√			√			√			√		√		√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:

* Leukapheresis will only be collected from participants who completed 2 injection visits and who agreed to provide this sample type and are eligible. For participants who do not provide leukapheresis samples, a larger blood draw will be done instead.

** For persons who were assigned female sex at birth and who are capable of becoming pregnant. A negative pregnancy test is required within 72 hours before leukapheresis. Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) or are in menopause, do not have to have pregnancy tests.

Group 8																					
			Time after first injection visit																		
Procedure	**Screening visit(s)**	**First injection visit**	**1 week**	**2 weeks**	**4 weeks**	**5 weeks**	**6 weeks**	**8 weeks**	**9 weeks**	**10 weeks**	**20 weeks**	**21 weeks**	**22 weeks**	**32 weeks**	**33 weeks**	**34 weeks**	**36 weeks**	**40 weeks**	**44 weeks**	**45 weeks**	**46 weeks**	**56 weeks**
Injection		√			√																	
Medical history	√																					
COVID-19 symptom check		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Complete physical	√																					√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√				√	√
Leukapheresis or larger blood draw*																					√	
Pregnancy test**	√	√			√																√**	
HIV testing	√									√			√			√					√	√
Risk reduction counseling	√			√			√			√			√					√		√		√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:

* Leukapheresis will only be collected from participants who completed 2 injection visits and who agreed to provide this sample type and are eligible. For participants who do not provide leukapheresis samples, a larger blood draw will be done instead.

** For persons who were assigned female sex at birth and who are capable of becoming pregnant. A negative pregnancy test is required within 72 hours before leukapheresis.

Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) or are in menopause, do not have to have pregnancy tests.

The amount of blood we will take has changed. It will be some amount between 7 mL and 225 mL (about 1 tablespoon to 15 tablespoons).

In the consent form you signed when you joined the study, we said that if you had an unusual reaction on your skin after a study injection, we may want to do a skin biopsy. We did do a skin biopsy on 2 Part B participants who had unusual reactions after their study injections. We will not do any more skin biopsies in this study.

4. **If you are in Group 7 or 8 and completed 2 injection visits earlier in the study, we would like to collect white blood cells from you using a procedure called leukapheresis.**

   The leukapheresis procedure collects more white blood cells than we can collect in an ordinary blood draw. During leukapheresis, the white blood cells are removed from the blood and the rest of the components of blood are put back into the body. Blood is made up of red cells that carry oxygen, white cells that fight infection, platelets that help form clots, and plasma, which is the fluid left over when all the cells are removed. Researchers will use white blood cell samples to look more deeply at immune responses. They will try to detect any rare cells that respond to the study vaccines, and whether these ways of making vaccines can stimulate the right kinds of cell responses. This information will guide the ways that vaccines are designed in the future. To get the number of white blood cells we need for this research, this procedure should take between 1-2 hours.

   We will ask you at the end of this consent form if you agree to the leukapheresis procedure. You can change your mind at any time. It is possible that you may not be eligible to do leukapheresis, even if you agree to the procedure. We can discuss the eligibility criteria with you now if you would like.

   The leukapheresis procedure will be done about 42-44 weeks after your last injection visit. Participants who do not have the leukapheresis procedure will instead have a larger blood draw of around 200 mL (about 15 tablespoons) 42-44 weeks after their last injection visit. To compare, people who donate blood in the US usually donate 500 mL (1 pint or 32 tablespoons) at a time.

   Some sites may send their participants to a different facility in the area to have the leukapheresis procedure done.

   Site: Sites must obtain copies of the informed consent form and any other educational materials that are available from the facilities where leukapheresis will be performed, so that they can be reviewed with potential participants during the study informed consent process.

   The leukapheresis procedure will be done at *[site: insert location]*. Your eligibility to have this procedure will be decided by the staff at our clinic and at the facility before you have the procedure. There will be another consent form for

you to review and sign at the facility. It will provide additional details about the procedure and any risks involved.

For the procedure, a clinician will insert a sterile needle into a vein in each of your arms. The needles are attached to tubes. Your blood will go out of your body through one tube and into a machine that separates the blood and takes out the white blood cells. After the white blood cells are taken out, the rest of the blood will go back into your body through the tube going into your other arm. Sometimes the fluid lost during the procedure is replaced by a sterile salt water solution, or a solution containing a protein called albumin. This protein is normally found in human blood. An anticoagulant may be added to your blood during the procedure. Anticoagulants prevent blood from clotting.

If you notice any symptoms during leukapheresis, please let the nurse know immediately. Usually, the symptoms can be reversed quickly by adding fluid or by slowing down the procedure. If there are any problems, the staff will use the appropriate medical procedures to treat you. It is normal to feel tired for up to 24 hours after having leukapheresis.

5. **Many things described in the informed consent form you signed when you joined the study remain the same.**

   These include:

   - The potential risks and benefits of being in the study;
   - Your rights and responsibilities in the study;
   - How your samples will be used;
   - What we will do if we find you have a health problem;
   - How we will protect your private information and who can access your study records;
   - Reasons we might take you out of the study;
   - What will happen if you get sick or injured during the study.
   - As before, there is no cost to you for being in the study. We will give you *[Site: Insert compensation]* for each study visit you complete. If you have the leukapheresis procedure, we will give you *[Site: Insert text]*.
   - In the consent form you signed when you joined the study, we told you about possible use of your samples and information in other studies. At the end of that consent form, you chose whether the HVTN could use your extra samples and information in other studies. The HVTN will continue to honor the choice you made in that consent form. You can change your mind if you want. Your

decision will not affect your being in this study or have any negative consequences here.

6. **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].

An institutional review board (IRB) is an independent committee established to help protect the rights of research subjects. If you have any questions about your rights as a research participant, and/or concerns or complaints regarding this research study, contact:

- By mail:

  Study Subject Adviser

  Advarra IRB

  6100 Merriweather Dr, Suite 600

  Columbia, MD 21044

- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: #######.

US sites may include, but are not required to: You may also contact the [name of local IRB/EC] at [insert contact information].

7. **In Section 3 of this form, we told you about the procedure of leukapheresis. If you agree to leukapheresis, please write your initials or make your mark in the box below. If you do not agree to leukapheresis, leave the box blank. You can change your mind after signing this form.**

☐ I agree to leukapheresis.

8. If you agree to continue in 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 stay in it. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to stay in this study.

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

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

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

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

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

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

Appendix K Low risk guidelines for US

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

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

A. For US volunteers NOT on stable Pre-exposure prophylaxis (PrEP)

1. SEXUAL BEHAVIORS

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

A. Have oral, vaginal or anal intercourse with an HIV-infected partner, or a partner who uses injection drugs
B. 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 a person born male with partner(s) born male (MSM) 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), OR

- 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 <u>not</u>:

- Inject drugs or other substances without a prescription
- Use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment, rendered the participant at greater than low risk for acquiring HIV infection.

  The investigator's judgment should consider local epidemiologic information about HIV prevalence in the area and community networks.

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

Acquired an STI (ie, new infection) in the **last 12 months**:

- Syphilis
- Gonorrhea
- Non-gonococcal urethritis
- Herpes Simplex Virus type 2 (HSV2)
- Chlamydia
- Pelvic inflammatory disease (PID)
- Trichomonas
- Mucopurulent cervicitis

- Epididymitis
- Proctitis
- Lymphogranuloma venereum
- Chancroid
- Hepatitis B

B. For US volunteers on Pre-exposure prophylaxis (PrEP)

1. PrEP ASSESSMENT

- Reports 6 months (180 days) or more of protective daily oral PrEP use
  - For persons born male at birth who have sex with persons born male at birth: reports equal to or greater than 70% when asked the following: "*Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications?"*
  - For people with a vagina having intra-vaginal intercourse: reports equal to or greater than 90% when asked the following: "*Thinking about the past 4 weeks, how many days per week were you able to take all your PrEP medications?"*
- Commits to maintaining protective PrEP use throughout trial

Note that some anti-retroviral medications at certain dose levels may potentially interfere with pseudoneutralization immunogenicity assays and could be exclusionary. Sites should consult the PSRT regarding eligible medications prior to enrolling persons on PrEP. The PrEP medication names and doses taken during screening and the trial should be recorded.

2. SEXUAL BEHAVIORS

Persons stably taking PrEP as described above for 6 months or longer are considered low risk of HIV infection, regardless of any sexual behavior that might otherwise be associated with high risk of HIV exposure.

3. NON-SEXUAL BEHAVIORS

In the **last 12 months** did 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.

Appendix L Approved birth control methods

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

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

You must agree to use effective birth control from at least 3 weeks before your first injection until 12 weeks after your last study injection.

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

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Male or female condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for one year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your “tubes tied”) or confirmed successful placement of a product that blocks the fallopian tubes;
- You are having sex only with a partner(s) assigned female sex at birth;
- You only have oral sex; or,
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, male and female condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

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

Appendix M Adverse events of special interest

AEs of special interest (AESI) for this protocol include but are not limited to potential immune-mediated diseases; representative examples of AESI are listed below. Updates to AESI will be provided as an appendix to the *HVTN 303 Study Specific Procedures.*

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

Appendix N Protocol team

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Appendix O 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 303 are described below.

Protocol history and modifications

Date: September 21, 2023

Protocol version: Version 2.0

Protocol modification: Full protocol amendment 1

Item 1 Added following title page: Boxed text summary of protocol version 2.0 rationale and design

Item 2 Updated in Section 1, *Executive summary*; Section 2, *Introduction;* Section 3, *Objectives and endpoints;* Section 5.1.3, *Leukapheresis Eligibility Criteria;* Section 6, *Statistical considerations;* Section 7, *Study product preparation, storage, and administration;* Section 8; *Clinical procedures;* New Appendix B, *Follow-up schedule of procedures for Part A (Groups 1-5) post termination of vaccinations;* New Appendix D, *Follow-up schedule of procedures for Part B (Tables for Group 6, Group 7, and Group 8) post termination of vaccinations;* New Appendix F, *Follow up visit windows post termination of vaccination;* New Appendix H, *Sample addendum to informed consent form for Part A (Group 1-5) post termination of vaccinations* and New Appendix J, *Sample addendum to informed consent form for Part B (Groups 6-8) post termination of vaccinations*: Language and content related to the updated study design and visit schedule due to vaccination termination

Item 3 Updated per Protocol Version 1.0, Clarification Memo 1, dated May 19, 2022

Item 4 Updated per Protocol Version 1.0, Letter of Amendment 1, dated July 12, 2022

Item 5 Updated per Protocol Version 1.0, Clarification Memo 2, dated July 29, 2022

Item 6 Updated per Protocol Version 1.0, Clarification Memo 3, dated August 16, 2022

Item 7 Updated per Protocol Version 1.0, Letter of Amendment 2, dated September 28, 2022

Item 8 Updated per Protocol Version 1.0, Letter of Amendment 3, dated December 1, 2022

Item 9 Updated per Protocol Version 1.0, Letter of Amendment 4, dated July 31, 2023

Item 10 Corrected throughout the protocol: Minor errors in grammar, typography, formatting

Item 11 Updated throughout the protocol: Section numbering and cross-references

Date: July 31, 2023

Protocol version: Version 1.0

Protocol modification: Letter of Amendment 4

Item 1 Removed in Appendix B, *Schedule of procedures for Part B (Tables for Group 6, Group 7 and Group 8)*: Blood collection for safety labs for any future visits.

Date: December 1, 2022

Protocol version: Version 1.0

Protocol modification: Letter of Amendment 3

Item 1 Revised in Section 8.3, *Reactogenicity Assessments*; Appendix A, *Schedule of procedures for Part A (Groups 1-5)*; Appendix B, *Schedule of procedures for Part B (Tables for Group 6, Group 7 and Group 8);* Appendix D, *Sample informed consent form for Part A (Groups 1-5)* and Appendix F, *Sample informed consent form for Part B (Groups 6-8)*: reactogenicity assessment period for the study

Date: September 28, 2022

Protocol version: Version 1.0

Protocol modification: Letter of Amendment 2

Item 1 Added in Section 9.1, *Adverse events*; Section 9.2.1, *Expedited reporting of adverse events to DAIDS* and *Acronyms and abbreviations:* language in response to comment received from FDA dated July 19, 2022

Item 2 Deleted in Section 1.5, *Study plan and schema table*: language from Table 1-1 footnotes

Item 3 Added in Section 3.3, *Exploratory objectives*: new objectives

Item 4 Added in Section 5.1.2, *Exclusion criteria*; and Section 5.2.1, *Delaying vaccinations for a participant*: considerations for timing of receipt of Monkeypox vaccines

Item 5 Added in Section 2.11.1, *Risks of the Vaccines;* New Section 2.11.3*, Other risks;* Section 8.3, *Reactogenicity assessments;* Appendix A, *Schedule of procedures for Part A (Groups 1-5);* Appendix B, *Schedules of procedures for Part B (Tables for Group 6, Group 7, and Group 8)*; Appendix D, *Sample informed consent form for Part A (Groups 1-5)* and Appendix E, *Sample informed consent form for Part B (Groups 6-8)*: language to implement a system for optional skin biopsies

Date: August 16, 2022

Protocol version: Version 1.0

Protocol modification: Clarification Memo 3

Item 1 Added in Section 9.6, *Total blood volume*: alternate laboratory specimen tube types allowed for research samples upon HVTN Laboratory Center approval

Date: July 29, 2022

Protocol version: Version 1.00

Protocol modification: Clarification Memo 2

Item 1 Revised in Section 8.3, *Reactogenicity Assessments*; Appendix A, *Schedule of procedures for Part A (Groups 1-5)* and *Appendix B, Schedule of procedures for Part B (Tables for Group 6, Group 7 and Group 8)*: participant contact on the day after vaccinations.

Item 2 Corrected in Section 8.3, *Reactogenicity Assessments*: title referring to Appendix A and Appendix B.

Date: July 12, 2022

Protocol version: Version 1.0

Protocol modification: Letter of Amendment 1

Item 1 Updated in Appendix C, *Visit Windows*: upper and lower allowable and target window dates for Part B (Groups 7-8)

Item 2 Updated in Appendix B, *Schedules of procedures for Part B (Tables for Group 6, Group 7 and Group 8)* and in Appendix E, *Sample informed consent form for Part B (Groups 6-8)*: footnotes related to pregnancy test requirement before leukapheresis

Item 3 Added in Appendix B, *Schedule of procedures for Part B (Tables for Group 6, Group 7 and Group 8)*: blood collection for CBC/Differential and ALT/Creatinine at visit # 118 in the table for Group 7

Item 4 Deleted in Appendix A, *Schedule of procedures for Part A (Groups 1-5)* and Appendix B, *Schedule of procedures for Part B (Tables for Group 6, Group 7 and Group 8)*: plasma collection

Item 5 Added in Section 8.2, *Definition of Study Day and Study Visit*: reference to Study Specific procedures to describe remote visits

Item 6 Updated in Appendix F, *Low risk guidelines for US*: PrEP assessment guidelines for US volunteers of PrEP

Item 7 Updated in Appendix I, *Protocol team*: names of team members

Date: May 19, 2022

Protocol version: Version 1.00

Protocol modification: Clarification Memo 1

Item 1 Added in Appendix A, Schedule of procedures for Part A (Groups 1-5) and Appendix B, Schedule of procedures for Part B (Tables for Group 6, Group 7 and Group 8): blood collection for Hepatitis B and Hepatitis C testing at the screening visit (Visit #01 fo Part A groups and visit #100 for Part B groups)

Item 2 Updated in Appendix A, Schedule of procedures for Part A (Groups 1-5) and Appendix B, Schedule of procedures for Part B (Tables for Group 6, Group 7 and Group 8): footnote #8 related to the pregnancy testing

Date: April 6, 2022

Protocol version: 1.0

Protocol modification: Not applicable

Original protocol