HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 300

A first-in-human Phase 1 clinical trial to evaluate the safety and immunogenicity of stabilized CH505 TF chTrimer in healthy, HIV-uninfected adult participants

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CLINICAL TRIAL SPONSORED BY

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Protocol Signature Page

A first-in-human Phase 1 clinical trial to evaluate the safety and immunogenicity of stabilized CH505 TF chTrimer in healthy, HIV-uninfected adult participants

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (U.S.) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (eg, U.S. National Institutes of Health, Division of AIDS) and institutional policies

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

DAIDS Protocol Number: HVTN 300

DAIDS Protocol Version: Version 2.0

Protocol Date: September 26, 2022

Acronyms and abbreviations

AAHI	Access to Advanced Health Institute
Ab	antibody
AESI	adverse event of special interest
AF	aqueous formulation
AoU	assessment of understanding
ALT	alanine transaminase
BAMA	binding antibody multiplex assay
BCR	B cell receptor
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
bnAb	broadly neutralizing antibody
cGMP	current Good Manufacturing Practice
CRS	clinical research site
DAIDS	Division of AIDS
EC	ethics committee
EIA	enzyme immunoassay
FDA	(US) Food and Drug Administration
GCP	Good Clinical Practice
HLA	human leukocyte antigen
IB	Investigator's Brochure
ICS	intracellular cytokine staining
IM	intramuscular
IND	investigational new drug (application)
IRB	institutional review board
IRM	immune response modifier
KI	knock-in
LABA	long-acting beta agonist
MO	medical officer
nAb	neutralizing antibody
NSAID	non-steroidal anti-inflammatory drug
PCR	polymerase chain reaction
PI	principal investigator
PSRT	Protocol Safety Review Team
RE	regulatory entity
SAE	serious adverse event
SAP	statistical analysis plan
SDMC	statistics and data management center

SICF	sample informed consent form
SMB	Safety Monitoring Board
SSP	Study Specific Procedures
SUSAR	suspected unexpected serious adverse reaction
TB	tuberculosis
TBS	Tris-NaCl buffer
TLR	toll-like receptor
UCA	unmutated common ancestor
ULN	upper limit of normal
USP	United States pharmacopeia
VISP	vaccine-induced seropositivity

Contents

1 Executive summary

1.1 Title

A first-in-human Phase 1 clinical trial to evaluate the safety and immunogenicity of stabilized CH505 TF chTrimer in healthy, HIV-uninfected adult participants

1.2 Design

This is an open-label Phase 1 study to evaluate the safety and immunogenicity of CH505 TF chTrimer admixed with 3M-052-AF + Alum or 3M-052-AF alone. The primary hypothesis is that the CH505 TF chTrimer vaccine will expand B cell precursor lineages capable of ultimately producing autologous and heterologous Tier 2 broadly neutralizing antibodies (bnAbs).

1.3 Study products

- **Vaccine product:** CH505 TF chTrimer: a stabilized chimeric SOSIP Env trimer protein with the N-terminal sequence of CH505 TF gp120 Env transplanted into the BG505 SOSIP sequence. To be administered as 300 mcg admixed with 3M-052-AF ± Alum adjuvant.

- **Adjuvant 1:** 3M-052-AF is an aqueous formulation (AF) of the small molecule imidazoquinoline, that works as a toll-like receptor (TLR)7/8 agonist. To be administered as 5 mcg admixed with CH505 TF chTrimer, with or without Alum

- **Adjuvant 2:** Aluminum hydroxide suspension (Alum) to be administered as 500 mcg (aluminum content) admixed with 3M-052-AF and CH505 TF chTrimer.

- **Vaccine/adjuvant combination:** administered as two separate intramuscular (IM) injections into the deltoid muscle of each arm.

- **Tris-NaCl buffer** (TBS): diluent for Part A

- **Sodium Chloride for injection, 0.9% Unites States pharmacopeia (USP):** diluent for Part B

1.4 Study population

Healthy adults ages 18 through 55 years.

1.5 Study plan and schema table

Participants will receive CH505 TF chTrimer at doses of 300 mcg, administered via intramuscular injections into the deltoid muscle. Participants will be evaluated for safety and immune responses through blood collection at specified timepoints throughout the study. The study schema is below:

Table 1-1 Schema

Part A									
Study arms	**N**	**Protein antigen**	**Adjuvant**	**Route**	**Month 0 (Day 0)**	**Month 2 (Day 56)**	**Month 4 (Day 112)**	**Month 8 (Day 224)**	**Month 12 (Day 364)**
Group 1	12 *	CH505 TF chTrimer (300 mcg)	3M-052-AF (5 mcg) + Alum (500 mcg)	IM	✓	✓	✓	✓	✓
Part B									
Study arms	**N**	**Protein antigen**	**Adjuvant**	**Route**	**Month 0 (Day 0)**	**Month 2 (Day 56)**	**Month 4 (Day 112)**	**Month 8 (Day 224)**	**Month 12 (Day 364)**
Group 2	12	CH505 TF chTrimer (300 mcg)	3M-052-AF (3 mcg)	IM	✓	✓	✓	✓	✓
Group 3	12	CH505 TF chTrimer (300 mcg)	3M-052-AF (3 mcg) + Alum (500 mcg)	IM	✓	✓	✓	✓	✓
Group 4	12	CH505 TF chTrimer (300 mcg)	3M-052-AF (5 mcg)	IM	✓	✓	✓	✓	✓
Total	48								

Notes:

*In Part A, up to 18 participants could have been enrolled, if needed, to have at least 12 participants contribute to the immunogenicity analyses.

Study product is 1 mL in volume injected as half of the total volume (0.5 mL) intramuscularly into each deltoid muscle (both left and right) by needle and syringe.

Part A: Enrollment was restricted to one participant per day for the first five participants and enrollment was paused after the first five participants are enrolled. The Protocol Safety Review Team (PSRT) reviewed cumulative safety information for all participants recorded through the visit scheduled 2 weeks post first vaccination for the first five participants and determined it was safe to proceed with full enrollment.

There was a pre-planned safety review after the 5th participant received their third vaccination. No participants received the 4th and 5th vaccination until PSRT evaluation of cumulative safety data 2 weeks following the Month 4 (3rd) study injection for the first five participants enrolled in the study, after it was determined it was safe to proceed with the remaining vaccinations.

The protocol team convened after the 12th participant received their third vaccination to determine if further participants should be enrolled. Specific scenarios which could have necessitated enrollment of additional participants in order to prevent loss of statistical power of the study include (but are not limited to) the following: loss-of-participants due to moving, withdrawal of consent, inability to complete fine needle aspiration, or variations in the clinical care due to unpredictable events. Because of the early discontinuation of a participant due to a panic attack after vaccination 1, an additional participant was enrolled as a replacement, for a total of 13 enrolled in Part A. No additional participants were enrolled beyond that.

Part B: Enrollment in Part B will be stepwise by group. Enrollment in Group 2 will be restricted to one participant per day for the first five participants and then enrollment will be held. The Protocol Safety Review Team (PSRT) will review cumulative safety information recorded through the visit scheduled 2 weeks post-first vaccination for the first five participants and will determine whether it is safe to proceed with full enrollment in Group 2, then Group 3 and then Group 4.

Participants will be followed for safety through the date of a contact to assess for adverse events of special interest (AESI) 12 months after the last vaccination received.

1.6 Duration per participant

Up to 24 months. Participants will have up to 18 months of scheduled clinic visits. There will be a contact for AESI assessment at 12 months after their final vaccination.

1.7 Estimated total study duration

Anticipate up to around 48 months if the study enrolls a total of 48 participants, and up to 56 months if the study enrolls more than 48 participants (eg, if

participants dropout at a rate higher than anticipated, or miss key visits due to unforeseen circumstances). The estimate of the study duration includes enrollment, planned safety holds, enrollment of additional participants beyond 48, if this occurs, and follow-up.

1.8 Study sites

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

2 Introduction

One of the major obstacles to developing an efficacious preventive HIV-1 vaccine is the challenge of inducing broadly neutralizing antibodies (bnAbs). The approach in this study involves recreating the Env immunogens that induced a CD4 binding site (CD4bs) bnAb in an HIV-infected individual by making sequential recombinant Envs from the HIV strain that infected the individual and using these Envs for vaccination (1, 2). A candidate vaccine could use transmitted/founder virus envelopes to, at first, stimulate the beginning stages of a bnAb lineage, and subsequently boost with evolved Env variants to recapitulate the high level of somatic mutation needed for affinity maturation and bnAb activity. The B-cell lineage vaccine strategy thus includes designing immunogens based on unmutated ancestors as well as intermediate ancestors of known bnAb lineages. The goal of such a strategy is to selectively drive desired bnAb lineages by giving a survival advantage to specific bnAb clones that are typically subdominant in HIV-infected persons.

To this end, Haynes et al have defined the natural sequence of Envs that induced bnAb lineages in select HIV-infected individuals who make bnAbs. From an HIV-infected African individual designated CH505, sequential Envs and bnAbs were isolated over time and the co-evolution of the CH103 CD4bs bnAb lineage was mapped (1). Among CH505 Env mutants, candidate Env antigens were selected for optimal affinity with different steps of the CH103 lineage. The CH505 TF (transmitter-founder) Env was found to react optimally with the unmutated common ancestor (UCA) of the CH103 lineage.

This study will test a next generation immunogen in the CH505 TF family, a stabilized chimeric SOSIP trimer (CH505 TF chTrimer) that elicited neutralizing antibody (nAb) and B cell responses in preclinical testing superior to the CH505 TF monomer currently being tested in HVTN 115 (B Haynes, personal communication). Specifically, when combined with 3M-052-AF 5 mcg in alum, the CH505 TF chTrimer induced tier 2 autologous CH505 neutralizing antibodies with antibodies targeted to both the CD4 binding site and to the V3-glycan (base of the V3 loop) bnAb site. Structural studies of the V3-glycan antibodies suggested they were similar to PGT124 type of V3-glycan bnAb precursors. In addition, this study will help determine if the preclinical metrics used for this immunogen should be applied to future HIV vaccine candidates. Specifically, if CH505 TF chTrimer elicits a high frequency of CD4bs-directed B cells, elicits high binding and autologous tier 2 neutralization titers, and/or expands pools of B cells targeting either the CD4 binding site and the base of the V3 loop bnAb site, this will suggest that the preclinical testing models are correct and can be applied more generally across the HIV vaccine development pipeline.

2.1 Rationale for evaluation of CH505 TF chTrimer

The stabilized chimeric SOSIP envelope (Env) trimer protein is derived from the CH505 TF protein sequence. This immunogen, hereafter referred to as CH505 TF chTrimer, is a new generation of CH505 immunogen designed to elicit the CH103-class of CD4bs bnAbs. This class of bnAbs is an excellent target for vaccine development because of the lower degree of somatic hypermutation in the CH103 bnAb lineage (1) and the lower number of improbable mutations required to achieve neutralization breadth (3). Moreover, when formulated in the TLR7/8 agonist 3M-052-AF, CH505 TF chTrimer also induced autologous nAbs to other epitopes in rhesus macaques, including the V3-glycan bnAb site similar to PGT 124 bnAbs. This study follows on the HVTN 115 trial that is testing the ability of CH505 TF gp120 Env to initiate the CH103-type of bnAb lineage in vaccinees. Preliminary data from HVTN 115 Part A showed that most vaccinees expanded populations of B cells that bound to the CH505 TF Env protein but not to a variant at amino acid position 371 that eliminates the binding of the CH103 UCA (unpublished data), suggesting that the correct lineage may have been engaged by the CH505 TF gp120, as demonstrated in non-human primates (4).

CH505 TF chTrimer was developed using BG505 SOSIP as the starting point. The portion of CH505 TF gp120 Env N-terminal to the α5 helix was transplanted onto the BG505 SOSIP gp41 sequence, resulting in a chimeric protein that formed trimers. CH505 TF chTrimer is antigenic for V2 apex, base of the V3 loop bnAb site CD4bs, and gp120-gp41 interface bnAbs, and lacks binding to non-neutralizing antibodies against the C1, V2, co-receptor binding site, and V3 regions. The addition of E64K and A316W mutations eliminated antibody recognition of V3 and the co-receptor binding site. CH505 TF chTrimer protein bound to the CH103 UCA at Kd = 171 nM apparent affinity (5).

Binding affinity of CH103 lineage members to CH505 TF chTrimer correlated with the neutralization titer of the same antibodies against CH505.TF pseudovirus.

Preclinical data: rabbits

Rabbits immunized six times, 4 weeks apart, with unstabilized (E64 / A316) or stabilized (E64K / A316W) CH505 TF chTrimer demonstrated the utility of the stabilizing mutations. No rabbits immunized with unstabilized CH505 TF chTrimer developed autologous tier 2 neutralizing antibodies. Critically, 6 of 8 (75%) immunized with stabilized CH505 TF chTrimer neutralized autologous tier 2 CH505.TF pseudovirus (6). Two rabbits developed autologous neutralizing antibodies after only two immunizations. Importantly, elicited antibodies were sensitive to mutation of both the CD4bs and to the N160 glycosylation site suggesting that if the evolution of these B cell lineages follows the same course as in natural human infection, this immunogen may initiate separate B cell lineages capable of giving rise to different bnAbs. The rabbit sera from these studies were tested against a panel of heterologous tier 2 viruses showing that two rabbits

developed heterologous tier 2 neutralizing antibodies; one rabbit serum neutralized 11 of 12 isolates. These antibodies from rabbit S402 mapped to the CD4bs and base of the V3 loop site, again suggesting that induction of multiple bnAbs is an important goal of a vaccination strategy (6).

Preclinical data: rhesus macaques

Immunogenicity of CH505 TF chTrimer was evaluated in a series of rhesus macaque studies designed to evaluate different adjuvants and different doses of CH505 TF chTrimer.

3M-052-SE Improves Tier 2 neutralizing antibodies in rhesus macaques: Eight rhesus macaques were immunized with CH505 TF chTrimer in combination with either GLA-SE (NHP 133) or 3M-052-SE (NHP 125) as an adjuvant. All macaques immunized using the 3M-052-SE formulation generated autologous tier 2 neutralizing antibodies after 4 immunizations, while only 3 of 4 macaques in the GLA-SE group developed autologous tier 2 neutralizing antibodies and only after 6 immunizations. After six immunizations, antibody titers were higher in the 3M-052 group (Mann-Whitney, $p < 0.05$) (Figure 2-1).

After five immunizations, 3 of 4 macaques immunized with CH505 TF chTrimer formulated with 3M-052-SE weakly neutralized 4-6 heterologous tier 2 pseudoviruses in the HIV pseudovirus global panel (7), and the sixth immunization increased heterologous neutralization breadth in two macaques.

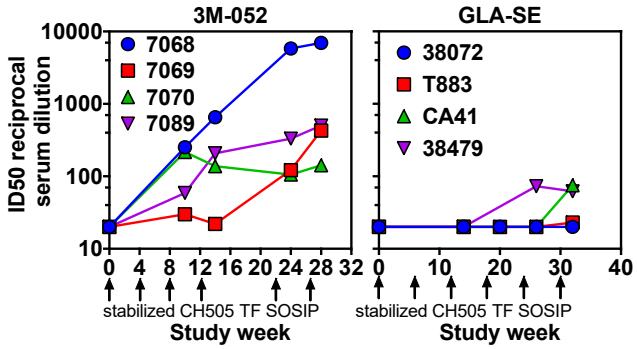

Figure 2-1 CH505 TF chTrimer induced autologous tier 2 neutralizing antibodies in rhesus macaques. Each line represents the ID50 for an individual macaque. Arrows indicate immunization timepoints. Titers against murine leukemia virus were <20.

3M-052-SE + CH505 TF chTrimer Generates Multiple bnAb lineages: The autologous neutralizing antibodies were mapped using CH505.TF pseudoviruses with knockout mutations in bnAb sites. Removal of the V1V2 glycan site reduced or knocked out neutralization in several macaques, a phenomenon also seen in CH103 VH knock-in mice and in other macaque studies (6). One macaque showed an increase in neutralization with the removal of glycans that block access

to the CD4bs which is an indication of the presence of CD4bs antibodies. Neutralization of CH505.TF was also reduced by the introduction of a N280D mutation within the CD4bs. In 3 of 4 macaque sera, neutralization was reduced by N160 virus Env mutations. Thus, CH505 TF chTrimer induced both CD4bs and N160 dependent (indicating binding at or near the Env apex glycan supersite) tier 2 neutralizing antibodies in macaques (6).

Tier 2 neutralizing antibodies in rhesus macaques: An alternative aqueous formulation (AF) of the 3M-052 adjuvant similar to the proposed product in this trial was evaluated with CH505 TF chTrimer in rhesus macaques (in study NHP 161). Across three different doses of CH505 TF chTrimer (33, 100 and 300 mcg), this regimen elicited tier 2 autologous neutralizing antibodies in 16 of 16 immunized animals (Figure 2-2). Figure 2-2 also provides a comparison of the 300 mcg CH505 TF chTrimer dose with 30 mcg 3M-052-SE (Group 2 in NHP 125).

	NHP 161																NHP 125			
	Group 1				Group 2				Group 3				Group 4				Group 2			
Virus Name	7349	7358	7359	7365	7348	7350	7354	7360	7355	7361	7357	7351	7338	7340	7352	7364	7068	7069	7070	7089
CH0505.w4.3	5583	4880	2784	2863	32088	9216	16355	8366	26621	43740	29989	4435	1678	13886	4606	14952	26412	3779	1719	36374
CH0505TF	162	51	185	49	122	112	57	168	125	396	1247	144	55	176	170	972	5833	122	106	331
CH0505TF.gly4	4381	52	747	9985	602	189	51	39388	6172	43740	43740	757	658	97	145	2951	6584	381	96	43740

Figure 2-2 NHP 125 and NHP 161 Plasma Neutralization Post 5th Immunization
NHP 161 Group 1: 33 mcg in 5 mcg 3M-052-AF + 500 mcg Alum
NHP 161 Group 2: 100 mcg in 5 mcg 3M-052-AF + 500 mcg Alum
NHP 161 Group 3: 300 mcg in 5 mcg 3M-052-AF + 500 mcg Alum
NHP 161 Group 4: 300 mcg in 5 mcg 3M-052-SE
NHP 125 Group 2: 300 mcg in 30 mcg 3M-052-SE

Yellow: ID50>20; Orange: ID50>200; Red: ID50>1000

Structural studies of antibodies induced in NHP 125 showed antibodies binding either to the CD4 binding site or to the V3-glycan bnAb site, the latter of which are similar to human PGT124 bnAbs in angle of approach. The CD4bs autologous neutralizing antibodies were somewhat different from CH103-like antibodies with regard to interactions with the V5 loop. Thus, immunogenicity information was derived from structural studies, which showed the CH505 chTrimer induced PGT124-like V3-glycan antibodies that also were dependent on N160 (as was the original human PGT124) (8), and CD4 bs antibodies that interact with the V5 loop.

Protection against intrarectal SHIV Challenge: After the immunization regimens were complete, protection against intrarectal (IR) SHIV challenge was evaluated in animals combined from studies NHP 125, NHP 161 and NHP 161.1. These animals received weekly IR SHIV challenges for 9 weeks (weeks 0-8). After a four-week rest (week 9-12), weekly challenges were resumed at weeks 13-16 for

an additional four challenges. For evaluation of results animals immunized with CH505 TF chTrimer (all doses) and 3M-052 adjuvant (either formulation) were combined and grouped as either high or low bnAb responders based on pre-challenge neutralizing titers against SHIV CH505.375H (Figure 2-3). After nine challenges all 9 control monkeys had developed viral titers, whereas six of seven high bnAb responders and two of 15 low responders were protected from infection. After all thirteen challenges were complete, one additional macaque in the high neutralizing antibody responder group became infected (Figure 2-3).

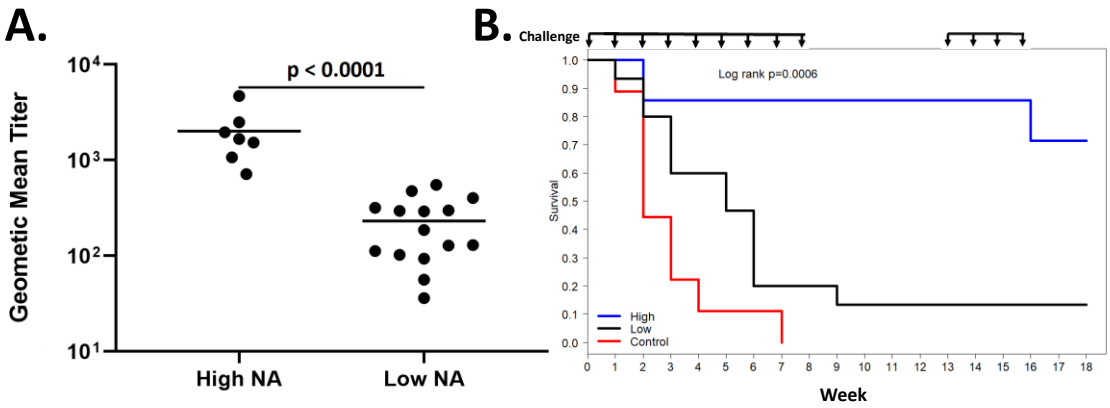

Figure 2-3 Protection from SHIV challenge. CH505 TF chTrimer-immunized animals from studies NHP 125, NHP 161, and NHP 161.1 received intrarectal SHIV challenges every week for nine weeks, followed by a three-week rest, and then four more weekly challenges. Nine negative control animals also received SHIV challenges. (A) Animals from all groups were divided into high and low responders based on pre-challenge titers. (B) After 9 challenges, 5 of 7 high-responders were protected from infection, and 2 of the 15 low responders remained protected.

Preclinical data: knock-in mice

CH505 TF chTrimer has been evaluated in over 100 mice at 25 mcg doses. In one of these studies (VMU108), seven CH103 VH + VL UCA knock-in (KI) mice were immunized with 25 mcg CH505 TF chTrimer + poly I:C adjuvant. An adjuvant-only group did not neutralize CH505TF.gly4 variant virus (ie, CH505TF lacking glycans at 462, 460, and 276 that are known to impede CD4bs bnAbs). After five immunizations, no sera from the adjuvant-only- immunized mice neutralized CH505.TF, while 5 of 7 immunized with CH505 TF chTrimer plus adjuvant neutralized CH505TF.gly4 virus. Thus, CH505 TF chTrimer induced candidate precursors of CD4bs bnAbs in CH103 UCA KI mice.

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

2.2 Clinical Experience with Recombinant Env Vaccinations

Vaccine mixtures of gp140 Env oligomers, including trimeric forms, have been evaluated in healthy, HIV-uninfected participants in previous DAIDS-sponsored studies. Table 2-1 summarizes completed Phase 1 clinical trials with gp140 subtype C and subtype B Env proteins. Overall, almost 240 participants received these protein vaccines either co-administered with a DNA or MVA vaccine prime or as a protein boost. The safety profile of these products was similar to that observed for gp120 Env products. The vaccines were generally well tolerated, with no vaccine related SAEs (9-11).

Table 2-1 Completed studies with HIV Env oligomers and trimers

Protocol	Immunogen	Dose (N)	Adjuvant	Schedule	Safety data available
HVTN 049	gp140 (oligomeric, clade B)	100 mcg (80)	MF59C.1	Month 0, 3, 9 (2 protein boosts, IM)	YES
HVTN 073E	gp140 (oligomeric, clade C)	100 mcg (17)	MF59C.1	Month 0, 3 (2 protein boosts, IM)	YES (9)
HVTN 086	gp140 (oligomeric, clade C)	100 mcg (124)	MF59C.1	Month 3, 6 (2 protein boosts, IM)	YES (10)
HVTN 088	gp140 (oligomeric, clade C)	100 mcg (16)	MF59C.1	Month 0, 6	YES (11)

Note: IM: intramuscular

Recent progress in the design, selection, and preclinical evaluation of stabilized soluble native-like Env trimers have led to development of new immunogens capable of maintaining multimeric configurations. Table 2-2 and the following paragraphs summarize ongoing clinical trials with this new generation of Env proteins. Safety data from these studies are anticipated and we aim to incorporate updates in the IND submission. See below for summaries of safety information; for more details of these studies please consult the Investigator's Brochures.

Table 2-2 Ongoing studies with HIV Env trimers

Protocol	Immunogen	Dose (N), route	Adjuvant	Schedule	Safety data (related)
HVTN 122	gp145 C.6980 (oligomers, > 50% trimer)	300 mcg (25) 100 mcg (15) IM	Alum (500 mcg)	Month 0, 2, 6	No SAEs One Grade 3 induration No product-associated early terminations
IAVI W001 (NCT03699241)	BG505 SOSIP.664	30 mcg (20) 100 mcg (20) 300 mcg (10) IM	AS01$_B$	Month 0, 2, 6	No SAEs No Grade 3 or 4 AEs No Early Terminations
HVTN 137 (Part A)	BG505 SOSIP.664	100 mcg + 1 mcg 3M-052-AF+Alum (5), IM 100 mcg + 5 mcg 3M-052-AF+Alum (10), IM	3M-052-AF (1 or 5 mcg) + Alum (500 mcg)	Month 0, 2, 6 (optional)	No related SAEs or AESIsTwo Grade 3 Local Reactogenicity Erythema/Induration Two early terminations
HVTN 137 (Part B, Group 4)	BG505 SOSIP.664	100 mcg (20), IM	5 mcg 3M-052-AF + 500 mcg Alum	Month 0, 2, 6	No related SAEs or AESIs
HVTN 300 (Part A)	CH505 TF chTrimer	300 mcg (12), IM	3M-052-AF (5 mcg) + Alum (500 mcg)	Month 0, 2, 4, 8, 12	No related SAEs, AESIs

HVTN 122: The gp145 C.6980 immunogen is an HIV Env trimer developed by the United States Military HIV Research Program (MHRP), constructed with modifications to facilitate trimer assembly. The safety and immunogenicity of the predominantly trimeric gp145 C.6980 was evaluated in HVTN 122 at 100 mcg and 300 mcg protein dose admixed intramuscularly with aluminum hydroxide adjuvant (months 0, 2, 6). While analysis of the trial data is still ongoing, no serious adverse events related to study product administration were reported. HVTN 122 is closed and no early terminations associated with an adverse event deemed related to study product have been reported. There has been one report of Grade 3 induration. No related SAEs have been reported. 60% (n=27) of participants experienced one or more AEs, of which only one AE (mild injection site pruritis) was deemed related to study product.

IAVI W001: BG505 SOSIP.664 gp140, the native-like stabilized SOSIP trimer sharing the gp41 sequence and highly similar biophysical and antigenicity properties with the CH505 TF chTrimer (12, 13). This immunogen is currently being evaluated in IAVI W001 (NCT03699241), a randomized double-blinded, placebo-controlled, dose-escalation Phase 1 clinical trial evaluating safety and immunogenicity of recombinant HIV BG505 SOSIP.664 gp140 vaccine

formulated with $AS01_B$ (GlaxoSmithKline [GSK]) administered intramuscularly. Blinded safety data as of May 25, 2020 includes information for 12 volunteers in Group 1 (30 mcg in the US), 1 volunteer in Group 2 (100 mcg in the US), 12 volunteers in Group 4 (30 mcg in Kenya) and 12 volunteers in Group 5 (100 mcg in Kenya). The trial is ongoing and remains blinded to investigational product administration. As of May 25, 2020, there have been three reported serious adverse events, with one volunteer suffering multiple fractures following a road accident, another suffering a soft tissue injury while working at a construction site and a third volunteer requiring surgery for acute appendicitis. These events were all deemed unrelated to the investigational product. One potential immune-mediated disease has been reported; this was judged to be unrelated to the investigational product as signs of the illness were recorded prior to enrollment. No incident HIV infections, deaths, or pregnancies have been reported. Six volunteers have reported grade 3 reactogenicity events that resolved without sequelae. No grade 3 or higher hematology, chemistry or urine laboratory values have been reported. No volunteers have been discontinued from the trial due to adverse events. Two scheduled interim Safety Monitoring Committee (SMC) meetings have occurred, during which the SMC has reviewed all the available safety data and recommended continuation of the study, with dose escalation in both the US and Kenyan sites.

HVTN 137 Part A: Part A is a first-in-human, double-blinded dose escalation study testing the combination of 100 mcg BG505 SOSIP.664 gp140 with 3M-052-AF at two doses (1 mcg and 5 mcg) and 500 mcg Alum. The study is ongoing and remains blinded to within-group treatment assignment. As of April, 2022, both the 1 mcg 3M-052-AF group (6 participants total, 5 receiving protein/adjuvant, 1 receiving placebo) and the 5 mcg 3M-052-AF group (11 participants total, 10 receiving protein/adjuvant, 1 receiving placebo) completed enrollment. Part A of the protocol specified that participants would receive two total doses, one at month zero and the other at month two. Five out of six participants received two doses of the 1 mcg dose (one participant discontinued for reasons unrelated to vaccination) and 10 out of 11 participants received two doses of the 5 mcg dose. One participant in the 5 mcg dose group (Group 2) decided to discontinue vaccination due to Grade 3 induration/erythema first noted on Day 8 post-vaccination. The maximum size of both the induration and erythema was measured at 17 x 17 cm on Day 8 (Grade 3). The erythema resolved over three days and the induration subsided to less than 5 cm on Day 11 (Grade 1). The induration was measured at 2 x 2 cm on Day 14 but did not completely resolve until 41 days post-injection. At no point was the pain/tenderness greater than mild and the participant continued to work. It is unknown at present whether this participant received placebo or study product. Another participant in Group 2 experienced two days of Grade 3 induration and erythema from Days 6-7 post-vaccination that resolved completely by Day 8. In consultation with the Protocol Safety Review Team, the participant did receive the second dose, which was uneventful. In addition, 4 participants reported Grade 3 systemic reactogenicity events. All 4 participants experienced severe fatigue and myalgia which lasted for

1-2 days. Additionally, 2 participants experienced severe myalgia, arthralgia, chills and headache that lasted for a day.

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

HVTN 137 Part B is evaluating the safety and immunogenicity of 100 mcg BG505 SOSIP.664 gp140 in combination with 3 TLR agonists, including 5 mcg 3M-052-AF + 500 mcg Alum, and Alum alone. Enrollment is now complete with 88 participants enrolled. Given that the trial remains blinded across 4 treatment arms, interpretation of the blinded safety data from HVTN 137 Part B is challenging, but there have been no related SAEs, AESIs, deaths, or unplanned study pauses.

Overall, the BG505 SOSIP.664 with 3M-052-AF was generally well tolerated, with no unsolicited Grade 3 or 4 adverse events, no related SAEs, AESIs, or deaths, and no unplanned study pauses. Other than the persistent erythema in one of the Group 2 participants described above, all reactogenicity symptoms resolved within 14 days and generally within seven days. For more detailed information, please see the Investigator's Brochure.

HVTN 300 (Part A): As of September 7, 2022, the study is ongoing and all 13 participants have received the first vaccination, 10 participants have received the second vaccination, 9 participants have received the third vaccination, 9 participants have received the fourth vaccination, and 6 participants have received the fifth vaccination. Five participants have discontinued further vaccinations (1 due to a panic attack after the first injection (this participant had a history of panic attacks before being part of the study), 3 due to reactogenicity events, and 1 was lost to follow-up); Out of these 5 participants, 2 terminated from the study early (panic attack and lost to follow-up) and 3 have remained in the study for follow-up.

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

All participants reported some systemic reactogenicity during the course of the trial to date, mostly mild to moderate. For example, 11 of 13 participants experienced systemic reactogenicity after the first dose. Five (5) participants reported Grade 3 (severe) systemic reactogenicity during the trial through September 7, 2022. One (1) of these 5 participants received a subsequent vaccination without any Grade 3 reactogenicity, one (1) experienced an additional

Grade 3 systemic reactogenicity symptom, one (1) has not yet received a subsequent vaccination, and two (2) declined to receive additional vaccinations after their first Grade 3 systemic reactogenicity event. All Grade 3 events resolved within the 7-day reactogenicity period; the longest duration for severe reactogenicity was 2 days.

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

In conclusion, multiple variants of the HIV Env protein including trimeric formulations have been evaluated in clinical trials with an acceptable safety profile. The Env protein is relatively well conserved functionally albeit with amino acid sequence variability associated with strain variants. This characteristic is predicted to result in a similar safety profile for different Env antigens, including novel stabilized SOSIP trimers. Although two grade 3 local erythema/induration events occurred on days 6 and 8 in the 10 participants in the HVTN137—Part A who received the 5mcg dose of 3M-052-AF plus alum, no grade 3 local reactogenicity was observed in 18 participants in Part B or the participants in HVTN300--- Part A. Additionally, the bilateral administration of a split dose HVTN300 is different from the unilateral administration in HVTN137. We therefore believe that the reactogenicity profile of the regimen in Part B will be adequately captured with a standard 7 day reactogenicity monitoring period (see Section 8.3 below).

2.2.1 Evaluation of 3M-052-AF + Alum

3M-052-AF contains 3M-052 and an emulsifier in an aqueous formulation. 3M-052 is a TLR7 and TLR8 agonist (TLR7/8 agonist) that induces production of cytokines in vitro from immune cells, such as dendritic cells, macrophages, and monocytes (14-17).

Nonclinical studies in guinea pigs, rats, rabbits, and rhesus macaques comparing the immunogenicity of the related SOSIP protein BG505 SOSIP.664 formulated with a diverse range of adjuvants have consistently shown that the protein adjuvanted with 3M-052-AF + Alum elicits neutralizing and binding antibodies more quickly, to higher peak magnitudes, and with greater durability than any of the other protein-adjuvant combinations tested.

In rhesus macaques, among eight adjuvants combined with an Env gp140 immunogen, the alum/TLR7 were the most potent in assays characterizing both Ab and cellular responses. This hierarchy of potency was sustained throughout the longitudinal follow up. The overall effect of the alum/TLR combination was to boost Env binding Ab titers 3-10-fold compared to alum alone (18). Data presented in Figure 2-1 also confirm that the qualitative antibody response induced by 3M-052-AF is different as higher levels of autologous tier 2 neutralizing antibodies were induced when compared with GLA-SE.

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

2.2.2 Rationale for evaluation of 3M-052-AF alone (addition of Part B)

3M-052-AF adjuvant was evaluated in a cynomolgus macaque study with and without Alum in a study of a coronavirus vaccine candidate. This receptor-binding domain (RBD) sortase A-conjugated ferritin nanoparticle (RBD-scNP) vaccine consists of a 24-mer SARS-CoV-2 RBD nanoparticle conjugated to a ferritin scaffold (19).

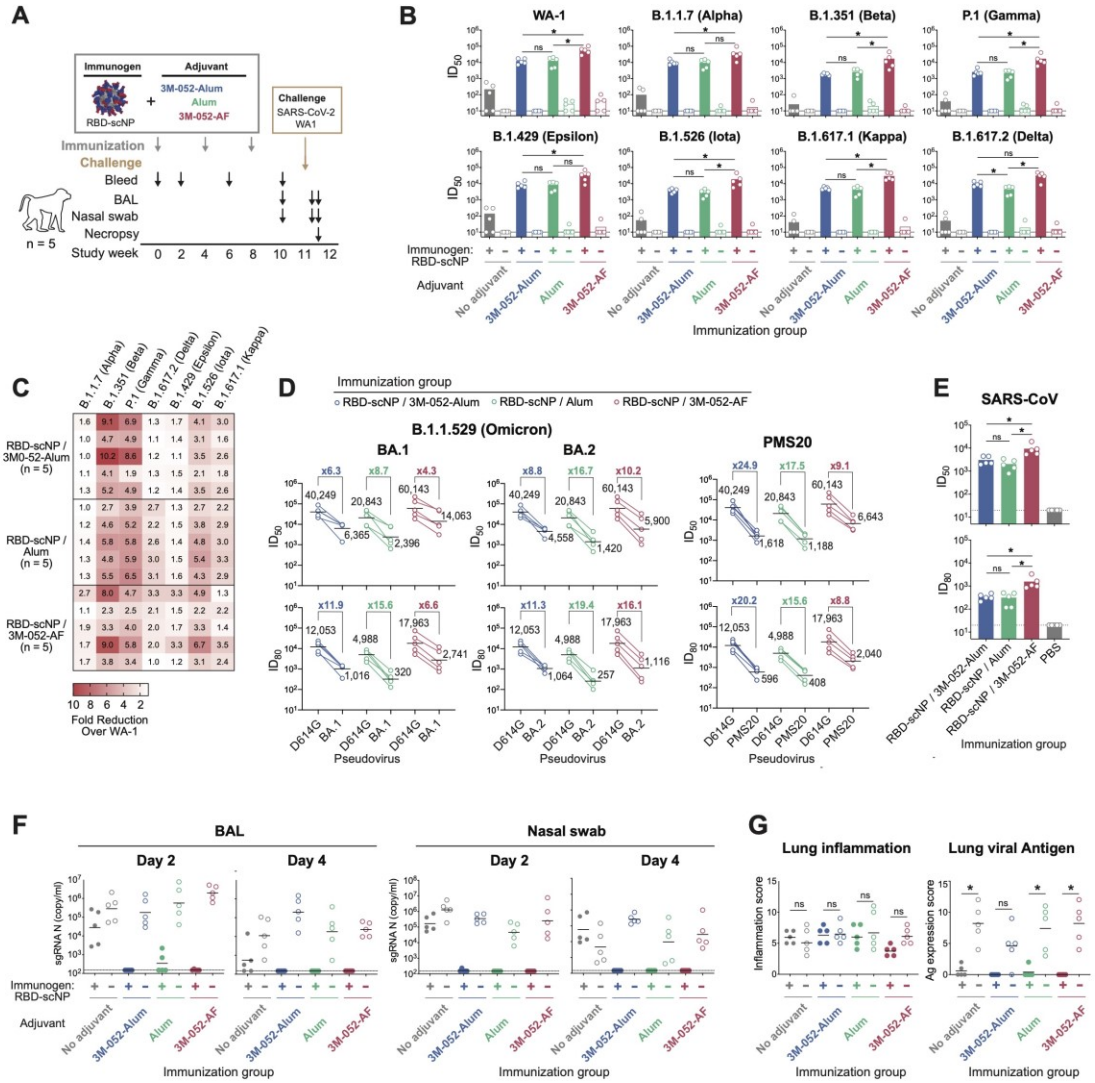

Figure 2-4 Neutralizing antibodies and in vivo protection elicited by RBD-scNP vaccine formulated with three different adjuvants.

(A) Schematic of the vaccination and challenge study. Cynomolgus macaques (n=5 per group) were immunized intramuscularly 3 times with 100 μg of RBD-scNP adjuvanted with 3M-052-AF-Alum, Alum, 3M052-AF, or PBS control. Animals injected with adjuvant alone or PBS were set as control groups. Monkeys were then challenged with SARS-CoV-2 WA-1, collected for blood, Bronchoalveolar lavage (BAL) and nasal swab samples, and necropsied for pathologic analysis. **(B)** Neutralization titers of plasma antibodies (post-3rd immunization) against pseudovirus of

SARS-CoV-2 variants in 293T-ACE2-TMPRSS2. **(C)** Reduction of ID50 titers against variants shown as fold reduction compared to the titers against WA-1. **(D-E)** Neutralization titers of plasma antibodies (post-3rd immunization) against pseudoviruses of the SARS-CoV-2 Omicron (BA.1 and BA.2) variants, SARS-CoV-2 PMS20 variant, and SARS-CoV in 293T-ACE2 cells. For SARS-CoV-2 variants, the geometric mean titers and the fold reduction compared to D614G are shown. **(F)** SARS-CoV-2 N gene sgRNA in BAL and nasal swab samples collected on day 2 and 4 post-challenge. Dashed line indicates limit of the detection. **(G)** Histopathological analysis. Lung sections from each animal were scored for lung inflammation by haematoxylin and eosin (H&E) staining, and for SARS-CoV-2 nucleocapsid antigen (Ag) expression by immunohistochemistry (IHC) staining.ns, not significant, *P<0.05, Wilcoxon rank sum exact test.

The RBD-scNP (100 μg) was administered intramuscularly in the right and left quadriceps at 3 timepoints with or without adjuvant as defined in Figure 2-4, Panel A. Surprisingly we found that 3M-052-AF in each assay out-performed or was not inferior to the other formulations (Figure 2-4). In the cynomolgus monkey study (Figure 2-4 Panel A), the antibodies induced by 3M-052-AF adjuvant with the RBD-sortase conjugated ferritin nanoparticle (scNP) induced higher neutralizing antibodies for many SARS-CoV-2 variants (Figure 2-4 Panel B). Of great interest is the finding that 3M-052-AF alone induced higher titers to Omicron compared to 3M-052/Alum or Alum alone (Figure 2-4). After 2 or 3 immunizations, the combination of 3M-052/Alum was the lower value for Omicron neutralization and 3M-052-AF alone was the higher titer. Panels F and G of Figure 2-4 demonstrate the results of challenge of the monkeys after three immunizations and show protection with all adjuvant formulations with the least complete protection in the Alum alone group. All adjuvant combinations were well tolerated in this study. There were no deaths. Animals were monitored for safety and serum chemistry, and hematology parameters were measured routinely. Mild eosinophilia was observed in 6 of the 35 monkeys, which did not correlate with a particular adjuvant regimen and was not observed in the group receiving RBD-scNP + 3M-052-AF. No other vaccine or adjuvant related serum chemistry or hematology changes were observed. No weight loss or adverse effects were observed.

The above data provide the rationale for evaluating 3M-052-AF alone as an alternative to the combination of 3M-052-AF and Alum for use in HIV-1 vaccine trials. While these data were generated with a SARS-CoV-2 RBD immunogen, both RBD and HIV-1 Env are highly glycosylated trimers, and effective elicitation of neutralizing antibodies to these viruses may therefore be supported by similar adjuvant regimens. Additionally, the monoclonal antibodies with the most potent neutralization isolated from the memory B cells of SARS-CoV-2 vaccine recipients have substantial somatic hypermutation, further supporting the concept that the underlying immunologic mechanisms that lead to improved quality of the neutralizing antibody response are likely to apply to both SARS-CoV-2 and HIV (20).

3M-052-AF is a lipid-modified synthetic imidazoquinoline that is a TLR7/8 agonist. The lipid tail of 3M-052-AF was designed to minimize systemic distribution, and this tail associates with a phospholipid excipient, 1,2-Distearoyl-

sn-glycero-3-phosphoglycerol (DSPG) to create an aqueous nanosuspension formulation (14). In a mouse study of 3M-052 formulated with phosphatidylcholine (a similar phospholipid) instead of DSPG, subcutaneous administration of 25 mcg of 3M-052 in mice did not induce circulating TNFα, indicating an absence of a systemic proinflammatory effect (15). Additionally, in a study performed by Charles River for 3M, localization of radiolabeled 3M-052 (350 mcg) formulated in sesame oil was measured after subcutaneous administration in Sprague Dawley Rats. In this study, concentrations of total radioactivity at the dose site were more than 100-fold higher than the concentrations of radioactivity observed in the sampled tissues at all times, up to 14 days. Notably, clinical safety of the DSPG component of 3M-052-AF has been demonstrated in the liposomal formulation of amphotericin B (Ambisome) which contains DSPG. Ambisome has reduced incidence of adverse events compared to amphotericin B deoxycholate upon i.v. administration (19, 21). Even though the evaluation of 3M-052-AF biodistribution (without Alum) is not yet available, data with other formulations of 3M-052 without Alum, as mentioned above, indicate that the activity of this adjuvant is predominantly localized.

2.3 Rationale for Schedule

To match the schedule used in other comparable trials (HVTN 115) and pre-clinical studies, the CH505 TF chTrimer protein will be administered IM into both deltoids in a volume of 1 mL divided into two 0.5 mL injections administered in the left and right deltoid at a dose of 150 mcg per deltoid (total dose 300 mcg) at months 0, 2, 4, 8, and 12. The rationale for splitting the dose between deltoids is based upon both theoretical and practical considerations. Theoretically, having more lymph nodes engaged in the germinal center process should allow for more clonal selection and competition, leading to additional somatic hypermutation necessary for bnAb maturation. Practically, splitting the dose should decrease the potential for localized reactogenicity in this first in human trial and facilitate sampling of lymph nodes via Fine Needle Aspiration in HVTN 300 Part A (see Section 2.6). Additionally, in pre-clinical studies in rhesus macaques, antibody titers rose with each subsequent injection up to five boosts. This is in contrast to previous experience with other adjuvants.

2.4 Rationale for Dose of Protein

The 300 mcg dose of protein has been selected on the basis of promising preclinical data generated from rhesus macaques (see Section 2.1, Figure 2-2 and Figure 2-3) and reassuring safety data generated from analogous studies of recombinant trimers used in clinical trials. These studies include IAVI W001 (300 mcg) and HVTN122 (300 mcg) (see Section 2.2).

Clinical Data: We anticipate summaries of preliminary clinical safety data from the HVTN 122 and IAVI W001 studies to support the protein trimer dose selected for this study and the IND application.

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

2.5 Rationale for Dose of Adjuvant

The 5 mcg dose of 3M-052-AF adjuvant has been selected based on previous preclinical experience (see Section 2.1, Figure 2-2 and Figure 2-3) and dose selection in HVTN 137 (see Table 2-2). The 500 mcg dose of alum (ie, aluminum content by weight) adjuvant also matches the regimen in HVTN 137.

The 3 mcg dose of 3M-052-AF was selected for use in HVTN 300 Part B in order to compare 3 mcg and 5 mcg doses to determine if the reactogenicity profile seen with 5 mcg of 3M-052-AF/Alum can be reduced with 3 mcg 3M-052-AF (± Alum) while maintaining similar adjuvant activity. Results from this trial will be compared to data from Part A of the HVTN 300 trial.

2.6 Rationale for Fine Needle Aspiration

To improve assessment of the effects of CH505 TF on B cell lineage development, this protocol will employ fine needle aspiration (FNA) of an axillary lymph node proximal to the site of vaccine administration in study participants enrolled in Part A (Appendix A). FNA will be performed after the 3rd dose of CH505 TF chTrimer has been administered. The germinal center response, which occurs exclusively in the lymphatic tissue, is critical for directing B cell lineage development. Havenar-Daughton and colleagues have employed repeated direct probing of germinal center responses by fine needle lymph node aspirates in nonhuman primate (NHP) studies and found that induction of neutralizing antibodies against Tier 2 autologous HIV strains best correlated with germinal center B cell magnitude and Env-specific CD4+ Tfh cells (22). Similar sampling strategies are under evaluation in licensed vaccine and early phase human HIV vaccine studies and are planned in this protocol (23).

2.7 Rationale for Leukapheresis

To assess the expansion of rare precursor B cell populations that occur at a frequency of approximately ~1/20 million cells, large numbers of peripheral blood mononuclear cells (PBMCs) are required. Leukapheresis can provide 6-10 billion PBMCs without depleting red blood cells or other blood components. There are approximately 1 million PBMCs per milliliter of blood and therefore peripheral blood sampling at volumes associated with typical blood donation (~500 mL) would not provide sufficient PBMC numbers. Furthermore,

leukapheresis is associated with fewer adverse events when compared to blood donation. Hence this protocol will employ leukapheresis in study participants enrolled in Part A (Appendix A) to ensure adequate specimens for analysis of rare precursors. Leukapheresis will be performed at screening and after the 4th dose of CH505 TF chTrimer has been administered. The rationale for performing leukapheresis three weeks after the 4th dose is based upon detailed preclinical investigations into the kinetics of the germinal center response in rhesus macaques (see Section 2.1 and (24)).

2.8 Risks and benefits

2.8.1 Potential risks

In addition to risks associated with 3M-052-AF + Alum adjuvants (see Section 2.2), frequent potential side effects resulting from other intramuscular (vaccines) and subcutaneous (local anesthetic prior to FNA) injections include stinging, discomfort, redness of skin, or mild bruising at injection site.

Frequent potential side effects resulting from other intramuscular (vaccines) and subcutaneous (local anesthetic prior to FNA) injections include stinging, discomfort, redness of skin, or mild bruising at injection site.

Less frequent side effects from other intramuscular vaccines include: fever, chills, rash, aches and pains, nausea, dizziness and fatigue. If these occur, they will be monitored, but with other vaccines, these effects are generally short term, mild to moderate in severity, and usually do not require treatment.

Rare risks with any injection procedure include infection at the site of injection. Signs of infection at the injection site include severe pain, erythema, induration, warmth or drainage. There are rare reports of anaphylaxis with many vaccines. There may be side effects from the study products which may be serious or life threatening that we do not know about yet.

If antibodies elicited by the study vaccine result in a positive or indeterminate HIV test (ie, vaccine-induced seropositivity or VISP), this may have a negative impact on employment, health care, insurance, travel, and personal relationships. HIV PCR will be used to exclude or confirm HIV infection.

Alum adjuvant: For more than 90 years, Alum has been used as an adjuvant in commercial vaccines. Hundreds of millions of people have gotten vaccines containing Alum. Side effects are generally limited to minor local reactions at the injection site.

Blood drawing may cause pain, bruising, fainting, and, rarely, infection at the site where the blood is taken. Leukapheresis can also cause pain, bruising, and, rarely

infection. Additional risks may depend on local leukapheresis procedures. Fainting can also occur, but occurs less frequently than with blood drawing.

Fine needle aspiration of lymph nodes can uncommonly cause pain, bruising, and/or bleeding at the site where the needle is inserted. Similar to any procedure where a needle is inserted into the skin (such as vaccination, as described above), there is a rare risk of localized infection. The local anesthetic rarely can cause arm numbness or weakness.

Risks during Pregnancy: Because possible effects of the study vaccine on a fetus or nursing infant are unknown, persons assigned female sex at birth who have reproductive potential will be tested for pregnancy at screening and prior to administration of each dose of study vaccine. Such persons will be asked to notify the site immediately if they suspect or learn they are pregnant during this study. In case of pregnancy, participants will continue to be followed for safety and the participant will not receive any additional vaccinations. The participant will be contacted about the outcome of a pregnancy that begins during the study.

Other Risks: The medical tests performed as part of this research protocol may result in new diagnoses or abnormal values without clinical significance ("false positives"). Depending on the medical findings and consequences of being provided with the results of these tests, the study participant may view this as either a risk or a benefit. Any such information will be shared and discussed with the participant and, if requested by the participant, may be forwarded to the primary health care provider for further workup and management.

Participants in this study risk experiencing discrimination or other personal problems that may result from study participation itself: these are known collectively as negative social impacts. The HVTN clinical research site (CRS) is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding ways to assist a participant dealing with a negative social impact, a designated HVTN Core representative can be contacted.

2.8.2 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.

2.9 Assessment of immunogenicity endpoints

The primary immunogenicity endpoint assay will measure the frequency of CH505TF- and epitope-specific memory B cells after vaccination). Evaluation of cellular responses will be performed on all samples after vaccination and compared to each participant's baseline. Multiparameter flow cytometry will be used to assess the frequency of CH505TF- and epitope-specific IgG+ B cells. The antigen-specific B cell phenotyping assays will be performed at the Fred Hutch HVTN laboratory.

Lymphocytes from an axillary lymph node obtained by fine needle aspiration (FNA) of the node will be examined for a germinal center phenotype (germinal center B cell) via flow cytometry three weeks after the 3rd vaccination.

Additional assays may be performed on selected responders and at additional timepoints at the discretion of Protocol Team leadership based on its evaluation of primary results following the third vaccination (see Section 3.1).

3 Objectives and endpoints for Part A and Part B

3.1 Primary objectives and endpoints

Primary objective 1:

To evaluate the ability of the vaccine regimen to elicit CD4 binding-site, V2 apex and V3 glycan (bnAb region at the base of the V3 loop), and CH505TF-specific memory B cells

Primary endpoint 1:

Flow cytometry analysis of the frequency of the CD4 binding-site, V2 apex and V3 glycan (bnAb region at the base of the V3 loop), and CH505TF-specific IgG+ B cells

Primary objective 2

To evaluate the ability of the vaccine regimen to elicit autologous tier 2 virus neutralizing antibodies

Primary endpoint 2:

Response rate and magnitude of serum antibody neutralization of vaccine-matched tier 2 HIV-1 strains as measured by TZM-bl assay

Primary objective 3:

To evaluate the safety and tolerability of a 300 mg dose of CH505 TF chTrimer admixed with different adjuvant regimens

Primary endpoint 3:

Local and systemic reactogenicity signs and symptoms will be collected for a minimum of seven days following receipt of any study vaccine.

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 twelve months following any receipt of study product. Additionally, all adverse events will be collected for 30 days after any receipt of study vaccination.

3.2 Secondary objectives and endpoints

Secondary objective 1:

To evaluate HIV-1 binding antibody responses elicited by CH505 TF chTrimer admixed in 3M-052-AF + Alum or 3M-052-AF (without alum)

Secondary endpoint 1:

Response rate and magnitude of serum IgG binding antibodies as assessed by binding Ab multiplex assay (BAMA)

Secondary objective 2:

To evaluate the ability of the vaccine regimen to elicit HIV-1 specific heterologous tier 2 neutralizing antibodies

Secondary endpoints 2:

Response rate and magnitude of serum antibody neutralization of heterologous HIV-1 strains as measured by TZM-bl assay

3.3 Exploratory objectives

Exploratory objective 1:

To map the epitope specificity of autologous and heterologous tier 2 virus neutralizing activity

Exploratory endpoints 1:

Response rate and magnitude of serum antibody neutralization of epitope knock-out mutants of CH505 TF and heterologous tier 2 viruses

Exploratory objective 2:

Determine the B cell repertoire of peripheral blood B cells in each group of vaccinees

Exploratory endpoints 2:

Detection of induced V3 glycan and CD4 binding site epitope binding antibodies.

Exploratory objective 3:

Determine the transcriptome of vaccine and adjuvant induced peripheral blood immune cells in each group of vaccinees

Exploratory endpoints 3:

Detection of origin of V3-glycan and CD4 binding site antibodies from either extrafollicular B cells or follicular B cells.

Additional exploratory objectives:

To conduct analyses related to furthering the understanding of HIV, immunology, vaccines, and clinical trial conduct, including but not exclusive to B cell repertoire analysis (including analysis of rare B cell lineages associated with bnAb precursors), and assessment of lymph node aspirate for germinal center activity, including cellular phenotyping and mutational frequency analysis suggestive of somatic hypermutation and affinity maturation following immunization.

4 Laboratory strategy

The primary goal of HVTN 300 is to determine whether the CH505 TF chTrimer immunogen combined with a 3M-052-AF adjuvant can drive the expansion of B cell lineages similar to those known to be associated with broadly neutralizing antibodies induced during natural HIV-1 infection. To this end, HVTN immunogenicity assays will be used to evaluate induction of vaccine and epitope-specific B cells and binding antibodies as well as serum neutralization. These data will also be used to identify and prioritize samples for in-depth immunogenicity analyses, including B cell receptor sequencing of epitope-specific B cells, epitope-mapping of neutralizing antibody responses, and characterization of isolated mAbs. Specifically, flow cytometry will be used to characterize the vaccine-specific B cells using fluorescently-labeled Env probes in both the periphery and the regional lymph node. Furthermore, by using different fluorescently-labeled Env probes with minimal mutations in epitopes of interest, CD4-binding site and epitope-specific lineages of B cells will be identified for downstream analysis. These rare epitope-specific populations of B cells will be enumerated, characterized by surface expression markers, and sorted for BCR sequencing. In addition, the sera from participants will be tested for neutralization not only against the autologous CH505TF pseudovirus, but also a panel of pseudoviruses with engineered mutations specifically designed to identify antibodies that are able to neutralize UCA-sensitive pseudoviruses in an epitope-specific manner. The pace of the development of both B cell isolation techniques and the generation of specific pseudoviruses intended to identify specific antibody lineages is rapid. The 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/en/science.html.

5 Study design

This is an open-label Phase 1 study to examine the safety and immunogenicity of the CH505 TF chTrimer vaccine with 3M-052-AF +/- Alum adjuvant in healthy adults. The primary hypothesis is that the CH505 TF chTrimer will expand CH103-like B-cell precursors.

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: 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. 18-55 years old, inclusive, on day of enrollment.

3. Available for clinic follow-up through the last clinic visit, willing to undergo lymph node fine needle aspiration and leukapheresis, and willing to be contacted 12 months after the last vaccine administration.

4. Agrees not to enroll in another study of an investigational agent during participation in the trial.

5. In good general health according to the clinical judgement of the site investigator.

6. Physical examination and laboratory results without clinically significant findings that would interfere with assessment of safety or reactogenicity in the clinical judgement of the site investigator.

7. Assessed as low risk for HIV acquisition per low risk guidelines (see Appendix E), 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 PrEP as prescribed for 6 months or longer.

8. Hemoglobin >12.5 mg/dL to 18 mg/dL

9. White blood cell (WBC) count > 3,500/mm^3

10. Platelets ≥125,000 /mm^3

11. Alanine aminotransferase (ALT) < 2.5 x ULN based on the institutional normal range

12. Serum creatinine ≤1.1 x ULN based on the institutional normal range

13. Blood pressure in the range of 90 to < 160 mmHg systolic and 50 to < 100 mmHg diastolic.

14. Negative results for HIV infection by an FDA-approved enzyme immunoassay (EIA) or chemiluminescent microparticle immunoassay (CMIA).

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

16. Negative for Hepatitis B surface antigen.

17. For a volunteer capable of becoming pregnant:

    - Volunteers who were assigned female sex at birth and are of reproductive potential must agree to use effective means of birth control from at least 21 days prior to enrollment through 8 weeks after their fifth vaccination timepoint (see Appendix G).

    - Has negative β-HCG (human chorionic gonadotropin) pregnancy test (urine or serum) on day of enrollment.

5.1.2 Exclusion criteria

1. Volunteer who is breast-feeding or pregnant.

2. Previous or current recipient of an investigational HIV vaccine (previous placebo recipients are not excluded).

3. Systemic glucocorticoid use equal to or greater than prednisone 10 mg/day within 3 months prior to enrollment, congenital or acquired immunodeficiency or other systemic medication use likely to impair immune response to vaccine in the opinion of the site investigator.

4. Blood products or immunoglobulin within 16 weeks prior to enrollment; receipt of immunoglobulin within 16 weeks prior to enrollment requires PSRT approval.

5. Receipt of any live attenuated vaccine within 4 weeks prior to enrollment.

6. Receipt of any vaccines that are not live attenuated within 14 days prior to enrollment; replication incompetent vaccines such as the Jynneos vaccine for the prevention of monkeypox disease are not considered to be live vaccines.

7. ACAM2000 vaccine for Monkeypox received within 30 days prior to enrollment or receipt of study product, or if ACAM2000 received greater than 30 days prior to enrollment or receipt of study product, vaccination scab still present; or planned administration within 30 days after enrollment or receipt of study product.

8. Initiation of antigen-based immunotherapy for allergies within the previous year (stable immunotherapy is not exclusionary); inclusion of participants who initiated immunotherapy within the previous year requires PSRT approval.

9. Receipt of 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.

10. Serious reactions to vaccines that preclude receipt of study injections as determined by the principal investigator or designee. History of serious reaction (eg. hypersensitivity, anaphylaxis) to any vaccine or any component of the study vaccine, including imidazoquinolone (eg, imiquimod).

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

12. Idiopathic urticaria within the past year.

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

14. Seizure disorder; febrile seizures as a child or seizures secondary to alcohol withdrawal more than 5 years ago are not exclusionary.

15. Asplenia or functional asplenia.

16. Active duty and reserve US military personnel.

17. 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).

18. Asthma is excluded if the participant has ANY of the following:
    1. Required either oral or parenteral corticosteroids for an exacerbation two or more times within the past year; OR
    2. 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
    3. 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
    4. 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]);
    5. 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.

19. A participant with a history of an immune-mediated disease, either active or remote. Specific examples are listed in Appendix H (AESI index). Not exclusionary: 1) remote history of Bell's palsy (>2 years ago) not associated with other neurologic symptoms, 2) mild psoriasis that does not require ongoing systemic treatment

20. History of allergy to local anesthetic (Novocaine, Lidocaine).

21. Investigator concern for difficulty with venous access based upon clinical history and physical examination. For example, history of IV drug abuse or substantial difficulty with previous blood draws.

5.2 Participant departure from vaccination schedule or withdrawal

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), immune targeting monoclonal antibodies or other immunomodulators (other than nonsteroidal anti-inflammatory drugs [NSAIDs]), with the exception that study injection may continue per 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; (Note: ACAM2000 vaccine for Monkeypox received within 30 days prior to enrollment or receipt of study vaccine; or if ACAM2000 received greater than 30 days prior to enrollment or receipt of study vaccine and vaccination scab still present; or planned administration within 30 days after enrollment or receipt of study vaccine).

- Receipt of any vaccines that are not live attenuated vaccines within 2 weeks prior to study vaccine administration. Replication incompetent vaccines such as the Jynneos vaccine for the prevention of monkeypox disease are not considered to be live vaccines.

Vaccinations should not be administered outside the visit window period specified in Appendix C without PSRT approval.

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 exception of fever and subjective local and systemic symptoms. For grade 3 injection site erythema and/or induration, upon review, the PSRT may allow continuation of vaccination (except with review by the PSRT)

- 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 (25), is provided below:

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 PEF, hypoxemia)

b. Reduced 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.
- Newly disclosed AESI (see Appendix H)
- 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 300 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 for up to 12 months following their last study vaccine administration. 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 or until the final scheduled clinic visit, whichever comes first. 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 IND 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, CBC with differential, serum chemistry, physical examination, and if indicated, a pregnancy test (see Appendix A and Appendix B). 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

6.1 Sample size justification and accrual

Recruitment will target enrolling a total of 48 healthy, HIV-uninfected adult participants in 4 groups.

In Part A, recruitment will target enrolling 12 healthy, HIV-uninfected adult participants in Group 1. The protocol team will convene after the 12th participant has received their third vaccination to determine if further participants should be enrolled to evaluate immunogenicity. Examples of reasons that might necessitate enrollment of additional participants are provided in Section 1.5. If some participants from the original number of 12 enrolled will not contribute data to some of the immunogenicity analyses, the protocol team may enroll additional fully evaluable participants, up to 6 additional participants, or a total of 18 participants enrolled, with the goal of at least 12 contributing to the final immunogenicity analyses.

In Part B, recruitment will target enrolling a total of 36 healthy, HIV-uninfected adult participants in Groups 2, 3, and 4 (n = 12 participants per group). Enrollment in Groups 2, 3, and 4 will be stepwise by group, starting with Group 2, continuing with Group 3, and finishing with Group 4.

Every study participant will be followed for an AESI contact 12 months after the last vaccination received (for example, following participants until Month 16 if they receive 3 vaccinations, or Month 24 if they receive 5 vaccinations).

Detail about the pace of enrollment can be found in Section 1.5.

Since enrollment is concurrent with receiving the first vaccination, all participants will provide some safety data. However, for immunogenicity analyses, data may be missing for a variety of reasons, including participants terminating from the study early, problems in shipping specimens, or high assay background. Immunogenicity data from 17 Phase 1 and 2 Phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of September 2014), indicate that 17% is a reasonable estimate for the rate of missing data.

6.1.1 Power calculations for immunogenicity for Part A

One primary goal of the statistical analysis for Part A is to assess whether the vaccine regimen evaluated in Group 1 elicits CD4 binding-site, V2 apex and V3 glycan epitope (bnAb region at the base of the V3 loop), and CH505TF-specific memory B cells. The primary endpoint will be the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope (bnAb region at the base of the V3 loop), and CH505TF-specific IgG+ B cells at baseline and after vaccination, as measured by flow cytometry. Another primary goal is to evaluate the ability of the vaccine

regimen to elicit autologous tier 2 virus neutralizing antibodies, as measured using the TZM-bl assay.

The statistical analysis will first compare the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope, and CH505TF-specific IgG+ B cells at baseline with those observed after vaccination. These comparisons will use Wilcoxon signed-rank tests. Should the vaccine regimen elicit a desired immune response, we expect the frequencies of CD4 binding-site or V2 apex or V3 glycan epitope or CH505TF-specific IgG+ B cells to increase after vaccinations, and the tests will be one-sided. An additional analysis will compare the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope, and CH505TF-specific IgG+ B cells at different time points after vaccination using two-sided Wilcoxon signed-rank tests.

The statistical analysis will also perform response positivity calls for each study participant by comparing B cells frequencies at baseline to B cells frequencies post-vaccination using Fisher's exact tests. The statistical analysis will next assess whether the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope, and CH505TF-specific IgG+ B cells differ between time points post-vaccination. These comparisons will be performed using two-sided Fisher's exact tests.

We will next estimate the response rate at each post-vaccination time point by the empirical proportion of positive calls among study participants and build an associated 95% confidence interval.

We computed the power of a one-sided Wilcoxon signed-rank to detect a significant difference between the frequencies of CD4 binding-site, or V2 apex or V3 glycan epitope (bnAb site at the base of the V3 loop), or CH505TF-specific IgG+ B cells observed at baseline with that observed at one time point post-vaccination. The power was evaluated as a function of (1) the effect size, defined here as the average change (increase) in the frequencies of (eg) CD4 binding-site or V3 glycan (base of the V3 loop) binding-IgG+ B cells after vaccination relative to baseline, and (2) the standard deviation (SD) of the difference between pre- and post-vaccination frequencies. The calculations made the following assumptions:

- The average increase in the frequency of CD4 binding-site IgG+ B cells post-vaccination relative to baseline in study participants ranges between 0 and 5%.

- The standard deviation of the frequency of CD4 binding-site IgG+ B cells post-vaccination relative to baseline in study participants ranges between 0.5% and 4%.

- The increase in the frequency of CD4 binding-site IgG+ B cells after vaccination relative to baseline is normally distributed. Departure from this assumption is not expected to impact the power of the study because the Wilcoxon signed-rank test is nonparametric.

The calculations considered samples of size n = 12 and n = 10 to assess power when the data set is complete (n = 12) and when 2 out of the 12 participants (or, equivalently, an attrition rate of approximately 17%; n = 10) are lost to follow up before the post-vaccination time point.

The power function of the test under these different scenarios is presented in Figure 6-1. For example, if the standard deviation of the change in the frequency of binding IgG+ B cells is SD = 2%, the calculation indicates that a sample of size n = 10 achieves almost 80% power to detect a significant increase at the 5% significance level if the average increase in the proportion of binding IgG+ B cells is 1.5%. With a sample of size n = 12 (no loss to follow-up), the test has more than 80% power to detect a significant difference under the same scenario.

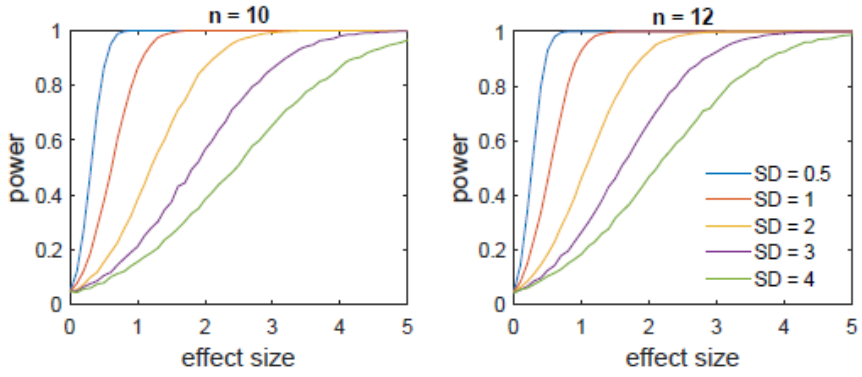

Figure 6-1 Power of a one-sided Wilcoxon signed-rank test comparing the frequency of CD4 binding-site or V2 apex or V3 glycan epitope (bnAb site at the base of the V3 loop) CH505TF-specific IgG+ B cells post-vaccination relative to baseline. The power was calculated as a function of (1) the effect size, defined as the average difference between the frequencies of B cells pre- and post-vaccination; (2) the standard deviation (SD) of the difference between the pre- and post-vaccination B cell frequencies; and (3) the sample size (n = 10 and n = 12).

6.1.2 Power calculations for immunogenicity for Part B

The goal of Part B is to evaluate whether the three vaccine regimens tested in Groups 2, 3, and 4 elicit CD4 binding-site, V2 apex and V3 glycan epitope (bnAb region at the base of the V3 loop), and CH505TF-specific memory B cells. The primary endpoint will be the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope (bnAb region at the base of the V3 loop), and CH505TF-specific IgG+ B cells at baseline and after vaccination, as measured by flow cytometry. Part B will also evaluate whether the vaccine regimens elicit autologous tier 2 virus neutralizing antibodies using the TZM-bl assay.

The statistical analysis will first compare the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope, and CH505TF-specific IgG+ B cells at baseline with those observed after vaccination. These comparisons may be performed within each treatment group (ie, Group 2, 3, and 4) using Wilcoxon signed-rank tests. Should the vaccine regimens elicit a desired immune response, we expect the

frequencies of CD4 binding-site or V2 apex or V3 glycan epitope or CH505TF-specific IgG+ B cells to increase after vaccinations, and the tests will be one-sided. Additional analyses will compare the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope, and CH505TF-specific IgG+ B cells at other time points following vaccination; these tests will be two-sided because the direction of the effect may not be known.

The statistical analysis will also perform response positivity calls for each study participant by comparing B cells frequencies at baseline to B cells frequencies post-vaccination using Fisher's exact tests. The statistical analysis will next assess whether the frequencies of CD4 binding-site, V2 apex and V3 glycan epitope, and CH505TF-specific IgG+ B cells differ between time points post-vaccination. These comparisons will be performed using two-sided Fisher's exact tests. We will next estimate the response rate at each post-vaccination time point within each group by the empirical proportion of positive calls among study participants. Associated 95% confidence intervals will also be built.

We evaluated the power of a one-sided Wilcoxon signed-rank to detect a significant difference between the frequencies of CD4 binding-site, or V2 apex or V3 glycan epitope (bnAb site at the base of the V3 loop), or CH505TF-specific IgG+ B cells observed at baseline with that observed at one time point post-vaccination in one of the treatment group (ie, either Group 2, 3, or 4; see Section 6.1.1) and when two groups are combined (eg, Groups 2 and 4; see results below). The power was evaluated as a function of (1) the effect size, defined here as the average change (increase) in the frequencies of (eg) CD4 binding-site or V3 glycan (base of the V3 loop) binding-IgG+ B cells after vaccination relative to baseline, and (2) the standard deviation (SD) of the difference between pre- and post-vaccination frequencies. The calculations made the following assumptions:

- The average increase in the frequency of CD4 binding-site IgG+ B cells post-vaccination relative to baseline in study participants ranges between 0 and 5%.

- The standard deviation of the frequency of CD4 binding-site IgG+ B cells post-vaccination relative to baseline in study participants ranges between 0.5% and 4%.

- The increase in the frequency of CD4 binding-site IgG+ B cells after vaccination relative to baseline is normally distributed. Departure from this assumption is not expected to impact the power of the study because the Wilcoxon signed-rank test is nonparametric.

The calculations considered samples of size n = 12 and n = 10 to assess power when the data set is complete (n = 12) and when 2 out of the 12 participants (or, equivalently, an attrition rate of approximately 17%; n = 10) are lost to follow up before the post-vaccination time point. The power function of the test under these different scenarios when carried with each group separately (i.e., within either

Group 2, or 3, or 4) is discussed in Section 6.1.1 in the context of Group 1 (Part A); see also Figure 6-1.

The statistical power of statistical tests for the comparison of immunogenicity data measured at two time points within participants could be increased by combining relevant treatment groups together. For example, we could combine Groups 2 and 4 together to assess whether vaccination with 3M-052-AF induced any immune responses, regardless of dose. The power of the Wilcoxon signed-rank test for sample sizes of n = 20 (eg, Groups 2 and 4 combined when 4 out of the 24 participants (or, equivalently, an attrition rate of approximately 17% per group) are lost to follow up) and n = 24 (eg, Groups 2 and 4 combined when no participant is lost to follow-up) are presented in Figure 6-2.

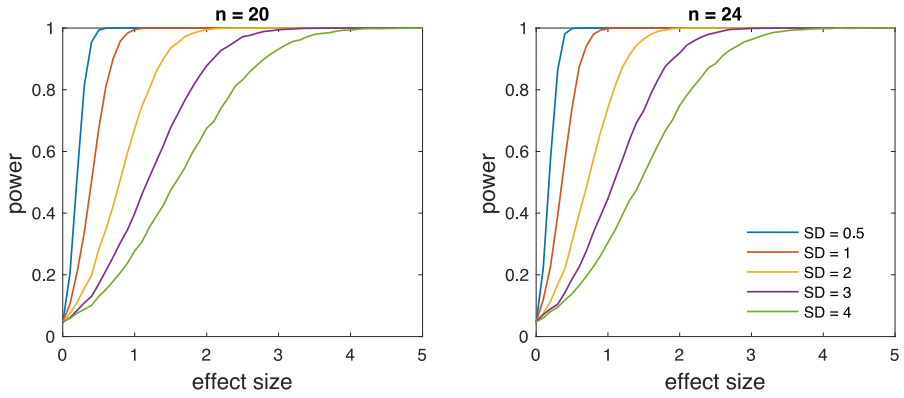

Figure 6-2 Power of a one-sided Wilcoxon signed-rank test comparing the frequency of CD4 binding-site or V2 apex or V3 glycan epitope (bnAb site at the base of the V3 loop) CH505TF-specific IgG+ B cells post-vaccination relative to baseline. The power was calculated as a function of (1) the effect size, defined as the average difference between the frequencies of B cells pre- and post-vaccination; (2) the standard deviation (SD) of the difference between the pre- and post-vaccination B cell frequencies; and (3) the sample size in the combined two groups (n = 20 and n = 24).

The statistical analysis of immunogenicity data measured in Part B will also include the following group comparisons; some of these comparisons may include data from Group 1 (Part A):

- Group 2 versus Group 3 post baseline to assess the effect of Alum (at a dose of 500 mcg) on immune responses in participants vaccinated with 3M-052-AF at a dose of 3 mg/kg.
- Group 1 versus Group 4 post baseline to assess the effect of Alum (at a dose of 500 mcg) on immune responses in participants vaccinated with 3M-052-AF at a dose of 5 mg/kg.
- Group 1 versus Group 3 post baseline to assess whether vaccination with a dose of 3 mcg versus 5 mcg of 3M-052-AF induces different immune responses in participants vaccinated with Alum (at a dose of 500 mcg).

- Group 2 versus Group 4 post baseline to assess whether vaccination with a dose of 3 mcg versus 5 mcg of 3M-052-AF induces different immune responses in participants who were not vaccinated with Alum.

The effect of vaccination with Alum as well as that of the dose of 3M-052-AF on immune responses may be performed using nonparametric one-way analysis of variance (ANOVA) (eg, Wilcoxon rank sum tests). To increase the power of statistical tests, these comparisons may be carried out by combining all four groups together and using nonparametric two-way ANOVA (eg, Friedman-type or Skillings-Mack tests) that include vaccination with Alum and vaccination with 3M-052-AF as factors.

Since participants will not be randomized between Groups 1, 2, 3, and 4 at enrollment, comparisons of immunogenicity endpoints between treatment groups will be cautiously interpreted. Differences in baseline characteristics may be examined to determine whether differences between groups could have been induced by (eg) differences in the demographics of study participants between treatment groups.

6.1.3 Power calculations for safety for Part A

The goal of the safety evaluation for Part A is to identify safety concerns associated with vaccine administration in Group 1. The ability of the study to detect serious adverse events (SAEs) (see Section 9.2) 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 this single-arm study (n = 12), there is at least a 90% chance of observing at least 1 event if the true rate of such an event is 17.5% or more; and there is at least a 90% chance of observing no events if the true rate is 0.87% or less. Safety data will be evaluated using historical controls. As a reference, in HVTN vaccine trials conducted in the US from April 2008 through March 2018, about 1% of participants who received placebos experienced an SAE.

Binomial probabilities of observing 0, 1 or more, and 2 or more events among 12 participants receiving study vaccine are presented in Table 6-1 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among a group of 12 study participants for different true event rates

True event rate (%)	arm size	0 events	1+ events	2+ events
1	12	0.89	0.11	0.01
4	12	0.61	0.39	0.08
10	12	0.28	0.72	0.34
20	12	0.07	0.93	0.73
30	12	0.01	0.99	0.91

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 6-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method for CI described in Agresti and Coull formula 2 (26). If none of the 12 participants receiving the study vaccine experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 24.2%.

Table 6-2 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints in a group of 12 study participants

Observed event rate	95% Confidence interval (%)
0/12	[0 ; 24.2]
1/12	[1.5 ; 35.4]
2/12	[4.7 ; 44.8]

6.1.4 Power calculations for safety for Part B

The goal of the safety evaluation for Part B is to identify safety concerns associated with vaccine administration in Groups 2, 3, and 4. The ability of the study to detect serious adverse events (SAEs) 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 group in Part B (n = 12), there is at least a 90% chance of observing at least 1 event if the true rate of such an event is 17.5% or more; and there is at least a 90% chance of observing no events if the true rate is 0.87% or less. For two vaccine groups of Part B combined (eg, Groups 2 and 3; n = 24), there is a 90% chance of observing at least 1 event if the true rate of such an event is 9.2% or more; and there is a 90% chance of observing no events if the true rate is 0.43% or less. For all three vaccine groups of Part B combined (n = 36), there is a 90% chance of observing at least 1 event if the true rate of such an event is 6.2% or more; and there is a 90% chance of observing no events if the true rate is 0.29% or less. For the four vaccine groups of Parts A and B combined (n = 48), there is a 90% chance of observing at least 1 event if the true rate of such an event is 4.7% or more; and there is a 90% chance of observing no events if the true rate is 0.21% or less.

Binomial probabilities of observing 0, 1 or more, and 2 or more events among the 12 participants of each group (Groups 2, 3, or 4) are presented in Table 6-1 for a range of possible true adverse event rates. Binomial probabilities of observing 0, 1 or more, and 2 or more events among the 36 participants of Groups 2, 3, and 4 combined are presented in Table 6-3 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-3 Probability of observing 0 events, 1 or more events, and 2 or more events, among groups of 12, 24, 36, and 48 study participants for different true event rates

True event rate (%)	group size	0 events	1+ events	2+ events
1	12	0.89	0.11	0.01
4	12	0.61	0.39	0.08
10	12	0.28	0.72	0.34
20	12	0.07	0.93	0.73
30	12	0.01	0.99	0.91
1	24	0.79	0.21	0.02
4	24	0.38	0.62	0.25
10	24	0.08	0.92	0.71
20	24	0.005	1.00	0.97
30	24	0.000	1.00	1.00
1	36	0.70	0.30	0.05
4	36	0.23	0.77	0.43
10	36	0.02	0.98	0.89
20	36	0.00	1.00	1.00
30	36	0.00	1.00	1.00
1	48	0.62	0.38	0.08
4	48	0.14	0.86	0.58
10	48	0.01	0.99	0.96
20	48	0.00	1.00	1.00
30	48	0.00	1.00	1.00

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 6-4 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate in one group (eg, Group 2, or 3, or 4; n = 12), or 2 groups (eg, Groups 2 and 3; n = 24), or 3 groups (Groups 2, 3, and 4; n = 36), or 4 groups (Groups 1through 4; n = 48). Calculations are done using the score test method for CI described in Agresti and Coull formula 2 (26). If none of the 48 participants receiving any the study vaccines experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 7.4%.

Table 6-4 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints in n = 12, or n = 24, or n = 36, or n = 48 study participants

Observed event rate	95% Confidence interval (%)
0/12	[0.0 ; 24.2]
1/12	[1.5 ; 35.4]
2/12	[4.7 ; 44.8]
0/24	[0.0 ; 13.8]
1/24	[0.7 ; 20.2]
2/24	[2.3 ; 25.8]
0/36	[0.0 ; 9.6]
1/36	[0.5 ; 14.2]
2/36	[1.5 ; 18.1]
0/48	[0.0 ; 7.4]
1/48	[0.4 ; 10.9]
2/48	[1.2 ; 14.0]

6.2 Statistical analyses

This section describes the final study analysis, unblinded as to treatment arm assignment. All data from enrolled participants will be analyzed regardless of how many vaccinations they received. All analyses will be performed using SAS and R.

No formal multiple-comparison adjustments will be employed for multiple safety endpoints, multiple primary immunogenicity endpoints, or secondary endpoints. Statistical tests for comparisons of primary and secondary endpoints, including two- and one-sided tests, will use a significance level (alpha) of 5%.

6.2.1 Baseline demographics

Participants' baseline characteristics will be summarized using descriptive statistics.

6.2.2 Immunogenicity analyses

The frequencies of CD4 binding-site, V2 apex and V3 glycan epitope (bnAb region at the base of the V3 loop), and CH505TF-specific IgG+ B cells elicited by the vaccine measured by flow cytometry, the response rate and magnitude of binding measured by the BAMA assay, and the area-under-the-magnitude-breadth curve (AUC) (27) to panels of viral isolates measured by the HIV-1 neutralization TZM-bl assay, will be summarized using descriptive statistics. Data will be tabulated and graphically displayed by visit number and by number of doses received.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response. Crude response rates will be presented with their corresponding 95% confidence interval estimated based on the score test method (26). The Barnard's exact tests may be employed to compare the response rates of certain immune responses between the test vaccine and other candidates in the CH505 family.

Quantitative assay endpoints (eg, frequencies of memory B-cells) will be summarized by time point. Nonparametric paired comparisons will be performed using Wilcoxon signed-rank tests, and the Wilcoxon rank sum test will be used to compare independent samples. More details of the analyses of each assay will be provided in the statistical analyses plan (SAP).

6.2.3 Safety Analyses

Reactogenicity: The number and percentage of subjects experiencing each type of reactogenicity sign or symptom will be tabulated by severity. For a given sign or symptom, each subject's reactogenicity will be counted once under the maximum severity for all assessments.

Adverse Events: AEs will be coded into Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. The number and percentage of subjects experiencing each specific AE will be tabulated by severity and relationship to study vaccine. For the calculations in these tables, each subject's AE will be counted once under the maximum severity or strongest recorded causal relationship to treatment. A complete listing of AEs for each subject will provide details including severity, relationship to treatment, onset, duration and outcome.

7 Study vaccine preparation, storage, and administration

7.1 Vaccine Regimen

The schedule of vaccination is shown in Section 1 and additional information is given below.

Part A:

Group 1

Treatment 1 (T1)- (300mcg) CH505TF chTrimer admixed with (5mcg) 3M-052-AF + (500 mcg) Aluminum Hydroxide Suspension (Alum), to be administered as two 0.5 mL doses intramuscularly (IM) at months 0, 2, 4, 8 and 12.

Part B:

Group 2

Treatment 2 (T2) - 300 mcg CH505TF chTrimer admixed with (3mcg) 3M-052-AF to be administered as two 0.5 mL doses intramuscularly (IM) at months 0, 2, 4, 8, and 12.

Group 3

Treatment 3 (T3) – 300 mcg CH505TF chTrimer admixed with (3mcg) 3M-052-AF + (500 mcg) Aluminum Hydroxide Suspension (Alum) to be administered as two 0.5 mL doses intramuscularly (IM) at months 0, 2, 4, 8, and 12.

Group 4
Treatment 4 (T4) - 300mcg CH505TF chTrimer admixed with (5mcg) 3M-052-AF to be administered as two 0.5 mL doses intramuscularly (IM) at months 0, 2, 4, 8, and 12.

7.2 Study Product Formulation and Storage

7.2.1 CH505 TF chTrimer

CH505 TF chTrimer drug product is formulated in 20 mM Tris, 100mM NaCl, pH 7.5. The visual appearance of the formulated product is clear to slightly opalescent, colorless to slightly yellow, and essentially free from visible particles. The drug product is supplied as a frozen liquid in 2 mL Type 1 glass vials. Each

vial contains 0.75 mL of formulated CH505 TF chTrimer at a concentration of 0.8 mg/mL and is stored at ≤ -65°C.

7.2.2 3M-052-AF (Labeled as AP 60-702)

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

7.2.3 Aluminum Hydroxide Suspension (Alhydrogel®)

The Aluminum Hydroxide Suspension (Alum) will be provided in 3 mL Type 1 glass vials. Each vial contains 0.7 mL fill volume at a concentration of 5 mg/mL aluminum. Alum appears as an opaque, white gelatinous precipitate in aqueous suspension. The product is stored refrigerated at 2-8° C. Do not freeze.

7.2.4 Tris-NaCl buffer (TBS) (Diluent) for Part A

The Tris-NaCl buffer will be used as a diluent for mixing with adjuvants. The sterile buffer will be provided in 2 mL Type 1 glass vials. Each vial contains a fill volume of 1.1 ± 0.1 mL. Tris-NaCl buffer is a clear to opalescent, colorless to slightly yellow liquid, without any visible particulates. The product is stored at 2-8° C.

7.2.5 Sodium Chloride for injection, 0.9% USP (Diluent) for Part B

Sodium Chloride for injection, 0.9% USP must be stored as recommended by the manufacturer.

7.3 Product Preparation

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

Any unused portion of study product will not be used for another participant. Empty vials, unused portion of entered vials, or unused prepared study product should be discarded in a biohazard container and disposed of in accordance with institutional or pharmacy policy.

7.3.1 Group 1: CH505 TF chTrimer 300 mcg admixed with (5 mcg) 3M-052-AF + (500 mcg) Aluminum Hydroxide Suspension (Alum)

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

- Remove one vial of 3M-052-AF, one vial of TBS, and one vial of Alum from the refrigerator. Resuspend Alum by inverting the vial for 30-60 seconds.
- Remove one vial of CH505 TF chTrimer from the freezer and allow to thaw at room temperature. Resuspend the protein by inverting the vial for 30-60 seconds.

Preparation of Adjuvants:

- Withdraw 0.4 mL of Alum and inject into the 3M-052-AF vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

Preparation of CH505 TF chTrimer:

- Withdraw 0.64 mL TBS and inject into the empty sterile vial (mixing vial).
- Withdraw 0.56 mL of CH505 TF chTrimer and inject into mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.3 mL of the adjuvant mixture and inject into the mixing vial.
- Mix by gently inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.5 mL of the mixed preparation into two separate syringes, for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.
- The admixed product is stable for 8 hours at room temperature.

7.3.2 Group 2: CH505 TF chTrimer 300 mcg admixed with (3 mcg) 3M-052-AF

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

- Remove one vial of 3M-052-AF and an adequate volume of Sodium Chloride for injection, 0.9% USP (Diluent)
- Remove one vial of CH505 TF chTrimer from the freezer and allow to thaw at room temperature. Resuspend the protein by inverting the vial for 30-60 seconds.

Preparation of Adjuvants

- Withdraw 0.4 mL of of Sodium Chloride and inject into the vial with 3M-052-AF.
- Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

Preparation of CH505 TF chTrimer:

- Withdraw 0.76 mL of Sodium Chloride and inject into the empty sterile vial (mixing vial).
- Withdraw 0.56 mL of CH505 TF chTrimer and inject into mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.18 mL of the 3M-052-AF admixture and inject into the mixing vial.
- Mix by gently inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.5 mL of the mixed preparation into two separate syringes, for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.
- The admixed product is stable for 8 hours at room temperature.

7.3.3 Group 3: CH505 cTrimer 300 mcg admixed with (3 mcg) 3M-052-AF + (500 mcg) Aluminum Hydroxide Suspension (Alum)

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

- Remove one vial of 3M-052-AF, an adequate volume of Sodium Chloride , and one vial of Alum from the refrigerator. Resuspend Alum by inverting the vial for 30-60 seconds.
- Remove one vial of CH505 TF chTrimer from the freezer and allow to thaw at room temperature. Resuspend the protein by inverting the vial for 30-60 seconds.

Preparation of Adjuvants:

- Withdraw 0.16 mL of 3M-052-AF and inject into the empty sterile vial.
- Withdraw 0.16 mL of of Sodium Chloride and inject into vial with 3M-052-AF.

- Withdraw 0.4 mL of Alum and inject into the 3M-052-AF vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

Preparation of CH505 TF chTrimer:

- Withdraw 0.64 mL of Sodium Chloride and inject into the empty sterile vial (mixing vial).
- Withdraw 0.56 mL of CH505 TF chTrimer and inject into mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.3 mL 3M-052-AF admixture and inject into the mixing vial.
- Mix by gently inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.5 mL of the mixed preparation into two separate syringes, for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.
- The admixed product is stable for 8 hours at room temperature.

7.3.4 Group 4: CH505 chTrimer 300 mcg admixed with (5 mcg) 3M-052-AF

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

- Remove one vial of 3M-052-AF and an adequate volume of Sodium Chloride
- Remove one vial of CH505 TF chTrimer from the freezer and allow to thaw at room temperature. Resuspend the protein by inverting the vial for 30-60 seconds.

Preparation of Adjuvants:

- Withdraw 0.4 mL of Sodium Chloride and inject into the 3M-052-AF vial.
- Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously. Allow formulation to incubate at room temperature for 30 minutes.

Preparation of CH505 TF chTrimer:

- Withdraw 0.64 mL of Sodium Chloride and inject into the empty sterile vial (mixing vial).

- Withdraw 0.56 mL of CH505 TF chTrimer and inject into mixing vial. Mix by gently swirling and inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.3 mL of the 3M-052-AF admixture and inject into the mixing vial.
- Mix by gently inverting the vial about 5 times. Do not shake vigorously.
- Withdraw 0.5 mL of the mixed preparation into two separate syringes, for study participant administration. Prior to administration, gently invert the syringe to resuspend admixed product.
- The admixed product is stable for 8 hours at room temperature.

7.3.5 Labeling

Label the study product as follows:

- Participant identifier(s)
- CH505 TF chTrimer + Adjuvant(s)
- Final volume (mL)
- Route (IM)
- Beyond use date and time
- “Syringe 1 of 2” and “Syringe 2 of 2”
- Any additional information required by jurisdiction

7.4 Study Vaccine Administration

Study product will be administered as two separate 0.5 mL injections intramuscularly into both deltoid muscles (both left and right) by needle and syringe.

If an injection needs to be administered in an alternate body site (eg, lateral thigh) due to a medical contraindication, it should not be administered in the same deltoid as the other injection. If no injection can be administered in either deltoid muscle, the injections should be administered in separate alternate body sites. The appropriate study staff should document this clearly. Under this circumstance, this is NOT a protocol violation.

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

7.5 Acquisition of study products

CH505 TF chTrimer was CGMP manufactured by Vetter Development Services in Skokie, IL and will be provided by Duke University (Durham, NC, USA).

The 3M-052-AF adjuvant was CGMP manufactured and will be provided by AAHI (Seattle, WA, USA).

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

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

Sodium Chloride (NaCl) will be locally sourced by the CRSs.

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

7.6 Study Vaccine Accountability

The HVTN CRS pharmacist is required to maintain complete records of all study products.

7.7 Final Disposition of study product

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

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 and Appendix B) 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.

Leukapheresis specimens for baseline comparison will be drawn during the screening period if participant meets all additional eligibility criteria and wishes to join the study (Section 5.1.1 and 8.5).

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

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 C), procedures for a study visit can be completed over multiple days.

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.

Guidance for participants scheduling licensed vaccines or allergy immunotherapy: In order to prevent interference with reactogenicity assessment, participants who plan to receive licensed vaccines or allergy immunotherapy should be counseled to avoid scheduling receipt of these substances, when possible, within the 2-week interval after study-product administration. To avoid unnecessary delays in study-product administration, participants should be counseled to avoid scheduling receipt of these substances before study-product administration (see Section 5.2.1).

Post-Vaccine Administration Follow-Up in Clinic: Following each vaccine administration, participants will be observed for a minimum of 60 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.

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 and erythema. Participant diaries will be reviewed by a clinician and reconciled for accuracy and completeness. No attribution assessment will be performed for systemic reactogenicity events reported in the participant diary without additional evaluation of the participant by clinician.

Participants will be given a thermometer for oral temperature measurement, a ruler, and provided access to the electronic diary. The participant will use the diary to record daily their highest temperature as well as local and systemic signs and symptoms for 7 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.

Remote or in-person contact between the participant and the site staff should take place at least once on the third- or fourth-day following vaccination. The reactogenicity assessment period is seven days following vaccine administration. Clinicians will follow and collect resolution information for any reactogenicity signs and symptoms that have not resolved within 7 days.

Any postvaccination 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.

8.4 Lymph node FNA

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

Post-procedural safety assessments will be performed in accordance with the standard practices of the participating provider and/or facility. The participant will be advised to contact the study site if they experience severe pain, fever (post-

procedural body temperature ≥ 38.0°C or 100.4°F) or other evidence of infection (inflammation and/or pus) at the aspiration site, or arm numbness or weakness.

Eligibility for lymph node FNA:

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

8.5 Leukapheresis

Collection of PBMC via leukapheresis will be performed in accordance with the standard practices of the participating apheresis center. Typically, in this procedure, approximately 6 liters of blood will be processed over about 1.5 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 adverse events following the procedure.

Eligibility for leukapheresis:

- Prior to leukapheresis, participant must meet all apheresis center requirements for this procedure.
- Participants who can become pregnant must have a negative pregnancy test within 48 hours prior to the procedure.

8.6 Visit windows and missed visits

The schedule of visits and evaluations performed at each visit are shown in Appendix A and Appendix B. Visit windows are shown in Appendix C. The procedures for documenting missed visits and out of window visits are described in the HVTN 300 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.

8.7 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 and Appendix B. The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see Study Specific Procedures [SSP]), which is able to distinguish vaccine-induced antibody responses from actual HIV infections.

Participants will be promptly informed and counseled if they become HIV-infected during the study and 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 vaccine induced seropositivity (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 offered poststudy HIV diagnostic testing (per the HVTN poststudy HIV testing algorithm) periodically free of charge as medically/socially indicated (approximately every 6 months) unless or until HIV antibody testing is no longer standard in clinical settings.

- Unless they 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 principal investigator should consider if the following assessments are

appropriate: end-of-study HIV test, CBC with differential, serum chemistry, physical examination, and if indicated, a pregnancy test (see Appendix A and Appendix B). For participants with HIV infection, please see Section 5.2.2. If the site principal investigator has questions regarding a termination visit they should consult with the PSRT.

8.9 AESI contact

CRS staff will contact study participants 12 months after the last vaccination to collect the information listed below. If indicated, the participant may be asked to come in for a clinical assessment which may also include referrals for AESI assessment. AESIs are described further in Appendix H.

- Confirmation of vital status; if deceased, attempt to learn cause and date of death
- If participant is alive, record the participant's responses to the following:
- SAEs
- AESI (a sample list of AESIs is provided in Appendix H). AESIs are reported regardless of relationship to study product(s);
- MAAEs, defined as any adverse events leading to an unscheduled visit to a healthcare professional which are reported regardless of relationship to study product(s);
- New diagnosis of HIV infection; and
- Pregnancies and outcomes, including congenital anomalies/birth defects.
- All such events will be recorded and adverse events will be assessed for relationship to study vaccines.

9 Safety and adverse events

9.1 Adverse events

Unsolicited AEs will be collected over a period of 30 days after each vaccination. All collected AEs are captured in the clinical database on the appropriate 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 9.7), (3) if the AE meets the criteria for an MAAE, and (4) if the AE is a potential immune-mediated disease that may be listed as an AE of special interest (AESI). A sample list of AESIs is provided in Appendix H.

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

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

Adverse events (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 300 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 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);

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 subject 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 adverse event that at occurrence represents an immediate risk of death to the subject. 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 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 expedited AE (EAE) reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE form https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting.

The SAE Reporting Category will be used throughout the study. After completion of the study the 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: CH505 TF chTrimer, administered in combination with 3M-052-AF (aqueous formulation), and Aluminum hydroxide suspension (Alum). There is no placebo product administered in this protocol.

9.3 Safety monitoring

9.3.1 Protocol Safety Review Team (PSRT)

The PSRT comprises the Study Chairs, participating CRS PI(s), HVTN Protocol Team Leader, DAIDS Medical Officer, HVTN Clinical Safety Specialist (CSS), and other Study Clinicians. The PSRT will review study safety information on a weekly basis through 2 weeks after the last participant receives the final study injection. Because participants can be enrolled in the study with a Grade 1 ALT value (up to <2.5 x ULN), the PSRT will monitor all ALT values on all study participants throughout the trial. 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 300 PSRT.

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

9.4 Total blood volume

Required blood volumes per visit are shown in Appendix A and Appendix B. 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 as 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. Alternate tube types may be used under certain circumstances (eg, ACD tube shortages) upon approval of the HVTN Laboratory Center. Refer to the HVTN 300 Specimen Collection SSP for more information.

9.5 Initial safety review for Part A

Enrollment was restricted to one participant per day for the first 5 participants and then enrollments were held. The PSRT reviewed cumulative safety information recorded through the visit scheduled 2 weeks post first vaccination and determined it was safe to proceed with full enrollment.

9.6 Interim Safety Review for Part A

There was a pre-planned safety review after the 5th participant in Part A received their third vaccination. The PSRT reviewed cumulative safety information recorded through the visit scheduled 2 weeks post third vaccination and decided it was safe to proceed with the remaining vaccinations. No participant received the fourth or fifth vaccination until the PSRT gave express permission.

9.7 Initial safety review for Part B

Enrollment in Part B will be stepwise by group. Enrollment in Group 2 will be restricted to one participant per day for the first 5 participants and then enrollment will be held. The PSRT will review cumulative safety information recorded through the visit scheduled 2 weeks post first vaccination for the first 5 participants and determine whether it is safe to proceed to full enrollment in Group 2 and then followed by enrollment in Group 3 and then Group 4.

9.8 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 300 PSRT AE review are summarized in Table 9-1. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the HVTN 300 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 9-1 Pause Rules Table

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 you need 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).

9.8.1 Plan for review of pause rules

For all safety pauses, HVTN Core notifies the HVTN 300 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 300 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 300 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 300 PSRT notification or prompt HVTN 300 PSRT AE review is triggered, HVTN Core notifies the HVTN 300 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 300 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.

9.9 Study termination

This study may be terminated early by the determination of the HVTN 300 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.

9.10 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given, but remaining visits and study procedures should be completed unless medically contraindicated or applicable regulations require termination from the study. During follow-up in persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome. Pregnancies and pregnancy outcomes will be reported as described in the HVTN 300 SSP section on Pregnancy Management and Reporting. 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) (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 300 *Study Specific Procedures*. Regarding protocol registration, sites should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

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 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 (SICF) in Appendix D and Appendix E describes the investigational vaccine 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 level of understandability.

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 subjects 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.

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 accord with the language in the sample informed consent forms (Appendix D and Appendix E) 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 forms for the main study (see Appendix D and Appendix E).

This research may relate to HIV, vaccines, the immune system, and other diseases. This could include genetic testing and, potentially, genome-wide studies. This research is done only to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other 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

Visit Number		01	02	03	04	05	06	07	08	09	10	11	12	AESI[15]
Study Week			0	2	8	10	16	18	32	34	52	54	80	104
Study Month			0	0.5	2	2.5	4	4.5	8	8.5	12	12.5	18	24
Study Day		-56 to 0	0	14	56	70	112	126	224	238	364	378	560	728
Procedure		Screen[1]	**Vac 1**		**Vac 2**		**Vac 3**		**Vac 4**		**Vac 5**			
Study procedures														
Assessment of Understanding		√												
Informed consent		√												
Medical history[2]		√												
Physical exam[3]		√	√	√	√	√	√	√	√	√	√	√	√	
Contraception status assessment[4]		√	√		√		√		√		√			
Risk reduction counseling[5]		√	√	√	√	√	√	√	√	√	√	√	√	
Concomitant medications[6]		√	√	√	√	√	√	√	√	√	√	√	√	
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√		
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[7]			√		√		√		√		√			
Reactogenicity assessment[8]			√		√		√		√		√			
Clinical labs	Tube													
Pregnancy test (urine or serum)[9, 10]		√	√		√		√	√	√	√	√		√	
HBsAg/anti-HCV[10]	SST	5												
HIV screening test[10, 11]	SST	5												
CBC/Differential[10]	EDTA	5		5		5		5		5		5		
ALT & Creatinine[10]	SST	5		5		5		5		5		5		
HIV diagnostic test	EDTA						10		10		10		20	
Research samples[12]														
PBMC for assays & storage	ACD			136		136		136				178.5	178.5	
Serum for assays and storage	SST		34	34		34		34		34		34	34	
Leukapheresis			√[13]							√				
Fine Needle Aspiration[14]								√						
Daily volume (mL)		20	34	180	0	180	10	180	10	44	10	222.5	232.5	
56-day total volume (mL)		20	54	234	234	360	190	370	10	54	10	232.5	232.5	

Green shading = Vaccination Visit; Blue shading = FNA visit

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

[2] **Medical history**: A complete medical history is performed during screening. At enrollment and at subsequent visits, an interim medical history may be performed.

[3] 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.

[4] **Contraception status assessment** is required only for participants who were assigned female sex at birth and are capable of becoming pregnant.

[5] **Risk reduction counseling** per CRS Standard Operating Procedures.

[6] **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.

[7] **Vaccination (in clinic assessments)**: At least 60 minutes after each vaccination and prior to clinic discharge, participants will have vital signs taken, the injection site will be assessed, and systemic symptoms will be assessed.

[8] **Reactogenicity**: Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 until resolution.

[9] **Pregnancy test**: For participants assigned female sex at birth. Pregnancy test may be performed on 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. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[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] **Leukapheresis**: May be performed prior to enrollment visit once the participant has been found to have met all protocol enrollment criteria (see Section 5.1.1 and 5.1.2) and leukapheresis eligibility criteria (see Section 8.5). A pregnancy test (see also footnote 9) must be performed and confirmed negative within 48 hours prior to leukapheresis.

[14] **Fine Needle Aspiration:** Target for FNA is three weeks post 3rd vaccination. A pregnancy test (see also footnote 9) must be performed and confirmed negative within 48 hours prior to lymph node cell collection.

[15] **AESI contact:** CRS staff will contact study participants 12 months after the last vaccination received to collect the information listed in Section 8.9.

Appendix B Schedule of procedures for Part B

Visit Number		01	02	03	04	05	06	07	08	09	10	11	12	AESI[13]
Study Week			0	2	8	10	16	18	32	34	52	54	80	104
Study Month			0	0.5	2	2.5	4	4.5	8	8.5	12	12.5	18	24
Study Day		-56 to 0	0	14	56	70	112	126	224	238	364	378	560	728
Procedure		Screen[1]	**Vac 1**		**Vac 2**		**Vac 3**		**Vac 4**		**Vac 5**			
Study procedures														
Assessment of Understanding		√												
Informed consent		√												
Medical history[2]		√												
Physical exam[3]		√	√	√	√	√	√	√	√	√	√	√	√	
Contraception status assessment[4]		√	√		√		√		√		√			
Risk reduction counseling[5]		√	√	√	√	√	√	√	√	√	√	√	√	
Concomitant medications[6]		√	√	√	√	√	√	√	√	√	√	√	√	
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√		
AESIs/MAAEs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[7]			√		√		√		√		√			
Reactogenicity assessment[8]			√		√		√		√		√			
Clinical labs	Tube													
Pregnancy test (urine or serum)[9, 10]		√	√		√		√		√		√		√	
HBsAg/anti-HCV[10]	SST	5												
HIV screening test[10, 11]	SST	5												
CBC/Differential[10]	EDTA	5		5		5		5		5		5		
ALT & Creatinine[10]	SST	5		5		5		5		5		5		
HIV diagnostic test	EDTA						10		10		10		20	
Research samples[12]														
PBMC for assays & storage	ACD		136	136		136		136		136		178.5	178.5	
Serum for assays and storage	SST		34	34		34		34		34		34	34	
Daily volume (mL)		20	170	180	0	180	10	180	10	180	10	222.5	232.5	
56-day total volume (mL)		20	190	370	370	360	190	370	10	190	10	232.5	232.5	

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

[2] **Medical history**: A complete medical history is performed during screening. At enrollment and at subsequent visits, an interim medical history may be performed.

[3] 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.

[4] **Contraception status assessment** is required only for participants who were assigned female sex at birth and are capable of becoming pregnant.

[5] **Risk reduction counseling** per CRS Standard Operating Procedures.

[6] **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.

[7] **Vaccination (in clinic assessments)**: At least 60 minutes after each vaccination and prior to clinic discharge, participants will have vital signs taken, the injection site will be assessed, and systemic symptoms will be assessed.

[8] **Reactogenicity**: Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 until resolution.

[9] **Pregnancy test**: For participants assigned female sex at birth. Pregnancy test may be performed on 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. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[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] **AESI contact:** CRS staff will contact study participants 12 months after the last vaccination received to collect the information listed in Section 8.9.

Appendix C Visit windows for Part A and Part B

Visit Number	Visit Type	Lower Allowable Window (-)	Lower Target Window (-)	Target Day*	Upper Target Window (+)	Upper Allowable Window (+)
01.0	Screening	-56	-	0	-	+0
02.0	**Enrollment/Vaccination 1**	**-**	**-**	**0**	**-**	**-**
03.0	2 weeks post-vaccination 1		-4	14	+4	+7
04.0	**Vaccination 2**		**-7**	**56**	**+9**	**+14**
05.0	2 weeks post-vaccination 2		-4	70	+4	+7
06.0	**Vaccination 3**	**-21**	**-14**	**112**	**+14**	**+21**
07.0**	2 weeks post-vaccination 3 Primary Immunogenicity	-	-4	126	+4	+21
08.0	**Vaccination 4**	**-28**	**-14**	**224**	**+14**	**+28**
09.0	2 weeks post-vaccination 4	-	-4	238	+4	+7
10.0	**Vaccination 5**		**-28**	**364**	**+28**	
11.0	2 weeks post-vaccination 5		-4	378	+4	+7
12.0	Final Visit	-28	-14	560	+14	+28
AESI	AESI Contact	-28	-14	728	+14	+28

* All target dates are relative to Day 0, with the exception of the post-vaccination visits, visits 3.0, 5.0, 7.0, 9.0, and 11.0 which are relative to the vaccination immediately preceding the visit.

** Target date for FNA is three weeks post 3rd vaccination, see HVTN 300 Study Specific Procedures (SSP) for more details.

Appendix D Sample informed consent form for Part A

Title: A first-in-human Phase 1 clinical trial to evaluate the safety and immunogenicity of stabilized CH505 TF chTrimer in healthy, HIV-uninfected adult participants
HVTN protocol number: HVTN 300
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 18-55, HIV negative and healthy.
- The purpose of this study is to see how a person's immune system responds to the study vaccine.
- The study will also see if the study vaccine is safe to give to people and does not make people too uncomfortable.
- You will be in this study for up to 18 months of clinic visits, with a follow up contact 1 year after your last vaccination to check on your health.
- Procedures will include blood draws and injections of study vaccine, as well as the collection of white blood cells and cells from your lymph nodes. White blood cells will be collected by a procedure called leukapheresis. Lymph node cells will be collected by a doctor using a very thin needle guided by ultrasound. We will tell you more about these procedures later in this consent form.
- There are risks from participating. The most common risks are:
    - Because this vaccine has not been given to people yet, we do not know what all of the risks may be.
    - Taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding.
    - Leukapheresis and collecting cells from your lymph node can cause pain, bruising, and rarely an infection.
    - We will tell you more information about risks later in this consent form.

- We do not expect the study vaccine 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 an HIV vaccine. HIV is the virus that causes AIDS.

Up to 18 people will take part in this study. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

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

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

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

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

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

3. **This study vaccine is experimental.**

    We are testing an experimental vaccine called CH505 TF chTrimer. It is a man-made piece of protein that looks like a protein found on the outside of HIV. This protein is mixed with an adjuvant called 3M-052-AF + Alum. An adjuvant is something added to a vaccine to help the immune system respond better. From here on, we will call the protein-adjuvant combination "the study vaccine". We do not know if the study vaccine will be safe to use in people, or if it will work to prevent HIV infection. This study vaccine is used only in research studies.

    The protein part of the study vaccine was developed by the Duke Human Vaccine Institute and is being provided by Duke University in Durham, North Carolina, USA. 3M-052 was developed by 3M Corporation, headquartered in St. Paul, Minnesota, USA. In this study, 3M-052-AF is being provided by the Infectious Disease Research Institute (IDRI) in Seattle, Washington. Alum is being provided by the Division of AIDS (DAIDS) at the US National Institutes of Health in Rockville, Maryland, USA.

The study vaccine has not been given to people before. The protein part of the vaccine has been engineered to stay in a shape much like natural HIV. In this way it is different from proteins used in some other HIV study vaccines.

3M-052 is one of many similar products developed by 3M Corporation to treat skin conditions, tumors, and to make vaccines more effective. It is an experimental product. It is designed to stimulate parts of the immune system that recognize invaders like viruses. In this study, 3M-052-AF is dissolved in water and is mixed with Alum. Alum is an approved adjuvant that has been used in commercial vaccines for more than 90 years.

A protein-adjuvant combination very similar to this study vaccine is being tested for the first time in humans in another HVTN study called HVTN 137. That study gives some people the study vaccine and some people a placebo which does not contain the study vaccine. As of November 4, 2020, 17 people have had 2 injections (15 people were given the study vaccine and 2 people were given a placebo). These injections have not caused any serious health problems.

In HVTN 137, two people had redness and swelling over a large area on their arm where they got the injection that started about 1 week after the injection. The redness lasted for about 2 to 3 days. For one of these people, the swelling went away within 2 to 3 days. The second person had significant swelling for about 3 days. The swelling went down but it took about 5 weeks to completely go away. They had mild pain which did not prevent them from going to work. Four other people had symptoms that did prevent them from going to work. Because the study is ongoing and is blinded, we do not know if these people got the study vaccine or placebo.

Risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, dizziness, and feeling tired. Vaccines can also cause redness or itching where you got the injection. Most side effects do not interfere with daily activities or make a person visit the doctor.

Rarely, a vaccine can cause an allergic reaction, including a rash, hives, or trouble breathing. Allergic reactions can be life-threatening. Tell us if you ever had a bad reaction to an injection or vaccine.

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, that will not affect your other care at this clinic and the benefits or rights you would normally have.

   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.

   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 also test you for Hepatitis B and Hepatitis C. 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.

   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 G, Approved birth control methods (for sample informed consent form), in this consent form, paste it below and delete paragraph below.

   You should not become pregnant during the study because we do not know how the study vaccine could affect a developing baby. For this reason, you must agree to use effective birth control from 21 days before your first injection until 8 weeks after the time of your fifth study injection (14 months). 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 up to [#] times over 18 months.**

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

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

   A study visit can be done in person at the clinic. They may also be done using tools such as phone calls, text messages, web platforms (sometimes called “telemedicine”) or email. A combination of these types of visits might also be used.

   You may have 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 two of the study procedures. We will tell you more about that below.

   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 vaccine on a schedule.**

    The study is designed to give participants injections of the study vaccine at 5 visits, according to the schedule in the table below. At these 5 visits, you will get 2 injections, 1 into the deltoid muscle of both upper arms. We will give the injections with a needle and syringe.

    Because this is the first time the study vaccine is being given to people, the study also has planned pauses when safety information will be reviewed to decide if it is safe to continue the study. The first pause will be after the first 5 participants have their first injection visit, to determine if the rest of the participants should be enrolled. The second pause will be after the 5th participant has their third injection visit. At this second pause, a decision will be made about whether it is safe for participants to continue with the 4th and 5th injection visits.

	First injection visit	2 months later	4 months later	8 months later	12 months later
Study vaccine	✓	✓	✓	✓	✓

    You will have to wait in the clinic for about an hour after getting injections to see if there are any problems. We will measure your temperature, blood pressure, heart and breathing rates. We will also look at your injection sites and ask how you feel. Then for that night and for 7 more days, you will use a secure online symptom log to keep track of how you are feeling. If you are unable or unwilling to use the online symptom log, please talk with us about other options that may be available. Within 4 days after each injection visit, we will contact you 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 the study vaccine, we will do the procedures shown in the following table.**

			Time after first injection visit										
Procedure	**Screening visit(s)**	**First injection visit**	**2 weeks**	**2 months**	**2½ months**	**4 months**	**4½ months**	**8 months**	**8½ months**	**12 months**	**12½ months**	**18 months**	**2 years**
Injection		√		√		√		√		√			
Medical history	√												
Complete physical	√											√	
Brief physical		√	√	√	√	√	√	√	√	√	√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√	
Lymph node cell collection							√						
Leukapheresis*		√							√				
Pregnancy test**	√	√		√		√	√	√	√	√		√	
HIV testing	√					√		√		√		√	
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√

* Leukapheresis may be done at the First injection visit or before enrollment if a person wishes to join the study and is eligible.

** For persons who were assigned female sex at birth and who are capable of becoming pregnant. A negative pregnancy test is required within 48 hours before lymph node cell collection and leukapheresis. Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records), do not have to have pregnancy tests.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 233 mL (about 1 tablespoon to a little less than one cup). Your body will make new blood to replace the blood we take out.

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

Leukapheresis procedure:

This procedure collects large amounts of white blood cells. Blood is made up of red cells that carry oxygen, white cells that fight infection, platelets that help form clots, and plasma, which is the fluid left over when all the cells are removed. During leukapheresis, white blood cells are removed and the rest of the blood is put back into the body.

The leukapheresis procedure will be done at *[site: insert location]*. Your eligibility to have this procedure will be assessed by 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. When the white blood cells are taken out, the rest of the blood will go back into

your body through the tube going into your other arm. Sometimes the fluid lost during the procedure is replaced by a sterile salt 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.

It is normal to feel tired for up to 24 hours after having leukapheresis.

The leukapheresis procedure will be done before your first injection visit and again about 2 weeks after your 4th injection visit. It will take about [Site: Insert timeframe] for the procedure.

We will give you [Site: Insert compensation] for having leukapheresis. This amount is to cover the costs of [Site: Insert text].

Risks of leukapheresis

Generally, the risks of leukapheresis include pain, bruising and rarely, infection. A less common risk is fainting. 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.

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.

Lymph node cell collection procedure

The formal name for this procedure is a Fine Needle Aspiration. This procedure collects cells from lymph nodes. Lymph nodes are one of the key places where immune responses develop and they are located in several places on the human body. In this procedure, a doctor will use a very thin needle guided by ultrasound to collect cells from a lymph node on your arm or in your arm pit.

The lymph node cell collection procedure will be done at *[site: insert location]*. Your eligibility to have this procedure will be assessed by the staff at our clinic during screening, and again 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.

To do the procedure, a clinician will find the appropriate lymph node by applying a cold gel to your skin and then pressing a hand-held ultrasound device against your skin. If necessary, a small area near the lymph node may be shaved. Once the node is found, a cleaning solution will be applied to the skin in that area. The area will be numbed by an injection of a local anesthetic, such as Lidocaine, which is like Novocaine that is used for dental procedures. The doctor will insert a very thin needle and use the images from the ultrasound to guide it into the lymph node. The needle may be moved up and down to collect cells from the lymph node. Fluid may also be

collected through the needle into a syringe or bottle. Each cell collection lasts about 10-15 seconds. It usually takes about 4 separate cell collections to get enough cells.

After the procedure is done, the area will be cleaned with warm water. You will be given a band-aid to cover the site where the needle was inserted.

Lymph node cell collection will happen about 3 weeks after your third injection visit. It will take about [site insert timeframe] for the procedure.

We will give you [Site: Insert compensation] for having a lymph node cell collection. This amount is to cover the costs of [Site: Insert text].

Risks of lymph node cell collection

The main risks of this procedure include pain, bruising, bleeding and infection where the needle is inserted. The cleaning solution or shaving the area may cause skin irritation. Injecting the local anesthetic may sting or burn for a little while until the numbing takes effect. Examining the lymph node area may be uncomfortable.

If discomfort, problems, or side effects happen during or after lymph node cell collection, the staff will use the appropriate medical procedures to treat you.

12. **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.

13. **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 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 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). It will involve 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.

14. **When your samples are no longer needed for this study, the HVTN will continue to store them. 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. *[Site: choose one of the following two sentences. African sites should choose the sentence referencing the repository in South Africa. All other sites should choose the sentence referencing the repository in the United States.]* Your samples will be stored in the HVTN repository in South Africa. Your samples will be stored in the HVTN repository in the United States.

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

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

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

Will the HVTN sell my samples and information? No, but the HVTN may share your samples with HVTN or other researchers. Once 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.

15. 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 online symptom log after the injection visits, that information only has your code number. Your data goes directly from the online symptom log 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,
- Any regulatory agency that reviews clinical trials,
- *US sites may, but are not required to include:* [Insert name of local IRB/EC] ,
- *US Sites include if this sIRB will be used:* Fred Hutchinson Cancer Research Center Institutional Review Board
- [Insert name of local and/or national regulatory authority as appropriate]
- The Duke Human Vaccines Institute, IDRI, DAIDS and people who work for them,

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

All reviewers will 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]

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 website will not include information that can identify you. The website may include a summary of the study results. You can search this website at any time.

16. 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 but it will be your choice. 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 also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study for up to 12 months if you choose. 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 stop your injections if you enroll in a different research study where you get another study product.

17. 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

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

In addition to the risks of the study vaccine 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 abnormal laboratory results

Minor changes in laboratory test results occasionally happen. This means that the test results can show something to be abnormal when it is not. If this happens, we will ask you to come back to the clinic to be retested. This may cause you to worry, and it may be inconvenient to come back to the clinic. If retesting confirms something to be abnormal, we will provide care or help you get the care you need.

Risks of routine medical procedures:

Routine medical procedures such as taking blood and giving injections 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 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 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 someone who should not may see your personal information. 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 vaccine will increase, decrease, or not change your risk of getting HIV if exposed. If you get HIV, we do not know how the study vaccine might affect your HIV infection or how long it takes to develop AIDS.

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

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

Benefits

19. 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 vaccine later becomes approved for sale, there are no plans to share any money with you.

Your rights and responsibilities

20. 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

21. 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.

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, and we may ask to take some blood samples. Whether you come for this last visit is up to you.

Injuries

22. 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 vaccine and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

Some of the study product providers have agreed to pay medical costs for study-related injuries that are determined to be caused by their own study products. If provider funds are not available or are not enough, or if the injury is determined to be caused by study procedures, the HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale-appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Questions

23. 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].

US sites include this paragraph if this sIRB will be used: This study has been reviewed and approved by a committee called the Fred Hutchinson Cancer Research Center Institutional Review Board. If you have questions about your rights as a research participant, or problems with or concerns about how you are being treated in this study, contact the Director at 206-667-5900 or irodirector@fredhutch.org.

You may also contact the [name of local IRB/EC] at [insert contact information].If you want to leave this study, contact
[name or title and telephone number of the investigator or other study staff].

Your permissions and signature

24. In Section 14 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.

25. 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 E Sample informed consent form for Part B

Title: A first-in-human Phase 1 clinical trial to evaluate the safety and immunogenicity of stabilized CH505 TF chTrimer in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 300

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 18-55, HIV negative and healthy.
- The purpose of this study is to see how a person's immune system responds to the study vaccine.
- The study will also see if the study vaccine is safe to give to people and does not make people too uncomfortable.
- You will be in this study for up to 18 months of clinic visits, with a follow up contact 1 year after your last vaccination to check on your health.
- Procedures will include blood draws and injections of study vaccine. We will tell you more about these procedures later in this consent form.
- There are risks from participating. The most common risks are:
    - Because this vaccine has only been given to 13 people in Part A of this study, we do not know what all of the risks may be
    - Taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding.
    - We will tell you more information about risks later in this consent form.
- We do not expect the study vaccine 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 an HIV vaccine. HIV is the virus that causes AIDS.

This study originally planned to give the study vaccine to 13 participants. Those participants have been enrolled. The researchers have now decided to make changes to the dose of the study products and to test these new doses alone and together in more participants. We will now call the first part of the study that enrolled 13 participants Part A, and the part that will enroll more participants will be Part B. Part B of this study will enroll about 36 people. You are being invited to participate in Part B.

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

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

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

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

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

3. **This study vaccine is experimental.**

    We are testing an experimental vaccine called CH505 TF chTrimer. We will call it the protein vaccine. It is a man-made piece of protein that looks like a protein found on the outside of HIV. This protein is being mixed with an adjuvant. An adjuvant is something added to a vaccine to help the immune system respond better. In Part A, the adjuvant was 3M-052-AF + Alum. In Part B, the adjuvant will be either 3M-052-AF + Alum or just 3M-052-AF alone. From here on, we will call the protein-adjuvant combinations "the study products". We do not know if the study products will be safe to use in people, or if they will work to prevent HIV infection. These study products are used only in research studies.

    The protein part of the study vaccine was developed by the Duke Human Vaccine Institute and is being provided by Duke University in Durham, North Carolina, USA. 3M-052 was developed by 3M Corporation, headquartered in St. Paul, Minnesota, USA. In this study, 3M-052-AF is being provided by the Access to Advanced Health

Institute in Seattle, Washington. Alum is being provided by the Division of AIDS at the US National Institutes of Health in Rockville, Maryland, USA.

The protein part of the vaccine has been engineered to stay in a shape much like natural HIV. In this way it is different from proteins used in some other HIV study vaccines.

3M-052 is one of many similar products developed by 3M Corporation to treat skin conditions, tumors, and to make vaccines more effective. It is an experimental product. It is designed to stimulate parts of the immune system that recognize invaders like viruses. In this study, 3M-052-AF is dissolved in water. Alum is an approved adjuvant that has been used in commercial vaccines for more than 90 years.

The protein and 3M-052-AF + Alum were given together to people for the first time in Part A of this study. As of September 7, 2022, all 13 people in Part A have gotten at least 1 injection, 10 have gotten at least 2 injections, 9 have gotten at least 3 injections, 9 have gotten 4 injections and 6 have gotten all 5 injections. These injections have not caused any serious health problems.

All people had some side effects that they described as mild to moderate. One (1) person had severe pain/tenderness in both the right and left injection sites 3 days following the fourth vaccination, though it lasted only one day. Five (5) people had severe side effects related to the vaccinations, including chills, headache, muscle aches, and generally feeling unwell. These severe side effects went away within 2 days. Of the 5 people who had severe side effects, 2 decided to stop getting study injections because they felt generally unwell after them and it was affecting their daily lives. Another 2 of the 5 people who had severe side effects got more injections; one of them had more severe side effects while the other did not. And one (1) of the 5 people who had severe side effects has not yet received another injection.

Two more people also decided to stop getting the study injections and dropped out of the study. One stopped because they experienced a panic attack after the first injection. However, this person had a history of panic attacks before being part of this study. The other did not return for their next injection visit and is no longer in the study. We do not know the reason for this.

A protein-adjuvant combination very similar to the combination in this study is being given to people for the first time in another study called HVTN 137. That study gives some people a study vaccine and some people a placebo which does not contain any study vaccine. The study has two parts, Part A and Part B. As of April 2022, 17 people in Part A have had 2 injections (15 people got the study vaccine and 2 people got a placebo). These injections have not caused any serious health problems.

In HVTN 137 Part A, two people had redness and swelling over a large area on their arm where they got the injection. The swelling started about 1 week after the injection. The redness lasted for about 2 to 3 days. For 1 of these people, the swelling went away within 2 to 3 days. The second person had significant swelling for about 3 days. The swelling went down but it took about 5 weeks to completely go away. They

had mild pain which did not prevent them from going to work. Four other people had symptoms that did prevent them from going to work. All 4 felt severely tired and generally unwell. Two of them also had severe muscle and joint pain and 1 had severe headache and chills. For these 4 people, all of these symptoms lasted 1-2 days. Because the study is ongoing and is blinded, we do not know if these people got the study vaccine or placebo.

HVTN 137 was later updated to offer people in Part A a third injection and 9 out of the 17 people agreed to it. The third injection has not caused any serious health problems.

In Part B of HVTN 137, 88 people have gotten either the study vaccine and adjuvant combinations or placebo injections. These injections have not caused any serious health problems.

Given the side effects experienced to date in HVTN 300 and HVTN 137 and in Part A of this study, we expect that many people in Part B of this study will have some side effects. The side effects we expect would be similar to those listed in the Risks of vaccines section below.

Risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, dizziness, and feeling tired. Vaccines can also cause redness or itching where you got the injection. Most side effects do not interfere with daily activities or make a person visit the doctor.

Rarely, a vaccine can cause an allergic reaction, including a rash, hives, or trouble breathing. Allergic reactions can be life-threatening. Tell us if you ever had a bad reaction to an injection or vaccine.

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, that will not affect your other care at this clinic and the benefits or rights you would normally have.

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.

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 also test you for Hepatitis B and Hepatitis C. 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.

   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 G, 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. For this reason, you must agree to use effective birth control from 21 days before your first injection until 8 weeks after the time of your fifth study injection (14 months). 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 up to [#] times over 18 months.**

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

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

   A study visit can be done in person at the clinic. They may also be done using tools such as phone calls, text messages, web platforms (sometimes called "telemedicine") or email. A combination of these types of visits might also be used.

   You may have 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 two of the study procedures. We will tell you more about that below.

   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 products on a schedule.**

    When you join the study, you will be assigned to 1 of 3 groups. Each group will get a different combination of the study products as shown in the table below. Each combination will have the same protein vaccine at the same dose that was given in Part A. Group 2's combination will have a lower dose of the adjuvant 3M-052-AF than was given in Part A and will not include Alum. Group 3's combination will also have a lower dose of 3M-052-AF than was given in Part A and will also have the same dose of Alum that was given in Part A. Group 4's combination will have the same dose of 3M-052-AF that was given in Part A, but will not include Alum.

    All groups will get injections of their study products at 5 visits, according to the schedule in the table below. At these 5 visits, you will get 2 injections, 1 into the deltoid muscle of both upper arms. We will give the injections with a needle and syringe.

In Part A of this study there were two pauses when safety information was reviewed to decide if it was safe to continue the study. There have been no serious health problems in Part A participants, so it was determined to be safe to continue.

Similarly, in Part B of the study, there will be a pause after the first 5 people in Group 2 have their first injection visit, to determine if the rest of the people in Part B should be enrolled.

Group	Study products	First injection visit	2 months later	4 months later	8 months later	12 months later
2	Study vaccine + lower dose study adjuvant	✓	✓	✓	✓	✓
3	Study vaccine + lower dose study adjuvant combined with Alum	✓	✓	✓	✓	✓
4	Study vaccine + higher dose study adjuvant	✓	✓	✓	✓	✓

You will have to wait in the clinic for about an hour after getting injections to see if there are any problems. We will measure your temperature, blood pressure, heart and breathing rates. We will also look at your injection sites and ask how you feel. Then for that night and for 7 more days, you will use a secure online symptom log to keep track of how you are feeling. If you are unable or unwilling to use the online symptom log, please talk with us about other options that may be available. Within 4 days after each injection visit, we will contact you 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 the study vaccine, we will do the procedures shown in the following table.**

	Time after first injection visit												
Procedure	**Screening visit(s)**	**First injection visit**	**2 weeks**	**2 months**	**2½ months**	**4 months**	**4½ months**	**8 months**	**8½ months**	**12 months**	**12½ months**	**18 months**	**2 years**
Injection		√		√		√		√		√			
Medical history	√												
Complete physical	√											√	
Brief physical		√	√	√	√	√	√	√	√	√	√		
Blood drawn	√	√	√		√	√	√	√	√	√	√	√	
Pregnancy test*	√	√		√		√		√		√		√	
HIV testing	√					√		√		√		√	
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√

* For persons who are capable of becoming pregnant. Persons who have had a hysterectomy (removal of the uterus), 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. It will be some amount between 10 mL and 250 mL (about 1 tablespoon to a little more than one cup). Your body will make new blood to replace the blood we take out.

We will review test 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. **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.

13. **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 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 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). It will involve 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.

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

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

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

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

Will the HVTN sell my samples and information? No, but the HVTN may share your samples with HVTN or other researchers. Once 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.

15. **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 online symptom log after the injection visits, that information only has your code number. Your data goes directly from the online symptom log 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,
- Any regulatory agency that reviews clinical trials,
- *US sites may, but are not required to include:* [Insert name of local IRB/EC] ,

- *US Sites include if this sIRB will be used:* Fred Hutchinson Cancer Research Center Institutional Review Board
- [Insert name of local and/or national regulatory authority as appropriate]
- The Duke Human Vaccines Institute, AAHI, DAIDS and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will 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]

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. The Web site may include a summary of the study results. You can search this Web site at any time.

16. **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 but it will be your choice. 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 also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study for up to 12 months if you choose. 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 stop your injections if you enroll in a different research study where you get another study product.

17. **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

18. **There are other risks to being in this study.**

    In addition to the risks of the study vaccine 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 abnormal laboratory results

    Minor changes in laboratory test results occasionally happen. This means that the test results can show something to be abnormal when it is not. If this happens, we will ask you to come back to the clinic to be retested. This may cause you to worry, and it may be inconvenient to come back to the clinic. If retesting confirms something to be abnormal, we will provide care or help you get the care you need.

    Risks of routine medical procedures:

    Routine medical procedures such as taking blood and giving injections 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 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 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 someone who should not may see your personal information. 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 vaccine will increase, decrease, or not change your risk of getting HIV if exposed. If you get HIV, we do not know how the study vaccine might affect your HIV infection or how long it takes to develop AIDS.

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

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

Benefits

19. **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 vaccine later becomes approved for sale, there are no plans to share any money with you.

Your rights and responsibilities

20. **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

21. **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.

    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, and we may ask to take some blood samples. Whether you come for this last visit is up to you.

Injuries

22. **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 vaccine and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

    Some of the study product providers have agreed to pay medical costs for study-related injuries that are determined to be caused by their own study products. If provider funds are not available or are not enough, or if the injury is determined to be caused by study procedures, the HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale-appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Questions

23. **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].

    US sites include this paragraph if this sIRB will be used: This study has been reviewed and approved by a committee called the Fred Hutchinson Cancer Research Center Institutional Review Board. If you have questions about your rights as a research participant, or problems with or concerns about how you are being treated in this study, contact the Director at 206-667-5900 or irodirector@fredhutch.org.

    You may also contact the [name of local IRB/EC] at [insert contact information].If you want to leave this study, contact
    [name or title and telephone number of the investigator or other study staff].

Your permissions and signature

24. **In Section 14 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.

25. **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 F Low risk guidelines (for U.S. studies)

The following are intended as guidelines for the investigator to help identify potential vaccine trial participants at "low risk" for HIV infection. These guidelines are based on behaviors within the last 6-12 months prior to enrollment; however, it may be appropriate to consider a person's behavior over a longer period of time than specified to assess the person's likelihood of maintaining low risk behavior. Some volunteers may not be appropriate for enrollment even if they meet these guidelines. These guidelines should be supplemented and interpreted with local epidemiologic information about HIV prevalence in your area and community networks. The investigator may review the risk level of any volunteer with the site PI and/or the Protocol Safety Review Team.

A volunteer may be appropriate for inclusion if he/she meets these guidelines:

1. Sexual behaviors

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

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

AND

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

In the **last 6 months**:

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

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

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

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

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

AND

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

2. Non-sexual behaviors

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

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

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

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

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

For US volunteers on Pre-exposure prophylaxis (PrEP)

1. PrEP ASSESSMENT

- Reports equal to or greater than six consecutive months of protective PrEP use
- Commits to maintaining protective PrEP use throughout trial
- Participant 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?"*

2. SEXUAL BEHAVIORS

- Persons stably taking PrEP as prescribed above for 6 months or longer are considered low risk of HIV infection, regardless of any sexual behavior that might otherwise be associated with high risk of HIV exposure.

3. NON-SEXUAL BEHAVIORS

In the **last 12 months** did 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 G 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 vaccine could affect the developing baby.

You must agree to use effective birth control from 21 days before your first injection until 8 weeks after the time of your fifth study injection (14 months).

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 H 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 300 Study Specific Procedures.*

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

Appendix I Protocol team

Protocol leadership

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Community Advisory Board (CAB) members	Harrison Knowlton Boston HIV Research CAB	*Community engagement unit representative*	Gail Broder HVTN Core, Fred Hutch
	John Isaac Boston HIV Research CAB	*Community educator/recruiter*	Adrianna Boulin Fenway Health

Appendix J 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 300 are described below.

Protocol history and modifications

Date: September 26, 2022

Protocol version: Version 2.0
Protocol modification: Full protocol amendment 1

Item 1 Updated in Section 1, *Executive summary*; Section 2.2, *Clinical experience with recombinant Env vaccines*; Section 2.5, *Rationale for Dose of Adjuvant*; Section 3, *Objectives and Endpoints for Part A and Part B*; Section 5, *Study design*; Section 6, *Statistical considerations*; Section 7, *Study vaccine preparation, storage, and administration*; Section 9.7, *Initial safety review for Part B, Group 2*; A new Appendix B, *Schedule of procedures for Part B* and a new Appendix E, *Sample informed consent (SICF) for Part B*: Information related to the addition of Part B and recruitment of additional participants

Item 2 Updated in Section 2.2, *Clinical experience with recombinant Env vaccines*: Updated safety data for HVTN 137 and HVTN 300 Part A

Item 3 Added in Section 2.2, *Clinical experience with recombinant Env vaccines*: Rationale for 7-day reactogenicity monitoring period

Item 4 Revised in Section 5.1.2, *Exclusion criteria*: Exclude volunteers with congenital or acquired immunodeficiency

Item 5 Updated in Section 1.3, *Study products*; Section 7.2.4, *Tris-NaCl buffer (TBS) (Diluent) for Part A*; new Section 7.2.5, *Sodium Chloride for injection, 0.9% USP (Diluent) for Part B* and Section 7.3, *Product preparation*: Sodium Chloride diluent for Part B

Item 6 Clarified in Section 5.2.2, *Discontinuation of study vaccine administration*: Assessment of grade 3 adverse event (AE)

Item 7 Clarified in Section 8.7, *Monitoring for HIV infection*: Included reference to HVTN HIV testing algorithm

Item 8 Added in Section 10.1, *Protocol conduct*: Stipulation for Protocol monitoring

Item 9 Revised in Section 1.5, *Study plan and schema table*; Section 9.5, *Initial safety review for Part A* and Section 9.6, *Interim safety review for Part A*: Updated safety reviews

Item 10 Added in Section 5.1.2, *Exclusion criteria*; Section 5.2.1, *Delaying vaccinations for a participant* and in Section 8.3, *Reactogenicity assessments*: considerations for timing of receipt of vaccines for Monkeypox

Item 11 Corrected in Appendix A, *Schedule of procedures for Part A*: corrected errors in blood volume.

Item 12 Revised in Section 7.6, *Study vaccine accountability*: Deleted language related to randomization

Item 13 Revised in Section 2.8.1, *Potential Risks*: Included reference to risks associated with 3M-052-AF + Alum

Item 14 Updated Appendix I, *Protocol Team:* Included new team members

Item 15 Updated in Title page; Appendix J, *Version history*; *Acronyms and abbreviations* and Section 12, *Literature cited*: Contents of this amendment

Item 16 Updated throughout the Protocol: Name of the study product provider

Item 17 Updated throughout the Protocol: Formulation of the adjuvant 3M-052-AF

Item 18 Corrected throughout the Protocol: Minor errors in grammar, typography, formatting

Item 19 Updated throughout the Protocol: Section headers, Section sub- headers, Section numbering and cross-references

Item 20 Updated per Protocol Version 1.0, Letter of Amendment 1, dated Feb 09, 2021

Item 21 Updated per Protocol Version 1.0, Clarification Memo 1, dated Aug 16, 2022

Date: August 16, 2022

Protocol version: Version 1.0
Protocol modification: Clarification Memo 1

Item 1 Added in Section 9.4, *Total blood volume*: alternate laboratory specimen tube types allowed for research samples upon HVTN laboratory center approval

Date: February 09, 2021

Protocol version: Version 1.0
Protocol modification: Letter of Amendment 1

Item 1 Updated the Protocol Title page: added IND number 027197 for this protocol

Item 2 Clarified in Section 5.1.2, *Exclusion criteria*: criterion 8 referring to serious reactions to vaccines or any component of the study vaccine

Item 3 Added in Section 5.2.2, *Discontinuation of study vaccine administration*: definition of clinically significant type 1 hypersensitivity

Date: December 16, 2020

Protocol version: 1.0
Protocol modification: Original protocol