HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 305

A phase-1 open-label clinical trial to evaluate the safety and immunogenicity of synthetic DNAs encoding NP-GT8 and IL-12, with or without a TLR-agonist–adjuvanted HIV Env Trimer 4571 boost, in adults without HIV

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CLINICAL TRIAL SPONSORED BY

Division of AIDS (DAIDS)
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STUDY PRODUCTS PROVIDED BY

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Protocol Signature Page

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

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

DAIDS Protocol Number: HVTN 305

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Acronyms and abbreviations

AAHI	Access to Advanced Health Institute
Ab	antibody
ADCC	antibody-dependent cell-mediated cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
AE	adverse event
AESI	adverse event of special interest
AF	aqueous formulation
AoU	assessment of understanding
ALT	alanine transaminase
Alum	aluminum hydroxide suspension
Anti-HCV	anti-Hepatitis C antibody
AUC	area under the curve
β-HCG	beta human chorionic gonadotropin
BCR	B-cell receptor
BMI	body mass index
bnAb	broadly neutralizing antibody
BP	blood pressure
CAB	Community Advisory Board
CBC	complete blood count
CI	confidence interval
CMIA	chemiluminescent microparticle immunoassay
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS	clinical research site
CSS	clinical safety specialist
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS
DM	diabetes mellitus
EAE	expedited adverse event
EC	Ethics Committee
ECLIA	electrochemiluminescence
eGFR	estimated glomerular filtration rate
EIA	enzyme immunoassay
EMPEM	electron microscopy-polyclonal epitope mapping
Env	HIV/HIV-1 envelope protein
EP	electroporation
FDA	US Food and Drug Administration
FNA	fine-needle aspiration
GCP E6 (R2)	ICH Guideline for Good Clinical Practice

GINA	Genetic Information Nondiscrimination Act
GPP	Good Participatory Practices
HSV2	herpes simplex virus type 2
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICH	International Council for Harmonization
ICS	intracellular cytokine staining
ID	intradermal
IFN-gamma	interferon-gamma
IL-12	Interleukin 12
IM	intramuscular
IND	investigational new drug (application)
INR	international normalized ratio
IRB	Institutional Review Board
IUD	intrauterine device
LABA	long-acting beta agonist
MAAE	medically attended adverse event
MBL	mannose-binding lectin
MedDRA	Medical Dictionary for Regulatory Activities
MO	medical officer
MOP	Manual of Procedures
MSD	Meso Scale Discovery
MSM	person born male with partner(s) born male
nAb	neutralizing antibody
NAT	HCV nucleic acid test
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	US National Institutes of Health
NLT	native-like trimer
NSAID	nonsteroidal anti-inflammatory drug
OHRP	US Department of Health and Human Services Office for Human Research Protections
PAB	DAIDS Pharmaceutical Affairs Branch
PAGE	polyacrylamide gel electrophoresis
PBMC	peripheral blood mononuclear cell
PEF	peak expiratory flow
PI	principal investigator
PID	pelvic inflammatory disease
POPIA	Protection of Personal Information Act

PrEP	HIV pre-exposure prophylaxis
PSRT	Protocol Safety Review Team
PV	PENNVAX-B DNA vaccine
RAB	DAIDS Regulatory Affairs Branch
RBD	receptor binding domain
RE	Regulatory Entity
RM	rhesus macaque
RML	Regional Medical Liaison
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SAP	statistical analysis plan
sD-NP-GT8	synthetic-DNA–encoded nanoparticle GT8
SDMC	statistics and data management center
SHIV	simian-human immunodeficiency virus
SICF	sample informed consent form
SMB	Safety Monitoring Board
SOP	Standard Operating Procedures
SPT	DAIDS Safety and Pharmacovigilance Team
SSP	Study-specific Procedures
SUSAR	suspected unexpected serious adverse reaction
TDS	Ichor Medical Systems TriGrid Delivery System
TEM	transmission electron microscopy
TLR	toll-like receptor
ULN	upper limit of normal
VISP	vaccine-induced seropositivity
VRC	Dale and Betty Bumpers Vaccine Research Center
VSV	vesicular stomatitis virus
WBC	white blood cell
SWFI	sterile water for injection

Contents

1 Executive summary

1.1 Title

A phase-1 open-label clinical trial to evaluate the safety and immunogenicity of synthetic DNAs encoding NP-GT8 and IL-12, with or without a TLR-agonist–adjuvanted HIV Env Trimer 4571 boost, in adults without HIV.

This is a randomized trial to examine safety and immunogenicity of INO-6172 (synthetic DNAs encoding the GT8 nanoparticle and Interleukin-12), alone and with HIV envelope protein (Env) Trimer 4571 adjuvanted with 3M-052-AF + Alum. The primary hypothesis is that INO-6172 will elicit VRC01-class B-cell responses as well as antigen-specific T-cell responses.

1.2 Study products

- **INO-6172 (sD-NP-GT8 coformulated with IL-12 DNA (pGX6001)):** Synthetic-DNA–encoded nanoparticle GT8 (sD-NP-GT8) was developed by the Wistar Institute and consists of a single plasmid, pGX1072 (in pGX0001 vector backbone), encoding the soluble self-assembling nanoparticle vaccine, which is decorated with a germline-targeting, B-cell precursor activating immunogen GT8. pGX6001, developed by Inovio Pharmaceuticals, is derived from a pGX0003 backbone. pGX6001is a single plasmid, which contains a dual promoter system for the expression of both the IL-12 p35 and p40 genes, necessary for production of the active heterodimeric IL-12 protein. The plasmid ratio is 4:1 sD-NP-GT8: IL-12 DNA (0.8 mg pGX1072/0.2 mg pGX6001 or 0.2 mg pGX1072/0.05 mg pGX6001) per 0.1-mL injection.

- **Trimer 4571:** HIV-1 Env Trimer 4571 (VRC-HIVRGP096-00-VP) is a soluble protein that consists of BG505 DS-SOSIP.664 gp140 Env and is supplied as a sterile, aqueous, buffered solution filled into single-dose vials at a concentration of 500 mcg/mL and a volume of 1.2 mL in 3-mL glass vials. Trimer 4571 is provided by the Dale and Betty Bumpers Vaccine Research Center (VRC) and will be used at a dose of 100 mcg.

- **3M-052-AF:** This adjuvant is an aqueous formulation (AF) of the small molecule imidazoquinoline, which works as a toll-like receptor (TLR) 7/8 agonist. To be administered at 5 mcg admixed with 100 mcg Trimer 4571 and 500 mcg Alum.

- **Aluminum Hydroxide Suspension (Alum) adjuvant:** Alhydrogel at 500 mcg will be admixed with 5 mcg 3M-052-AF along with 100 mcg Trimer 4571.

1.3 Study devices

Electroporation device: The Inovio CELLECTRA Adaptive Constant Current Electroporation (EP) Device is a portable, battery-powered medical device designed to facilitate the introduction of DNA into skin through EP. The Inovio CELLECTRA 2000 will be used for intradermal (ID) delivery following Mantoux injection of the DNA vaccine, which is provided by Inovio Pharmaceuticals.

1.4 Study population

Forty-five healthy volunteers without HIV-1 aged 18 to 55 years, inclusive.

1.5 Study plan and schema table

Participants will receive INO-6172 (sD-NP-GT8 + IL-12 DNA) vaccinations at months 0, 1, and 3, with a total dose of 0.5 mg (Group 1) or 2 mg (Groups 2 and 3). For Group 1, sD-NP-GT8 DNA will be administered at a dose of 0.4 mg along with IL-12 DNA at a dose of 0.1 mg (0.5 mg total). For Groups 2 and 3, sD-NP-GT8 DNA will be administered at a dose of 1.6 mg along with 0.4 mg of IL-12 DNA (2 mg total). These will be administered intradermally via EP of the skin. Due to volume limitations of the device, the dose of the DNA will be administered as 2 separate intradermal (ID) injections. Each of 2 sites will receive 0.1 mL of study product (either the 1.0-mg/0.1-mL concentration or the 0.25-mg/0.1-mL concentration) via ID injection (Mantoux injection) bilaterally 1 on each upper arm. Following ID injections, EP will be performed with the Inovio CELLECTRA 2000 EP device.

A total of 18 out of 45 participants (Group 3) will also be administered Trimer 4571 at a total dose of 100 mcg at months 3 and 6. The Trimer dose is split over 2 injection sites. The injections are delivered intramuscularly (IM) via needle and syringe, 1 in each deltoid muscle. Participants will be evaluated for safety and immune responses through blood collection, lymph node fine-needle aspiration (FNA), and leukapheresis at specified timepoints throughout the study. The study schema is presented below in Table 1-1:

Table 1-1 Schema

				Injection schedule in months (days)			
Group	**N**	**Product/Dose**	**Route**	**Month 0 (Day 1)**	**Month 1 (Day 29)**	**Month 3 (Day 85)**	**Month 6 (Day 169)**
1**	9	INO-6172 (0.5 mg)	ID EP (2 injections; 1 into skin of each upper arm)	**X**	**X**	**X**	**--**
2§	18	INO-6172 (2 mg)	ID EP (2 injections; 1 into skin of each upper arm)	**X**	**X**	**X**	**--**
3§	18	INO-6172 (2 mg)	ID EP (2 injections; 1 into skin of each upper arm)	**X**	**X**	**X†**	**--**
		Trimer 4571 (100 mcg) + 3M-052 AF (5 mcg) / Alum (500 mcg)	IM (2 injections; 1 into each deltoid)	**--**	**--**	**X†**	**X**
Total	**45***						

Notes:

*Up to 9 additional participants may be enrolled (for a total of up to 54), if needed, to have approximately 45 participants contribute to immunogenicity analyses. Specific scenarios that could necessitate 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, missing vaccine visits, inability to complete FNA, or variations in the clinical care due to unpredictable events. Participants will not be replaced after completion of visit 6, the first FNA visit.

** Initial enrollment will be restricted to 1 participant per day for the first 5 participants in Group 1, and enrollment will pause after the first 5 participants are enrolled in Group 1. The Protocol Safety Review Team (PSRT) will review cumulative safety information for these first 5 participants recorded through the visit scheduled 2 weeks post–first vaccination and will determine whether it is safe to proceed with full enrollment in all groups.

†For the first five participants in Group 3, the Month 3 vaccination will be limited to 1 participant per day.

[§]FNA will not be conducted for Group 1 but may be optional for Groups 2 and 3 and will be offered to participants during screening. A minimum target of 9 participants in these groups will be asked to provide consent for receiving the FNA.

1.6 Duration per participant

The total duration per participant is 18 months, including 12 months of scheduled clinic visits (main study) followed by an adverse event of special interest (AESI) health contact at month 18.

1.7 Estimated total study duration

At least 22 months (includes enrollment, planned safety holds, follow-up, and AESI health contact).

1.8 Study sites

HIV Vaccine Trials Network (HVTN) clinical research sites (CRSs) in the US and South Africa, to be specified in the Site Announcement Memo.

2 Introduction

This first-in-human study will evaluate the safety and immunogenicity of a synthetic-DNA–encoded, self-assembling nanoparticle (sD-NP-GT8). NP-GT8 is an engineered outer domain of gp120 containing the CD4bs with an optimized human IgE-leader, which is displayed on a lumazine synthase scaffold to form a self-assembling 60-mer nanoparticle. NP-GT8 targets VRC01-class germline cells (1, 2). The immunization series will include priming with a synthetic DNA plasmid (pGX1072/sD-NP-GT8) encoding the nanoparticle displaying GT8, adjuvanted with a plasmid encoding human IL-12 (pGX6001) and boosting with sD-NP-GT8/pGX6001 alone and in combination with an HIV envelope soluble protein trimer, Trimer 4571, adjuvanted with 3M-052-AF + Alum. The DNAs will be delivered via ID EP, and the protein boost with Trimer 4571 will be administered via IM injection.

Preclinical data show that self-assembling nanoparticle vaccines enhance the kinetics of particle trafficking to the draining lymph nodes, and the uptake by the dendritic cells and macrophages (3-5). Multivalent antigen display on self-assembling nanoparticles can also enhance B-cell activation, thereby boosting the magnitude, affinity, and durability of humoral responses (3, 6, 7).

The long-term utility of this platform will be to employ sD-NP-GT8 as both a prime and boost with engineered booster immunogens to produce broadly neutralizing antibodies (bnAbs), to employ sD-NP primes and heterologous boosts for alternative epitopes, or potentially to employ sD-NP cocktails to provide diverse HIV isolates and generate broader responses.

Self-assembling antigens encoded into a DNA plasmid can be delivered in vivo, bypassing laborious in vitro protein purification steps. DNA is easily mass produced, temperature stable, easy to deliver clinically using the new ID EP technologies, and can be rapidly reformulated for new vaccine immunogens, making it an attractive vaccine technology for iterative HIV-1 vaccine studies (8, 9).

Advantages of the synthetic DNA vaccine platform with adjuvant IL-12 DNA (pGX6001), especially when administered in combination with native-like trimer (NLT) protein boosts, include:

- Potential to elicit more robust immune responses than DNA alone;
- Potential dose sparing of DNA vaccine required in the regimen; and
- Unique induction of cellular responses to act synergistically with induced humoral responses.

This clinical trial will inform future precursor-activating/germline-targeting/lineage B-cell–focused nanoparticles and DNA clinical studies. It will be important to discover the immune responses (both B cell and T cell) to the self-

assembling nanoparticles delivered by this advanced DNA platform, and to compare the responses to other studies using alternate methods of antigen delivery. In addition, this study can help answer the question in human clinical HIV-1 vaccinology of whether activation of bnAb precursors can be accomplished by repeated boosts with multiple engineered Envs delivered via DNA vaccination, or if a wild-type HIV-1 Env administered as a protein is capable of maturing and broadening desired antibody (Ab) responses.

Clinical evaluation of sD-NP-GT8 will allow examination of important goals in the HIV vaccine field, such as: (1) activation of CD4bs bnAb precursors by a self-assembling nanoparticle that is produced in vivo and can be trafficked in a mannose-binding lectin (MBL)–independent manner and (2) evaluation of advanced nucleic-acid–delivered self-assembling vaccines, which could change the clinical landscape for many future studies.

2.1 Rationale for evaluation of sD-NP-GT8

Single immunization of adjuvanted GT8 has been shown to be capable of priming germline VRC01-class responses. One example is the eOD-GT8-60mer, a self-assembling nanoparticle targeting VRC01-class precursors (4, 11-19). Preliminary results from a proof-of-concept trial (IAVI G001; clinicaltrials.gov identifier: NCT03547245) using vaccination with a recombinant eOD-GT8-60mer protein showed that VRC01-class precursor Abs were elicited (10), and these data strongly support the goals of this current protocol. We built upon this concept by developing DNA-launched nanoparticles encoding the GT8 immunogen (sD-NP-GT8). Vaccination with the sD-NP-GT8 nanoparticle elicits strong humoral and cellular responses. As with the recombinant protein, sD-NPs can induce similar levels of immunogenicity and develop human Ab responses in human Ab repertoire mice ((4) and unpublished).

2.2 Preclinical data: mice, guinea pigs and macaques

In a recent study in mice (4), we developed a synthetic-DNA–based nanoparticle platform (sD-NP) and observed in vivo assembly of nanoparticles as measured by muscle immunofluorescence, native polyacrylamide gel electrophoresis (PAGE) of muscle homogenates, transmission electron microscopy (TEM) of thin muscle sections and multimeric binding of MBL (see Figure 2-1) (4). This is the first time that in vivo assembly of a designed, self-assembling nanoparticle delivered by DNA has been demonstrated. sD-NP provides rapid seroconversion and is significantly more immunogenic than monomeric antigens in both the phase immediately postvaccination and the long-term timepoints. We also observed large dose-sparing effects, as a 25-fold lower dose of sD-NP can produce 10 times higher Ab titers compared to monomeric antigens. The sD-NP platform also produced strong CD8+ T cells as compared to recombinant protein with a number of distinct adjuvants.

The strong immunogenicity of the sD-NP platform was also demonstrated in guinea pigs (Figure 2-2) (4). Additionally, we observed that the sD-NP platform can function independently of the MBL-complement pathway, unlike HIV Env-decorated protein self-assembling nanoparticles. This observation is likely of interest for clinical translation of such vaccines, as approximately 5% to 20% of human populations have MBL deficiency (plasma MBL < 100 ng mL–1) (11). In published macaque studies by other groups, DNA/Env-protein coadministration at the same site during each vaccination can improve induced binding and neutralizing Ab (nAb) responses (12) and can also improve challenge outcomes (13).

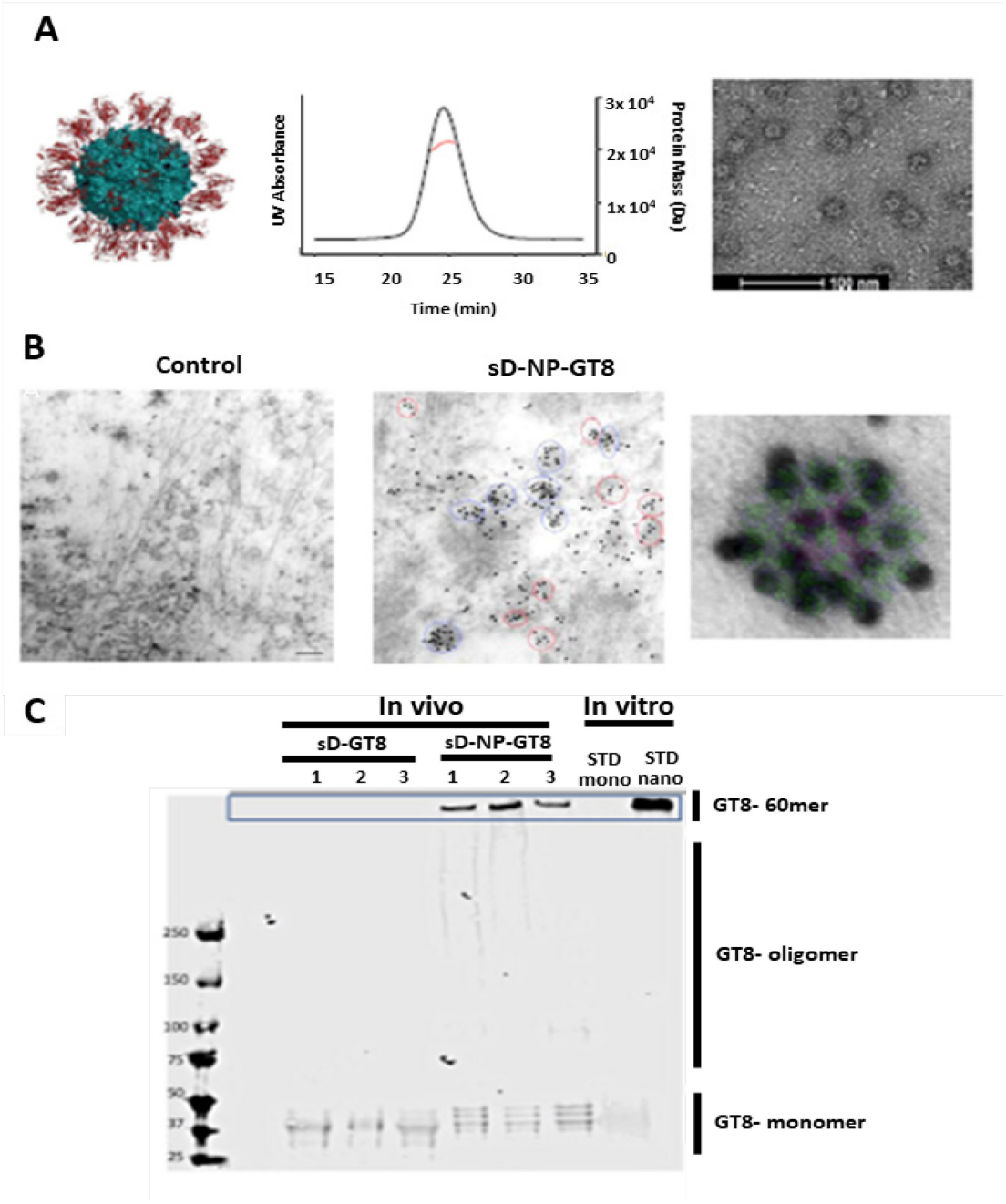

Figure 2-1 In vitro and in vivo assembly of sD-NP-GT8. (A) Nanoparticle structural model (left); size-exclusion chromatography and multiangle light scattering data with protein molecular weight on right y-axis (middle); negative stain EM imaging of NP-GT8 (right). (B) TEM images of muscle sections from mice injected with control or sD-NP-GT8 seven days postinjection that were immunolabeled with VRC01 and gold antihuman IgG. (C) Pseudonative PAGE analysis comparing migration of in vivo–produced sD-GT8 and sD-NP-GT8 to in vitro–produced SEC-purified recombinant GT8-monomer (labeled as STD mono) and eOD-GT8-60mer (labeled as STD nano) protein standards.

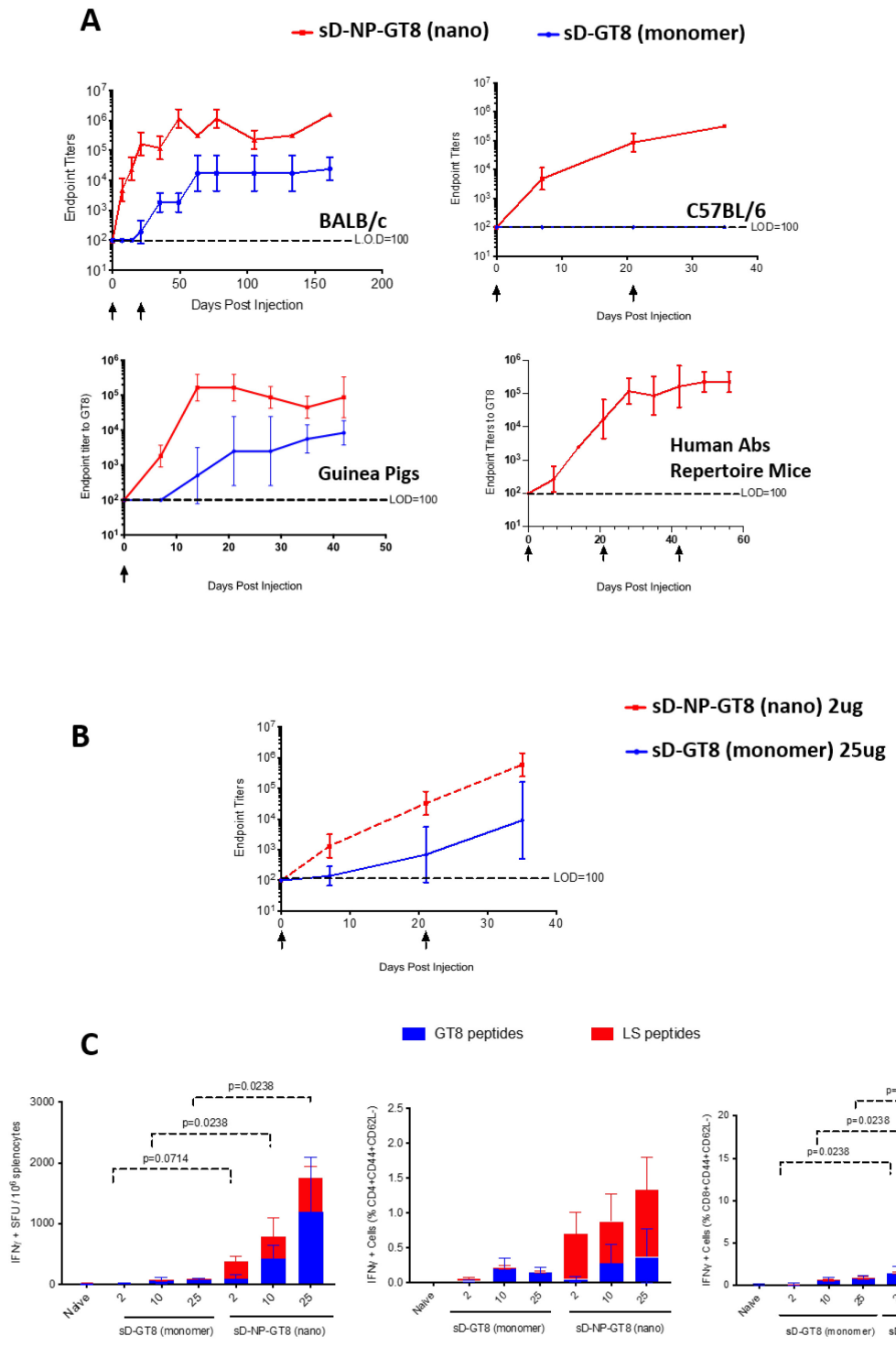

Figure 2-2 Enhanced immunogenicity of sD-NP-GT8. (A) Serology for 4 animal models immunized with sD-NP-GT8 compared to monomeric GT8 delivered by DNA. (B) Dose-sparing study showing that sD-NP-GT8 given at one-tenth of the dose induces > 1 log higher endpoint titers. (C) sD-NP-GT8 induces stronger CD4+ and CD8+ T cells than monomeric GT8.

2.3 Clinical experience with related DNA vaccines encoding HIV Env immunogens with and without IL-12 adjuvants

Table 2-1 Clinical trials with related DNA vaccines encoding HIV Env immunogens

Protocol	Products	N	Route of administration	Safety
HVTN 070 / NCT00528489	**PENNVAX-B DNA vaccine:** A mixture of 3 expression plasmids encoding HIV-1 clade B Env, Gag, and Pol **IL-12 plasmid DNA:** GENEVAX-IL-12-4532 expressing human IL-12 proteins p35 and p40 under separate regulatory control, formulated with bupivacaine **IL-15 DNA**	120	IM administration with needle and syringe	No severe systemic reactogenicity No related severe adverse events (AEs) One discontinuation due to possibly related AE (cervical radiculopathy) (14)
HVTN 080 / NCT00991354	**PENNVAX-B DNA vaccine:** A mixture of 3 expression plasmids encoding HIV-1 clade B Env, Gag, and Pol **IL-12 plasmid DNA:** GENEVAX-IL-12-4532, formulated with bupivacaine	48	IM administration with EP using VGX CELLECTRA EP system	No severe systemic reactogenicity No related severe AEs Two discontinuations due to injection-site pain (14)
HVTN 087 / NCT01578889	**HIV multiantigen DNA:** Consists of 2 plasmid DNA expression vectors, HIV-1 Gag/Pol and HIV-1 Nef/Tat/Vif, Env **IL-12 plasmid DNA:** GENEVAX IL-12-4532, formulated with bupivacaine **VSV-HIV Gag**	100	IM administration with EP using Ichor Medical Systems TriGrid Delivery System (TDS) EP device	No severe systemic reactogenicity No severe related AEs (15) (16)
HVTN 098 / NCT02431767	**PENNVAX-GP DNA vaccine:** Admixture of 2 plasmids encoding consensus clade A and C HIV-1 Env and 2 plasmids containing HIV-1 multiclade (A, B, C, and D) consensus Pol and Gag **IL-12 plasmid DNA:** Inovio INO-9012 expressing human IL-12 p35 and p40	94	IM and ID administration with EP using CELLECTRA 3P EP for ID delivery, and CELLECTRA 5P EP for IM delivery	No severe systemic reactogenicity No severe related AEs One participant discontinued due to pain, anxiety, and presyncopal episode (17, 18)

2.3.1 Safety of DNA Vaccines Encoding HIV Antigens Administered Via Electroporation

In general, DNA plasmids encoding HIV antigens coadministered with IL-12 plasmids via EP have a well- established track record for safety. The 4 most applicable trials are summarized in Table 2-1 above and include data from over 350 individuals. DNA vaccines were well tolerated, with no severe systemic reactogenicity and no severe related adverse events (AEs). Two participants who received IM injections discontinued further vaccinations due to pain at the injection site. It is important to note that there are differences between IM injection and ID injections followed by EP, with ID administration generally being less associated with injection-site pain.

Edupuganti et al reported on the safety, tolerability, and acceptability of IM and ID EP of the HIV-1 PENNVAX-GP DNA vaccine and IL-12 DNA in HVTN 098 (17). Systemic reactogenicity symptoms with IM injection of DNA vaccines and with ID injection of DNA vaccines did not differ (17). Injections were generally well tolerated but skin lesions (flat scars) occurred in 49% of participants with ID injections. These changes were found to be acceptable in 96% of participants and did not lead to vaccination discontinuation for any participant. Based on data from HVTN 098, there is no reason to suggest a different outcome. Accumulating safety data on trimer protein immunogens suggest no safety differences from monomers.

When the injection and EP are given in the skin, the needles from the EP device may leave marks, such as red bumps or scabs. The marks may heal later but may still leave light or dark spots or small scars. For some people, these marks have lasted 9 months or more (17). These marks tend to be more noticeable on darker skin.

In an ongoing phase 2/3 clinical trial (NCT04642638), a SARS-CoV-2 DNA vaccine is being administered with the aid of the Cellectra 2000 EP device. So far, over 3,200 participants have received over 9,300 active, blinded, or placebo product administrations via ID EP, across multiple countries. A few people have noticed skin discolorations after getting the vaccines, but so far, no health concerns have been reported. For more details, please see the Investigator's Brochure (IB).

2.3.2 Immunogenicity of DNA Vaccines Delivered Via EP

DNA vaccines can be efficiently introduced into cells via EP, as the transfection of DNA by in vivo EP allows entry of the macromolecules via a brief electrical field pulse. Previous approaches have used IM EP, but more recent approaches have delivered the DNA vaccine into the dermis (14, 15, 19). The dermis is an immunologic milieu rich in antigen-presenting cells (20).

Experience from the immunogenicity results from several prior clinical studies has informed the design of the current study. HVTN 070 and HVTN 080 tested the same PENNVAX-B DNA vaccine (PV), but with (HVTN 080) and without

(HVTN 070) IM EP (14). Comparing similar vaccine regimens between these trials (PV + IL-12) after the third vaccination, the HIV-specific CD4+ T-cell response rate increased from 19.2% to 80.8% and the CD8+ T-cell response rate increased from 6.9% to 51.9% when administered by EP. These results clearly demonstrated the markedly enhanced T-cell immunogenicity with EP, especially notable for CD8+ T cells that previously had not been induced well by DNA (21). The responses were primarily directed to Gag and Pol, and not to Env. Because of this, in HVTN 098, the Env DNA vaccine was modified, including 2 Env plasmids encoding clade A and C Env immunogens, and including a substitution of an optimized IgE leader sequence as well as the deletion of their cytoplasmic tail to improve surface expression (18). The dose of the Env plasmids was also increased relative to the Gag and Pol plasmids.

ID delivery is dose sparing. HVTN 098 compared IM and ID EP, with the EP administration at one-fifth the dose of the IM. CD4+ and CD8+ HIV-specific T-cell responses were equivalent for ID and IM, demonstrating the dose-sparing effect of ID EP, with the highest responses to Env. Unlike prior trials, Env-specific binding Ab responses were induced in the majority of vaccine recipients for both the ID and IM groups, with higher response rates and/or magnitudes for the ID + IL-12 group for some Env antigens. IgG-binding Abs to V1V2 Env antigens were detected in up to 56% of participants, with the highest responses from the ID + IL-12 group. Neutralizing Abs were mainly detected only to a tier-1A viral isolate. Only a few participants developed detectable Ab-dependent cellular cytotoxicity (ADCC), and these were mainly in the ID + IL-12 group. Thus, HVTN 098 demonstrated equivalent or sometimes superior immune responses for ID relative to IM (both including IL-12), and that the ID route was dose sparing.

Inclusion of IL-12 plasmid DNA. IL-12 induces the production of interferon-gamma (IFN-gamma), favoring the differentiation of T-helper cells (22, 23). Biologically active IL-12 has pleotropic effects but is generally considered to be a proinflammatory cytokine that biases the CD4+ T-cell response toward a Th1 phenotype. Previous studies have demonstrated that the inclusion of IL-12 (GENEVAX IL-12-4532) with HIV PENNVAX-B DNA was dose sparing (14). The inclusion of IL-12 did not affect the tolerability of an HIV DNA vaccine (24). Regarding the benefit of IL-12, in HVTN 080, the sample size of the group without IL-12 was small, likely reducing the ability to detect significant differences between the responses of the group with IL-12 and those of the group without IL-12. Although not statistically significant, the response rates including IL-12 were higher than those without IL-12 (CD4+ HIV-specific T-cell response rates increased from 44.4% to 80.8% and CD8+ increased from 33.3% to 51.9% after the third dose). In HVTN 098, the CD4+ HIV-specific T-cell response rate increased from 56.3% to 96.4% comparing the ID groups with and without IL-12 after the fourth dose. The binding Ab responses to Env for the ID groups with IL-12 and without IL-12 were similar after the fourth vaccination, but tended to be lower after the third vaccination for the group without IL-12, suggesting that the inclusion of IL-12 accelerated the response achieving near maximal Ab binding after the third rather than the fourth vaccination. The effect of IL-12 was examined in another HVTN study, HVTN 087, which tested increasing doses of

IL-12 included with DNA administered intramuscularly with EP for 3 doses followed by a vesicular stomatitis virus (VSV) boost (16). HIV-specific CD8+ T-cell response rates were generally increased when IL-12 was included, and the magnitude for these responses was significantly higher after the boost for the highest IL-12 dose group compared to the group without IL-12. Unexpectedly, the addition of IL-12 led to significantly decreased CD4+ HIV-specific T-cell response rates after the prime for the low- and medium-dose groups.

2.4 Safety of HIV Envelope proteins adjuvanted with 3M-052-AF + Alum: Clinical Experience

HVTN 137 (NCT04177355): HVTN 137 Part A is a first-in-human, double-blinded, dose-escalation study testing the combination of 100 mcg BG505 SOSIP.664 gp140 with 3M-052-AF at 2 doses, including 1 mcg (Group 1) and 5 mcg (Group 2), both combined with 500 mcg Alum. The study is ongoing and remains blinded to within-group treatment assignment. As of 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) have completed enrollment. Part A of the initial protocol specified that participants in Groups 1 and 2 would receive 2 total doses, one at month 0 and the other at month 2.

Five out of 6 participants received 2 doses of the 1-mcg dose (1 participant discontinued for reasons unrelated to vaccination) and 10 out of 11 participants received 2 doses of the 5-mcg dose. One participant in the 5-mcg dose group (Group 2) decided to discontinue vaccination due to Grade-3 induration/erythema first noted on Day 8 postvaccination. The maximum size of both the induration and erythema was measured at 17 x 17 cm on Day 8 (Grade 3). The erythema resolved over 3 days and the induration subsided to less than 5 cm on Day 11 (Grade 1). The induration was measured at 2 x 2 cm on Day 14 but did not completely resolve until 41 days postinjection. At no point was the pain/tenderness greater than mild, and the participant continued to work. It is unknown at present whether this participant received placebo or study product. Another participant in Group 2 experienced 2 days of Grade-3 induration and erythema from Days 6-7 postvaccination that resolved completely by Day 8. In consultation with the PSRT, 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. As of April 17, 2022, enrollment is complete and 88 participants have been enrolled. Of these 88 participants, 20 have been randomized to the 3M-052-AF group. All 88 participants have received at least two doses and 63 have received the third dose. 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. No additional grade 3 local reactogenicity events have been observed.

Overall, the BG505 SOSIP.664 with 3M-052-AF was generally well tolerated, with no unsolicited Grade-3 or -4 AEs, no related SAEs, AESIs, or deaths, and no unplanned study pauses. Other than the persistent erythema in 1 of the HVTN 137 Part A Group 2 participants described above, all reactogenicity symptoms resolved within 14 days and generally within 7 days. For more detailed information, please see the IB.

HVTN 300 (NCT04915768): This study is a first-in-human, unblinded trial testing a 300-mcg dose of a CH505 TF chTrimer (a stabilized, chimeric SOSIP Env trimer) in combination with 5 mcg 3M-052-AF + 500 mcg Alum delivered via split injection into both the right and left deltoids. The planned schedule is 5 total vaccinations at months 0, 2, 4, 8, and 12. 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 (5) participants have discontinued further vaccinations, 1 due to a panic attack after the first injection (this participant had a history of panic attacks before being part of the study), 3 due to reactogenicity events and 1 was lost to follow-up. Out of these 5, 2 terminated from the study early (panic attack and lost to follow-up) and 3 have remained in the study for follow-up.

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

Nearly 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 those participants received a subsequent vaccination without any Grade 3 reactogenicity, one participant experienced an additional repeat Grade 3 systemic reactogenicity symptom after a subsequent vaccination, one participant has not yet received a subsequent vaccination, and two (2) participants declined further 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.

2.5 Rationale for evaluation of 3M-052-AF + Alum adjuvant

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 (25-28).

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 elicits neutralizing and binding Abs more quickly, to higher-peak magnitudes, and with greater durability than any of the other protein-adjuvant combinations tested (29).

In rhesus macaques among 8 adjuvants combined with an Env gp140 immunogen, the Alum/TLR7 was 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-to-10-fold compared to Alum alone (30).

The 3M-052-AF adjuvant is a TLR7/8 agonist added to vaccine antigens to generate strong Ab responses in several nonhuman primate (NHP) models, and is currently being evaluated in HVTN 137 and HVTN 300 clinical trials (see Section 2.4). Combining 3M-052-AF with Alum results in a synergistic increase in immune responses to vaccine antigens in preclinical models, including Ab titers, CD4+ T-cell responses, infiltrating monocytes and dendritic cells, and B-cell activation when compared to either Alum or 3M-052-AF alone (25). In NHPs immunized with SARS-CoV-2, the receptor binding domain (RBD) formulated with 3M-052-AF combined with Alum and increased Ab titers, RBD binding and effector Abs, Th1 biased CD4+ T cells, and protection from SARS-CoV-2 viral challenge when compared to Alum alone (31).

For additional information, see the IB.

2.6 Rationale for schedule

Previous trials of DNA vaccines for HIV-1 Env immunogens have used a 0-, 1-, 3-, and 6-month vaccination schedule, exactly as proposed in this study. Those regimens were well tolerated and immunogenic (14, 15). Specifically for ID delivery, both Ab and T-cell responses were induced (17, 18). In HVTN 098, the fourth vaccination boosted the magnitude of total Ab response for all treatment groups against gp140 antigens from clades A and B as compared to the magnitude

of total Ab response after the third dose (18). By including a boost of Trimer 4571 at month 6 in Group 3, we can assess whether additional doses of protein and DNA lead to enhanced immunogenicity compared to DNA alone in this study (Group 2) and previous studies. By maintaining a standard schedule, data will be directly comparable to previous DNA vaccine studies.

2.7 Rationale for evaluation of boost with trimeric protein

Trimer 4571 is a stabilized NLT derived from the BG505 isolate in a gp140 format, modified with a different set of mutations to prevent CD4-induced conformational triggering (32-34). In proof-of-concept preclinical studies in guinea pigs, Trimer 4571, adjuvanted with Alum, induced autologous nAbs to BG505.W6M.C2 after the second administration, which increased further after the third administration. Rhesus macaques immunized via IM injection with research-grade Trimer 4571 produced nAbs to BG505.W6M.C2.T332N (see the IB for more details on these BG505 variants).

2.8 Rationale for targeting the same draining lymph node with both DNA and protein trimer

Direct targeting of the draining lymph node with a multipronged immune stimulus consisting of both a DNA vaccine and an adjuvanted protein vaccine led to improved outcomes in a variety of several preclinical models (12, 35-37). The most directly relevant study is that of Felber et al in rhesus macaques (37). This study compared vaccination with monomeric HIV envelope administered via both DNA and an adjuvanted protein delivered into the same limb or into contralateral limbs. This method allowed for a direct comparison of 2 injections with the same regimen (protein and DNA) at the same time in the same limb versus spacing simultaneous injections into contralateral limbs. Vaccination targeting the same draining lymph nodes led to improvement in a variety of immune outcomes, including functional Ab responses (increased ADCC activity and improved FcRIIIa binding) and cellular responses (CD4+ and CD8+). The proposed mechanism involves simultaneous priming of both CD4+ T cells (mostly induced by DNA) and Env-specific B cells (mainly induced by the adjuvanted protein component) in the same lymph node when injections were given in the same limb.

Most importantly, vaccination targeting the same draining lymph nodes resulted in superior protection against the simian-human immunodeficiency virus (SHIV) challenge (see Figure 2-3).

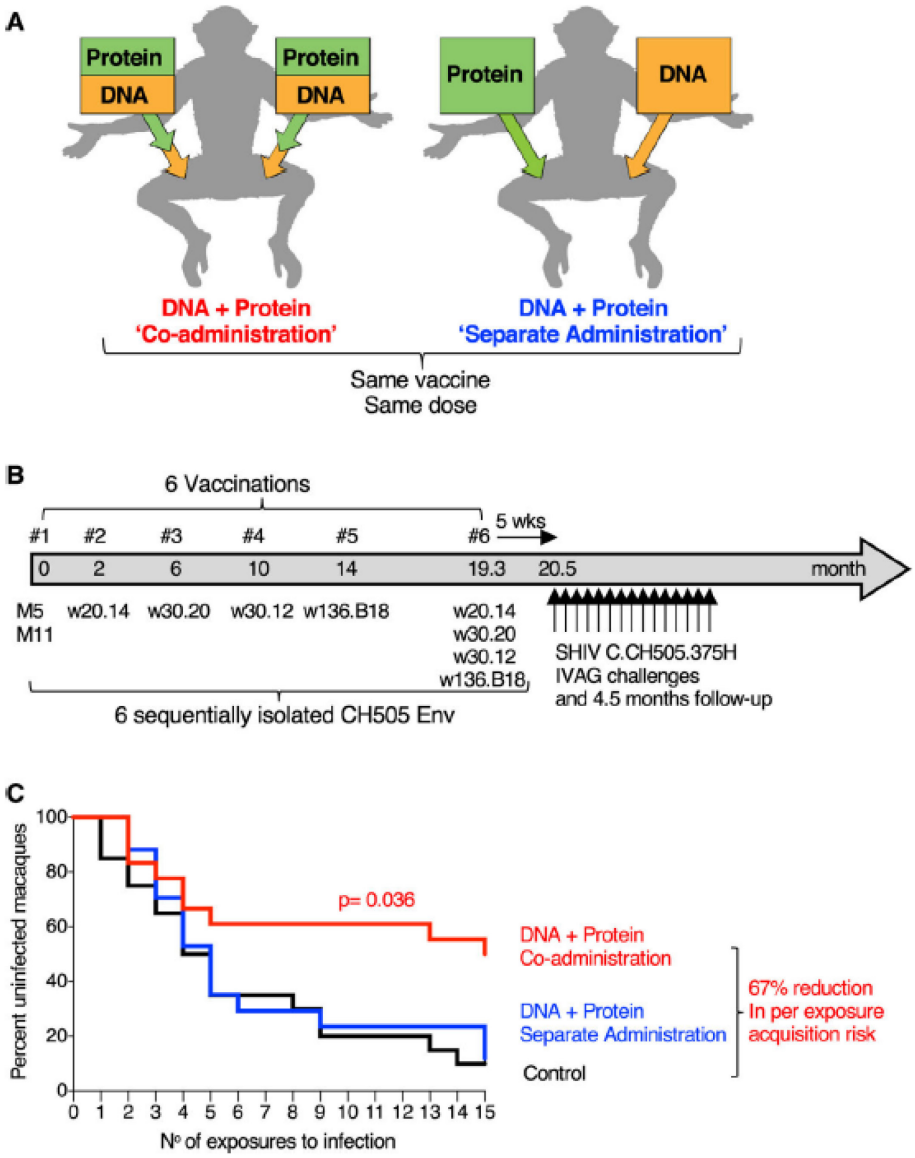

Figure 2-3 Coadministration group shows significant protection from SHIV.CH505 infection. (A) Schematic representation of vaccine delivery of the 2 components (DNA and protein) in the 2 vaccination regimens, "coadministration" in the same anatomical sites and "separate administration" in contralateral sites. The coadministration group received DNA delivered via IM EP, followed immediately by IM injection of the adjuvanted protein. The separate administration group received the vaccine components in different anatomical sites, with DNA delivered by IM EP in the left site and protein IM in the right site. The same vaccine components and the same total vaccine dose were used for both regimens. (B) Vaccination schedule indicating the sequentially isolated CH505 immunogens used. Five weeks after the last vaccination, the animals were exposed weekly to repeated low-dose vaginal challenges using SHIV.CH505. (C) Kaplan-Meier curves show the viral acquisition rate after repeated low-dose SHIV.CH505 challenges of the 2 vaccine groups (n = 18 and n = 17, respectively) and of the control group (n = 20). The RMs were exposed to 15 weekly intravaginal challenges. Infection was defined by 2 consecutive positive plasma viral load (VL) measurements. No RMs were censored. P value, exact log-rank test (37).

In keeping with the well tolerated safety profile of simultaneous delivery of HIV immunogens via both DNA+ envelope protein administration strategies (38) (39), no AEs were noted with either strategy (separation versus targeting the same draining lymph node), including assessments via clinical chemistry, complete

blood counts (CBCs), body weight, temperature, and regular physical exams by veterinarians. Additionally, theoretical concerns regarding increased local reactogenicity are mitigated by the observation that the DNA vector does not disperse from the site of injection (40) and that the DNA will be delivered intradermally, whereas the protein will be delivered intramuscularly.

2.9 Rationale for dose of Trimer 4571

The HIV-1 Env Trimer 4571 (VRC-HIVRGP096-00-VP) variant vaccine BG505 DS-SOSIP.664 consists of purified, formulated HIV-1 recombinant glycoprotein (gp140) as a soluble homotrimeric assembly of gp120-gp41 heterodimers, derived from the clade A HIV-1 strain BG505 and produced in a Chinese Hamster Ovary (CHO) DG44 cell line. Specifically, the BG505 DS-SOSIP.664 (Trimer 4571) possesses an engineered stabilizing disulfide bond between residues 201 and 433 (DS), maintaining the truncation at residue 664 in gp41, producing a soluble, cleaved Env trimer in a prefusion closed conformation. The ongoing and completed clinical trials with Trimer 4571 are listed in Table 2-2. Data on the safety and immunogenicity of Trimer 4571 + Alum from VRC 018 are available. No related SAEs were reported. The immunologic assays and analysis for this study are ongoing. Trimer 4571–specific Ab titers in serum samples were measured by electrochemiluminescence (ECLIA) using a Meso Scale Discovery (MSD) platform at baseline and at 2 weeks after the third product administration. Both the 100-mcg (n = 3) and 500-mcg (n = 5) doses elicited Trimer 4571–specific Abs, with geometric mean areas under the curve (AUCs) 8-fold and 40-fold over background, respectively. No substantial difference was observed between the 100-mcg and the 500-mcg dose, so the 100-mcg dose will be tested in this trial.

For additional information, see the IB.

Table 2-2 Clinical trials with Trimer 4571

Protocol	Study status	N receiving Trimer 4571 + Alum	Route of administration and dose	Schedule	Safety data (clinicaltrials.gov)
VRC 018 / NCT03783130	Completed	16	IM or subcutaneous (SC) at 100 mcg or 500 mcg Trimer with 500 mcg Alum	0, 2, 5 months	As of August 2021, final study data: no related SAEs, related grade AEs, or related MAAEs were reported or other unexpected reactions, and no study pause criteria were met at any time. The clinical experience with Trimer 4571 will be updated in the IB Version 4.0 expected in September 2022
NIAID 19-I-0069 / NCT03878121	Enrolling	Max 100	IM at 500 mcg Trimer with 500 mcg Alum	Boost post adenovirus vector prime	As of Jan 5, 2022, 12 participants have received study product. No SAEs, no SUSARs
NCT04985760	Enrolling	24	IM at 100 mcg or 500 mcg Trimer with 500 mcg Alum	0, 2 and 5 months	As of January 01, 2022, 5 participants have received either the 4571/alum or control. 2 participants have received two doses of study product and the remaining 3 have received a single dose. No related SAEs, no SUSARS

2.10 Rationale for dose of 3M-052-AF adjuvant

The 5-mcg dose of 3M-052-AF adjuvant has been selected based on preclinical data and clinical experience with other recombinant HIV envelope proteins in HVTN 137 and HVTN 300. The 500-mcg dose of Alum (ie, aluminum content by weight) adjuvant also matches the regimen in HVTN 137 and HVTN 300.

HVTN 137 has now completed enrollment in Part A and Part B. Based upon the available evidence, the HVTN 137 study team decided to proceed with a 5-mcg dose over a 1-mcg dose.

2.11 Rationale for FNA

To improve assessment of the effects of sD-NP-GT8 on B-cell lineage development, this protocol will employ FNA of an axillary lymph node proximal to the sites of vaccine administration. To assess the frequency of VRC01-class B cells from lymph nodes, FNA will be performed in a subset of participants in Groups 2 and 3 (at minimum 9 per group, half of each group). FNA will be performed approximately 3 weeks after both the second dose and the third dose of the sD-NP-GT8 immunogen. The germinal center response, which occurs exclusively in the lymphatic tissue, is critical for directing B-cell lineage development. Havenar-Daughton et al have employed repeated direct probing of germinal center responses by fine needle lymph node aspirates in NHP studies and found that the induction of nAbs against tier-2 autologous HIV strains best correlated with germinal center B-cell magnitude and Env-specific CD4+ Tfh cells (41). Similar sampling strategies are under evaluation in licensed vaccine and early-phase human HIV vaccine studies (42).

Fine needle aspirate of the axillary lymph node is a safe procedure with multiple studies reporting no significant complications with either FNA or core biopsy (a more invasive procedure than FNA) (43). The protocol team has developed eligibility requirements for the proposed procedures based upon guidelines from the American Society of Anesthesiologists, the American Board of Internal Medicine, the American Red Cross, the Society of Interventional Radiology, and a review of the scientific literature.

Specifically, it is a considered decision to include participants with obesity. The rate of obesity varies substantially by self-reported race with higher rates in populations identifying as Hispanic or non-Hispanic Black (44), and eligibility criteria related to obesity have been cited as a specific trial design barrier impeding the participation of women and underrepresented minorities (45). An extremely low rate of significant adverse events precludes identification of risk factors for poor outcomes, but we could find no evidence that Body Mass Index (BMI) is associated with increased complications with FNA. Additionally, diagnostic yields are higher in participants with obesity undergoing clinical FNA (46), an observation attributed to enhanced contrast between adipose and lymphatic tissue.

The team has also considered whether to include temporal limits on platelets/International Normalized Ratio (INR), and a temporal requirement to abstain from Non-steroidal anti-inflammatory drug (NSAID)/aspirin use prior to procedures. Routine measurement of INR has demonstrated no ability to identify patients who are at risk of periprocedural bleeding, even with higher risk procedures such as placement of central venous catheters (47). Additionally, platelets are assessed throughout the trial (including within 30 days of FNA). Hence the team has decided not to include INR measurement as a restriction. We also note that the trial eligibility criteria exclude persons with bleeding disorders (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions [see Section 5.1.2]). Interventional radiology guidelines state that aspirin and/or NSAIDS are not a contraindication to FNA (48).

2.12 Rationale for leukapheresis

To assess the expansion of rare precursor B-cell populations that occur at a frequency of approximately 1 out of 20 million cells, a large number of peripheral blood mononuclear cells (PBMCs) is required. Leukapheresis can provide 6 to 10 billion PBMCs without depleting red blood cells or other blood components. There are approximately 1 million PBMCs per milliliter of blood; 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 AEs when compared to blood donation. Hence, this protocol will employ leukapheresis in all study participants to ensure adequate specimens for analysis of rare precursors. Leukapheresis will be performed at screening and after the third dose of sD-NP-GT8 has been administered. The rationale for performing leukapheresis at 2 weeks after the third dose is based on previous studies that have assessed potentially rare VRC01-class memory B cells in the periphery (49).

Leukapheresis is a safe and well tolerated procedure with several studies reporting no significant late effects attributable to the procedure (50, 51). The protocol team has developed eligibility requirements for the proposed procedures (see Section 5.1) and leukapheresis eligibility criteria (see Section 8.6) as well as accounting for guidelines from the American Society for Apheresis, the American Society of Hematology, and review of the scientific literature.

The team has also considered whether to include temporal limits on platelets/INR, and a temporal requirement to abstain from NSAID/aspirin use prior to leukapheresis and has decided not to include these as restrictions (48). We note that the trial eligibility criteria exclude persons with bleeding disorders (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions (see Section 5.1.2). Assessment of patients post leukapheresis found the most frequent side effect (39%) was paresthesia due to citrate-related hypocalcemia, managed with oral calcium supplements and/or slower rates. No bleeding occurred (52). The study team conclude that adverse reactions with leukapheresis are similar to those reported for conventional PBMC collections. Routine measurement of INR has demonstrated no ability to identify patients who are at risk of periprocedural bleeding even with higher risk procedures such as placement of central venous catheter (47).

2.13 Risks and benefits

2.13.1 Potential risks

Table 2-3 Summary of potential risks of study products and administration

Risk type	Summary
Common	• Mild to moderate injection-site pain, tenderness, erythema, or swelling/induration/edema • Malaise/fatigue, myalgia, or headache in the first few days following injection • A vaccine-induced positive HIV Ab test result • Visible lesion(s) at the injection site, such as erythematous papules with eschar, hypopigmentation, hyperpigmentation, or scar due to EP
Less common	• Severe injection-site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, nausea, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Transient changes in clinical laboratory values • Injection-site hematoma, bruising/ecchymosis, other transient lesions, itching, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection-site reaction, such as sterile abscess or secondary bacterial infection • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis
Theoretical	• Muscle damage at the injection site • Electrical injury with EP • Disruption of implanted electrical devices • Exacerbation of cardiac arrythmia with EP • Effects on a participant's response to an approved HIV vaccine administered in the future • Effects on susceptibility to HIV if the participant is exposed to HIV • Effects on the course of HIV disease if the participant acquires HIV • Effects on the fetus and on pregnancy if the participant becomes pregnant

In addition to risks from ID injections and EP of DNA vaccines (see Section 2.3), frequent potential side effects resulting from subcutaneous (local anesthetic prior

to FNA) injections include stinging, discomfort, redness of skin, and mild bruising at injection site.

Blood drawing: May cause pain, bruising, fainting, and, rarely, infection at the site where the blood is taken.

Leukapheresis: Can cause hypocalcemia, 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.

FNA of lymph nodes: Can sometimes cause pain, bruising, and 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. Rarely, the local anesthetic 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 the 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 their 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 types of risks are known collectively as "negative social impacts." The HVTN CRS is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding ways to assist a participant dealing with a negative social impact, a designated HVTN Core representative can be contacted.

2.13.2 Benefits

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

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

3 Objectives and endpoints

3.1 Primary objectives and endpoints

Objectives	Endpoints
1) To evaluate the safety and tolerability of 3 doses of sD-NP-GT8 + IL-12 DNA adjuvant with or without 2 doses of Trimer 4571 + 3M-052-AF/Alum adjuvant	a) Local and systemic reactogenicity signs and symptoms will be collected for a minimum of 2 weeks following receipt of any study vaccine b) SAEs, medically attended adverse events (MAAEs), AESIs, and AEs leading to early participant withdrawal or permanent discontinuation will be collected throughout the study and for 12 months following any receipt of study products. Additionally, all AEs will be collected for 30 days after any receipt of study vaccination.
2) To evaluate the immunogenicity of 3 doses of sD-NP-GT8 + IL-12 DNA adjuvant with or without 2 doses of Trimer 4571 + 3M-052-AF/Alum adjuvant	a) Response rate and magnitude of HIV-1–specific binding Ab responses to eOD-GT8-60mer, eOD-GT8 monomer, or Trimer 4571 as assessed by multiplex assay 2 weeks following the third (Groups 1 and 2) and fourth (Group 3) vaccinations b) Response rate and magnitude of CD4+ and CD8+ T-cell responses, measured by flow cytometry, to HIV-1–specific Env peptide pools 2 weeks following the third (Groups 1 and 2) and fourth (Group 3) vaccinations
3) To evaluate the induction of VRC01-class B cells	a) Frequency of VRC01-class B cells from lymph nodes and periphery following the second and third vaccinations

3.2 Secondary objectives and endpoints

Objectives	Endpoints
1) To further evaluate the immunogenicity of 3 doses of sD-NP-GT8 + IL-12 DNA adjuvant with or without 2 doses of Trimer 4571 + 3M-052-AF/Alum adjuvant	a) eOD-GT8-60mer and monomer Ab response rate and magnitude measured by multiplex assay at 2 weeks following the first, second, and third immunizations between Group 1 and Group 2 to assess dose-sparing effects of self-assembling nanoparticle vaccines b) Quantify and compare antigen-specific germinal center Tfh and B-cell responses across groups at 3 weeks after the second and third vaccinations c) Evaluate nAb magnitude and breadth against tier-2 HIV-1 isolates after third and fourth vaccinations

3.3 Exploratory objectives

1. To clinically evaluate EP-injection–related skin changes for 6 months after the last study product administration and subjective assessment by participant of tolerability at 12 months after the last study product administration

2. Evaluate ADCC and antibody-dependent cellular phagocytosis (ADCP) after the last vaccination

3. Describe the binding specificities of sera using electron microscopy-polyclonal epitope mapping (EMPEM) at 2 weeks post–third and fourth vaccinations, if Env binding is observed

4. Evaluate Abs for cross-reactive binding to eOD-GT8 and Trimer 4571 6 weeks after the fourth vaccination and after 6 months post–fourth vaccination for Group 3

5. 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 immunogenicity endpoint assay will be a combination of measurements describing whether VRC01-like B-cell lineages are elicited and whether Env-specific CD4+ and CD8+ T cells are also elicited. Intracellular cytokine staining (ICS) assays will be performed at the Fred Hutch HVTN laboratory.

The detailed laboratory strategy and the technical details are described in the Central Assay Plan and will be updated as new reagents and techniques are incorporated into assay planning. This document will be available on the HVTN 305 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 study to examine the safety and immunogenicity of synthetic DNAs encoding NP-GT8 and IL-12 with or without a TLR-agonist–adjuvanted Env Trimer 4571 boost in adults without HIV. The primary hypothesis is that vaccination with a recombinant DNA vaccine encoding a germline-targeting epitope followed by a trimeric protein boost will be safe and immunogenic.

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

5.1.1 Inclusion criteria

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

2. 18 to 55 years old, inclusive, on day of enrollment.

3. Available for clinic follow-up through the last clinic visit and willing to be contacted at least 12 months after the last vaccine administration.

4. Willing to undergo leukapheresis (see Appendix A and Appendix B).

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

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

7. 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.

8. Assessed as low risk for HIV acquisition per low-risk guidelines (see Appendix F and Appendix G), agrees to discuss HIV-infection risks, agrees to risk-reduction counseling, and agrees to avoid behaviors associated with high risk of HIV

exposure through the final study visit. Low risk may include persons stably taking HIV pre-exposure prophylaxis (PrEP) as prescribed for 6 months or longer.

9. Hemoglobin:

    - ≥ 11.0 g/dL for volunteers who were assigned female sex at birth.

    - ≥ 13.0 g/dL for volunteers who were assigned male sex at birth and transgender males who have been on hormone therapy for more than 6 consecutive months.

    - ≥ 12.0 g/dL for transgender females who have been on hormone therapy for more than 6 consecutive months.

    - For transgender participants who have been on hormone therapy for less than 6 consecutive months, determine hemoglobin eligibility based on the sex assigned at birth.

10. Platelets = 125,000-550,000/mm^3.

11. White blood cell (WBC) count = 2,500-12,000/mm^3 (not exclusionary: if count greater than 12,000 with investigation showing general good health and PSRT approval). The Leukapheresis Center may impose a higher lower limit of 3,500/mm^3

12. Alanine aminotransferase (ALT) < 2.5 x upper limit of normal (ULN) based on the institutional normal range.

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

14. Corrected total serum calcium level of > 8.5 mg/dL

15. Blood pressure in the range of 90 to < 140 mmHg systolic and 50 to < 90 mmHg diastolic.

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

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

18. Negative for Hepatitis B surface antigen.

19. 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 last vaccination timepoint (see Appendix H).

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

5.1.2 Exclusion criteria

1. Volunteer who is breast-feeding or pregnant.

2. Morbid obesity. Enrollment of individuals with body mass index (BMI) that is ≥ 40, whom the site investigator assesses are in good health, may be considered by PSRT on a case-by-case basis.

3. Diabetes mellitus (DM). Type 2 DM, controlled with diet alone, or a history of isolated gestational diabetes are not exclusionary. Enrollment of individuals with Type 2 DM that is well-controlled on hypoglycemic agent(s) may be considered, provided the HgbA1c is ≤ 8% within the last 6 months (sites may draw these at screening).

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

5. 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.

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

7. Receipt of any live attenuated vaccine within 4 weeks prior to enrollment. (Note: ACAM2000 vaccine for Mpox (previously known as 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 prior to receipt of study vaccine and vaccination scab still present; or planned administration within 30 days after enrollment or receipt of study vaccine).

8. 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 Mpox disease are not considered to be live vaccines.

9. Non-HIV experimental vaccine(s) received within the last 1 year in a prior vaccine trial. Exceptions may be made by the HVTN 305 PSRT for vaccines that have subsequently undergone licensure or Emergency Use Authorization by the FDA or, if outside the United States, equivalent authorization by the national regulatory authority.

10. 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.

11. 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.

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

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

14. Idiopathic urticaria within the past year.

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

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

17. Asplenia or functional asplenia.

18. Active duty and reserve US military personnel.

19. 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, persons with any suicide attempt within the past one year (if between 1-2 years, consult PSRT) or cancer that, in the clinical judgment of the site investigator, has a potential for recurrence (excluding basal cell carcinoma).

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

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

    - Required either oral or parenteral corticosteroids for an exacerbation 2 or more times within the past year; OR

    - Needed emergency care, urgent care, hospitalization, or intubation for an acute asthma exacerbation within the past year (eg, would NOT exclude individuals with asthma who meet all other criteria but sought

urgent/emergent care solely for asthma medication refills or coexisting conditions unrelated to asthma); OR

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

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

- Uses more than 1 medication for maintenance therapy daily. Inclusion of anyone on a stable dose of more than 1 medication for maintenance therapy daily for greater than 2 years requires PSRT approval.

21. A participant with a history of an immune-mediated disease, either active or remote. Specific examples are listed in Appendix I (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.

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

23. 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.

24. Presence of implanted electronic medical device (eg, pacemaker, implantable cardioverter defibrillator).

25. Presence of surgical or traumatic metal implant in either upper arm and/or upper torso.

26. History of cardiac arrhythmia (eg, supraventricular tachycardia, atrial fibrillation) (Not excluded: sinus arrhythmia).

27. Tattoo overlying the injection sites preventing assessment of reactogenicity in the view of the investigator or skin condition at the injection sites.

28. History or presence of keloid scar formation or hypertrophic scar

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 prevaccination 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 Abs 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 Mpox received within 30 days prior to enrollment or receipt of study vaccine, or if ACAM2000 received greater than 30 days prior to enrollment, or prior to 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 14 days prior to study vaccine administration. Replication incompetent vaccines such as the Jynneos vaccine for the prevention of Mpox 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. Any vaccination(s) administered outside the visit window period specified in Appendix C without PRST approval should be reported as protocol deviations.

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 acquisition
- 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.

- 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, per the Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium criteria for anaphylaxis (19), is provided below:

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

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

  AND AT LEAST ONE OF THE FOLLOWING:

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

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

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

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

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

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

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

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

- PI assessment that it is not in the best interest of the participant to continue receiving study vaccine

- Newly disclosed AESI (see Appendix I)

- Coenrollment 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 305 PSRT)

- Participants discontinuing study vaccine for reasons other than HIV acquisition should be encouraged to participate in follow-up visits and procedures per the protocol for 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 acquires HIV during the course of the study, no additional study vaccine will be administered. Participants will be encouraged to continue scheduled study visits for 12 months following their last study vaccine administration. At postdiagnosis 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 (see Section 5.2.1). The site investigator can petition the PSRT for an out of window vaccination.

5.2.4 Discontinuation of study participation

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

- Participant voluntarily withdraws;

- CRS determines the participant is lost to follow-up;

- The investigational new drug (IND) application sponsor or regulatory authorities stop the study; or,

- PI assessment that it is not in the best interest of the participant to continue participation in the study or that the participant's compliance with the study is not sufficient.

If a participant terminates participation in the study early for any reason, the site PI should consider if the following assessments are appropriate: end-of-study HIV test, complete blood count (CBC with differential, serum chemistry [ALT and creatinine], physical examination, and if indicated, a pregnancy test [see Appendix A and Appendix B]). For participants living with HIV, please see

Section 8.8. If the site PI has questions regarding a termination visit, they should consult with the PSRT.

6 Statistical considerations

This phase-1 study will target recruiting a total of 45 adult participants without HIV aged 18 to 55 years.

In Group 1, a total of approximately 9 participants will receive 3 administrations of sD-NP-GT8 DNA at a dose of 0.4 mg, coformulated with IL-12 DNA at a dose of 0.1 mg at days 1, 29, and 85. Study products will be administered intradermally via EP of the skin on each upper arm.

In Group 2, a total of approximately 18 participants will receive 3 administrations of sD-NP-GT8 DNA at a dose of 1.6 mg, coformulated with IL-12 DNA at a dose of 0.4 mg at days 1, 29, and 85. Study products will be administered intradermally via EP of the skin on each upper arm.

In Group 3, a total of approximately 18 participants will receive 3 administrations of sD-NP-GT8 DNA at a dose of 1.6 mg, coformulated with IL-12 DNA at a dose of 0.4 mg at days 1, 29, and 85. These doses will be administered intradermally via EP of the skin on each upper arm. All participants in Group 3 will also receive 2 administrations of Trimer 4571 at a dose of 100 mcg adjuvanted with 5 mcg of 3M-052-AF + 500 mcg Alum via IM injections into the deltoid muscle at days 85 and 169.

The first 5 participants enrolled in the study will be assigned to Group 1. The PSRT will convene to review safety data recorded from these 5 participants through the visit scheduled 2 weeks post–first vaccination. If the PSRT determines that it is safe to proceed with enrollment, the remaining participants (approximately 40) will be randomized between Groups 1, 2, and 3.

Participants will be monitored for up to 18 months postenrollment. We anticipate that the study will take at least 22 months to be completed.

Participants will be evaluated for safety and immune responses through blood collection, lymph node FNA, and leukapheresis at specified timepoints throughout the study.

The study will target enrolling at least 50% of participants willing to undergo FNA in Groups 2 and 3. Participants enrolled in Group 1 will not undergo FNA. See Sections 1.5 and 2.11 for details.

Primary and secondary objectives will seek to characterize and compare immunogenicity endpoints between treatment groups. Since allocation ratios used to enroll participants may vary between groups during enrollment (eg, participants will be preferentially assigned to Groups 2 and 3 relative to Group 1 after Group 1 has enrolled its first 5 participants), group comparisons will be cautiously interpreted. Moreover, in order to mitigate potential biases, statistical analyses may adjust for potential confounders (eg, age) when comparing endpoints between groups.

6.1 Sample size justification and accrual

The protocol team will convene after the final enrolled participant has completed Visit 6 (first FNA visit) to determine if further participants should be enrolled to ensure adequate samples for immunogenicity analysis. Examples of reasons that might necessitate enrollment of additional participants are provided in Section 1.5. If some participants of the original 45 enrolled will not contribute data to some of the immunogenicity analyses, the protocol team may enroll up to 9 additional fully evaluable participants, or a total of 54 participants enrolled, with the goal of 45 contributing to the final immunogenicity analyses. Participants will not be replaced after completion of visit 6, the first FNA visit.

Every study participant will be followed up for an AESI contact at month 18.

Details 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, 2, and 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. Therefore, the sample-size calculations in Section 6.1.1 below account for about 17% of enrolled participants having missing data.

6.1.1 Power calculations for immunogenicity

The primary goal of the statistical immunogenicity analyses is to evaluate vaccine-induced immune responses in each treatment group by timepoint. The primary immunogenicity endpoints will be the response rate and magnitude of HIV-1–specific binding Ab responses, as measured by a multiplex assay; the response rate and magnitude of CD4+ and CD8+ T-cell responses to HIV-1–specific Env peptide pools, as measured by flow cytometry; and the frequency of VRC01-class B cells from lymph nodes and periphery following the second and third vaccinations, as measured by B-cell receptor (BCR) sequencing.

The primary and secondary statistical analyses of immunogenicity data will include the following comparisons:

- Group k postbaseline versus Group k at baseline to assess vaccine-induced immune responses within Group k, where $k = 1, 2, 3$. Groups 2 and 3 may be combined when comparing immune responses measured between baseline and Day 85 and those measured at baseline because participants in these 2 groups will have received the same products until Day 85.

- Group 1 versus Groups 2 and 3 combined to compare immune responses in the low- and high-dose groups (without administration of Trimer 4571) between Day 1 and Day 85.

- Group 1 versus Group 2 to compare immune responses in the low- and high-dose groups (without administration of Trimer 4571) post–Day 85.

- Group 2 versus Group 3 to compare immune responses with and without administration of Trimer 4571 post–Day 85.

Response magnitudes may be summarized using sample means and their two-sided 95% confidence intervals (CIs). Response rates will be estimated using empirical proportions; their two-sided 95% CIs will be constructed using the score method described in Agresti and Coull (1998) (53).

Comparisons of response magnitudes between timepoints within individual treatment groups or pooled treatment groups (eg, Groups 2 and 3 postbaseline versus baseline) will be carried out using Wilcoxon signed-rank tests. Comparisons of response magnitudes between 2 independent groups (eg, Group 2 versus Group 3) within timepoints will be done using Wilcoxon rank-sum tests.

The power achieved by the Wilcoxon rank-sum and the Wilcoxon signed-rank tests was computed as a function of the effect size for various sample sizes: n = 9, 15, 18, 30, 36, 45, including cases when no data are missing and cases when ~17% of the data are missing. These calculations also describe the power achieved by statistical comparisons of data measured in FNA samples if only 50% of participants enrolled in Groups 2 and 3 (ie, n = 9 participants per group or n = 18 in Groups 2 and 3 combined) consent to providing FNA samples. The calculations assumed that observations are normally distributed and that the number of observations in both groups or timepoints is identical.

The power function of the tests under these scenarios is presented in Figure 6-1. For instance, when comparing 2 independent groups of equal sizes n = 15 (eg, Groups 2 or 3 with about 17% loss to follow-up), the study achieves about 80% power to detect a significant difference at the 5% significance level using a two-sided Wilcoxon rank-sum test if the average increase in the standardized (Cohen's) effect size is about 1.1. For example, if the proportion of VRC01-class B cells two weeks after the second vaccination was 1.0% in Group 1 and 2.1% in Group 2, with a standard deviation of about 1.0% in both groups, the standardized effect size would be 1.1 = (2.1%-1.0%)/1.0%, and the study would have approximately 80% to detect a significant difference between the two groups using a two-sided Wilcoxon rank-sum test at the 5% significance level. With a sample size of n = 18 per group (eg, Groups 2 or 3 with no loss to follow-up), the test has about 80% power to detect a significant difference if the standardized effect size is 1.0. Thus, if the proportion of VRC01-class B cells two weeks after the second vaccination was 1.0% in Group 1 and 2.0% in Group 2, with a standard deviation of about 1.0% in both groups, the standardized effect size would be 1.0 = (2.0%-1.0%)/1.0%, and the study would have approximately 80% to detect a significant difference between the two groups using a two-sided Wilcoxon rank-sum test at the 5% significance level.

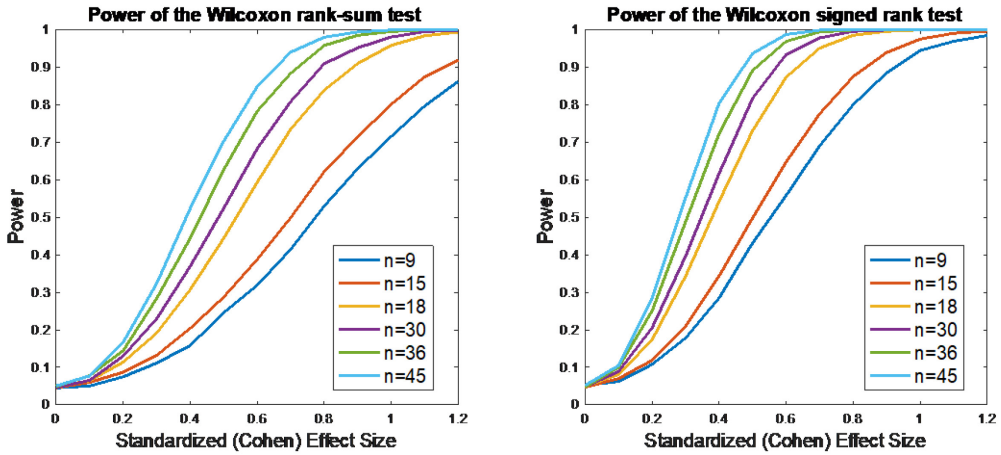

Figure 6-1 Left panel: Power of a two-sided Wilcoxon rank-sum test comparing 2 independent groups. Right panel: Power of a two-sided Wilcoxon signed-rank test comparing 2 groups of paired observations (eg, the frequency of VRC01-class B cells postvaccination and at baseline within a single group). The power was calculated as a function of (1) the standardized effect size, defined as the average difference between the 2 group means divided by the common group standard deviation (left-hand side) or as the mean difference divided by the standard deviation of the difference (right-hand side) and (2) the sample size (n = 9, 15, 18, 30, 36, and 45).

6.1.2 Power calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with vaccine administration. The ability of the study to detect 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 each treatment group of size n = 18 (Groups 2 or 3), there is at least a 90% chance of observing at least 1 event if the true rate of such an event is 12.5% or more, and there is at least a 90% chance of observing no events if the true rate is 0.53% or less. When pooling Groups 2 and 3 together (n = 36), there is at least a 90% chance of observing at least 1 event if the true rate of such an event is 6.5% or more, and there is at least a 90% chance of observing no events if the true rate is 0.29% or less. Safety data will be summarized as described in Section 6.4.3 and 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 events, 1 or more events, and 2 or more events among 9 (or 18 or 36 or 45) participants receiving the study vaccine are presented in Table 6-1 for a range of possible true AE rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events among a group of 9, 18, 36, or 45 study participants for different true event rates

True event rate (%)	Arm size	0 events	1+ events	2+ events
1	9	0.91	0.09	0.00
4	9	0.69	0.31	0.05
10	9	0.39	0.61	0.23
20	9	0.13	0.87	0.56
30	9	0.04	0.96	0.80
1	18	0.83	0.17	0.01
4	18	0.48	0.52	0.16
10	18	0.15	0.85	0.55
20	18	0.02	0.98	0.90
30	18	0.00	1.00	0.99
1	36	0.70	0.30	0.05
4	36	0.23	0.77	0.42
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	45	0.64	0.36	0.07
4	45	0.16	0.84	0.54
10	45	0.01	0.99	0.95
20	45	0.00	1.00	1.00
30	45	0.00	1.00	1.00

An alternative way of describing the statistical properties of the study design is in terms of the 95% CI for the true rate of an AE based on the observed data. Table 6-2 shows the two-sided 95% CIs for the probability of an event based on a particular observed rate. Calculations are done using the score test method for CIs described in Agresti and Coull formula 2 (53). If none of the 18 participants receiving the study vaccine in Groups 2 or 3 experience a safety event, the 95% two-sided upper confidence bound for the true rate of such events in the total vaccinated population is 17.6%. Table 6-2 also includes CIs for groups of size n = 9 (Group 1 alone), n = 36 (Groups 2 and 3 pooled together), and n = 45 (all 3 treatment groups combined).

Table 6-2 Two-sided 95% CIs for the probability of observing a safety event based on observing a particular rate of safety endpoints in a group of 18, 36, or 54 study participants

Observed event rate	95% CI (%)
0/9	[0 ; 29.9]
1/9	[2.0 ; 43.5]
2/9	[6.3 ; 54.7]
0/18	[0 ; 17.6]
1/18	[1.0 ; 25.8]
2/18	[3.1 ; 32.8]
0/36	[0 ; 9.6]
1/36	[0.5 ; 14.2]
2/36	[1.5 ; 18.1]
0/45	[0 ; 6.6]
1/45	[0.3 ; 9.8]
2/45	[1.0 ; 12.5]

6.2 Randomization

A participant's randomization assignment will be computer generated and provided to the HVTN CRS pharmacist through a Web-based randomization system. Pause rules discussed in Section 9.6 will be accounted for in this process. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments (except in emergency situations as specified in the HVTN MOP).

Enrollment will be restricted to 1 participant per day for the first 5 participants in Group 1 and pause once the first 5 participants are enrolled in Group 1. If the PSRT determines that it is safe to complete enrollment in all three groups, the remaining participants (approximately 40: n=4 in Group 1, n=18 in Group 2, and n=18 in Group 3) will be block randomized. Block size and allocation ratio will be determined based on group sizes.

The study will aim to ensure balanced representation with respect to sex assigned at birth. Sites will therefore be encouraged to enroll at least approximately 40% of each sex assigned at birth in the study.

6.3 Blinding

This is an open-label study. Participants and site staff will be unblinded to participants' group assignments. Laboratory program staff will be blinded to participants' group assignments during assay analysis, whenever feasible.

6.4 Statistical analyses

This section outlines the final study analysis, unblinded as to treatment arm assignment. All data from enrolled participants will be analyzed regardless of how many vaccinations each participant received. All analyses will be performed using SAS or R. More details about the statistical analyses will be described in the Statistical Analysis Plan (SAP).

6.4.1 Baseline demographics

Participants' baseline characteristics will be summarized using descriptive statistics.

6.4.2 Immunogenicity analyses

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

The goals of the primary and secondary immunogenicity analyses, including group comparisons, are presented in Section 3.

Discrete categorical immunogenicity endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response by timepoint and treatment group. Crude response rates will be presented with their corresponding 95% CI estimated based on the score test method (42). Barnard's exact tests may be employed to compare the response rates of certain immune responses between 2 treatment groups.

Quantitative assay endpoints (eg, frequencies of CD4-bs B cells as measured by flow cytometry) will be summarized by timepoint within treatment groups by tabulating univariate summary statistics (eg, mean, median, standard deviation, range) and plotting observations using boxplots. Comparisons of continuous outcome variables measured in 2 independent groups will be performed using the Wilcoxon rank-sum test. Comparisons of paired continuous outcome variables measured in 1 group will be performed using the Wilcoxon signed-rank test.

6.4.3 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.

AEs: 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 vaccinations is shown in Section 1 and additional information is given below.

Group 1

Treatment 1 (T1):

INO-6172, 0.5 mg to be administered as 2 separate ID injections (0.1 mL each) followed by EP using the CELLECTRA 2000 EP device at months 0, 1, and 3.

Group 2

Treatment 2 (T2):

INO-6172, 2 mg to be administered as 2 separate ID injections (0.1 mL each) followed by EP using the CELLECTRA 2000 EP device at months 0, 1, and 3.

Group 3

Treatment 3 (T3):

INO-6172, 2 mg to be administered as 2 separate ID injections (0.1 mL each) followed by EP using the CELLECTRA 2000 EP system at months 0, 1, and 3.

Trimer 4571, 100 mcg admixed with 3M-052-AF, 5 mcg and Alum, 500 mcg to be administered as 2 separate IM injections (0.2 mL each) at months 3 and 6.

7.2 Study product formulation and storage

7.2.1 INO-6172

INO-6172 Drug Product is sD-NP-GT8 coformulated with IL-12 DNA at a plasmid ratio of 4:1 (0.8 mg sD-NP-GT8 and 0.2 mg IL-12 DNA per 0.1 mL). The formulation is supplied in Water for Injection (WFI) at a concentration of 10 mg/mL, and a labeled volume of 0.4 mL in 2-mL glass vials. Vials may contain a volume greater than the labeled volume. INO-6172 is a clear, colorless solution essentially free of visible particulate matter. Store INO-6172 frozen at -25ºC to -15ºC. The study product is described in further detail in the IB.

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

Trimer 4571 will be provided as 3-mL, single-use glass vials with a labeled volume of 1.2-mL and a concentration of 500 mcg/mL. Each vial contains a sterile, aqueous, preservative-free, buffered solution that is clear and colorless. Some small, white, or translucent particles may be present.

Store Trimer 4571 frozen at -35ºC to -15ºC. Vials should not be refrozen after thaw. Thawed vials can be stored at 2ºC to 8ºC for up to 48 hours or at 15ºC to 27ºC for up to 24 hours. Vials are intended for single use only and do not contain preservatives. A single vial may not be used for multiple dose preparations. The study product is described in further detail in the IB.

7.2.3 3M-052-AF (labeled as AP 60-702)

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

7.2.4 Aluminum Hydroxide Suspension (Alum)

The Alum adjuvant is composed of Alhydrogel 2% (Brenntag Biosector, Frederikssund, Denmark). The Alum adjuvant is a sterile, pyrogen-free, off-white suspension filled into 3-mL glass vials with a labeled volume of 0.7 mL, diluted with water for injection to an aluminum concentration of 5 mg/mL.

Store Alum adjuvant refrigerated at 2°C to 8°C. Do not freeze. Vials are intended for single use only and do not contain preservatives. A single vial may not be used for multiple dose preparations. The study product is described in further detail in the IB.

7.3 Product preparation

Pharmacists must follow appropriate aseptic techniques 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 regarding 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 portions of entered vials, and unused prepared study product should be discarded in a biohazard container and disposed of in accordance with institutional or pharmacy policy.

7.3.1 INO-6172, 0.5 mg (Group 1)

INO-6172, 10 mg/mL will be further diluted to a final concentration of INO-6172, 2.5 mg/mL for Group 1 dosing.

1. Remove 1 vial of INO-6172, 10 mg/mL from the freezer and record this as the preparation start time on the Investigational Product Preparation Sheet.

2. Once contents of vial are thawed, withdraw 0.4 mL from the INO-6172 vial and inject into the sterile empty glass mixing vial. Note: Thawed vials of INO-6172 will appear clear and colorless, essentially free of visible particulate matter.

3. Withdraw 1.2 mL from the SWFI USP vial and inject into the sterile glass mixing vial, which contains 0.4 mL of INO-6172.
   Note: The final total volume in the sterile glass mixing vial should be 1.6 mL, for a final concentration of INO-6172, 2.5 mg/mL.

4. Gently swirl the INO-6172, 2.5 mg/mL in the sterile glass mixing vial.

5. Insert the sterile BD ½ mL Tuberculin Syringes with permanently attached needle, 27G x ½ inch (BD Reference Number 305620) into the sterile glass mixing vial containing INO-6172, 2.5 mg/mL and withdraw 0.1 mL. Place the needle cap back onto the needle. Repeat this step for a total of 2 syringes, each containing INO-6172, 2.5 mg/mL, 0.1 mL each.

6. Label the syringes according to Section 7.3.4 and include on the label “syringe 1 of 2” for the first syringe prepared and “syringe 2 of 2” for the second syringe prepared.

The prepared syringe(s) may be stored at room temperature and used within 4 hours of study product preparation time.

7.3.2 INO-6172, 2 mg (Group 2 and Group 3)

1. Remove 1 vial of INO-6172, 10 mg/mL from the freezer and record this as the preparation start time on the Investigational Product Preparation Sheet.

2. Once contents of vial are thawed, insert the sterile BD 0.5 mL Tuberculin Syringes with permanently attached needle, 27G x ½ inch (BD Reference Number 305620) into the sterile glass vial containing INO-6172, 10mg/mL and withdraw 0.1 mL. Place the needle cap back onto the needle. Repeat this step for a total of 2 syringes, each containing INO-6172, 10 mg/mL, 0.1 mL each. Note: Thawed vials of INO-6172 will appear clear and colorless, essentially free of visible particulate matter.

3. Label the syringes according to Section 7.3.4 and include on the label “syringe 1 of 2” for the first syringe prepared and “syringe 2 of 2” for the second syringe prepared.

The prepared syringe(s) may be stored at room temperature and used within 4 hours of study product preparation start time.

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

1. Thaw 1 vial of Trimer 4571 at ambient temperature (15˚C to 27˚C) for a minimum of 30 minutes. Vial should not be moved directly from a freezer to a refrigerator to thaw. Record this as the preparation start time. While vial is thawing proceed to the next steps.

2. Remove 1 vial of Alum adjuvant from the refrigerator and equilibrate at ambient temperature (15˚C to 27˚C) for a minimum of 15 minutes. Mix the vial of Alum adjuvant by gently inverting the vial 5 times.

3. Remove 1 vial of 3M-052-AF from the refrigerator and equilibrate at ambient temperature (15˚C to 27˚C) for a minimum of 15 minutes. Mix the vial of 3M-052-AF by gently inverting the vial 5 times.

4. Withdraw 0.2 mL of 3M-052-AF and inject into the sterile empty glass mixing vial.

5. Withdraw 0.2 mL of Alum and inject into the sterile glass mixing vial, which contains 0.2 mL of 3M-052-AF.

6. Mix and wait for 30 minutes.

7. Swirl the thawed, equilibrated Trimer 4571 vial for about 30 seconds with sufficient force to mix the solution while avoiding foaming. Do not shake the vial. If some white to translucent particles are observed, vials may be used for the preparation for IM administration.

8. Withdraw 0.4 mL of Trimer 4571 and inject into the sterile glass mixing vial, which contains 0.2 mL of 3M-052-AF, and 0.2 mL of Alum. The final volume in the sterile mixing vial will be 0.8 mL.

9. Invert mixing vial gently 5 times to mix. Withdraw 0.2 mL from the sterile glass mixing vial. Pull back on the syringe plunger to ensure all product is in the syringe. Discard preparation needle and either cap the syringe or attach a needle for IM administration, per institutional procedure. Repeat this step for a total of 2 syringes containing 0.2 mL each.

10. Label the syringes according to Section 7.3.4 and include on the label “syringe 1 of 2” for the first syringe prepared and “syringe 2 of 2” for the second syringe prepared. Invert each syringe gently 10 times to mix immediately prior to administration.

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

7.3.4 Labeling

Label the study product as follows:

- Participant identifier(s)
- Study product name
- Final volume (mL)
- Route (IM or ID)
- Beyond-use date and time
- Any additional information required by jurisdiction

7.4 Study vaccine administration

7.4.1 INO-6172

INO-6172, 0.5 mg and INO-6172, 2 mg will be administered as 2 separate 0.1-mL injections intradermally, bilaterally 1 on each arm at months 0, 1, and 3. Following ID injections of INO-6172, EP will be performed using the Inovio CELLECTRA 2000 EP Device.

The ID injections will be administered into the skin overlying the deltoid area of the arm. The needle will be inserted into the skin at a 5- to 15-degree angle to the skin and bevel side up until the bevel is seen to be fully under the skin. The syringe contents will be injected to form a small bleb. One injection should be completed (including EP) prior to administering the second injection.

7.4.2 Trimer 4571, 100 mcg admixed with 3M-052-AF, 5 mcg and Alum, 500 mcg

Trimer 4571, 100 mcg admixed with 3M-052-AF, 5 mcg and Alum, 500 mcg will be administered to Group 3 participants following administration of INO-6172 and at months 3and 6, as 2 separate 0.2-mL IM injections (1 in each arm) intramuscularly into the deltoid muscles by needle and syringe. The injection site for the protein injection (Trimer 4571) should be at least 2.5 centimeters (1 inch) from the ipsilateral EP injection site of INO-6172 (see 7.4.1). Immediately prior to administration, gently invert each syringe 10 times.

If an ID or IM injection needs to be administered to an alternate body site (eg, lateral thigh) due to a medical contraindication, it should not be administered in

the same deltoid as the injection site where the other half of the dose is administered, but rather in the thigh. 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

sD-NP-GT8 and IL-12 DNA are manufactured under quality controls and production protocols developed and tech transferred by Inovio Pharmaceuticals. INO-6172, the coformulation of sD-NP-GT8 with IL-12 DNA, is formulated under guidance provided by Inovio under subcontract to IDT and is being provided to HVTN to support this program under an IPCAVD grant to The Wistar Institute (Philadelphia, PA USA).

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

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

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.

Syringes, needles, SWFI, and other ancillary supplies will be locally sourced by the site.

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 CRSs, 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 CRSs, 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.

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

Leukapheresis specimens for baseline comparison will be drawn during the screening period if a participant meets all additional eligibility criteria and wishes to join the study (Sections 5.1.1 and 8.6).

8.2 Definition of “study day” and “study visit”

“Study Day 1” 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

Reactogenicity definition: 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.

The reactogenicity assessment period is the day of vaccination and 7 full days following vaccine administration for INO-6172 alone and 14 full days following vaccine administration for INO-6172 along with Trimer 4571 adjuvanted with 3M-052-AF/Alum and Trimer 4571 alone, adjuvanted with 3M-052-AF/Alum. Clinicians will follow and collect resolution information for any reactogenicity signs and symptoms that have not resolved within the reactogenicity assessment period.

Prevaccine administration: Medical history and evaluations, including vital signs and planned injection-site evaluations, are performed as described in Appendix A and Appendix B

Postvaccine administration in-clinic evaluation: Following the first vaccine administration of sD-NP-GT8, participants will be observed for a minimum of 60 minutes postadministration. For all subsequent injections, participants will be observed for a minimum of 30 minutes with the following exception: for Group 3, participants will be observed for 60 minutes postadministration of Trimer 4571 + 3M-052-AF + Alum. During this time, 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.

Postvaccine administration contact with participant: 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. Any postvaccination reaction that is 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.

Guidance for participants scheduling licensed vaccines or allergy treatments: In order to prevent interference with reactogenicity assessments, participants who plan to receive licensed vaccines or allergy treatments 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).

Symptom diaries: Participants are asked to record symptoms on a daily basis using an electronic participant diary (paper alternative is available). All participants will be given a thermometer for oral temperature measurement and a ruler, and will be provided access to the electronic diary (paper alternative available). All participants will be provided training on diary completion, proper thermometer usage, and how to use of the measuring device to measure any injection-site induration and/or erythema. Participants will use the diary to record daily their highest temperature as well as local and systemic signs and symptoms. For Groups 1 and 2, symptoms are recorded for the day of vaccination and for 7 full days following each vaccination. For Group 3 only, symptoms are recorded for the day of vaccination, for 7 full days following the first two vaccinations, and for 14 full days following the third and fourth vaccinations. Participant diaries will be reviewed by a clinician and reconciled for accuracy and completeness. Attribution assessment will be performed and recorded on a case report form (CRF) for systemic reactogenicity events reported in the participant diary after additional evaluation of the participant by clinician. Clinicians may photograph the injection site in order to document injection-site reactogenicity.

8.4 EP injection site assessment

To document the appearance of injection sites over time, the area to be injected will be assessed prior to receipt of an injection, and at subsequent scheduled

visits. The purpose of this assessment is to evaluate the appearance of the injection sites after time has allowed for healing. A description including type and size of any skin changes related to vaccination that are not described in Section 8.3 will be recorded. Clinicians may photograph the injection site in order to document EP injection-site skin changes. An abbreviated questionnaire to assess EP-related skin changes and tolerability will be asked at the AESI contact (Section 8.10)

8.5 Lymph node FNA

Tissue sampling of an axillary lymph node will be carried out percutaneously by FNA for participants who have opted for this procedure. A minimum of half of the participants in Groups 2 and 3 will undergo this procedure. 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 to 4 passes will be made to retrieve cytologic material.

Postprocedural 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 (postprocedural body temperature ≥ 38.0°C or 100.4°F), 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 nonsteroidal anti-inflammatory drugs (NSAID), aspirin, or other medications at doses 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 urine pregnancy test within 48 hours prior to the procedure

8.6 Leukapheresis

Collection of PBMCs via leukapheresis will be performed in accordance with the standard practices of the participating apheresis center. Typically, in this procedure, approximately 3 liters of blood will be processed using peripheral veins for venous access. The blood will be anticoagulated in accordance with standard practice of the apheresis center.

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

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 urine pregnancy test within 48 hours prior to the procedure

8.7 Visit procedures, schedule, windows, and missed visits

The schedules of visits and evaluations performed at each visit are shown in Appendix A (for participants undergoing lymph node FNA) and Appendix B (for participants not undergoing lymph node FNA). Visit windows are shown in Appendix C. The procedures for documenting missed visits and out-of-window visits are described in the HVTN 305 Study-specific Procedures (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.

8.8 Monitoring for acquisition of HIV

Study participants will be tested for presence of HIV 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 HVTN Laboratory Center Manual of Procedures (MOP), which is able to distinguish vaccine-induced Ab responses from the presence of actual HIV. Participants will be promptly informed and counseled if they acquire HIV during the study and will be referred for treatment (see Section 5.2.2).

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

Although the study vaccine will not cause HIV acquisition, it may induce Abs detectable by standard HIV 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 Ab testing outside of the HVTN CRS during study participation.

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

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

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

8.9 Early termination visit

If a participant terminates participation in the study early for any reason, the site PI should consider if the following assessments are appropriate: end-of-study HIV test, CBC with differential, serum chemistry (ALT and creatinine), physical examination, social-impact assessment, and, if indicated, a pregnancy test (see Appendix A and Appendix B). For participants living with HIV, please see Section 5.2.2. If the site PI has questions regarding a termination visit, they should consult with the PSRT.

8.10 AESI contact

CRS staff will contact study participants at month 18 to collect the information listed below and will ask an abbreviated set of EP-injection-site related skin changes and tolerability assessment questions that can be conducted over the phone. If indicated, the participant may be asked to come in for a clinical assessment, which may also include referrals for an AESI assessment. AESIs are described further in Appendix I.

- 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, EAEs;

AESIs (a sample list of AESI is provided in Appendix I). AESIs are reported regardless of relationship to study product(s);

Medically attended adverse events (MAAEs), defined as any AE leading to an unscheduled visit to a healthcare professional. MAAEs are reported regardless of relationship to study product(s);

New diagnosis of HIV acquisition; and

Pregnancies and outcomes, including congenital anomalies/birth defects.

All such events will be recorded and AEs will be assessed for relationship to study vaccines.

9 Safety and AEs

9.1 AEs

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) the AE meets the criteria for a safety pause/prompt AE review (see Section 9.6), (3) the AE meets the criteria for an MAAE, and (4) the AE is a potential immune-mediated disease that may be listed as an AESI. A sample list of AESIs is provided in Appendix I.

In addition, a limited set of AEs will be collected and reported until (but not including) the month 18 visit:

- SAEs/expedited adverse events (EAEs),
- AESIs,
- MAAEs, and
- AEs leading to early participant withdrawal or early discontinuation of study vaccine(s) administration.

AEs will be graded according to the 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 305 SSP).
- Do not grade creatinine clearance or estimated glomerular filtration rate (eGFR) based on the change from the baseline parameter. Do not grade on the basis of eGFR if there is clinical concern for acute 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 SAEs

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 AE 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 DAIDS Regulatory Support Center (RSC) website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

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

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

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

The SAE reporting category will be used throughout the study. After completion of the study, the Suspected Unexpected Serious Adverse Reaction (SUSAR) reporting category will be used if clinical staff becomes aware of an event on a passive basis.

The study products for which expedited reporting are required are:
- INO-6172,
- [Trimer 4571 + 3M-052-AF + Alum], and
- EP device.

There is no placebo product administered in this protocol. The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the FDA, in accordance with 21 CFR 312.32 (IND Safety Reports).

9.3 Safety monitoring

9.3.1 PSRT

The PSRT comprises: DAIDS Medical Officer (MO) representative, Protocol Chair and Cochair, Protocol Team Leader (PTL), Clinical Safety Specialist (CSS), and Regional Medical Liaison (RML). The protocol team's clinic coordinator, clinical data manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 305 PSRT meetings. The PSRT will review study safety information on a weekly basis through 2 weeks after the last participant receives the final study injection. Less frequent safety reviews will be conducted at the discretion of the PSRT.

9.3.2 HVTN Safety Monitoring Board

The Safety Monitoring Board (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 305 PSRT.

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

9.4 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, per American Red Cross guidelines for blood donation (https://www.redcrossblood.org/donate-blood/how-to-donate/eligibility-requirements.html).

9.5 Initial safety review

The trial will begin with enrollment in Group 1 only. Enrollment will be restricted to a maximum of 1 participant per day for the first 5 participants in Group 1. Enrollment will then be held. However, participants who have been enrolled will continue to receive vaccinations during the planned hold. The PSRT will review cumulative safety data reported through the visit scheduled 2 weeks post–enrollment. Upon PSRT determination that it is safe to proceed, full and unrestricted enrollments in all Groups may commence without restriction.

9.6 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 305 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 305 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

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** CSS/RML **Submit** CRFs immediately	Prompt PSRT AE review to consider a pause
AE, related (see Grade 3 exceptions below)	4 or 3	**Email** CSS/RML **Submit** CRFs immediately	Prompt PSRT AE review to consider a pause

Exceptions to 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/RML office phone upon learning of any unrelated participant deaths. The site will also email the CSS/RML and immediately submit CRFs. The CSS/RML will then promptly notify the PSRT.

If you need to contact the CSS/RML, refer to phone numbers and email addresses found on the HVTN 305 protocol home page on the HVTN Members' site at https://members.hvtn.org/protocols/hvtn305.

9.6.1 Plan for review of pause rules

For all safety pauses, HVTN Core notifies the HVTN 305 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 305 PSRT reviews safety data and decides whether the pause can be lifted or a 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 305 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 305 PSRT notification or prompt HVTN 305 PSRT AE review is triggered, HVTN Core notifies the HVTN 305 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 305 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.

9.7 Study termination

This study may be terminated early by the determination of the HVTN 305 PSRT, the NIH, the United States Department of Health and Human Services Office for Human Research Protections (OHRP), the FDA, or study product developers. In addition, the conduct of this study at an individual HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

9.8 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given but remaining visits and study procedures, including AESI health contact, should be completed unless medically contraindicated or applicable regulations require termination from the study. During follow-up of 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 305 SSP. If the participant is no longer pregnant, refer to Section 5.2.2.

10 Protocol conduct and informed consent

10.1 Protocol conduct

This research study will be conducted in the US and countries outside of the US, with funding from the NIH, among others. Due to this, the trial is subject to both US and local regulations and guidelines on the protection of human research subjects and ethical research conduct. Where there is a conflict in regulations or guidelines, the HVTN strives toward maximum protection of human research participants.

The study will comply with the International Council for Harmonization (ICH) Guideline for Good Clinical Practice (GCP 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 305 SSP. 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 (CABs) are required by DAIDS and supported at all HVTN research sites to ensure community input in accordance with Good Participatory Practices (GPP).

10.2 Compliance with NIH guidelines for research involving products containing recombinant or synthetic nucleic acid molecules

Because this study is evaluating products containing recombinant or synthetic nucleic acid molecules and is funded by the NIH, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (April 2019). Information about the study must be submitted to the Institutional Biosafety Committee (IBC) for each CRS. Investigators at each CRS are responsible for obtaining IBC approval per NIH guideline *Section IV-B7-a-(1).* IBC review and approval must be documented by the investigator and submitted as part of DAIDS's initial protocol registration for this trial before participants are enrolled at the CRS. If this protocol is amended, investigators should follow the requirements of their respective IBC.

10.3 Informed consent

The sample informed consent form (SICF) in Appendix D 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 SICF's level of comprehensibility.

If any new information is learned that might affect the participants' decisions to stay in the trial, this information will be shared with them. 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.

10.3.1 Screening consent form

Without a general screening consent, screening for a specific study cannot take place until the site receives protocol registration from the DAIDS RSC Protocol Registration Office. Sites should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

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

11 Exploratory studies

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

Only genetic testing that is in accordance with the language in the SICF (Appendix D) may be performed on samples.

11.1 Specimen storage and other use of specimens

The HVTN stores specimens from all study participants indefinitely unless a participant requests that specimens be destroyed or if destruction or a time limit for storage is required by IRB/EC or RE.

“Other use” of specimens is defined as “studies not covered by the protocol or the informed consent form for the main study” (see Appendix D).

This research may relate to HIV, vaccines, the immune system, or 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 will 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 an internet-based tool that documents participants’ 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: Participants undergoing FNA

Visit Number		01	02	03	04	05	06	07	08	09	10	11	12	AESI[18]
Study Week			0	2	4	6	7	12	14	15	24	26	52	78
Study Month			0	0.5	1	1.5	1.75	3	3.5	3.75	6	6.5	12	18
Study Day		-56 to 0	1	15	29	43	50	85	99	106	169	183	365	547
Procedure		Screen[1]	**Vac 1**		**Vac 2**		**FNA**	**Vac 3**		**FNA**	**Vac 4[17]**			
Study procedures														
Assessment of Understanding		√												
Informed consent		√												
Medical history[2]		√												
Physical exam[3]		√	√	√	√	√		√	√		√	√	√	
Contraception status assessment[4]		√	√		√			√			√			
Social impact assessment			√	√	√	√		√	√		√	√	√	
Social impact questionnaire					√			√					√	
Risk-reduction counseling[5]		√	√	√	√	√		√	√		√	√	√	
Concomitant medications[6]		√	√	√	√	√[7]	√	√	√[7]	√	√	√	√	
Adverse Events (AEs)			√	√	√	√		√	√		√	√		
AESIs/MAAEs/SAEs			√	√	√	√		√	√		√	√	√	√
Vaccination[8]			√		√			√			√			
Reactogenicity assessment[9]			√		√			√			√			
EP injection site assessment				√	√	√	√	√	√	√	√	√	√	√[18]
Clinical labs	Tube													
Pregnancy test (urine or serum) [10,11]		√	√		√		√	√	√	√	√			
HBsAg/anti-HCV[11]	SST	5												
HIV screening test[11,12]	SST	5												
Calcium[11]	SST	5												
CBC/Differential[11]	EDTA	5		5		5			5			5		
ALT & Creatinine[11]	SST	5		5		5			5			5		
HIV diagnostic test	EDTA							10				10	20	
Research samples[13]														
PBMC for assays & storage	ACD			42.5		136		136[14]				136	178.5	
Serum for assays and storage	SST	34		34		34			34			34	34	
Leukapheresis[15]		√							√					
Fine Needle Aspiration[16]							√			√				
Daily volume (mL)		59	0	86.5	0	180	0	146	44	0	0	190	232.5	
56-day total volume (mL)				145.5	145.5	325.5	325.5	326	370	370	0	190	232.5	

Green shading: Vaccination visit for all groups; **Blue shading:** Vaccination visit for Group 3 only.

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

[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, and vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck and 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 reports 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. Participants will also be provided with counselling about contraception.

[5] **Risk-reduction counseling** per CRS Standard Operating Procedures (SOP).

[6] **Concomitant medications,** including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots, are recorded during screening, at enrollment, and at each subsequent clinic visit.

[7] During concomitant medications check-in **participant is counseled to avoid medications that would increase the risk of bleeding for 7 days prior to FNA** (see Section 8.5).

[8] **Vaccination (in-clinic assessments):** At least 60 minutes after the first vaccination and after the fourth vaccination, and 30 minutes after for all other vaccinations. Prior to clinic discharge, the injection site and systemic symptoms will be assessed, and participants will have vital signs taken.

[9] **Reactogenicity:** Clinic staff will follow new or unresolved reactogenicity symptoms present at the final reactogenicity day (see Section 8.3 and HVTN 305 SSP)

[10] **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. For persons who are confirmed pregnant, pregnancy testing is not required unless clinically indicated.

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

[12] **HIV screening test:** See Section 5.1.1.

[13] **Research samples:** Blood-draw volumes for each tube type shown. Alternate tube types may be used under certain conditions (eg, product shortages) upon approval of the HVTN Laboratory Center. Refer to the Specimen Collection SSP for more information.

[14] **Blood draw** will be performed before vaccination.

[15] **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 8.6).

[16] **Fine Needle Aspiration:** Target for FNA is 3 weeks post–second and –third vaccinations.

[17] **Vaccination 4/Visit 10** will be conducted only for participants assigned to Group 3.

[18] **AESI contact:** CRS staff will contact study participants at month 18 to collect the information listed in Section 8.10. The EP-injection–site assessment will be an abbreviated questionnaire that can be conducted over phone.

Appendix B Schedule of procedures: Participants not undergoing FNA

Visit Number		01	02	03	04	05	06	07	08	09	10	11	12	AESI[16]
Study Week			0	2	4	6	7	12	14	15	24	26	52	78
Study Month			0	0.5	1	1.5	1.75	3	3.5	3.75	6	6.5	12	18
Study Day		-56 to 0	1	15	29	43	50	85	99	106	169	183	365	547
Procedure		Screen[1]	**Vac 1**		**Vac 2**			**Vac 3**			**Vac 4[15]**			
Study procedures														
Assessment of Understanding		√												
Informed consent		√												
Medical history[2]		√												
Physical exam[3]		√	√	√	√	√		√	√		√	√	√	
Contraception status assessment[4]		√	√		√			√			√			
Social impact assessment			√	√	√	√		√	√		√	√	√	
Social impact questionnaire					√			√					√	
Risk-reduction counseling[5]		√	√	√	√	√		√	√		√	√	√	
Concomitant medications[6]		√	√	√	√	√		√	√		√	√	√	
Adverse Events (AEs)			√	√	√	√		√	√		√	√		
AESIs/MAAEs/SAEs			√	√	√	√		√	√		√	√	√	√
Vaccination[7]			√		√			√			√			
Reactogenicity assessment[8]			√		√			√			√			
EP injection site assessment				√	√	√		√	√		√	√	√	√[16]
Clinical labs	Tube													
Pregnancy test (urine or serum) [9,10]		√	√		√			√	√		√			
HBsAg/anti-HCV[10]	SST	5												
HIV screening test· [10,11]	SST	5												
Calcium[11]	SST	5												
CBC/Differential[10]	EDTA	5		5		5			5			5		
ALT & Creatinine[10]	SST	5		5		5			5			5		
HIV diagnostic test	EDTA							10				10	20	
Research samples[12]														
PBMC for assays & storage	ACD			42.5		136		136[13]				136	178.5	
Serum for assays and storage	SST	34		34		34			34			34	34	
Leukapheresis[14]		√							√					
Daily volume (mL)		59	0	86.5	0	180		146	44		0	190	232.5	
56-day total volume (mL)				145.5	145.5	325.5		326	370		0	190	232.5	

Green shading: Vaccination visit for all groups; **Blue shading:** Vaccination visit for Group 3 only; **Gray shading:** FNA visit not applicable to this subgroup.

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

[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, and vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck and 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 reports 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. Participants will also be provided with counselling about contraception.

[5] **Risk-reduction counseling** per CRS Standard Operating Procedures (SOP).

[6] **Concomitant medications,** including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots, are recorded during screening, at enrollment, and at each subsequent clinic visit.

[7] **Vaccination (in-clinic assessments):** At least 60 minutes after the first vaccination and after the fourth vaccination, and 30 minutes after for all other vaccinations. Prior to clinic discharge, the injection site and systemic symptoms will be assessed and participants will have vital signs taken.

[8] **Reactogenicity:** Clinic staff will follow new or unresolved reactogenicity symptoms present at the final reactogenicity Day (see Section 8.3 and HVTN 305 SSP)

[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. For 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. Alternate tube types may be used under certain conditions (eg, product shortages) upon approval of the HVTN Laboratory Center. Refer to the Specimen Collection SSP for more information.

[13] **Blood draw** will be performed before vaccination.

[14] **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 8.6).

[15] **Vaccination 4/Visit 10** will be conducted only for participants assigned to Group 3.

[16] **AESI contact:** CRS staff will contact study participants at month 18 to collect the information listed in Section 8.10. The EP-injection–site assessment will be an abbreviated questionnaire that can be conducted over the phone.

Appendix C Visit windows

Visit #	Visit Type	Lower allowable window (-)	Lower target window (-)	Target day*	Upper target window (+)	Upper allowable window (+)up
01	Screening Leukapheresis	-56	-	0	-	+0
02	**Enrollment / Vaccination 1**	-	-	1	-	-
03	2 weeks postvaccination 1	-	-4	15	+4	+7
04	**Vaccination 2**	-	-	**29**	**+9**	**+28**
05	2 weeks postvaccination 2	-	-4	43	+4	+7
06*	**FNA** postvaccination 2	-	-4	50	+4	+10
07	**Vaccination 3**	-	-	**85**	**+14**	**+28**
08	2 weeks postvaccination 3 Leukapheresis Primary immunogenicity	-	-4	99	+4	+7
09*	**FNA** postvaccination 3	-	-4	106	+4	+10
10	**Vaccination 4 (Group 3 only)**	-	**-7**	**169**	**+14**	**+28**
11	2 weeks postvaccination 4 for Group 3; follow-up visit for Groups 1 and 2	-	-4	183	+4	+7
12	**Final clinic visit**	-28	-14	365	+14	+28
AESI**	AESI contact	-21	-14	547	+14	+28

All target dates are relative to Day 1, with the exception of visits, 3, 5, 8, and 11 (for Group 3 only) which are relative to the vaccination immediately preceding the visit.

*Visits 06 and 09 are only applicable to participants providing lymph node FNA collections. The FNA visits are relative to the preceding vaccination.

**This contact must be at least 12 months post last vaccination

Appendix D Sample informed consent form

Title: A phase 1 open-label clinical trial to evaluate the safety and immunogenicity of synthetic DNAs encoding NP-GT8 and IL-12, with or without a TLR-agonist-adjuvanted HIV Env Trimer 4571 boost, in adults without HIV

HVTN protocol number: HVTN 305

Site: [Insert site name]

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

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

Key information

- This is the first study in which one of the study vaccines will be given to people. This is also the first time these study products will be given together to people.
- Being in this research study is voluntary. It is your choice.
- You are being asked to take part in this study because you are aged 18 to 55, HIV negative, and healthy.
- The purpose of this study is to see if the study vaccines are safe, and how a person's immune system responds to them.
- You will be in this study for up to 12 months of clinic visits, with a follow-up contact at least 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. White blood cells will be collected by a procedure called leukapheresis. In this procedure, some blood will be drawn out of your body and passed through a device that will separate and save the white blood cells before returning the rest of the blood back into your body. You may also be asked if we can collect cells from your lymph nodes. 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.
- One of the study vaccines will be given with electroporation (EP). This procedure uses a handheld device to give an electrical pulse into the skin where the injection is given using 3 short needles. The pulse briefly opens tiny

pores in the cells where the injection is given. We will tell you more about this procedure later in this consent form.

- There are risks from participating:

The most common risks of taking blood and giving injections that we know about are bruising, pain, fainting, soreness, redness, swelling, itching, a sore, and bleeding.

Collection of white blood cells (leukapheresis) and collecting cells from your lymph node can cause pain, bruising, and rarely, an infection.

Because one of the vaccines has not been given to people before, and the other vaccine has only been given to a few people, we do not know what all of the risks may be.

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

The EP device can cause pain ranging from mild to severe that goes away quickly. It can also cause soreness, bruising, redness, swelling, itching, or hardness/stiffness in the arm where you got the EP. There is also a risk of marks on the skin, such as red bumps, scabs, and changes in skin color that may last for months.

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

About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test 2 HIV vaccines. HIV is the virus that causes AIDS.

Up to 54 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 critical questions.**

   - Are the study vaccines safe to give to people?

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

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

   - After getting the vaccines, do people develop a certain type of cell in their lymph nodes that researchers think are important for protecting against HIV?

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

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

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

3. The study vaccines are experimental.

We are testing 2 study vaccines.

One is INO-6172. We will call it the DNA vaccine. It is made up of two different pieces of DNA that were made in a laboratory. DNA is a natural substance found in all living things, including people and viruses. DNA tells cells to make proteins.

The second vaccine is called Trimer 4571. We will call it the protein vaccine. It is made with an adjuvant called 3M-052-AF + Alum. Adjuvants are products that help alert the immune system to have a stronger response.

Everyone in this study will get the DNA vaccine. It will tell your body to make small amounts of two different proteins. One of them is a protein that is found in HIV and the second protein is called IL-12 which is naturally found in people. Your body's immune system might recognize the HIV protein and prepare itself to fight HIV by making antibodies and T cells. IL-12 helps the immune system to make antibodies and T cells more efficiently. Antibodies and T cells are parts of an immune response that defend the body against diseases. DNA tells cells to make proteins. The DNA vaccine is very much like natural DNA, but it was made in a laboratory. You cannot get HIV or AIDS from the DNA vaccine or from the proteins the body makes in response to it.

Some people in the study will also get the protein vaccine. The protein vaccine is a small, lab-made piece of protein that looks like a part of a protein found on the surface of the HIV virus. The immune system might be able to see this protein piece and learn how to recognize HIV if you are ever exposed to it in the future.

The protein vaccine will be given with an adjuvant called 3M-052-AF + Alum. 3M-052 was originally developed by 3M Corporation. 3M-052 is one of many similar products developed by 3M Corporation to treat skin conditions and tumors, and to make vaccines more effective. It is designed to stimulate parts of the immune system that recognize invaders like viruses. But in this study, we will use the improved 3M-052-AF adjuvant.

The protein vaccine and the 3M-052-AF adjuvant have not been given together to people before. In this study, 3M-052-AF is dissolved in water and is mixed with Alum. Alum is made from Aluminum Hydroxide. Alum is an adjuvant with a long standing safety record that has been used in approved vaccines for more than 90 years

Where do the study products come from?

The DNA vaccine was developed by the Wistar Institute and Inovio Pharmaceuticals. It is provided for this study by the Division of AIDS (DAIDS) at the US National Institutes of Health (NIH) in Rockville, Maryland.

The protein vaccine was developed and is provided by the Dale and Betty Bumpers Vaccine Research Center (VRC) in Bethesda, Maryland, which is part of the NIH

3M-052 was developed by 3M Corporation in St. Paul, Minnesota USA. In this study, the 3M-052 is dissolved in water and this mixture is being provided by the Access to Advanced Health Institute (AAHI)in Seattle, Washington USA.

The Alum was manufactured by Brenntag Biosector in Frederikssund, Denmark. It is being provided by DAIDS at the US National Institutes of Health in Rockville, Maryland, USA.

What do we know about the study vaccines from other studies?

The DNA vaccine has not been given to people before. Similar HIV DNA vaccines with the same IL-12 adjuvant have been given to more than 350 people in other studies. In general, people who got the DNA vaccine in those studies were not too uncomfortable, and did not have any serious health problems related to the vaccine. Three participants had pain with the injection of the DNA vaccine, and decided not to get any more injections.

The protein vaccine combined with just Alum was given to people in 3 different ongoing studies. In the first study, VRC081, 16 people got the same protein vaccine mixed with the same Alum adjuvant that we will use in this study. Each person got 3 injections of the vaccine at the same dose or higher than the dose we will use in this study. The injections did not cause any serious health problems. Most people in that study had at least 1 of the side effects listed in the “Risks of Vaccines” described below. The participants said their side effects were mild, and they went away within about a week.

In the second study, NIAID 19-I-0069, 3 people got a single dose of this protein vaccine combined with Alum at a higher dose than the one we will use in this study. The injections did not cause any serious health problems. This study is still ongoing and plans to enroll up to 100 people.

In the third study, NCT04985760, 5 people got injections that had either, a) the protein vaccine mixed with Alum at the same dose or higher than the dose we will use in this study or, b) just the Alum. We do not yet know who got which products. Out of the 5 people, 2 received 2 injections, and 3 received 1 injection. The injections have not caused any serious health problems. This study is still ongoing and plans to enroll 32 people out of which 24 people will get the protein vaccine mixed with Alum.

Another protein vaccine and 3M-052-AF + Alum adjuvant combination is being tested for the first time in people in two other studies called HVTN 137 and

HVTN 300. As of September 7, 2022, 48 people in these 2 studies have had at least 1 study injection and at least 35 people have had 2 injections. These injections have not caused any serious health problems.

As of September 7, 2022, HVTN 300 has enrolled 13 people, all of whom received at least 1 injection. Ten (10) people received at least 2 injections, nine (9) received at least 4 injections, and six (6) have received all 5 injections.

All people in the study had at least some injection site reactions during the trial, mostly mild to moderate. One 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.

All people had some side effects that they described as mild to moderate. Five (5) people had severe side effects, including chills, headache, muscle aches, and generally feeling unwell. Two (2) of these 5 people decided to stop getting study injections because they felt generally unwell after injections, and it was affecting their daily lives. Another 2 of the 5 people who had severe side effects got more injections; one of them had more severe side effects, while the other did not. And 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.

HVTN 137 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 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. All 4 felt severely tired and generally unwell for up to 2 days. Two of them also had severe muscle and joint pain, severe headache and chills for a day. The study is ongoing and 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. In Part B of HVTN 137, 88 people received injections of either the study vaccine and adjuvant or the placebo. These injections have not caused any serious health problems.

Given the side effects experienced in HVTN 300 and HVTN 137, we can expect that many people in this study who receive the 3M-052-AF + Alum adjuvant will have some side effects. The side effects we expect would be similar to the *Risks of vaccines* listed below.

These are experimental HIV vaccines, which means we do not know if the vaccines will be safe to use in people, or if they will work to prevent HIV infection. These vaccines are used only in research studies.

Risks of vaccines:

All vaccines can cause fever, chills, headaches, rash, aches, pains, nausea, dizziness, and feeling tired. Vaccines can also cause tenderness, swelling, 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 have ever had a bad reaction to an injection or vaccine.

Risks of DNA vaccines:

More than 1,000 people have been given DNA vaccines being tested against HIV, and thousands of people have received other experimental DNA vaccines for other diseases. These other DNA vaccines have not caused serious health problems. We expect the risks of the DNA vaccine in this study to be similar to those of other DNA vaccines.

Risks of the protein vaccine:

This protein vaccine has been given to a small number of people so far and no serious health concerns have been reported. Because of this, we do not know all the side effects. There may be side effects, even serious or life-threatening ones, that we do not know about yet.

Risks of the study adjuvant:

The combination of the protein vaccine and adjuvant used in this study has not been given to people before, so we do not know all the risks. However, the adjuvant in combination with other similar protein vaccines is being given to people for the first time in other studies. A small number of people have received these vaccinations so far. A few of them have reported having some of the side effects described in 'Risks of vaccine', but no serious health concerns have been reported so far.

These are the side effects that 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.

4. The DNA vaccine is given using electroporation (EP).

Besides adjuvants, another way to improve immune responses to DNA vaccines is to use an EP device when giving the vaccine. EP gives an electric pulse using 3 short needles into the skin. The pulse briefly opens tiny pores in the cells where the injection is given. The DNA can enter the cells through these pores. Previous studies have shown that EP helped to get DNA into cells better. One study also showed increased immune responses to another experimental DNA vaccine.

Using EP in people is an experimental procedure, and the EP device is an experimental device. The EP device is only used in people in research studies.

EP is done in this study using a device called the CELLECTRA 2000 EP System. It was developed and is being provided for this study by Inovio Pharmaceuticals, Inc.

The EP device has been used with injections into the skin in at least 350 people. In another study, a DNA vaccine given together with an IL-12 DNA adjuvant was given into the skin with EP to 55 people. EP did not cause any serious health problems in these participants.

In a previous study using the EP device in healthy volunteers, some people said that they felt only a little discomfort, while others said it was very painful, even after the injections were over. However, only 3 out of 48 volunteers left the study because of pain and discomfort.

Another large study being done in multiple countries is currently giving EP with a DNA vaccine for SARS-CoV-2 (the virus that causes COVID-19). The study began in November 2020, and so far over 3,200 people have received more than 9300 injections with an EP device. A few people have noticed marks on the skin after getting the vaccines with EP, but no health concerns have been reported so far.

Risks of EP:

EP causes brief muscle contractions during the procedure. In previous studies using EP, people felt initial pain that ranged from mild to severe. For most people, the pain eased quickly. EP can also cause soreness, bruising, redness, swelling, itching, or hardness/stiffness in the upper arm where you got the injection. When the injection and EP are given to the skin, the needles may leave marks, such as red bumps or scabs. Later, the marks may heal, but may leave light or dark spots on the skin. In some people, these marks lasted 12 months after their last injection. We do not know if the marks went away later, because we did not follow the participants beyond that time. On darker skin, these marks tend to remain visible for longer, with less fading. We do not know how the skin at the injection site will change in appearance after EP with these study products because they have not been given with EP before. In addition, every person's skin can react differently. We will show you photos of people who got EP in a previous study to give you examples of what these injection marks can look like over time.

Site: Sites must review the sample photos of EP injection marks with potential participants during the study informed consent process.

On rare occasions, the EP device may cause infection at the part of your body where you got the injection.

Having the procedure or thinking about it may cause some stress and anxiety. If you feel anxious, please tell us and we will try to help you. In a previous study

where a DNA vaccine was given into the skin with EP, over 99% of the participants said they would recommend EP with an effective HIV vaccine to their family or friends if it felt the same as their study experience. Of these participants, 96% said that the appearance at injection sites was acceptable.

We do not know if EP will change the risks for the DNA vaccine with the IL-12 adjuvant. We do not know all the risks of EP because it has only been used in a limited number of people before this study and has not been used with these study products.

Joining the study

5. 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, it will not affect your other care at this clinic nor 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.

Site: The boxed text is for sites in South Africa.

> We check to make sure that you are not in more than one study by taking your fingerprint on an electronic system. This information is only accessed by a few members of the study team using a secure password.

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

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

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

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

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

We will also do blood tests. These tell us about the health of your kidneys and liver. We will ask you about medicines you are taking, including HIV pre-

exposure prophylaxis (PrEP). We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy.

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

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

7. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here for free.**

   For the care that we cannot give, we will help you get care elsewhere. For health problems unrelated to the study, we will not pay for care.

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

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

   It is important that you 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 last study injection at 6 months (9 months total). 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:

9. **You will come to the clinic for scheduled visits up to 12 times over 12 months.**

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

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

   You may have to come for more visits if you have a lab or health issue. We will also contact you 1 year after your last vaccination to check on your health. We may also contact you after the study ends (for example, to tell you about the study results). We may also contact you about other studies that you may want to join.

10. We will give you compensation for each study visit you complete.

This amount is to cover the costs of *[Site: Insert text]*. There is also additional compensation for the leukapheresis procedure and lymph node cell collection procedure. We will tell you more about the procedures below.

You will receive $xx.xx each time you have leukapheresis. This amount is to cover the costs of *[Site: Insert text]*. You will be paid _____ ***["following each completed visit", "monthly", "quarterly", "at the end of your participation in the research study", "following each completed visit or at the end of your participation in the research study, whichever you prefer"]***.

You will receive $xx.xx each time you have a lymph node cell collection. This amount is to cover the costs of *[Site: Insert text]*. You will be paid _____ ***["following each completed visit", "monthly", "quarterly", "at the end of your participation in the research study", "following each completed visit or at the end of your participation in the research study, whichever you prefer"]***.

The boxed text is for sites in the US

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).*

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

We will give you injections of the study vaccines at 3 or 4 visits, according to the schedule in the table below. All injections will be given into the upper arm with a needle and syringe. The first 5 participants to enroll in the study will be assigned to Group 1. If you are not one of them, you have a small chance (10%) of being assigned to Group 1. Your chance of being assigned to either group 2 or group 3 is slightly lower than 50%. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.* Your group assignment is completely random, like rolling dice or drawing straws.

If you are in Group 1 or Group 2, you will only get the DNA vaccine. You will have 3 injection visits where you get 1 injection in each arm, followed by EP into the skin of each arm.

If you are in Group 3, you will get the DNA vaccine at the first 2 injection visits. You will get 1 injection in each arm, followed by EP into your skin. At the third injection visit you will get the DNA vaccine injection in each arm, followed by EP into your skin, and you will also get a protein vaccine injection into the upper muscle of each arm. You will also have a fourth injection visit where you get just the protein vaccine into the upper muscle of each arm.

Because this is the first time the DNA vaccine is being given to people, only 1 participant in Group 1 will get the study vaccine each day until the first 5 participants have gotten the DNA vaccine. After this, we will not enroll anyone else into the study until a safety review is completed. A team of experts who are not part of the study will look at the information about the first 5 people's responses to the vaccine and decide if it is safe to continue the study. If it is, the rest of the participants in all groups will be enrolled. Since this is the first time the DNA and protein vaccines are given as a combination, at the Month 3 injection visit, only 1 participant in Group 3 will get the study vaccinations each day for the first five participants to reach this study visit, so that we can closely monitor their safety.

Group #	Number of People	Products	Dose	Where	How	First injection visit	1 month later	3 months later	6 months later
1	9	DNA vaccine	Lower dose	Skin of both upper arms	Needle & syringe + EP	✓	✓	✓	
2	18	DNA vaccine	Higher dose	Skin of both upper arms	Needle & syringe + EP	✓	✓	✓	
3	18	DNA vaccine	Higher dose	Skin of both upper arms	Needle & syringe + EP	✓	✓	✓	
		Protein vaccine	--	Muscle of both upper arms	Needle & syringe			✓	✓

For injections of the DNA vaccine between the layers of the skin (known as intradermal injections), we will give the injection and then press the EP device firmly against that same area. The device inserts 3 very short needles into your skin. We will activate the device and a very small amount of electricity will be sent in 4 short pulses from the needles into your arm. Each of these pulses will last less than 1 second. Your arm will move because of the electrical pulses.

This injection procedure will take less than 1 minute. During the procedure and right after, you will feel some pain or discomfort. The intensity of that feeling lessens or may go away within a couple of minutes. After that, your arm may be sore for a day or two.

People in Group 3 will also get injections of the protein vaccine and study adjuvant. These are given in the traditional way vaccines are given, using a needle and syringe to give the vaccine in the muscle of the upper arm.

You will have to wait in the clinic for about 30 minutes to an hour after getting your injections to see if there are any problems.

- If you are in Group 1 or Group 2, you will wait in the clinic for 60 minutes after your first injection and for 30 minutes after all other injections.
- If you are in Group 3, you will wait for 60 minutes after your first, third and fourth injections and for 30 minutes after your second injection.

You will also use a secure electronic symptom log to keep track of how you are feeling. We call this symptom log an eDiary.

- If you are in Group 1 or Group 2, after all injections you will use the eDiary to keep track of how you are feeling for that day and for 7 more days.
- If you are in Group 3, after just the first 2 injections you will use the eDiary to keep track of how you are feeling for that day and for 7 more days. For the other injections, you will use the eDiary for that day and for 2 more weeks.

If you are unable or unwilling to use the eDiary, 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 us if you have any issues or concerns after getting an injection.

As part of this research, you may need to use an app on your phone to access the eDiary. While using this app, information about you may be collected and shared with the researchers or people outside of the study who have been approved by the researchers. These data might include personal health information and information about your use of the app, such as the amount of time you spend on each screen. The eDiary does not collect personal information about your activities over time or from other websites or online services. It also does not allow third parties to collect that information. A complete description of the data collection and sharing can be found in the privacy policy associated with the app. If you would like to read these documents, the study doctor can tell you how to access this information.

The privacy policy may include statements that limit your rights if you are harmed by using the app in this study. You do not release the study doctor, sponsor, this institution, or the research staff for responsibilities from mistakes. You also do not waive any of your rights as a research participant.

We will ask you questions about any skin changes related to the EP procedure. We may ask to take photos of your arm where you got the injection or EP. This may help us learn more about reactions to the study vaccines or the EP procedure. We will not take photos of your face. Your name and other identifying information will not be included with the photos or in publications. The photos will only be used to research the safety of the vaccine and EP.

12. We will do the procedures shown in the following table.

			Time after first injection visit										
Procedure	**Screening visit(s)**	**First injection visit**	**2 weeks**	**1 month**	**1½ months**	**1¾ months**	**3 months**	**3½ months**	**3¾ months**	**6 months***	**6½ months**	**12 months**	**1 year + 6 months**
Injection(s)		√		√			√			√			
Medical history	√												
Complete physical	√											√	
Brief physical		√	√	√	√		√	√		√	√		
Blood drawn	√		√		√		√	√			√	√	
Lymph node cell collection**						√			√				
Leukapheresis	√							√					
Pregnancy test***	√	√		√		√	√	√	√	√			
HIV testing	√						√				√	√	
Risk reduction counseling	√	√	√	√	√		√	√		√	√	√	
Interview / questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	
Skin assessment			√	√	√	√	√	√	√	√	√	√	√
Health contact													√

* The gray shading for the visit at 6 months is only for participants assigned to Group 3.

** Lymph node cell collections are an optional procedure for people in Groups 2 and 3.

*** Applies only to persons who were assigned female sex at birth and who are capable of becoming pregnant. Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) 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 44 mL and 258 mL (about 3 tablespoons to about 1 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.

Site: Sites must obtain copies of the informed consent form and any other educational materials that are available from the facilities where leukapheresis and FNA will be performed, so that they can be reviewed with potential participants during the study informed consent process.

Leukapheresis procedure (for all participants):

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 from a vein in one arm and the rest of the blood is put back into a vein on the other arm.

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. After the white blood cells are taken out, the rest of the blood will go back into your body through the tube going into your other arm. Sometimes the fluid lost during the procedure is replaced by a sterile salt water solution, or a solution containing a protein called albumin. This protein is normally found in human blood. An anticoagulant may be added to your blood during the procedure. Anticoagulants prevent blood from clotting.

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 third injection visit. It will take about *[Site: Insert timeframe]* for the procedure.

Risks of leukapheresis

Generally, the risks of leukapheresis include pain, bruising, and rarely, infection. Rarely, albumin can cause an allergic reaction. If the leukapheresis procedure has to be stopped, it could result in the loss of up to 1 cup of blood. Your body makes new blood within 2 weeks.

During leukapheresis, you may be given a blood thinner to prevent blood clots in the machine. This may cause you to have low calcium levels in your blood for a short time. Sometimes people with low calcium levels feel numbness and tingling in their body. Low calcium can be corrected by replacing what is lost with a calcium tablet during or shortly after the procedure. The leukapheresis centers know how to treat low calcium, so please let the staff know if you feel numbness or tingling. Very rarely low calcium levels can cause heart problems, but to our knowledge, this procedure should not cause you to have heart problems.

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 (optional for Groups 2 and 3):

People in Groups 2 and 3 will be asked to consider an additional procedure. The formal name for this procedure is fine needle aspiration (FNA). 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 clinician will use a very thin needle guided by ultrasound to collect cells from a lymph node on your arm or in your armpit. The researchers would like to have at least half of the people in Groups 2 and 3 agree to these cell collections.

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. We will also tell you about some medications you should avoid before having the procedure.

To do the procedure, a clinician will find the appropriate lymph node by applying a cold gel to your skin and then pressing a handheld ultrasound device against your skin. If necessary, they may shave a small area near the lymph node. Once the node is found, they will apply a cleaning solution to the skin in that area. They will numb the area with an injection of a local anesthetic, such as Lidocaine; this is like Novocaine which is used for dental procedures. They will insert a very thin needle and use the images from the ultrasound to guide it into the lymph node. They may move the needle 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 to 15 seconds. It usually takes about 4 separate cell collections to get enough cells.

After the procedure is done, the clinician will clean the area with warm water and give you a band-aid to cover the site where the needle was inserted.

Lymph node cell collection will be done twice, about 3 weeks after your second and third injection visits. It will take about *[Site: insert timeframe]* for the procedure.

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. Rarely the local anesthetic can cause your arm to feel numb or weak. 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.

13. 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.

14. The HVTN will test your samples to see how your body, including your immune system, responds to the study vaccines.

We will send your samples (without your name or other identifying information) to labs 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 vaccines.

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

These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

When your samples are no longer needed for this study, the HVTN will continue to store them.

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

15. When samples are no longer needed for this study, the HVTN may want 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 it. 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 anyone else that 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. 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 IRB or EC
- 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.

16. We will do our best to protect your private information.

US 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 eDiary after the injection visits, that information will only have your code number, not your name. Your data goes directly from the eDiary 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 NIH and its study monitors;
- The US Food and Drug Administration (FDA);
- Any regulatory agency that reviews clinical trials;
- [Insert name of local IBC];
- US sites may, but are not required to include: [Insert name of local IRB/EC] ;
- Advarra IRB;
- [Insert name of local and/or national regulatory authority as appropriate];
- The Wistar Institute, Inovio Pharmaceuticals, AAHI and people who work for them;
- The HVTN and people who work for them;
- The HVTN Safety Monitoring Board (SMB); and
- The US Office for Human Research Protections (OHRP).

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]

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

To help protect your privacy, we have a Certificate of Confidentiality from the US government. With the certificate, we do not have to release information about you to someone who is not connected to the study, such as the courts or police. However, we cannot withhold information from the US government because it funds this research. You can still give information about yourself and your study participation to others.

The results of this study may be published. No publication will use your name or identify you personally.

We may share information from the study with other researchers. We will not share your name or information that can identify you.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

17. 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 and complete the other study procedures, 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 if you agree.

We will stop your injections if you get HIV. We will also take fewer samples and may not perform some of the procedures we told you about earlier. We will help you get care and support. We will counsel you about having HIV and about telling your partner(s). *Site: Modify the following sentence as appropriate.* We will not provide or pay for your HIV care. We will encourage you to stay in the study for up to 12 months after your last injection. It is up to you whether you choose to stay or leave. 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.

18. 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

19. 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 that we know about. There may be other risks not listed below, 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 for someone to see your personal information even if they should not be able to. 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 that 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.

US Sites, include the following paragraph.

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 making decisions about giving you health insurance or offering you work. GINA does not help or protect you against discrimination by companies that sell life, disability, or long-term care insurance.

Unknown risks:

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

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

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

Benefits

20. 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

21. 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

22. 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.

Whether we take you out of the study or you leave on your own, we will ask you to come to the clinic one last time for a physical exam, a questionnaire, and a

pregnancy test, if indicated. We may ask to take some blood samples. Whether you come for this last visit is up to you.

Injuries

23. 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.*Next paragraph for non-US sites.*

Sites: adjust the language in this paragraph so it is applicable to your site. Note that the ABPI guidelines apply to South Africa only. In this study, our clinic has insurance to cover your medical treatment in the case of a study-related injury. We will follow the Association of the British Pharmaceutical Industry guidelines for payment of study-related injury. We can give you a copy of these guidelines. In rare cases, the insurance funds may not be enough.

In this study, Inovio Pharmaceuticals, Inc. will pay the cost of medical care that arises from injuries caused by the EP device and the DNA vaccine, INO-6172. AAHI will pay the cost of medical care from injuries caused by the 3M-052-AF adjuvant. For injuries caused by other study products or study procedures, the HVTN has limited funds to pay medical costs that it determines are reasonable. If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

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

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

Questions

24. 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:

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

By mail:
Study Subject Adviser

Advarra IRB

6100 Merriweather Dr., Suite 600

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

Please reference the following number when contacting the Study Subject Adviser: Pro000xxx.

Remainder of section for South African sites: The study has been structured in accordance with the Declaration of Helsinki (last updated October 2013), which deals with the recommendations guiding doctors in biomedical research involving human participants, *the Ethics in Health Research: Principles, Structures and Processes Second Edition 2015* and *Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa.* We can provide you with copies of these guidelines if you wish to review them. In addition, the recent Protection of Personal Information Act (POPIA) ensures that all South African institutions conduct themselves in a responsible manner when collecting, processing, storing, and sharing another entity's personal information by holding them accountable should they abuse or compromise your personal information in any way.

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].

You can reach a study staff member 24 hours a day at [telephone number].

If you have questions about this trial, you should first discuss them with your doctor or the EC (contact details as provided on this form). After you have consulted your doctor or the EC, and if they have not provided you with answers to your satisfaction, you should write to the South African Healthcare Products Regulatory Authority (SAHPRA) at:

The Chief Executive Officer
Dr Boitumelo Semete-Makokotlela
South African Health Products Regulatory Authority Private Bag X828
PRETORIA
0001
Tel: (012) 501 0410/13
e-mail: Boitumelo.Semete@sahpra.org.za

Your permissions and signature

25. In Section 12 of this form, we told you about an optional sample collection procedure if you are in Groups 2 or 3. Please write your initials or make your mark in the box next to the procedure if you agree to have this done, or leave it blank if you do not want to provide these extra samples. You can change your mind after signing and dating this form. There will also be an additional consent form to sign and date at the time the procedure is done.

☐ I agree to lymph node cell collection.

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

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

OR

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

OR

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

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

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

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

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

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

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

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

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

Appendix E Sample consent form for use of samples and information in other studies

Title: A phase 1 open-label clinical trial to evaluate the safety and immunogenicity of synthetic DNAs encoding NP-GT8 and IL-12, with or without aTLR-agonist-adjuvanted HIV Env Trimer 4571 boost, in adults without HIV

HVTN protocol number: HVTN 305

Site: [Insert site name]

Key Information

These are some of the things you should know about the use of your samples and information for other studies:

- The extra samples will be labeled with a code number. They will not be labeled with your name. The extra samples are stored in a secure place. At your request, the HVTN will destroy all your extra samples. You can still join the main study even if you do not agree to use of your extra samples in other studies.

- Researchers may do genetic testing on your samples, which could include genome wide studies. It is unlikely, but these tests could show you may be at risk for certain diseases. In the very unlikely event that others found out, this could lead to discrimination or other problems.

- You will not be paid or otherwise benefit from allowing your extra samples to be used in other studies.

The rest of this form gives more information about use of your extra samples for other studies. Please read it carefully.

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

1. Do I have to agree?

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

2. Where are the samples stored?

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

the sentence referencing the repository in the United States.] Your samples will be stored in the HVTN repository in South Africa. Your samples will be stored in the HVTN repository in the United States.

3. How long will the samples be stored?

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

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

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

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

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

6. Will the HVTN sell my samples and information?

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

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

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

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

The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with anyone who does not need to know your name. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

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

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

Researchers may also do genetic testing on your samples.

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

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

10. What are the risks of genetic testing?

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

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

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- The researcher’s Institutional Review Board or Ethics Committee
- Any regulatory agency that reviews clinical trials
- The people who work with the researcher

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

Questions

12. If you have questions or problems about allowing your samples and information to be used in other studies, use the following important contacts.

If you have questions about the use of your samples or information or if you want to change your mind about their use, contact
[name or title and telephone number of the investigator or other study staff].

If you think you may have been harmed because of studies using your samples or information, contact
[name or title and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact
[name or title and telephone number of person on IRB/EC .

The study has been structured in accordance with the Declaration of Helsinki (last updated October 2013) which deals with the recommendations guiding doctors in biomedical research involving human participants, the Ethics in Health Research: Principles, Structures and Processes Second Edition 2015, and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa. We can provide you with copies of these guidelines if you wish to review them. In addition, the recent Protection of Personal Information Act (POPIA) ensures that all South African institutions conducts themselves in a responsible manner when collecting, processing, storing and sharing another entity's personal information by holding them accountable should they abuse or compromise your personal information in any way.

You can reach a study staff member 24 hours a day at [telephone number].

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Healthcare Products Regulatory Authority (SAHPRA) at:

The Chief Executive Officer
Dr Boitumelo Semete-Makokotlela
South African Health Products Regulatory Authority Private Bag X828
PRETORIA
0001
Tel: (012) 501 0410/13
e-mail: Boitumelo.Semete@sahpra.org.za

13. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track

of your choice about how your samples and information can be used. You can change your mind after signing this form.

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

OR

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

OR

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

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

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

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

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

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

Appendix F Low-risk guidelines for US

The following are intended as guidelines for the investigator to help identify potential vaccine trial participants at "low risk" for HIV infection. These guidelines are based on behaviors within the last 6 to 12 months prior to enrollment; however, it may be appropriate to consider a person's behavior over a longer period of time than specified in order 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 (PSRT).

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

For US volunteers NOT on stable Pre-exposure prophylaxis (PrEP)

1. SEXUAL BEHAVIORS

In the last 12 months, did not:

- Have oral, vaginal, or anal intercourse with an HIV-infected partner or a partner who uses injection drugs

- Give or receive money, drugs, gifts, or services in exchange for oral, vaginal, or anal sex

AND

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

In the **last 6 months,** had 4 or fewer partners of the opposite birth sex for vaginal and/or anal intercourse, OR

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

- Had 2 or fewer MSM partners for anal intercourse and had no unprotected anal sex with MSM, OR

- Had unprotected anal intercourse with only 1 MSM partner within a monogamous relationship lasting at least 12 months (during which neither partner had any other partners). If the monogamous relationship ended, the

volunteer may then have had protected anal intercourse with 1 other MSM partner (total 2 or fewer partners in the last 12 months), OR

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

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

AND

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

2. NONSEXUAL BEHAVIORS

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

- Inject drugs or other substances without a prescription
- Use cocaine, methamphetamine, or excessive alcohol that, in the investigator's judgment, rendered the participant at greater-than-low risk for acquiring HIV infection.

The investigator's judgment should consider local epidemiologic information about HIV prevalence in the area and community networks.

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

Acquired an STI (ie, new infection) in the **last 12 months**:

- Syphilis
- Gonorrhea
- Nongonococcal 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)

- **PrEP ASSESSMENT**
- Reports 6 months (180 days) or longer of protective PrEP use
- For daily oral PrEP use:
    - For persons AMAB who have sex with persons AMAB: Reports equal to or greater than 70% when asked the following: "*Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications?"*
    - For people with a vagina having intravaginal intercourse: Reports equal to or greater than 90% when asked the following: "*Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications?"*
- For event-driven (on-demand or "2-1-1") PrEP use[1] in persons AMAB who have sex with persons AMAB: Reports use consistent with the guidance of the professional education organization, the International Antiviral Society–USA (IAS-USA):
    - For individuals with frequent use (> 15 pills per month): At least 80% of condomless sex acts are covered with on-demand PrEP at the recommended dose schedule
    - For individuals with less frequent use (≤ 15 pills per month):
        - A past history of high adherence (> 90%)

        - Commitment to use on-demand PrEP for *all* condomless sex acts at the recommended dose schedule

- For long-acting agents, such as injectable cabotegravir, please consult with the PSRT.

- Commits to maintaining protective PrEP use throughout trial

SEXUAL BEHAVIORS

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

NONSEXUAL BEHAVIORS

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

- Inject drugs or other substances without a prescription

- Use cocaine, methamphetamine, or excessive alcohol that, 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 Low-risk guidelines for South Africa

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 12 months prior to enrollment; however, it may be appropriate to consider a person's behavior 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 epidemiological information about the 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 (PSRT).

Assessment of sexual behaviors

Consider whether a volunteer would be appropriate for inclusion if, within 12 months prior to enrollment, the person:

- Abstained from penile/vaginal and penile/anal intercourse, or
- Was in a mutually monogamous relationship with a partner with a known HIV-uninfected status, or
- Had 1 partner believed to be HIV-uninfected with whom he/she regularly used condoms for penile/vaginal or penile/anal intercourse.

Exclude a volunteer if:

Within 12 months prior to enrollment: a history of newly acquired syphilis; gonorrhea; chlamydia; trichomoniasis; active HSV lesions; chancroid; genital warts of the labia minora, vagina, or cervix; or any other symptomatic genital warts.

Appendix H 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.

It is important that you 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 your last study injection at 6 months (9 months total).

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 I Adverse events of special interest (AESIs)

AESIs for this protocol include, but are not limited to, potential immune-mediated diseases; representative examples of AESIs are listed below. Updates to AESIs will be provided as an appendix to the *HVTN305 Study Specific Procedures (SSPs).*

<table>
<tr><th>Neuroinflammatory disorders</th><th>Musculoskeletal disorders</th><th>Skin disorders</th></tr>
<tr><td>• Cranial nerve disorders, including paralyses/paresis (eg, Bell’s palsy)
• Optic neuritis
• Multiple sclerosis
• Transverse myelitis
• Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
• Acute disseminated encephalomyelitis, including site specific variants: eg, noninfectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis
• Myasthenia gravis, including Lambert-Eaton myasthenic syndrome
• Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy).
• Narcolepsy</td><td>• Systemic lupus erythematosus and associated conditions
• Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome
• Idiopathic inflammatory myopathies, including dermatomyositis
• Polymyositis
• Antisynthetase syndrome
• Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still’s disease
• Polymyalgia rheumatica
• Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
• Psoriatic arthropathy
• Relapsing polychondritis
• Mixed connective tissue disorder</td><td>• Psoriasis
• Vitiligo
• Erythema nodosum
• Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)
• Alopecia areata
• Lichen planus
• Sweet’s syndrome
• Localized Scleroderma (Morphea)
• Cutaneous lupus erythematosus

Metabolic disorders
• Addison’s disease
• Autoimmune thyroiditis (including Hashimoto thyroiditis)
• Diabetes mellitus type I
• Grave's or Basedow’s disease</td></tr>
<tr><th>Vasculitides</th><th>Blood disorders</th><th>Others</th></tr>
<tr><td>• Large vessels vasculitis including: giant cell arteritis such as Takayasu’s arteritis and temporal arteritis.
• Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener’s granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger’s disease (thromboangiitis obliterans), necrotizing vasculitis and antineutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet’s syndrome, leukocytoclastic vasculitis.</td><td>• Autoimmune hemolytic anemia
• Autoimmune thrombocytopenia
• Antiphospholipid syndrome
• Pernicious anemia
• Autoimmune aplastic anemia
• Autoimmune neutropenia
• Autoimmune pancytopenia

Gastrointestinal disorders
• Celiac disease
• Crohn’s disease
• Ulcerative colitis
• Ulcerative proctitis

Liver disorders
• Autoimmune cholangitis
• Autoimmune hepatitis
• Primary biliary cirrhosis
• Primary sclerosing cholangitis</td><td>• Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
• Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy)
• Autoimmune myocarditis/cardiomyopathy
• Sarcoidosis
• Stevens-Johnson syndrome
• Sjögren’s syndrome
• Idiopathic pulmonary fibrosis
• Goodpasture syndrome
• Raynaud’s phenomenon</td></tr>
</table>

Appendix J Protocol team

Protocol leadership

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		Technical editor	Sydney Strauss HVTN LOC, Fred Hutch

Appendix K 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 305 are described below.

Protocol history and modifications

Date: Jan 06, 2023

Protocol version: Version 2.0

Protocol modification: Full protocol Amendment 1

Item 1 Added in Section 5.1.1, *Inclusion criteria;* Appendix A, *Schedule of Procedures: Participants undergoing FNA and* Appendix B*, Schedule of procedures: Participants not undergoing FNA* : serum calcium

Item 2 Revised in Section 5.1.1, *Inclusion criteria*: criterion for blood pressure

Item 3 Removed in Section 9.1, *AEs*: grading Creatinine

Item 4 Added in Section 2.13.1, *Potential Risks* and in Appendix D, *Sample informed consent form (SICF)*: risk of hypocalcemia

Item 5 Added in Appendix D, *Sample informed consent form (SICF)*: study related injury compensation

Item 6 Updated in Section 5.1.2, *Exclusion criteria* and Section 5.2.1, *Delaying vaccinations for a participant*: nomenclature for Monkeypox

Item 7 Corrected in Section 7.1, *Vaccine regimen*: injection volume

Item 8 Updated in Appendix C, *Visit windows*: interval between last vaccination and AESI contact

Item 9 Corrected in Appendix C, *Visit windows*: Lower target window

Item 10 Updated in Title page and Appendix K, *Version History*: IND number and contents of this amendment

Item 11 Corrected throughout the Protocol: minor errors in grammar, typography, formatting

Date: Sep 21, 2022

Protocol version: v1.0

Protocol modification: None