HIV VACCINE
TRIALS NETWORK

PROTOCOL
HVTN 304

A phase 1 open-label clinical trial to evaluate the safety and immunogenicity of synthetic DNAs encoding a native-like HIV Env Trimer and Interleukin-12 (INO-6160), alone or in a prime-boost regimen with 3M-052-AF + Alum adjuvanted VRC HIV Env Trimer 4571 in adult participants without HIV

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Division of AIDS (DAIDS)
National Institute of Allergy and Infectious Diseases (NIAID)
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Bethesda, Maryland, USA

STUDY PRODUCTS PROVIDED BY

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Bethesda, Maryland, USA

Inovio Pharmaceuticals, Inc.
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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 (U.S.) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Council on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (eg, U.S. National Institutes of Health, Division of AIDS) and institutional policies

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

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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
ALT	alanine transaminase
Alum	aluminum hydroxide suspension
ANCA	anti-neutrophil cytoplasmic antibody
AVEG	AIDS Vaccine Evaluation Group
AoU	assessment of understanding
BAMA	binding antibody multiplex assay
BCR	B-cell receptor
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
bnAb	broadly neutralizing antibody
BRR	Bill of Rights and Responsibilities
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
DHHS	Department of Health and Human Services
DM	diabetes mellitus
EAE	expedited adverse event
EC	Ethics Committee
ECLIA	Electrochemiluminescence
eGFR	estimated glomerular filtration rate
EIA	enzyme immunoassay
Env	HIV envelope protein
EP	electroporation

FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GEE	generalized estimating equation
GINA	Genetic Information Nondiscrimination Act
GPP	Good Participatory Practices
HCV	Hepatitis C antibody
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICABA	infected cell antibody-binding assay
ID	intradermal
IFN-gamma	interferon-gamma
IM	intramuscular
IND	investigational new drug application
IRB	Institutional Review Board
IUD	intrauterine device
LABA	long-acting beta agonist
MAAE	medically attended adverse event
MAR	missing at random
MCAR	missing completely at random
MedDRA	Medical Dictionary for Regulatory Activities
MO	Medical Officer
MSD	Meso Scale Discovery
nAb	neutralizing antibody
NAT	nucleic acid test
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NLT	native-like trimer
NSAID	non-steroidal anti-inflammatory drug
OHRP	Office for Human Research Protections
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PI	principal investigator
PID	pelvic inflammatory disease
PrEP	HIV pre-exposure prophylaxis
PSRT	Protocol Safety Review Team
PV	PENNVAX-B DNA vaccine

RAB	DAIDS Regulatory Affairs Branch
RE	Regulatory Entity
RSC	Regulatory Support Center
SAE	serious adverse event
sD	synthetic DNA
SDMC	Statistics and Data Management Center
SHIV	simian-human immunodeficiency virus
SICF	sample informed consent form
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
SSP	Study-specific Procedures
SUSAR	suspected unexpected serious adverse reaction
Th1	type 1 T helper
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
WFI	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 a native-like HIV Env Trimer and Interleukin-12 (INO-6160), alone or in a prime-boost regimen with 3M-052-AF + Alum adjuvanted VRC HIV Env Trimer 4571 in adult participants without HIV.

1.2 Design

This is a randomized open-label trial to examine the safety and immunogenicity of INO-6160 (synthetic DNAs encoding a native-like HIV Env Trimer and Interleukin-12), alone or in a prime-boost regimen with VRC HIV Env Trimer 4571 adjuvanted with 3M-052-AF + Alum. The primary hypothesis is that the vaccine regimen will elicit HIV-1 envelope protein–specific binding antibody (Ab) and T-cell responses.

1.3 Study products, diluents, and electroporation device

- **INO-6160: sD-NLT-AB05 co-formulated with IL-12 DNA (pGX6001)**: sD-NLT-AB05 consists of a single plasmid, pGX1060 (in pGX0001 vector backbone), encoding a soluble stabilized native-like trimer derived from clade A isolate BG505. pGX6001 (pGX0003 vector backbone), contains a dual promoter system for expression of both the human IL-12 p35 and p40 genes necessary for production of the active heterodimeric IL-12 protein. The plasmid ratio for the coformulated drug product is 4:1 (0.8 mg pGX1060/0.2 mg pGX6001) per 0.1 mL/1 mg injection. The coformulation, INO-6160, in water-for-injection (WFI), is supplied at a concentration of 10 mg/mL and a volume of 0.4 mL in 2-mL glass vials.

- **Trimer 4571**: HIV-1 Env Trimer 4571 (VRC-HIVRG096-00-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 adjuvant**: This adjuvant is an aqueous formulation (AF) of the small molecule imidazoquinoline, which acts as a toll-like receptor (TLR) 7/8 agonist. 3M-052-AF is supplied at a concentration of 50 mcg/mL and a fill volume of 0.4 mL in 2-mL glass vials.

- **Aluminum Hydroxide Suspension, Adjuvant**: Aluminum hydroxide suspension (Alum) is composed of Alhydrogel 2% (Brenntag Biosector, Frederikssund, Denmark) diluted with WFI to a concentration of 5 mg/mL. It is supplied as a sterile, pyrogen-free suspension filled into single-dose vials at a volume of 0.7 mL.

- **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 and is provided by Inovio Pharmaceuticals.

1.4 Study participants

20 healthy volunteers without HIV, 18 through 55 years of age.

1.5 Study plan and schema table

Participants will be evaluated for safety and immune responses through blood collection at specified timepoints throughout the study. The study schema is below:

Table 1-1 Schema

				Injection Schedule			
Group	**N**	**Product/Dose**	**Route**	**Month 0**	**Month 1**	**Month 3**	**Month 6**
1	10	INO-6160 / 2.0 mg	ID EP	X	X	X	X
2	10	INO-6160 / 2.0 mg	ID EP	X	X	X	X
		Trimer-4571 / 100 mcg 3M-052-AF (5 mcg) + Alum (500 mcg)	IM	--	--	X	X
Total	**20***						

Table 1-1 Notes: The dose of INO-6160, 2 mg, will be administered as 2 separate intradermal (ID) injections because of the volume limitation of the device for intradermal injection. Each of 2 sites will receive 0.1 mL via ID injection (Mantoux injection) bilaterally, one on each upper arm. Following ID injections, EP will be performed with the Inovio CELLECTRA 2000 device. The dose of Trimer 4571, 100 mcg, will be administered as 2 injections delivered intramuscularly (IM) via needle and syringe in both deltoid muscles. For all injections, if administration into the deltoid is contraindicated, the thigh muscle can be used (see Section 7.3.4).

* Up to 5 additional participants may be enrolled (for a total of 25), if needed, with a goal of approximately 20 participants to contribute to the 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, or variations in the clinical care due to unpredictable events. Participants will not be replaced after visit 5, the visit 2 weeks post second vaccination, and replacement will require the assent of Protocol team leadership.

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

1.6 Duration per participant

12 months of scheduled clinic visits (main study) and an AESI health contact at month 18.

1.7 Estimated total study duration

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

1.8 Study sites

The HIV Vaccine Trials Network (HVTN) Clinical Research Sites (CRSs) will be located in the US and will be further specified in the Site Announcement Memo.

2 Introduction

This first-in-human clinical trial will examine the safety and immunogenicity of a vaccination regimen with a synthetic DNA–encoded stabilized HIV-1 Env Native-Like Trimer (sD-NLT-AB05 adjuvanted with IL-12 DNA), alone or in combination with HIV-1 Env Trimer 4571, adjuvanted with 3M-052-AF + Alum, as a boost. The DNA vectors will be delivered via ID EP. Vaccination with Native-Like Trimer (NLT) via a DNA platform (as opposed to recombinant protein) eliminates the need to express and purify NLTs in vitro, saving time and lowering costs.

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

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

The clinical trial will inform future NLT and DNA clinical studies for both HIV vaccines and non-HIV vaccines, such as sD-NLT germline-targeting trimers to activate specific bnAb precursor lineages, sD-NLT heterologous boosting to broaden antibody recognition, and sD-NLT prime with protein NLT boosts with combinations of NLTs based on more diverse HIV isolates to generate broader responses.

2.1 Rationale for evaluation of sD-NLT-AB05 with IL-12 adjuvant and Trimer 4571 adjuvanted with 3M-052-AF + Alum in a prime boost regimen

2.1.1 sD-NLT-AB05 with IL-12 adjuvant

Native-Like Env Trimers (NLTs): HIV envelope protein (Env) is a conformationally dynamic structure that is a key target for HIV vaccine development (1, 2). Preclinical studies demonstrate that recombinant NLTs, which resemble ‘native’ Env on the HIV virion surface, display surfaces recognized by all lineages of broadly neutralizing antibodies (bnAbs) capable of neutralizing HIV while simultaneously ensuring that many non-neutralizing epitopes remain hidden (3, 4). Stabilized NLTs can be engineered by incorporation of mutations to favor the prefusion state conformation. Recently, we optimized NLTs for an advanced synthetic DNA vaccine delivery platform (sD-

NLT). In the sD-NLT formulation, NLT immunogens can be encoded in synthetic DNA cassettes as transgenes in a clinical plasmid (pVax).

sD-NLT-AB05 encodes for an Env trimer derived from the HIV-1 clade A strain BG505(5). The DNA platform is thus referred to as "sD-NLT-AB05." It is a gp140 format modified through a set of directed mutations to produce a stable NLT exposing epitopes targeted by bnAbs. It also occludes distracting epitopes targeted by non-neutralizing Abs (eg, on the V3 loop and inner domain surface) (5, 6). Similarly, the VRC-HIVRGP096-00-VP Env trimer (**Trimer 4571**) is a stabilized NLT, also derived from BG505 in a gp140 format, modified with a different set of mutations to prevent CD4-induced conformational triggering (7-9). Specifically, the sD-NLT-AB05 encoded trimer and Trimer 4571 share the following amino acid sequence changes: SOS stabilizing mutations (501C, 605C), gp41 stabilizing mutation (559P), glycan introduction mutation (332N), and improved furin cleavage site mutations (RRRRRR between 507 and 512). They differ in 2 sets of changes: Trimer 4571 contains disulfide mutations (201C, 433C) and sD-NLT-AB05 contains 11 changes to improve biophysical properties (T106E, M271I, F288L, N363Q, F519S, A561P, V570H, R585H, L568D, R304V and A319Y). The sD-NLT-AB05 and Trimer 4571 harbor the same bnAb epitopes and differ only by a small set of mutations, which are not anticipated to affect the B-cell or T-cell responses.

IL-12 induces the production of interferon-gamma (IFN-gamma), favoring the differentiation of type 1 T helper (Th1) cells (10, 11). Biologically active IL-12 has pleotropic effects but is generally considered to be a pro-inflammatory cytokine that biases the CD4+ T-cell response towards a Th1 phenotype. Previous studies have demonstrated that the inclusion of plasmid-encoded IL-12 (GENEVAX IL-12-4532) with HIV PENNVAX-B DNA was dose sparing (12). The inclusion of IL-12 DNA did not affect the tolerability of an HIV DNA vaccine (13).

2.1.2 Trimer 4571 adjuvanted with 3M-052-AF + Alum

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

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

Nonclinical studies in guinea pigs, rats, rabbits, and rhesus macaques comparing the immunogenicity of the related SOSIP protein BG505 SOSIP.664, formulated with a diverse range of adjuvants, have consistently shown that the protein

adjuvanted with 3M-052-AF + Alum elicits neutralizing and binding Abs more quickly, to higher peak magnitudes, and with greater durability than any of the other protein-adjuvant combinations tested (see IB).

In rhesus macaques, among 8 adjuvants combined with an Env gp140 immunogen, the Alum/TLR7 agonist 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 3-10-fold compared to Alum alone (18).

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 it 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 (14). In NHPs immunized with SARS-CoV-2 RBD formulated with 3M-052 combined with Alum, several enhanced immune responses were observed, including 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 (19).

For additional information, see the IB.

2.2 Preclinical data with sD-NLT-AB05: mice and rabbits

In preclinical animal models, DNA-encoded NLTs are capable of inducing autologous neutralizing immune responses, and DNA-encoded NLTs act synergistically with recombinant protein NLTs in prime-boost regimens. NLTs formulated as synthetic DNA and delivered by electroporation have not been evaluated in humans.

Preclinical studies in mice demonstrated that sD-NLT-AB05 can assemble in vivo with a native-like antigenic profile similar to the same recombinant NLT protein produced in vitro (see Figure 2-1, and reference (6)).

This is the first time NLTs have been shown to assemble directly in vivo when launched from synthetic DNA by an advanced EP device. DNA-encoded NLTs folded properly in vivo and induced the appropriate humoral response, as demonstrated by the observation that sD-NLT-AB05 induces autologous tier-2 neutralizing antibodies (nAbs) in mice. Comparison of protein versus DNA NLT vaccinations in mice demonstrated induction of trimer-binding antibodies by both vaccine platforms. High levels of CD4+ and CD8+ T-cell responses, as well as autologous tier-2 BG505.T332N neutralization was observed in mice immunized

with sD-NLT-AB05 (Figure 2-2 A and B). However, recombinant protein failed to generate either CD8+ T-cell responses or any tier-2 responses in mice, as shown by us and others (20). Neutralizing clones have been isolated and Cryo-EM employed to molecularly map the murine neutralizing epitope targeted by vaccination with sD-NLT-AB05, which is also a target of protective nAbs in primates (21, 22) (Figure 2-3 A and B). sD-NLT-AB05 has also been observed to generate autologous tier-2 neutralizing responses in rabbits (unpublished data, see the IB). In published macaque studies by other groups, DNA/Env protein co-administration at the same site during each vaccination can improve induced binding and nAb responses (23), as well as challenge outcome (24).

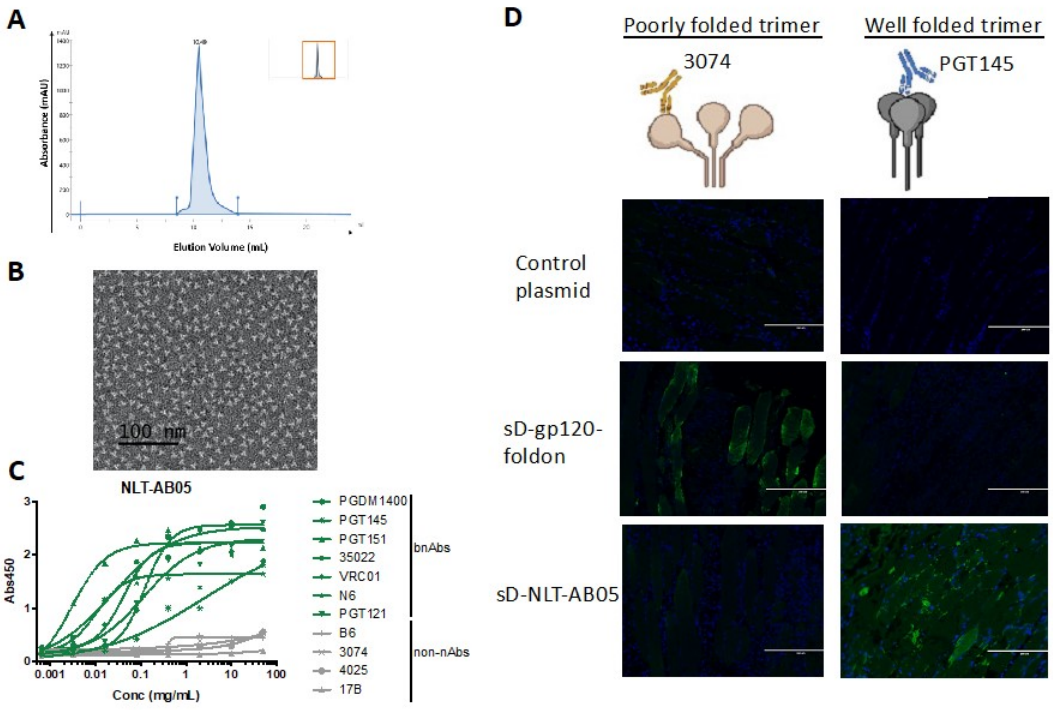

Figure 2-1 In vitro and in vivo characterization of sD-NLT-AB05. (A) Size-exclusion chromatography elution showing homogenous trimers are produced (B) Negative stain electron microscopy imaging of NLT-AB05 (C) Antigenic profile of NLT-AB05 (D) Muscle sections from mice treated intramuscularly with DNA-encoded BG505.gp120.foldon or sD-NLT-AB05 Stained with known monoclonal Abs recognizing either well folded trimers (PGT145) or poorly folded trimers (3074). Staining with non-nAb 3074 indicates poorly folded trimer while staining with bnAb PGT145 indicates well-folded trimer.

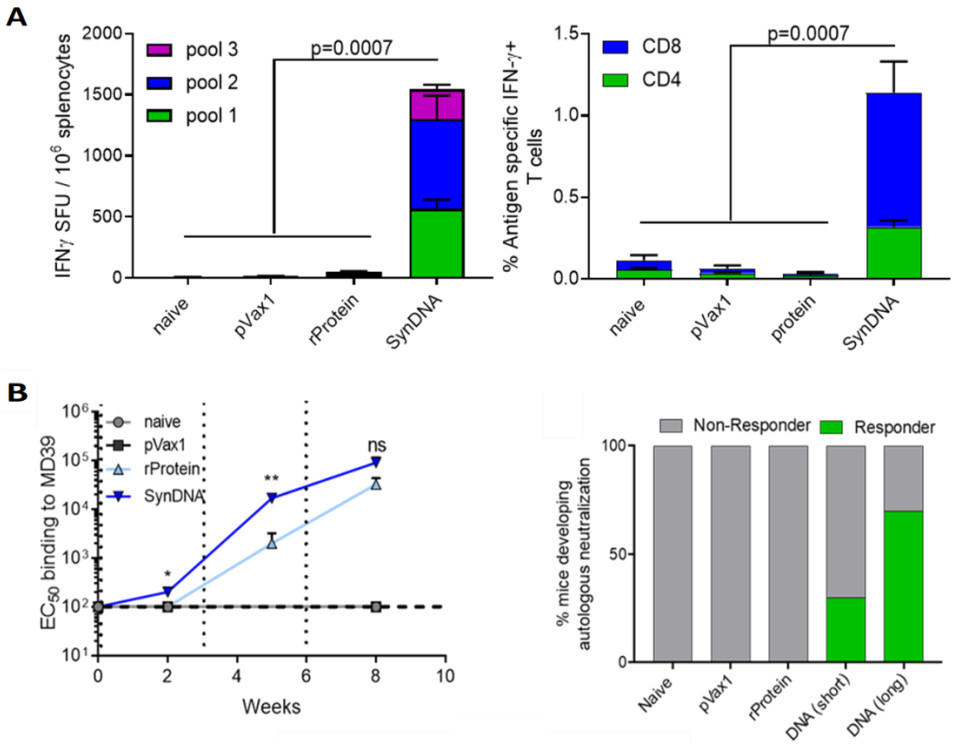

Figure 2-2 Immunogenicity of sD-NLT-AB05. (A) Comparison of BG505 Env-specific cellular responses in naïve mice or mice immunized with pVAX plasmid backbone, Sigma Adjuvant System (RIBI)-co-formulated protein BG505.MD39, or sD-NLT-AB05 without adjuvant by IFNγ ELIspot assay (left panel) or intracellular cytokine staining (right panel). Cells were stimulated with overlapping 15-mer peptide pools for wild-type BG505 gp140 divided into 3 pools from N-terminus to C-terminus. (B) Left panel: Binding antibodies induced by sD-NLT-AB05 comparable to RIBI-co-formulated protein BG505.MD39 immunizations. Right panel: Unique induction of autologous tier-2 neutralizing responses by sD-NLT-AB05 in contrast to recombinant protein. DNA (short) = immunization at weeks 0, 3, 6. DNA (long) = immunization at weeks 0, 3, 16.

A

ID50 Titers		Engineered Pseudovirus				
		BG505.T332N	T332N.S241N	T332N.T465N	MG505H3	MLV
Mouse ID	1	330	597	<45	<45	<45
	2	943	860	72	<45	<45
	3	>1215	>1215	<45	78	<45
	4	731	963	<45	78	<45
	5	>1215	>1215	<45	<45	<45
	6	>1215	>1215	<45	<45	<45
	7	>1215	>1215	70	<45	<45
	8	570	489	<45	<45	<45
	9	782	814	185	<45	<45
	10	594	439	<45	<45	<45
	11	796	832	<45	<45	<45
	12	>1215	>1215	123	<45	<45
	13	>1215	>1215	<45	<45	<45

IC50 (ug/mL)	C05	C22	C24	C33	C37
BG505.T332N	0.039	0.174	0.095	1.332	0.047
BG505.T332N.T465N	>50	>50	2.1	>50	>50
MLV	>50	>50	>50	>50	>50

B

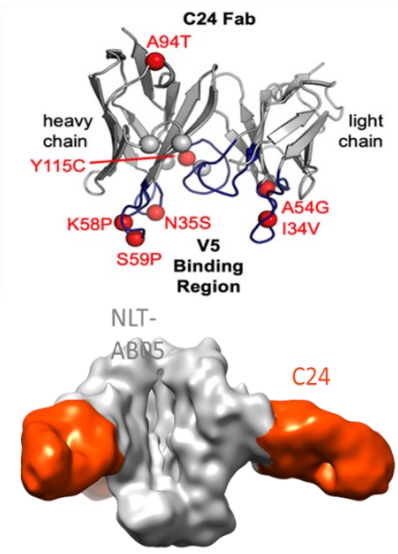

Figure 2-3 Immunogenicity of sD-NLT-AB05. (A) Neutralization ID50 titers of heat-inactivated mice sera for sD-NLT-AB05 immunized mice with strong neutralizing activity against listed pseudovirus isolates. Isolated nAbs using antigen-specific B-cell sorting of sD-NLT-AB05 immunized mice. IC50 values of trimer-specific murine mAbs for BG505.T332N, BG505.T332N.T465N or MLV. (B) Structural model of nAb clone C24 with positions mutated from the germline sequences are displayed in red. Cryo-EM structure of C24 in complex with BG505.MD39.

2.3 Clinical Experience with related DNA vaccines encoding HIV Env immunogens with and without IL-12 adjuvant

Prior clinical studies (HVTN 070, HVTN 080, HVTN 087, and HVTN 098) have demonstrated the safety of DNA-encoded HIV Env immunogens delivered via EP and demonstrated consistent induction of Ab and CD4+ T-cell and CD8+ T-cell responses. Key features of these trials and summaries of safety are shown in Table 2-1. HVTN 098 tested optimized cocktail formulations administered via a device enhanced for ID EP delivery and resulted in an efficient platform, generating a potent, dose-sparing, and highly tolerable ID-based vaccine approach in humans. These studies established the importance of the optimized plasmid IL-12 for ID HIV DNA vaccine immune potency, response rates, and memory (25, 26).

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

Study / ClinicalTrials.gov identifier	Products	Sample size (N)	Route of administration	Safety	Reference
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 One discontinuation due to possibly related adverse event (cervical radiculopathy)	Kalams, 2013 (12)
HVTN 080 / NCT00991354	**PENNVAX-B DNA vaccine** **IL-12 plasmid DNA:** GENEVAX-IL-12-4532, formulated with bupivacaine	48	IM administration with electroporation using VGX CELLECTRA electroporation system	No severe systemic reactogenicity No related severe adverse events Two discontinuations due to injection site pain	Kalams, 2013 (12)
HVTN 087 / NCT01578889	**HIV multiantigen DNA:** consists of 2 plasmid DNA expression vectors, HIV-1 *gag*/*pol* and HIV-1 *nef*/*tat*/*vif*, *env* (clade B). **IL-12 plasmid DNA:** GENEVAX IL-12-4532, formulated with bupivacaine **VSV-HIV Gag**	100	IM administration with electroporation using Ichor Medical Systems TriGrid Delivery System (TDS) EP device	No severe systemic reactogenicity. No severe related adverse events. Three discontinuations due to injection site pain.	Elizaga, 2018 (27) (28)
HVTN 098 / NCT02431767	**PENNVAX-GP DNA Vaccine:** admixture of 2 plasmids encoding consensus clade A and C HIV-1 envelope protein 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 electroporation using CELLECTRA 3P EP for ID delivery and CELLECTRA 5P EP for IM delivery.	No severe systemic reactogenicity. No severe related adverse events. One participant discontinued due to pain, anxiety and presyncopal episode	Edupuganti, 2020 (26) De Rosa 2020 (25)

2.3.1 Safety

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 300 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 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 in HVTN 098 (26). Systemic reactogenicity symptoms with IM injection of DNA vaccines and with ID injection of DNA vaccines did not differ (26). 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.

When the injection and EP are given in the skin, the needles 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 (26). 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 using 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 IB.

2.3.2 Immunogenicity

EP improves immunogenicity of DNA vaccines. 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 (12). Comparing the comparable 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 have not been induced well by DNA. The responses were primarily directed to Gag and Pol, and not to Env. Because of this, in HVTN 098, the Env expressing DNA vaccine was modified, including two env plasmids encoding clade A and C Env immunogens, and including a substitution of an

optimized IgE leader as well as the deletion of their cytoplasmic tail to improve surface expression (25). 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 EP and intradermal EP, with the ID EP administration at 1/5th the dose of the IM EP. 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 highest responses for the ID + IL-12 group. Neutralizing antibodies were mainly detected only to a tier 1A viral isolate. Only a few participants developed detectable antibody-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 the ID route was dose-sparing.

Inclusion of IL-12 plasmid DNA . Regarding the benefit of IL-12, in HVTN 080, the sample size for the group without IL-12 was small, likely reducing the ability to detect significant differences. Although not statistically significant, the response rates including IL-12 were higher than 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 without and with IL-12 after the fourth dose. The binding Ab responses to Env were similar for the ID groups with and without IL-12 after the fourth vaccination but tended to be lower after the third vaccination without IL-12, suggesting that inclusion of IL-12 accelerated the response, achieving near maximal after the third rather than the fourth. The effect of IL-12 was examined in another HVTN study, HVTN 087, that tested increasing doses of IL-12 included with DNA administered IM with EP for 3 doses followed by vesicular stomatitis virus (VSV) boost (28). 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, 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 Clinical experience with HIV Envelope proteins adjuvanted with 3M-052-AF + Alum: safety data

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 27, 2022, both the 1 mcg 3M-052-AF group (6 participants total; 5 receiving protein/adjuvant, 1 receiving placebo) and the 5 mcg 3M-052-AF group (11 participants total; 10 receiving protein/adjuvant, 1 receiving placebo) completed enrollment. Part A of the 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 post-vaccination. The maximum size of both the induration and erythema was measured at 17 x 17 cm on Day 8 (Grade 3). The erythema resolved over 3 days and the induration subsided to less than 5 cm on Day 11 (Grade 1). The induration was measured at 2 x 2 cm on Day 14 but did not completely resolve until 41 days post-injection. At no point was the pain/tenderness greater than mild and the participant continued to work. It is unknown at present whether this participant received placebo or study product. Another participant in Group 2 experienced 2 days of Grade 3 induration and erythema from Days 6-7 post-vaccination 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.

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), adverse events of special interest (AESIs), or deaths in either group after this third dose. Both local and systemic reactogenicity was 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 27, 2022, Part B is fully enrolled but remains blinded. 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 one of the HVTN

137 Part A Group 2 participants described above, all reactogenicity symptoms resolved within 14 days and generally within 7 days. For more detailed information, please see the IB.

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

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

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

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

2.5 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 (12, 27). Specifically for ID delivery, both Abs and T-cell responses were induced (25, 26). In HVTN 098, the fourth vaccination boosted the magnitude of total Ab response for all treatment

groups against gp140 antigens from clade A and B as compared to the magnitude of total Ab response after the third dose (25).

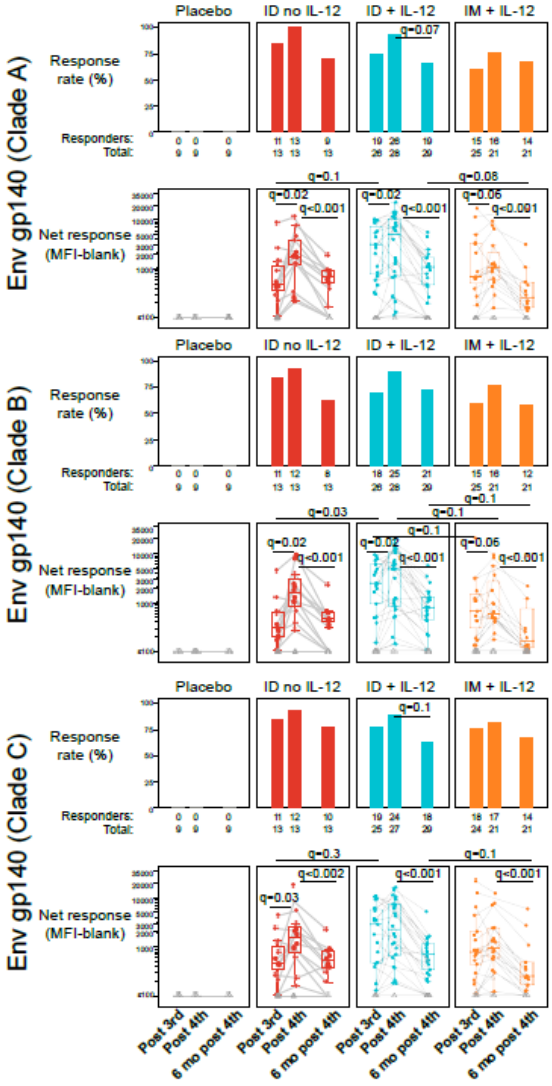

Figure 2-4 IgG binding antibody responses as measured by binding antibody multiplex assay (BAMA) against consensus Env gp140 antigens for clades A, B and C. Assays were performed 2 weeks after the third and fourth vaccinations and 6 months after the fourth vaccination. Positive responses are shown in filled circles in color, negative responses are shown in open gray triangles. Box plots represent the distribution for the positive responders only. Bar plots show response rates. Numbers below the bars indicate numbers of positive responders and total participants. Positive response rates were compared using the Fisher's exact test for unpaired data (between treatment groups) and the McNemar test for paired data (between visits). Response magnitudes among positive responders were compared using the Wilcoxon rank sum test for unpaired data and the Wilcoxon signed rank test for paired data. All p-values are two-sided. False-discovery rate adjusted q values were calculated to account for multiple antigens, multiple timepoints, or treatment groups (Figure from supplemental data (25)).

By including 2 boosts of Trimer 4571 at months 3 and 6 in Group 2, we can assess whether additional doses of protein and DNA lead to enhanced

immunogenicity compared to DNA alone in this study (Group 1) and previous studies. By maintaining a standard schedule, data will be directly comparable to previous DNA vaccine studies.

2.6 Rationale for Dose of Trimer 4571 and 3M-052-AF + Alum adjuvant

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 is 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 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

Study	ClinicalTrials.gov NCT # / Study Status	Participant HIV status	Number receiving Trimer 4571 + Alum	Route / Dose	Schedule	Safety data (ClinicalTrials.gov)
VRC 018	NCT03783130 / Completed	Negative	16	IM or SC at 100 or 500 mcg Trimer and 500 mcg Alum	0, 2, 5 months	As of August 2021, final study data: no related SAEs, related grade 4 AEs, or related MAAEs were reported or other unexpected reactions, and no study pause criteria were met at any time.
NIAID 19-I-0069	NCT03878121 / Enrolling	Negative	Max 100	IM at 500 mcg Trimer with 500 mcg Alum	Boost post Adenovirus vector prime	As of Jan 5, 2022, 12 participants have received study product. No SAEs, no SUSARs.
Therapeutic immunization study (NETI) PI: M Choudhary, Univ of Pittsburg	NCT04985760 Enrolling	Positive	24	IM at 100 or 500 mcg Trimer and 500 mcg Alum	0, 2, 5 months	As of Jan 1, 2022, 5 participants have received Trimer 4571. No related SAEs, no SUSARs.

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.

2.7 Rationale for Targeting Same Draining Lymph Node with both DNA and Protein

Direct targeting of the draining lymph node with a multipronged immune stimulus consisting of both a DNA vaccine and an adjuvanted protein vaccine leads to improved outcomes in a variety of several preclinical models (23, 29-31). The most directly relevant study is that of Felber and colleagues in rhesus macaques (31). It involved a comparison of vaccination with monomeric HIV envelope administered via both DNA and an adjuvanted protein delivered either into the same limb or into contralateral limbs, allowing 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 lead to an improvement in a variety of immune outcomes, including functional Ab responses (both increased ADCC activity and improved FcγRIIIa binding) and cellular responses (both 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 simian-human immunodeficiency virus (SHIV) challenge (see Figure 2-5).

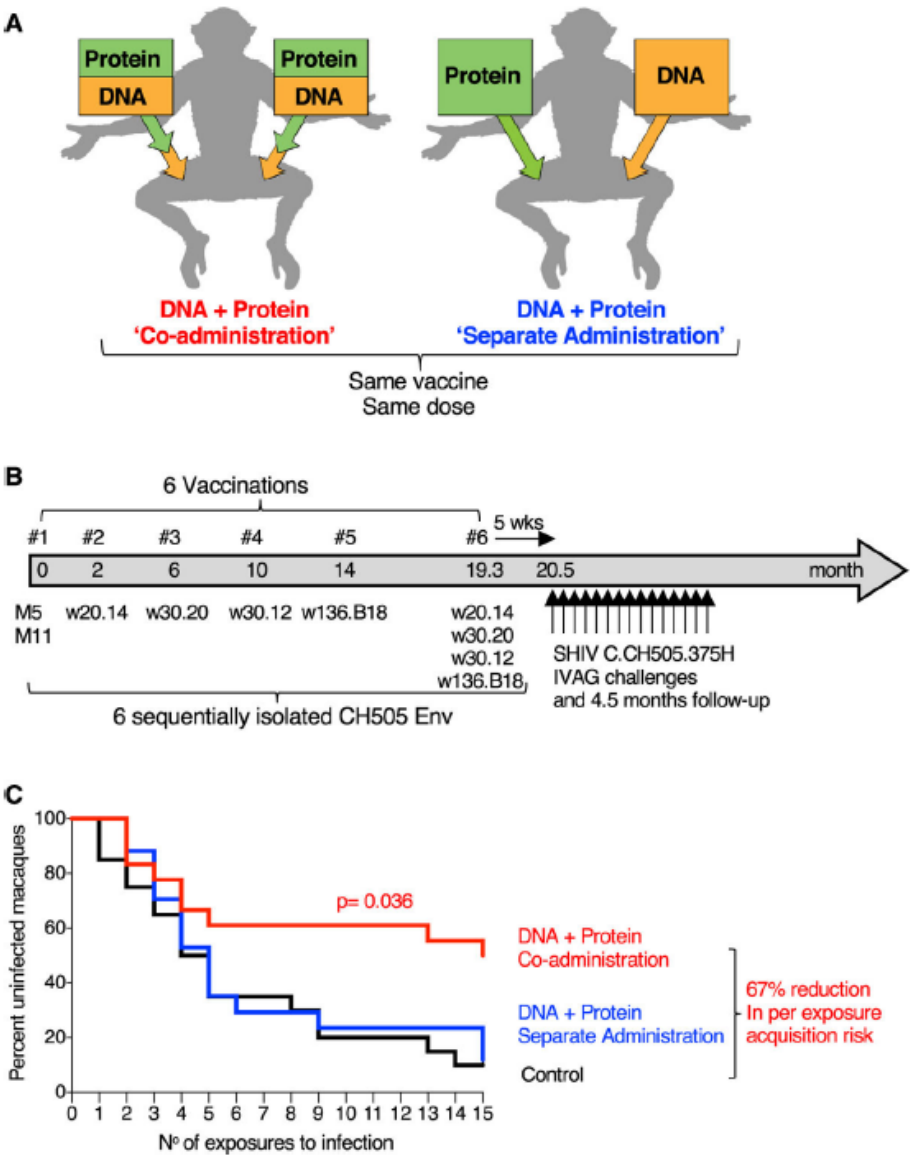

Figure 2-5 Coadministration Group Shows Significant Protection from SHIV.CH505 Infection
(A) Schematic representation of vaccine delivery of the 2 components (DNA and protein) in the 2 vaccination regimens, "Coadministration" in the same anatomical sites and "separate administration" in contralateral 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 two vaccine groups (n = 18 and 17, respectively) and the control group (n = 20). The RMs were exposed to 15 weekly intravaginal challenges. Infection was defined by 2 consecutive positive plasma VL measurements. No RMs were censored. P-value, exact log-rank test. Figure from Felber et al (31).

In keeping with the well-tolerated safety profile of simultaneous delivery of HIV immunogens via both DNA+ envelope protein administration strategies (32, 33),

no AEs were noted with either strategy (separate versus 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 (34) and the DNA will be delivered intradermally whereas the protein will be delivered intramuscularly.

2.8 Risks and benefits

2.8.1 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 antibody test result • Visible lesion(s) at the injection site, such as erythematous papules with eschar, hypopigmentation, hyperpigmentation, or scar related to the EP injection
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 by EP • 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 infection/disease if the participant is infected with HIV • Effects on the fetus and on pregnancy

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

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

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

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

2.8.2 Benefits

Study participants will not receive direct health 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 infection.

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

3 Objectives and endpoints

3.1 Primary objectives and endpoints

Objectives	Endpoints
1. To evaluate the safety and tolerability of 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant followed by 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant alone or in combination with Trimer 4571 adjuvanted with 3M-052-AF + Alum	a) Local and systemic reactogenicity signs and symptoms will be collected for a minimum of 2 weeks following receipt of any study vaccine b) Serious adverse events (SAEs), medically attended adverse events (MAAEs), adverse events of special interest (AESIs) and AEs leading to early participant withdrawal or permanent discontinuation will be collected throughout the study and for 12 months following any receipt of study product. Additionally, all adverse events will be collected for 30 days after any receipt of study vaccination.
2. To evaluate the immunogenicity of 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant followed by 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant alone or in combination with Trimer 4571 adjuvanted with 3M-052-AF + Alum	a) Response rate and magnitude of vaccine-matched IgG binding Ab responses as assessed by multiplex assay 2 weeks following the fourth vaccination 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 fourth vaccination

3.2 Secondary objectives and endpoints

Objectives	Endpoints
1. To further evaluate and compare the ability of 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant followed by 1 or 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant alone or in combination with Trimer 4571 adjuvanted with 3M-052-AF + Alum to elicit humoral immune responses	a) Neutralizing Ab magnitude and breadth against autologous and tier 1a HIV-1 isolates as assessed by TZM-bl neutralization assay following the third and fourth vaccinations b) Response rate, magnitude, and epitope specificity of HIV-1 specific IgG binding Ab responses as assessed by multiplex assay 2 weeks following third vaccination
2. To further evaluate and compare the ability of 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant alone or in combination with Trimer 4571 adjuvanted with 3M-052-AF + Alum to elicit cellular immune responses	a) 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 third vaccination

Objectives	Endpoints
3. To evaluate the durability of cellular and humoral immune responses elicited by 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant followed by 2 doses of sD-NLT-AB05 + IL-12 DNA adjuvant alone or in combination with Trimer 4571 adjuvanted with 3M-052-AF + Alum	a) Magnitude and response rate of CD4 + and CD8+ T-cell responses measured by flow cytometry, to HIV-1-specific Env peptide pools 6 months post last vaccination b) Response rate and magnitude of HIV-1 specific IgG binding Ab responses as assessed by multiplex assay 6 months post last vaccination c) Neutralizing Ab magnitude and breadth against autologous tier 2 HIV-1 isolates as assessed by TZM-bl neutralization assay 6 months post last vaccination

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. To evaluate the response rate and magnitude of HIV-1 specific IgG binding Ab responses as assessed by multiplex assay 2 weeks following second vaccination.

3. To evaluate the 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 second vaccination.

4. To evaluate the frequency of Env-specific B cells measured by flow cytometry 2 weeks following the second, third, and fourth vaccinations and 6 months post last vaccination.

5. To evaluate serum Ab specificities and elicitation of trimer-degrading Abs using polyclonal epitope mapping as assessed by Electron Microscopy 2 weeks post third and fourth vaccinations.

6. To evaluate the neutralizing Ab magnitude and breadth against heterologous and tier 2 HIV-1 isolates as assessed by TZM-bl neutralization assay following the third, and fourth vaccinations.

7. To evaluate of HIV-1 specific IgG binding Ab responses to the trimer base 2 weeks following second, third, and fourth vaccinations.

8. To evaluate Ab avidity and Fc Receptor functions such as FcR binding, ADCC, antibody-dependent cellular phagocytosis (ADCP), and infected cell antibody-binding assay (ICABA) after the second, third, and fourth vaccinations.

9. To evaluate B-cell receptor (BCR) repertoires and sequences (including analysis of rare B-cell lineages associated with bnAb precursors), including cellular phenotyping and mutational frequency analysis suggestive of somatic hypermutation and affinity maturation following immunization.

10. To characterize monoclonal Abs derived from BCR sequences from Env-specific B cells.

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

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

4 Laboratory strategy

4.1 Assessment of immunogenicity endpoints

The primary goal of HVTN 304 is to determine whether the sD-NLT-AB05/ IL-12 DNA alone or in a prime-boost regimen with VRC Env Trimer 4571 adjuvanted with 3M-052-AF + Alum will elicit HIV-1 envelope protein specific binding antibody (Ab) and T-cell responses. To this end, HVTN immunogenicity assays will be used to measure both Env-binding Abs and both CD4+ and CD8+ Env-specific T cells.

For the primary endpoints, vaccine matched HIV-specific binding Ab responses will be assessed by multiplex assay, and response rate and magnitude of CD4 + and CD8+ T-cell responses will be assessed by flow cytometry. These data will also be used to identify and prioritize samples for in-depth immunogenicity analyses (secondary and exploratory endpoints, see Sections 3.2 and 3.3).

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

The laboratory strategy and the technical details are described in the Central Assay Plan and will be updated as new reagents and techniques are incorporated into assay planning. This document will be available on the protocol webpage. Descriptions of the standard HVTN laboratory assays can be found online at https://www.hvtn.org/content/dam/hvtn/scientific-programs/hvtn-laboratory-assay-descriptions.pdf.

5 Study design

This is a randomized trial to examine the safety and immunogenicity of INO-6160 (sD-NLT-AB05/ IL-12 DNA) alone or in combination with Trimer 4571 adjuvanted with 3M-052-AF + Alum in healthy adults. The primary hypothesis is that INO-6160 alone or in combination with Trimer 4571 adjuvanted with 3M-052-AF + Alum vaccinations can induce seroconversion binding antibody responses and induce antigen specific T-cell responses (both CD4+ and CD8+).

5.1 Study population

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

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

5.1.1 Inclusion criteria

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

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

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

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

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

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

7. Assessed as low risk for HIV acquisition per low risk guidelines (see Appendix D), agrees to discuss HIV infection risks, agrees to risk reduction counseling, and

agrees to avoid behavior associated with high risk of HIV exposure through the final study visit. Low risk may include persons stably taking PrEP as prescribed for 6 months or longer.

8. Hemoglobin:

    - ≥ 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 men who have been on hormone therapy for more than 6 consecutive months
    - ≥ 12.0 g/dL for transgender women 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 their sex assigned at birth

9. 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).

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

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

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

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

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

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

16. Negative for Hepatitis B surface antigen.

17. For a volunteer capable of becoming pregnant:

    - Volunteers who were assigned female sex at birth and are of reproductive potential must agree to use effective means of birth control from at least 21

days prior to enrollment through 8 weeks after their last scheduled fifth vaccination timepoint (see Appendix E).

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

- Volunteers who were assigned female sex at birth must also agree not to seek pregnancy through alternative methods, such as oocyte retrieval, artificial insemination or in vitro fertilization through 8 weeks after their last scheduled vaccination timepoint.

5.1.2 Exclusion criteria

1. Volunteer who is breast-feeding or pregnant.

2. Body mass index (BMI). Enrollment of individuals with BMI ≥ 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 diet alone or 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. Congenital or acquired immunodeficiency, including systemic medication use likely to impair immune response to vaccine in the opinion of the site investigator such as glucocorticoid use equal to or greater than prednisone 10 mg/day within 3 months prior to enrollment.

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.

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

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

10. Receipt of non-HIV experimental vaccine(s) received within the last 1 year. Exceptions may be made by the PSRT for vaccines that have subsequently undergone licensure or Emergency Use Authorization by the FDA or, if outside the United States, by the national regulatory authority or World Health Organization. For volunteers who have received control/placebo in an experimental vaccine trial, the PSRT will determine eligibility on a case-by-case basis.

11. 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.

12. 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.

13. History of serious reaction (eg, hypersensitivity, anaphylaxis) to any related vaccine or component of the study vaccine regimen.

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

15. Idiopathic urticaria within the past year.

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

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

18. Asplenia or functional asplenia.

19. Active duty and reserve US military personnel.

20. 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).

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

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

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

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

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

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

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

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

- Treatment with systemic glucocorticoids (eg, prednisone or other glucocorticoid), immune targeting monoclonal antibodies or other immunomodulators (other than nonsteroidal anti-inflammatory drugs [NSAIDs]), with the exception that study injection may continue per principal investigator (PI) discretion if the next study injection occurs at least 2 weeks following completion of glucocorticoid treatment;

- Receipt of any live attenuated vaccines within 4 weeks prior to study vaccine administration; (Note: ACAM2000 vaccine for Monkeypox received within 30 days prior to enrollment or receipt of study vaccine; or if ACAM2000 received greater than 30 days prior to enrollment or receipt of study vaccine and vaccination scab still present; or planned administration within 30 days after enrollment or receipt of study vaccine).

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

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

5.2.2 Discontinuation of study vaccine administration

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

- SAE that is subsequently considered to be related to vaccination

- Pregnancy (regardless of outcome)

- HIV infection

- Grade 3 AE assessed as related to study vaccine with the exception of fever and subjective local and systemic symptoms. For grade 3 injection site

erythema and/or induration, upon review, the PSRT may allow continuation of vaccination.

- Grade 4 AE assessed as related to study vaccine
- Clinically significant type 1 hypersensitivity associated with study vaccine

For ease of reference and review, the clinically significant type 1 hypersensitivity definition, as per the Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium criteria for anaphylaxis (35), is provided below:

> *1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)*
>
> *AND AT LEAST ONE OF THE FOLLOWING:*
>
> > *a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)*
> >
> > *b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)*
>
> *2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient [participant] (minutes to several hours):*
>
> > *a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)*
> >
> > *b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)*
> >
> > *c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)*
> >
> > *d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)*
>
> *3. Reduced BP after exposure to known allergen for that patient [participant] (minutes to several hours). Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline*

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

- Newly disclosed AESI (see Appendix F)

- 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 304 PSRT)

Participants discontinuing study vaccine for reasons other than HIV infection should be encouraged to participate in follow-up visits and procedures per the protocol for at least 12 months following their last study vaccine administration. At the discretion of the CRS clinician and the PSRT (for composition of PSRT see Section 9.3), some clinic procedures and sample collections may be modified or discontinued.

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

5.2.3 Participant departure from vaccine schedule

If a participant misses a scheduled vaccination, they are still eligible to receive future vaccinations.

5.2.4 Discontinuation of study participation

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

- Participant voluntarily withdraws;

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

- The investigational new drug (IND) 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, CBC with differential, serum chemistry, physical examination, and if indicated, a pregnancy test (see Appendix A). For participants with HIV infection,

please see Section 8.6. If the site PI has questions regarding a termination visit, they should consult with the PSRT.

6 Statistical considerations

6.1 Sample size justification and accrual

Recruitment will target enrolling 10 healthy, adult participants without HIV per group for the 2 study groups receiving either 2 doses of INO-6160 followed by 2 doses of INO-6160 alone or in combination with Trimer adjuvanted with 3M-052-AF + Alum (for study schema see Table 1-1). Up to 5 additional participants, or a total of 25 participants, may be enrolled to ensure sufficient samples for immunogenicity analyses, with a goal of at least 20 contributing to the final analyses. Examples of reasons that might necessitate enrollment of additional participants are provided in Section 1.5.

Since enrollment is concurrent with receiving the first vaccination, all participants will provide some safety data. It is possible, however, for immunogenicity data to be missing; previous HVTN and AIDS Vaccine Evaluation Group (AVEG) studies suggest 10% is a reasonable estimate for the rate of missing data. For this reason, the sample size calculations account for 10% of enrolled participants having missing data for the primary immunogenicity endpoints. This 10% missingness accounts for both post-processing variables (eg, problems with samples) and is mitigated by the potential for replacing participants who do not continue with the study.

6.1.1 Power calculations for immunogenicity

The main goal for the analysis is to evaluate the difference of the frequency and magnitude of HIV-1 specific binding Ab responses, and the rate of CD4+ and CD8+ T-cell responses between the 2 study groups, following the third and fourth vaccination. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% confidence intervals (CIs) for the response rate based on observing a particular rate of responses in the study groups is shown in Table 6-1. The table indicates n = 9 per group, assuming a 10% loss of data.

Table 6-1 Two-sided 95% CIs for the true response rate based on observing a particular rate of responses in the study groups, n = 9 (total 10 with 10% missing)

N of responses	Observed response rate (%)	95% CI (%)	
0/9	0	0	37.1
1/9	11.1	0.6	49.3
2/9	22.2	3.9	59.8
3/9	33.3	9	69.1
4/9	44.4	15.3	77.3
5/9	55.6	22.7	84.7
6/9	66.7	30.9	91
7/9	77.8	40.2	96.1
8/9	88.9	50.7	99.4
9/9	100	62.9	100

The estimated differences in response rate, as well as their 95% CIs are shown in Table 6-2. Due to the relatively small sample sizes, it is recommended (36) to construct the two-sided response rate difference CIs with Agresti-Caffo interval (37) that is less conservative. The group with higher response rate is assumed to have positive response rate of 50%, 70%, and 90%, and the assumed range of response rate differences between 2 groups is 0% to 50%, 70%, and 90%, respectively. With sample size of 10 for each group, the statistically significant difference in response rates of approximately 50% (44.4%-55.6%) can be detected while controlling Type-I error rate at 5%.

Table 6-2 Observed rate difference between study groups and their two-sided 95% confidence intervals from 10 total study participants per group. Assuming 10% missing rate for immunogenicity endpoints, n = 9 (total 10 with 10% missing).

Group 1 positive response	Group 2 positive response	Observed rate difference (%)	Rate difference 95% CI (%) (Agresti-Caffo)	
5	5	0	-41.6	41.6
5	4	11.1	-32.5	50.7
5	3	22.2	-22.7	59.1
5	2	33.3	-12.2	66.8

Group 1 positive response	Group 2 positive response	Observed rate difference (%)	Rate difference 95% CI (%) (Agresti-Caffo)	
5	1	44.4	-0.9	73.6
5	0	55.6	11.5	79.4
7	7	0	-37.2	37.2
7	6	11.1	-29.6	47.8
7	5	22.2	-21.3	57.7
7	4	33.3	-12.2	66.8
7	3	44.4	-2.4	75.1
7	2	55.6	8.2	82.7
7	1	66.7	19.7	89.4
7	0	77.8	32.3	95
9	9	0	-24	24
9	8	11.1	-19.3	37.5
9	7	22.2	-13.1	49.5
9	6	33.3	-5.8	60.4
9	5	44.4	2.4	70.3
9	4	55.6	11.5	79.4
9	3	66.7	21.4	87.7
9	2	77.8	32.3	95

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 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. The SAEs will be monitored following each dose. For the first 2 doses, study participants in both groups will receive the same sD-NLT-AB05 + IL-12 DNA adjuvant; thus, the safety sample size can be doubled. Specifically, for this two-arm study with sample size n = 10 per group (n = 20* for safety after the first 2 doses), there is at least a 90% chance of observing at least 1 event if the true rate of such an event is 20.6% (10.9%*) or more and there is at least a 90% chance of observing no events if the true rate is 1.05% (0.53%*) or less. Safety data will be evaluated using historical controls. As a reference, in HVTN vaccine trials conducted in the US from April 2008 through March 2018, about 1% of

participants who received placebos experienced an SAE. Binomial probabilities of observing 0 events, 1 or more events, and 2 or more events among 10 (20*) participants receiving study vaccine are presented in Table 6-3 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 6-3 Probability of observing 0 events, 1 or more events, and 2 or more events among a group of 10 (20* for the first two doses) study participants for different true event rates.

True event rate (%)	Group size	0 events	1+ events	2+ events
1	10	0.9	0.1	0
4	10	0.66	0.34	0.06
10	10	0.35	0.65	0.26
20	10	0.11	0.89	0.62
30	10	0.03	0.97	0.85
True event rate (%)	**Safety size for the first two doses***	**0 events**	**1+ events**	**2+ events**
1	20*	0.82*	0.18*	0.02*
4	20*	0.44*	0.56*	0.19*
10	20*	0.12*	0.88*	0.61*
20	20*	0.01*	0.99*	0.93*
30	20*	0*	1*	0.99*

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 6-4 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. If none of the 10 (20* for safety after first 2 doses) participants receiving the study vaccine experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 32.6% (19.4%*).

Table 6-4 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints in a group of 10 study participants (n*=20 for safety after first two doses).

Observed event	n	95% CI (%)	
0	10	0	32.6
1	10	0	42.9
2	10	4.9	52.2
3	10	10.6	60.8
Observed event	**n***	**95% CI (%)**	
0	20*	0*	19.4*
1	20*	0*	25.7*
2	20*	1.8*	31.6*
3	20*	4.6*	37.1*

6.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through the Statistics and Data Management Center's (SDMC) Web-based randomization system. The randomization will be done in blocks to ensure balance across study groups. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments.

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 describes the final study analysis. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. The analysis is a modified intent-to-treat analysis in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of the brief length of time between randomization and enrollment—typically no more than 4 working days—very

few such individuals are expected. All analyses will be performed using SAS and R.

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

6.4.1 Analysis variables

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

6.4.2 Baseline demographics

Participants' baseline characteristics will be summarized using descriptive statistics.

6.4.3 Safety/tolerability analysis

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

Reactogenicity: The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. Wilcoxon rank sum tests will be used to test for differences in severity between groups.

AEs and SAEs: AEs will be coded into Medical Dictionary for Regulatory Activities (MedDRA)–preferred terms. The number and percentage of participants 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.

6.4.4 Immunogenicity analyses

6.4.4.1 Statistical Analysis to address primary objectives

The first component of the analysis will entail comparing the frequency of HIV-1–specific binding Ab responses 2 weeks following the fourth vaccination. Comparison between the 2 vaccine groups will use Fisher's exact test, while

McNemar's test will be used for paired data (between visits), with a significant difference declared if the 2-sided p-value is ≤ 0.05.

To compare the magnitude of HIV-1–specific binding Ab responses 2 weeks following the fourth vaccination. Between the 2 vaccine groups, a two-sided Wilcoxon rank sum test with 5% type-I error rate will be performed. Wilcoxon signed rank test with 5% type-I error rate will be performed for paired data.

With the response rates of CD4+ and CD8+ T cell to HIV-1 specific Env peptide pools 2 weeks following the fourth vaccination, the 2 vaccine groups will be compared using Fisher's exact test, and McNemar's test will be used for paired data), with a significant difference declared if the 2-sided p-value is ≤ 0.05. For each T-cell subset, the positivity call for each peptide pool will include a multiple-comparison adjustment for the number of peptide pools used in the assay using the discrete Bonferroni adjustment. The magnitude of response will be analyzed with two-sided Wilcoxon rank sum test for independent data and Wilcoxon signed rank test for paired data, with 5% type-I error rate. Graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm, and timepoint, with a box plot of data from positive responders superimposed on the individual data values. Statistical testing comparing the magnitudes will be based on positive responders only.

6.4.4.2 General approach for secondary and exploratory 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 they received. Additional analyses may be performed, limited to participants who received all scheduled injections per protocol. Assay results that are unreliable, from specimens collected outside of the visit window, or from HIV-infected participants are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks, or less, prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample post enrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% CI estimates calculated using the score test method (38). Fisher's exact tests will be used to compare the response rates of between 2 vaccine groups, with a significant difference declared if the 2-sided p-value is ≤ 0.05.

For quantitative assay data (eg, magnitude of HIV-1 Env-specific binding Ab responses), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. The difference between arms at a specific timepoint will be tested with a nonparametric Wilcoxon rank sum test if

the data are not normally distributed and with a 2-sample t-test if the data appear to be normally distributed. An appropriate data transformation (eg, log10 transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance). More sophisticated analyses employing repeated measures methodology (for example, repeated measures analysis of variance or generalized estimating equations) may be utilized to incorporate immune responses over several timepoints and to test for differences over time. However, inference from such analyses would be limited by the small sample size of this study. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05$.

6.4.4.3 Missing Data

Based upon previous AIDS Vaccine Evaluation Group and HVTN trials, missing 10% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with nonparametric tests and generalized linear models fit by generalized estimating equation (GEE) methods, missing data need to be missing completely at random (MCAR). MCAR assumes that the probability of an observation being missing does not depend upon the observed responses or upon any unobserved covariates but may depend upon covariates included in the model (eg, missing more among whites than nonwhites). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then nonparametric tests and GEE methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests. These models will include as covariates all available baseline predictors of the missing outcomes.

If a substantial amount of immunogenicity data is missing (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right-censored, then the linear mixed effects models of Hughes (39) will be used because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE (40) methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

6.4.5 Analyses prior to end of scheduled follow-up visits

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

6.4.5.1 Safety

During the course of the trial, analyses of safety data will be prepared approximately every 4 months for review by the Safety Monitoring Board (SMB). Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 304 PSRT. The HVTN leadership must approve any other requests for safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity

A statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed. The HVTN Laboratory Program will review the analysis report prior to distribution to the protocol chairs, Division of AIDS (DAIDS), vaccine developer, and other key HVTN members and investigators. Distribution of reports will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the scheduled follow-up visits. Any analyses conducted prior to the end of the study should not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments.

7 Study vaccine preparation, storage, and administration

7.1 Vaccine Regimen

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

Group 1

Treatment 1 (T1):

INO-6160, 2 mg dose, to be administered as 2 separate intradermal (ID) injections (0.1 mL each) followed by electroporation (EP) using the CELLECTRA 2000 EP device at months 0, 1, 3, and 6.

Group 2

Treatment 2 (T2):

INO-6160, 2 mg dose, 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, 3 and 6.

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

7.2 Study Product Formulation and Storage

7.2.1 INO-6160

INO-6160 Drug Product is sD-NLT-AB05 coformulated with IL-12 DNA at a plasmid ratio of 4:1 (0.8 mg sD-NLT-AB05 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 size glass vials. Vials may contain a volume greater than the labeled volume.

Store INO-6160 frozen at -25°C to -15°C. 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 a preservative. 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 WFI 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 technique and sterile preparation procedures/guidance as outlined in USP <797>, utilizing a pharmacy biosafety cabinet/isolator or better. Local regulations and site institutional policies and procedures for use of personal protective equipment, such as gloves, gowns, masks, and safety glasses, must be followed. Pharmacists should follow the requirements of their country, their institution, and their pharmacy regulatory authority regarding these procedures.

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

7.3.1 INO-6160, 2 mg (Group 1 and Group 2)

1. Remove 1 vial of INO-6160, 10 mg/mL from the freezer and record this as the study product preparation start time.

2. Once contents of vial are thawed, insert the sterile BD 0.5 mL Tuberculin Syringe with permanently attached needle, 27G x ½ inch (BD Reference Number 305620), into the sterile glass vial containing INO-6160, 10 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-6160, 10 mg/mL, 0.1mL each.

3. Label the syringes according to Section 7.3.3 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 syringes may be stored at room temperature and used within 4 hours of study product preparation start time.

7.3.2 Trimer 4571, 100 mcg, admixed with 3M-052-AF, 5 mcg, and Alum, 500 mcg (Group 2)

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 the 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 7.3.3 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.

7.3.3 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

7.3.4 Study Vaccine Administration

7.3.4.1 INO-6160

INO-6160, 2 mg, will be administered as 2 separate 0.1 mL injections intradermally, bilaterally, one on each arm at months 0, 1, 3 and 6. Following ID injections of INO-6160, EP will be performed using the Inovio CELLECTRA 2000 EP Device.

The ID injections will be administered in 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.

If an injection cannot be given in the upper arm due to a medical contraindication, it should be administered in the thigh (not in the contralateral upper arm). If both upper arms are unsuitable for injection, the injections should be administered in the thigh. The appropriate study staff should document this clearly.

7.3.4.2 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 2 participants at months 3 and 6, following administration of INO-6160, as 2 separate 0.2 mL injections (1 in each arm) intramuscularly into the deltoid muscles by needle and syringe. Immediately prior to administration, gently invert each syringe 10 times.

If an injection cannot be given in a deltoid muscle due to a medical contraindication, it should be administered in the thigh (not in the contralateral deltoid). If both deltoids are unsuitable for injection, the injections should be administered in the thigh muscle. The appropriate study staff should document this clearly.

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.

7.4 Acquisition of study products

sD-NLT-AB05 and IL-12 DNA are manufactured under quality controls and production protocols developed and tech transferred by Inovio Pharmaceuticals. INO-6160, the co-formulation containing sD-NLT-AB05 and 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 are manufactured by Leidos Biomed (Vaccine Research Center, Frederick, MD, USA). Trimer 4571 will be provided by Dale and Betty Bumpers Vaccine Research Center (VRC), and Alum will be provided by DAIDS, the National Institute of Allergy and Infectious Diseases (NIAID), the US National Institutes of Health (NIH) (Rockville, MD, USA).

3M-052-AF is manufactured and provided by Access to Advanced Health Institute (AAHI) (Seattle, Washington, USA).

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

7.5 Study Vaccine Accountability

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

7.6 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

All required clinic and laboratory procedures for each study visit are summarized in Appendix A, Schedule of 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) 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 Assessment of Understanding (AoU) will be completed prior to enrollment. Records will be kept documenting the reason(s) that screened participants did not enroll.

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 B), 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 14 full days following vaccine administration. Clinicians will follow and collect resolution information for any reactogenicity signs and symptoms that have not resolved within the reactogenicity assessment period.

Pre-vaccine Administration: Intercurrent illness assessment and evaluations (including vital signs and planned injection-site evaluation) are performed prior to each vaccine administration.

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

Post-vaccine administration in-clinic evaluation: Following the enrollment vaccine administration, participants will be observed for a minimum of 60 minutes post study product administration. For all subsequent administrations, participants will be observed for a minimum of 30 minutes with the following exception: for Group 2, participants will be observed for 60 minutes following the first administration of Trimer 4571 + 3M-052 AF + Alum scheduled for Month 3. 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.

Post-vaccine 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 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.

Symptom diaries: Participants are asked to record symptoms on a daily basis using an electronic participant diary (eDiary). A paper alternative is available. All participants will be given a thermometer for oral temperature measurement, a ruler, and access to the eDiary (paper alternative available). All participants will be provided training on diary completion, proper thermometer usage, and the use of the measuring device to measure any injection site induration and/or erythema. Participants will use the diary to record daily their highest temperature as well as local and systemic signs and symptoms for the day of vaccination and 14 full days following each vaccine administration. 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.

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 reactogenicity. An abbreviated assessment will take place during the AESI contact (Section 8.8). Please see HVTN 304 Study-specific Procedures (SSP) for further details.

8.5 Visit windows and missed visits

The schedule of visits and evaluations performed at each visit is shown in Appendix A. Visit windows are shown in Appendix B. The procedures for documenting missed visits and out of window visits are described in the HVTN 304 SSP. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

8.6 Monitoring for HIV infection

Study participants will be tested for HIV infection periodically throughout the study per the HVTN HIV testing algorithm as noted in Appendix A. 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 infection in accordance with the most recent Centers for Disease Control and Prevention HIV counseling guidelines.

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

- Participants will be counseled to avoid HIV 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 the participants request that their names be removed, the names of all participants in HVTN studies are entered into a secure VISP registry in order to verify that an individual received an HIV vaccine (and therefore has the

potential for VISP) and to qualify former participants for post-study HIV testing to distinguish between VISP and HIV infection. Information in the VISP registry is not used for research.

8.7 Early termination visit

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

8.8 AESI contact

CRS staff will contact study participants 12 months after the last vaccination to collect the information listed below and will perform an abbreviated EP-injection–site assessment 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 AESI assessment. AESIs are described further in Appendix F.

- 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;
  - AESIs (a sample list of AESIs is provided in Appendix F). AESIs are reported regardless of relationship to study product(s);
  - Medically attended adverse events (MAAEs), defined as any AEs leading to an unscheduled visit to a healthcare professional, which are reported regardless of relationship to study product(s);
  - New diagnosis of HIV infection; and
  - Pregnancies and outcomes, including congenital anomalies/birth defects.

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

9 Safety and adverse events

9.1 Adverse events

Unsolicited AEs will be collected over a period of 30 days after each vaccination. All collected AEs are captured in the clinical database on the appropriate CRF. Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting (see Section 9.2.1), (2) 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 Appendix F.

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

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

9.2 Serious adverse events

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

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

- *Death,*
- *A life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization,*
- *A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,*
- *Congenital anomaly/birth defect.*

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse."

"Life-threatening" refers to an 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 Regulatory Support Center (RSC) website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

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

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited 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 and investigational device for which expedited reporting are required are:

- INO-6160,
- [Trimer 4571 + 3M-052-AF + Alum], and
- EP Device.

The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the FDA in accordance with 21 CFR 312.32 (IND Safety Reports).

9.3 Safety monitoring

9.3.1 Protocol Safety Review Team (PSRT)

The PSRT comprises: DAIDS Medical Officer, Protocol Chair, Protocol Cochair, Protocol Team Leader, and Clinical Safety Specialist (CSS). Other members of the Protocol Team (Clinic Coordinator, Clinical Data Manager, Clinical Trial Manager, vaccine developer representative(s), and others) may also be included in HVTN 304 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 (SMB)

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

The SMB reviews safety data (including cumulative reactogenicity events, AEs, laboratory safety data, and individual SAE reports) approximately every 4

months. The SMB conducts additional special reviews at the request of the HVTN 304 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. 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

Enrollment will be restricted to 1 participant per day for the first 5 participants and then enrollment will pause. Participants who have been enrolled will continue to receive vaccinations during the pause. The PSRT will review cumulative safety information recorded through the visit scheduled 2 weeks post first vaccination for the first 5 participants and will determine whether it is safe to proceed with full enrollment.

9.6 Safety pause and prompt PSRT AE review

The PSRT (see Section 9.3.1) 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 an immediate safety pause or prompt HVTN 304 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 304 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**	Any	**Phone** 24/7 Safety Phone immediately **Email** vtn.clin.safety.spec@hvtn.org **Submit** CRFs immediately	Immediate pause and PSRT review
AE, **related** (see Grade 3 exceptions below)	4 or 3	**Email** CSS **Submit** CRFs immediately	Prompt PSRT AE review to consider a pause

*Once a CRF is submitted to the database that meets a Pause Rule, the CSS receives an immediate email alert in order to follow up with the site and inform the PSRT.

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

- Injection site pain/tenderness
- Fatigue
- Generalized myalgia
- Generalized arthralgia
- Chills
- Headache
- Nausea (unless IV rehydration required)

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

If you need to contact the CSS, refer to phone numbers and email addresses found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn304).

9.6.1 Plan for review of pause rules

For all safety pauses, HVTN Core notifies the HVTN 304 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 304 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the SMB if necessary. HVTN Core notifies the participating HVTN CRSs, HVTN Regulatory Affairs, DAIDS PAB, DAIDS RAB, and DAIDS SPT of the decision regarding resumption or discontinuation of

study vaccinations. Based on the HVTN 304 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 304 PSRT notification or prompt HVTN 304 PSRT AE review is triggered, HVTN Core notifies the HVTN 304 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 304 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 304 PSRT, the NIH, the US Department of Health and Human Services (DHHS) 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 the 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 304 SSP.

10 Protocol conduct and informed consent

10.1 Protocol conduct

This research study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) (ICH E6 (R2)), HVTN and DAIDS policies and procedures as specified in the *HVTN Manual of Operations* and the 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 304 SSP.

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

10.2 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, 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 C describes the investigational vaccine and all aspects involved in study participation. Documentation of appropriate informed consent must be in place prior to conducting study procedures with participants. Periodic assessment of participants' continued understanding of key study concepts and informed consent must also be documented. Study sites are strongly encouraged to have their local

CABs review their site-specific consent forms. This review should include, but should not be limited to, issues of cultural competence, local language considerations, and the level of understandability.

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

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

10.3.1 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 accord with the language in the SICF (Appendix C) 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 C).

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

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

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

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Appendix A Schedule of procedures

Visit Number		01	02	03	04	05	06	07	08	09	10	AESI[13]
Study Week			0	2	4	6	12	14	24	26	52	78
Study Month			0	0.5	1	1.5	3	3.5	6	6.5	12	18
Study Day		-56 to 1	1	15	29	43	85	99	169	183	365	547
Procedure		Screen[1]	**Vac 1**		**Vac 2**		**Vac 3**		**Vac 4**			
Study procedures												
Assessment of Understanding (AoU)		√										
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				√	√	√	√	√	√	√	√	√
Clinical labs	Tube											
Pregnancy test (urine or serum) [9, 10]		√	√		√		√		√			
HBsAg/anti-HCV [10]	SST	5										
HIV screening test [10, 11]	SST	5										
CBC/Differential [10]	EDTA	5		5		5		5		5		
ALT & Creatinine [10]	SST	5		5		5		5		5		
HIV diagnostic test	EDTA						10		10		20	
Research samples[12]												
PBMC for assays & storage	ACD	136				136	51	136		136	178.5	
Serum for assays and storage	SST	34				34	34	34		34	34	
Daily volume (mL)		190	0	10	0	180	95	180	10	180	232.5	
56-day total volume (mL)		190	190	200	200	380	275	455	10	190	232.5	

Blue shading: Vaccination Visit

[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, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck, lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin. At other visits, a **targeted physical exam** will be performed as needed, based on participant report or indications of illness.

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

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

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

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

[8] **Reactogenicity**: Reactogenicity assessments performed daily from **the day of vaccination through** 14 full days following vaccine administration. (see Section 8.3)

[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] **AESI contact:** CRS staff will contact study participants 12 months after the last vaccination received to collect the information listed in Section 8.8. The EP-injection–site assessment will be an abbreviated form that can be conducted over the phone.

Appendix B Visit windows

Visit Number	Visit Type	Lower Allowable Window (-)	Lower Target Window (-)	Target Day*	Upper Target Window (+)	Upper Allowable Window (+)
01.0	Screening	-56	-	-	-	-
02.0	**Enrollment / Vaccination 1**	**-**	**-**	**1**	**-**	**-**
03.0	2 weeks post-vaccination 1		-4	15	+4	+7
04.0	**Vaccination 2**		**-**	**29**	**+9**	**+28**
05.0	2 weeks post-vaccination 2		-4	43	+4	+7
06.0	**Vaccination 3**		**-**	**85**	**+14**	**+28**
07.0	2 weeks post-vaccination 3	-	-4	99	+4	+7
08.0	**Vaccination 4**	**-**	**-7**	**169**	**+14**	**+28**
09.0	2 weeks post-vaccination 4	-	-4	183	+4	+7
10.0	6 months post vaccination 4	-28	-14	365	+14	+28
AESI*	Final Visit	-21	-14	547	+14	+28

All target dates are relative to Day 1, with the exception of the post-vaccination visits, visits 3, 5, 7, and 9 which are relative to the vaccination immediately preceding the visit.

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

Appendix C Sample informed consent form

Title: A phase 1 open-label clinical trial to evaluate the safety and immunogenicity of synthetic DNAs encoding a native-like HIV Env Trimer and Interleukin-12 (INO-6160), alone or in a prime-boost regimen with 3M-052-AF + Alum adjuvanted VRC HIV Env Trimer 4571 in adult participants without HIV

HVTN protocol number: HVTN 304

Site: [Insert site name]

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

Key information

- This is the first study in which one of the study vaccines will be given 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 age 18-55, do not have HIV, and are generally healthy.
- The purpose of this study is to see if the study vaccines are safe and to see 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 1 year after your last vaccination to check on your health.
- Procedures will include blood draws and injections of study vaccines.
- 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. We will tell you more about this procedure and its risks later in this consent form.
- There are risks from participating. We will tell you more about all of them later in this consent form. The most common risks are as follows:
  - Taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding.
  - 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 12 months or longer.

  - 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 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 25 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 three 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 vaccines? (Your immune system protects you from infections and disease.)

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

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

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

3. **The study vaccines are experimental.**

   We are testing 2 study vaccines and a study adjuvant.

   One is called INO-6160. We will call it the DNA vaccine. It is made up of 2 different pieces of DNA, sD-NLT-AB05 + interleukin-12 or IL-12. This DNA is very much like natural DNA, but it was 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 study vaccine is called Trimer 4571. We will call it the protein vaccine. It is a small, lab-made piece of protein that looks like a part of a protein found on the surface of the HIV virus. In this study, it is being mixed with an adjuvant called 3M-052-AF and another adjuvant called Alum. We will call the combination of these 2 adjuvants the study adjuvant. Adjuvants are products that help alert the immune system to have a stronger response. 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. This study is using the improved 3M-052-AF adjuvant. It is dissolved in water and 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.

Everyone in this study will get the DNA vaccine. It will tell your body to make small amounts of 2 different proteins. One of them is a protein that is found in HIV, and the second protein is called IL-12. IL-12 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 make antibodies and T cells more efficiently. Antibodies and T cells are parts of an immune response that defends the body against diseases. 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 and the study adjuvant. The immune system might be able to see this piece of protein and learn how to recognize HIV if you are ever exposed to it in the future.

Where do the study products come from?

The DNA vaccine was developed by the Wistar Institute (Philadelphia, Pennsylvania) and formulated by Inovio Pharmaceuticals. It is provided by the Wistar Institute through a grant from the NIH.

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

The 3M-052-AF adjuvant was developed, manufactured and is being provided by the Access to Advanced Health Institute (AAHI) in Seattle, Washington. Alum is being provided by the Division of AIDS (DAIDS) in Bethesda, Maryland, which is part of the NIH.

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 4 other studies. In general, people who got DNA vaccines in those studies were not too uncomfortable and did not have any serious health problems related to the vaccine. Six 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 as part of 2 different ongoing studies. In one study, VRC018, 16 people got the same protein vaccine mixed with the Alum adjuvant that we will use in this study. Each person got 3 injections of the vaccine and adjuvant at the same dose or higher than the

dose we will use in this study. The injections did not cause any serious health problems. Most people in that study had at least one of the side effects listed in the *Risks of vaccines* described below. They said the 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 the same protein vaccine combined with Alum at a higher dose than the one we will use in this study. These injections did not cause any serious health problems. This study is still ongoing and plans to enroll up to 100 people.

Another protein vaccine and the same 3M-052-AF + Alum adjuvant being used in this study are being tested for the first time in people in 2 other studies called HVTN 137 and HVTN 300. As of Spring 2022, 48 people in these 2 studies have had at least 1 study injection and at least 45 people have had 2 injections. These injections have not caused any serious health problems.

HVTN 300 enrolled 13 people. All people in the study had at least some injection site reactions during the trial, mostly mild to moderate. One (1) person had severe pain/tenderness in both the right and left injection sites 3 days following the fourth vaccination, though it lasted only one day.

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. These severe side effects went away within 2 days. Of the 5 people who had severe side effects, 2 decided to stop getting study injections because they felt generally unwell after injections and it was affecting their daily lives. Another 2 of the 5 people who had severe side effects got more injections; one of them had more severe side effects while the other did not. And one (1) of the 5 people who had severe side effects has not yet received another injection.

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

One person enrolled in HVTN 137 had some redness in the area where they got the injection that lasted for about 3 days. They also had significant swelling on their arm for about 4 days. It took about 6 weeks for the swelling to completely go away. They also had mild pain, which did not prevent them from going to work, and the pain went away on its own. This person decided to stop getting any more injections. There were 4 people who experienced stronger side effects. These included chills, headache, muscle aches, and generally feeling unwell. Because the study is ongoing and is blinded, we do not know if these people got the study vaccine with the adjuvant or a placebo (sterile salt water with no vaccine in it).

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

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

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 the "*Risks of vaccines"* section above, but no serious health concerns have been reported so far.

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

4. **The DNA vaccine is given using electroporation.**

Besides adjuvants, another way to improve immune responses to DNA vaccines is to use EP. EP uses an electrical pulse to briefly open tiny pores in the cells. The DNA can enter the cells through these pores. EP has been used for many years in the laboratory to get DNA or other substances into cells. 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. 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, together with IL-12 adjuvant, were given into the skin with EP to 55 people. EP has not caused 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 9,300 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 and scabs. Later the marks may heal but might 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/friends if it felt the same as their study experience, and 96% of these participants said that the appearance at injection sites was acceptable.

We do not know if EP will change the risks for the DNA vaccine. 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 not with this DNA vaccine.

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

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

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

   Site: Remove item 8 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 you are not eligible for the study, even if you want to.

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 E, 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 (for a total of 9 months). We will talk to you about effective birth control methods. They are listed on a handout that we will give to you.

   You also should not begin the process to have your eggs collected until 8 weeks after your last scheduled study injection. If this is something you are considering, please discuss it with your study doctor and your fertility specialist.

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 10 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 contact you 12 months 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).

10. **We will pay you for each study visit you complete.**

    This payment is to cover the costs of [Site: insert text].

Payments you receive for being in the study may be taxable. We may need to ask you for your Social Security number for tax reasons.

You do not have to pay anything to be in this study. *Site: Insert any costs to participants (eg, birth control costs for female participants who could become pregnant).*

11. **We will give you the study vaccines on a schedule.**

We will give participants injections of the study vaccines at 4 visits, according to the schedule in the table below. All participants will get 2 injections of the DNA vaccine with a needle and syringe into the skin of both upper arms followed by electroporation at all 4 vaccination visits. People in Group 2 will also get 2 injections of the protein vaccine plus the study adjuvant with a needle and syringe into the upper arm muscle of both arms at the last 2 vaccination visits. If for some reason we cannot give an injection in your arm, we may give it in the thigh instead.

You have an equal chance of being assigned to Group 1 or Group 2. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.* Whether you are assigned to Group 1 or 2 is completely random, like rolling dice or drawing straws.

Because this is the first time the DNA vaccine is being given to people, only 1 participant will get the study vaccine each day, until a total of 5 participants across both groups have been vaccinated. After this, we will pause the study for a safety review by a team of experts who are not part of the study. They will look at information about participant responses to the study vaccines and decide if it is safe to continue the study. If it is, the rest of the participants will be enrolled.

Group	Vaccine type	Where	How	First injection visit	1 month later	3 months later	6 months later
1	DNA vaccine	Skin of both upper arms	Needle & syringe with EP	✓	✓	✓	✓
2	DNA vaccine	Skin of both upper arms	Needle & syringe with EP	✓	✓	✓	✓
	Protein vaccine plus adjuvant	Muscle of both upper arms	Needle & syringe			✓	✓

The injections of the DNA vaccine will be given between the layers of the skin (known as intradermal injections with a needle and syringe. Then we will press the EP device firmly against that same area. The device will insert 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 the electrical pulses.

The injection procedure with EP 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 and may go away in a couple of minutes. After that, your arm may be sore for a day or two.

You will have to wait in the clinic for about an hour after your first study injection of the DNA vaccine and your first study injection of the protein vaccine plus adjuvant to see if there are any problems. For the other injections, you will have to wait for about 30 minutes. Then for that night and for 14 more days, you will use a secure electronic symptom log to keep track of how you are feeling. We call this symptom log an eDiary. 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, we 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 changes on your skin related to the EP procedure. We may ask to take photos of your arm where you got an 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 study vaccines and EP.

12. **We will do the procedures shown in this table.**

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

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

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 243 mL (2 teaspoons to about 1 cup). These samples are below the maximum of 500 mL that is allowed when people donate blood. Your body will make new blood to replace the blood we take out within 4 to 8 weeks.

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

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.

13. **We will counsel you about protecting yourself from HIV.**

We will ask you questions about your risk factors for being exposed to HIV, such as sexual behavior, alcohol, and drug use. We will talk with you about ways to keep your risk of exposure to 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.

15. **When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.**

    The HVTN calls these samples "extra samples." The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this form. If you have any questions, please ask.

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

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

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

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

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

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

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

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

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

Researchers may also do genetic testing on your samples.

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

If you agree, your samples could also be used for genome-wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included.

Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

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

People who may see your information are:

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

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

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

Site: Check HIPAA authorization for conflicts with this section.

All of your samples and most of your study records will be labeled with a code number. Samples and study records are kept in secure locations. When you provide information in the online symptom log after the injection visits, that information only has your code number. Your data goes directly from the 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;
- *US sites may, but are not required to include:* [Insert name of local IRB/EC] ;
- Advarra IRB;
- *Site:* [Insert name of local IBC] ;
- [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; and
- The US Office for Human Research Protections.

All reviewers will keep your records private.

We cannot guarantee absolute privacy. If you have a medical condition that we are required to report by law, then some of your information may be shared. At this clinic, we have to report the following information:

Site: Include any public health or legal reporting requirements. Bulleted examples should include all appropriate cases (reportable communicable disease, risk of harm to self or others, etc.) If your site does not have public health or legal reporting requirements, you may delete the last sentence in the paragraph above, along with the bullets below.

- [Item 1]
- [Item 2]
- [Item 3]

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, but it will be your choice. You may complete study procedures unless there is a medical reason not to or applicable regulations require you leave the study. If you leave the study while you are pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery. We will also contact you to check on your health 12 months after your last injection.

We will stop your injections if you get HIV. We will also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study for up to 12 months if you choose. We will also contact you to check on your health 12 months after your last injection. 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.

If we stop your injections, we may ask you to stay in the study to complete other study procedures for up to 12 months after your last injection, if you agree to do so.

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 we know about. There may be other risks, even serious ones. We will tell you if we learn anything new that may affect your decision to stay in the study.

Risks of abnormal laboratory results:

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

Risks of routine medical procedures:

Routine medical procedures such as taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, stinging, swelling, itching, a sore, bleeding, and rarely infection where you got the injection. Taking blood can cause a low blood cell count (anemia), making you feel tired.

Personal problems/discrimination/testing HIV antibody positive:

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

Most vaccines cause the body to make antibodies to prevent infection. Your body may make antibodies to HIV because you received an HIV study vaccine. Those antibodies could cause you to test positive on some types of HIV tests, even if you do not have HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccine, a routine HIV test done outside this clinic is likely say you have HIV, even if you don’t. For this reason, you should get HIV tests only at this clinic. Our tests can tell the difference between true HIV infection and a positive result caused by the study vaccine. If you have VISP, we can arrange free HIV testing for as long as you need it.

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

Site: Modify the following paragraph if applicable. If someone believes you have HIV, you could face discrimination and other problems. In some countries, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If you have VISP, you will not be able to donate blood or organs. Your family and friends may treat you differently. We will give you a brochure that tells you more about VISP, and how you can avoid some of these problems.

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

You should tell the delivery staff if you have VISP. However, you may still be tested for HIV using the antibody test when you deliver your baby. If your test is positive, your baby may be started on antiretroviral treatment when it is not needed. If this happens, we can arrange for you and the baby to have tests that can distinguish true HIV infection from VISP. If you or the baby continue to have VISP, we can arrange this testing for free for as long as it is needed.

Embarrassment/anxiety:

You may feel embarrassed when we ask questions about HIV risks, such as sex and using drugs. Also, waiting for HIV test results could make you feel anxious. You could feel worried if test results show that you have HIV. If you feel embarrassed or anxious, please tell us and we will try to help you.

Risks of disclosure of your personal information:

Although the risk is very low, it is possible that someone who should not may see your personal information. If that happens, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information.

Risks of genetic testing:

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

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

Unknown risks:

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

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

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

Benefits

20. The study may not benefit you.

We do not expect the study vaccines to benefit you in any way. However, being in the study might still help you in some ways. The counseling that you get as part of the study may help you avoid getting HIV. The lab tests and physical exams might detect health problems you don't yet know about.

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

This study may help in the search for a vaccine to prevent HIV. However, if the study vaccines later become approved for sale, there are no plans to share any money with you.

Your rights and responsibilities

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.

We will ask you to come to the clinic one last time for a physical exam, 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.

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-6160. 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].

If you have any questions about your rights as a research participant, or problems with or concerns about how you are being treated in this 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: [Advarra to provide].

US sites may include if applicable: You may also contact the [name of local IRB/EC] at [insert contact information].

If you want to leave this study, contact [name or title and telephone number of the investigator or other study staff].

Your permissions and signature

25. In Section 15 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used. You can change your mind after signing this form.

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

OR

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

OR

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

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

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

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

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

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

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

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

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

Appendix D Low-risk guidelines for US

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

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

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

1. SEXUAL BEHAVIORS

In the **last 12 months,** did <u>not</u>:

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

AND

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

In the **last 6 months**:

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

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

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

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

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

AND

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

2. NON-SEXUAL BEHAVIORS

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

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

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

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

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

- Chancroid
- Hepatitis B

For US volunteers on PrEP

1. PrEP ASSESSMENT

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

[1] See Study-specific Procedures (SSP) for additional guidance on on-demand PrEP.

2. SEXUAL BEHAVIORS

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

3. NON-SEXUAL BEHAVIORS

In the last 12 months, did not:

- Inject drugs or other substances without a prescription

- Use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment, rendered the participant at greater than low risk for acquiring HIV infection.

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

Appendix E Approved birth control methods

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

You should not become pregnant during the study because we do not know how the study vaccine could affect the developing baby.

You must agree to use effective birth control from 21 days before your first injection until 8 weeks after the time of your last study injection at 6 months (for a total of 9 months).

Effective birth control means using any of the following methods every time you have sex:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- External or internal 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, external and internal condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

If you join the study, we will test you for pregnancy at some visits, including before each study injection.

Appendix F Adverse events of special interest

Adverse events of special interest (AESI) for this protocol include but are not limited to potential immune-mediated diseases; representative examples of AESI are listed below. Updates to AESI will be provided as an appendix to the HVTN 304 Study-specific Procedures (SSP).

Neuroinflammatory disorders

- Cranial nerve disorders, including paralyses/paresis (eg, Bell's palsy)
- Optic neuritis
- Multiple sclerosis
- Transverse myelitis
- Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
- Acute disseminated encephalomyelitis, including site specific variants: eg, non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis
- Myasthenia gravis, including Lambert-Eaton myasthenic syndrome
- Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy).
- Narcolepsy

Musculoskeletal disorders

- 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

Skin disorders

- Psoriasis
- Vitiligo
- Erythema nodosum
- Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)
- Alopecia areata
- Lichen planus
- Sweet's syndrome
- Localized Scleroderma (Morphea)
- Cutaneous lupus erythematosus

Metabolic disorders

- Addison's disease
- Autoimmune thyroiditis (including Hashimoto thyroiditis)
- Diabetes mellitus type I
- Grave's or Basedow's disease

Vasculitides

- Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis.
- Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.

Blood disorders

- 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

Others

- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
- Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy)
- Autoimmune myocarditis/cardiomyopathy
- Sarcoidosis
- Stevens-Johnson syndrome
- Sjögren's syndrome
- Idiopathic pulmonary fibrosis
- Goodpasture syndrome
- Raynaud's phenomenon

Appendix G Protocol team

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Appendix H 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 304 are described below.

Protocol history and modifications

Date: January 11, 2023

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

Item 1 Revised in Section 5.1.1, *Inclusion criteria*, inclusion criterion #13 :blood pressure range

Item 2 Deleted in Section 9.1, *Adverse events*: creatinine as an exception to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events

Item 3 Revised in Section 9.6, *Safety pause and prompt PSRT AE review,* Table 9-1: pause rules for related SAEs

Item 4 Revised in Appendix C, *Sample informed consent form*, item 23: study-related injury language and role of Inovio Pharmaceuticals, Inc. and Access to Advanced Health Institute (AAHI)

Item 5 Revised in Appendix B, *Visit windows*: lower allowable window for AESI contact

Item 6 Minor formatting changes throughout

Item 7 Added to Appendix H*, Version history*: contents of this amendment

Date: November 3, 2022

Protocol version: 1.0

Original protocol