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STATISTICAL ANALYSIS PLAN FOR SAFETY

Protocol HVTN 304 (v2.0)

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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Author:

Legal Name	Lorel Schmitzberger
Job Title	Statistical Research Associate III
Signature & Date	See eTMF Signature Manifest

Approval:

Legal Name	Yunda Huang
Job Title	Associate Professor
Signature & Date	See eTMF Signature Manifest

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SAP Modification History

The version history of, and modifications to, this statistical analysis plan are described below.

SAP Version	Modification
1.0	Initial
2.0	Updated exploratory endpoint 1 per Clarification Memo 1 Added baseline reactogenicity definition to Section 10.3

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Table of Contents

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1 LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
EAE	Expedited Adverse Event
EP	Electroporation
FSR	Final Study Report
RSC	Regulatory Support Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMB	Safety Monitoring Board

2 OVERVIEW

The following describes the Statistical Analysis Plan (SAP) for the analysis of safety data from HVTN 304 for Safety Monitoring Board (SMB) reports and the Final Study Report (FSR) for Safety.

3 PROTOCOL SUMMARY

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.

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.

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.

Study participants

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

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:

				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***						

* 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.

Duration per participant

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

Estimated total study duration

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

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.

4 SAFETY OBJECTIVES AND ENDPOINTS

4.1 Primary safety 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.

4.2 Exploratory safety objectives

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

5 COHORT DEFINITION

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. 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.

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data.

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6 POTENTIAL CONFOUNDERS

Characterization of the safety of the vaccine is susceptible to confounding by adverse events not related to the vaccine that by chance occur more often in one arm of the trial than another. Therefore, analyses involving adverse events will incorporate the reported relationship to product as assessed by HVTN staff.

7 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.

8 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.

9 SAMPLE SIZE

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 1 for a range of possible true AE rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 1 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 2 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 2 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints in a group of 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*

10 STATISTICAL 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.

10.1 Analysis variables

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

10.2 Baseline demographics

Participants' baseline characteristics will be summarized using descriptive statistics.

10.3 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. Baseline reactogenicity grade is collected at the post-vaccine administration in-clinic evaluation.

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.

10.4 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 endpoint assessments. In particular, early analyses by treatment assignment require careful consideration and should be made available on a need-to-know basis only.

During the course of the trial, analyses of treatment-blinded 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.

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11 SAFETY TABLES, PARTICIPANT LISTINGS, AND FIGURES

11.1 List of Tables

The following tables are included in the SMB reports and FSR for Safety:

- Enrollment Report
- Demographics and Vaccination Frequencies
- Overall Protocol Status
- Maximum Local and Systemic Reactogenicity Summaries
- Adverse Events by Body System and Severity – By Decreasing Frequency
- Adverse Events by Preferred Term and Severity – By Decreasing Frequency – Includes Severe, Life-threatening or Fatal Events Only
- Adverse Events by Preferred Term and Severity – By Decreasing Frequency – Includes Events of All Severities
- Adverse Events by Preferred Term and Relationship to Study Product – By Decreasing Frequency – Includes Related Events Only
- Adverse Events by Preferred Term and Relationship to EP Device – By Decreasing Frequency – Includes Events Related to EP Device Only
- Adverse Events by Preferred Term and Relationship to Study Product – By Decreasing Frequency – Includes Events of Any Relationship
- Expedited Adverse Events (EAEs) Reported to the Regulatory Support Center (RSC)
- Pregnancy Listing

Additional tables included in the FSR for Safety:

- Adverse Events of Special Interest (AESIs)
- Social Impact Summary
- End of Study HIV Diagnostic Testing Results (Vaccine-Induced Seropositivity/Reactivity)
- Local Lab Value Summary Statistics
- Local Laboratory Values Meeting Grade 1 AE Criteria or Above
- EP Accessibility Questionnaire Results

11.2 List of Participant Listings

These participant listings are included in the SMB reports:

- Discontinuation Status
- Pregnancies
- Severe or Life-Threatening Local and Systemic Reactogenicities
- Moderate Erythema and Induration
- Expedited Adverse Events (EAEs)
- Adverse Events of Special Interest (AESIs)
- Severe, Life-Threatening, or Fatal Adverse Events
- Adverse Events with Relationship to Study Product

- Adverse Events with Relationship to EP Device
- HIV Infection Results from Lab and Reported by Site
- Device-Only Participants
- Device-Only Participants Adverse Events

11.3 List of Figures

These graphs are included in the SMB reports and FSR for Safety:

- Maximum Local Reactogenicities
- Maximum Systemic Reactogenicities
- Boxplots for hemoglobin, platelets, WBC, neutrophils, lymphocytes, ALT, and creatinine at baseline and each post-vaccination follow-up visit

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Reason for signing: Approved	Name: Lorel Schmitzberger Role: I am an author of the document. Date of signature: 22-Aug-2024 20:00:22 GMT+0000

Reason for signing: Approved	Name: Yunda Huang Role: I reviewed and approved the document. Date of signature: 23-Aug-2024 22:49:38 GMT+0000

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