Fred Hutch Cancer Center

SCHARP

STATISTICAL ANALYSIS PLAN FOR HVTN SAFETY

Protocol HVTN 300 (v2.0)

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

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Statistical Analysis Plan for Safety

Protocol: HVTN 300 (v2.0)

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SAP Modification History

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

[*Record revisions to the SAP. Specify the SAP version number, and major changes made. Do not include interim versions, i.e., only include whole numbers (1.0, 2.0, etc.). Add rows as needed to the following table.*]

SAP Version	Modification
1.0	Initial
2.0	Updated to accommodate Part B and Part C through Protocol v3.0
3.0	Update to remove Part C as Protocol v3.0 (Part C) was not approved (refer to Protocol bulletin #30)
4.0	Update *Contents of TLFs in SMB and Safety FSR *Baseline definition for safety lab values *Reactogenicity Onset/Resolution date
5.0	Update *using the lasted template *Section 4 to include only safety objective/endpoint *remove Social Impact table as it is not collected in the CRF *update schema table based on Letter of Amendment 1

Table of Contents

1 LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Experience
EAE	Expedited Adverse Experience
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 and tolerability data from HVTN 300 for Safety Monitoring Board (SMB) reports and the Final Study Report (FSR) for Safety. As detailed in SCHARP SOP-0013, revision 9.0 (effective date: February 1, 2023), this SAP is required prior to the first analysis and must be approved by the protocol team chair and the lead protocol statistician. The plan will be reviewed and updated prior to the first SMB report and before the final analysis with all major revisions of the plan archived.

3 PROTOCOL SUMMARY

3.1 Title

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

3.2 Design

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

3.3 Study products and route of administration

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

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

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

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

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

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

3.4 Study population

Healthy adults ages 18 through 55 years.

3.5 Study plan and schema table

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

Table 1-1 Schema

Part A									
Study arms	**N**	**Protein antigen**	**Adjuvant**	**Route**	**Month 0 (Day 0)**	**Month 2 (Day 56)**	**Month 4 (Day 112)**	**Month 8 (Day 224)**	**Month 12 (Day 364)**
Group 1	12*	CH505 TF chTrimer (300 mcg)	3M-052-AF (5 mcg) + Alum (500 mcg)	IM	✓	✓	✓	✓	✓
Part B									
Study arms	**N**	**Protein antigen**	**Adjuvant**	**Route**	**Month 0 (Day 0)**	**Month 2 (Day 56)**	**Month 4 (Day 112)**	**Month 8 (Day 224)**	**Month 12 (Day 364)**
Group 2	12	CH505 TF chTrimer (300 mcg)	3M-052-AF (3 mcg)	IM	✓	✓	✓	✓	✓
Group 3**	12	CH505 TF chTrimer (300 mcg)	3M-052-AF (2.2 mcg) + Alum (556mcg)	IM	✓	✓	✓	✓	✓
Group 4	12	CH505 TF chTrimer (300 mcg)	3M-052-AF (5 mcg)	IM	✓	✓	✓	✓	✓
Total	48								

*In Part A, up to 18 participants may be enrolled, if needed, to have at least 12 participants contribute to the immunogenicity analyses.
** Please note that on October 13, 2023, errors were identified in the study injection preparation section for Group 3 (see Section 7.3.3), due to which the participants in Group 3 received 2.2 mcg of 3M-052-AF (instead of 3 mcg) and 556 mcg of Alum (instead of 500 mcg). As there were no identified safety concerns among Group 3 participants, and to maintain consistency in the study data, the HVTN 300 PSRT decided that the study should continue using the same preparation instructions, resulting in CH505TF chTrimer vaccination with 2.2 mcg of 3M-052-AF and 556 mcg of Alum for Group 3 participants, even though they were not the exact intended doses.

3.6 Duration per participant

Up to 24 months. Participants will have up to 18 months of scheduled clinic visits. There will be a contact for an adverse events of special interest (AESI) assessment at 12 months after their final vaccination.

3.7 Estimated total study duration

Anticipate up to around 60 months if the study enrolls a total of 48 participants, and up to 56 months if the study enrolls more than 48 participants (eg, if participants dropout at a rate higher than anticipated, or miss key visits due to unforeseen circumstances). The estimate of the study duration includes enrollment, planned safety holds, enrollment of additional participants beyond 48, if this occurs, and follow-up.

3.8 Study sites

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

4 SAFETY OBJECTIVES AND ENDPOINTS

Primary objective 3:
To evaluate the safety and tolerability of a 300 mg dose of CH505 TF chTrimer admixed with different adjuvant regimens

Primary endpoint 3:
Local and systemic reactogenicity signs and symptoms will be collected for a minimum of seven days following receipt of any study vaccine.

Serious adverse events (SAEs), medically attended adverse events (MAAEs), adverse events of special interest (AESIs) and AEs leading to early participant withdrawal or permanent discontinuation which will be collected throughout the study and for twelve months following any receipt of study product. Additionally, all adverse events will be collected for 30 days after any receipt of study vaccination.

5 COHORT DEFINITION

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

In Part A, recruitment will target enrolling 12 healthy, HIV-uninfected adult participants in Group 1. The protocol team will convene after the 12th participant has received their third vaccination to

determine if further participants should be enrolled to evaluate immunogenicity. Examples of reasons that might necessitate enrollment of additional participants are provided in Section 1.5. If some participants from the original number of 12 enrolled will not contribute data to some of the immunogenicity analyses, the protocol team may enroll additional fully evaluable participants, up to 6 additional participants, or a total of 18 participants enrolled, with the goal of at least 12 contributing to the final immunogenicity analyses.

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

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

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

No randomization will be performed in this open-label study. Since participants will not be randomized between Groups 1 through 4 at enrollment, comparisons of immunogenicity or safety endpoints between treatment groups will be cautiously interpreted.

8 BLINDING

This is an open-label study and all participants are unblinded to the study products administered.

9 SAMPLE SIZE

9.1 Power calculations for safety for Part A

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

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

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

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

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 2-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method for CI described in Agresti and Coull formula 2 (1). If none of the 12 participants receiving the study vaccine experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 24.2%.

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

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

9.2 Power calculations for safety for Part B

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

Binomial probabilities of observing 0, 1 or more, and 2 or more events among the 12 participants of each group (Groups 2, 3, or 4) are presented in Table 2-1 for a range of possible true AE rates. Binomial probabilities of observing 0, 1 or more, and 2 or more events among the 36 participants of Groups 2, 3, and 4 combined are presented in Table 2-3 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 2-3 Probability of observing 0 events, 1 or more events, and 2 or more events, among groups of 12, 24, 36, and 48 study participants for different true event rates

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

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an AE based on the observed data. Table 2-4 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate in one group (eg, Group 2, or 3, or 4; n = 12), or 2 groups (eg, Groups 2 and 3; n = 24), or 3 groups

(Groups 2, 3, and 4; n = 36), or 4 groups (Groups 1through 4; n = 48). Calculations are done using the score test method for confidence interval described in Agresti and Coull formula 2 (44). If none of the 48 participants receiving any the study vaccines experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 7.4%.

Table 2-4 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints in n = 12, or n = 24, or n = 36, or n = 48 study participants

Observed event rate	95% Confidence interval (%)

0/12	[0.0 ; 24.2]
1/12	[1.5 ; 35.4]
2/12	[4.7 ; 44.8]
0/24	[0.0 ; 13.8]
1/24	[0.7 ; 20.2]
2/24	[2.3 ; 25.8]
0/36	[0.0 ; 9.6]
1/36	[0.5 ; 14.2]
2/36	[1.5 ; 18.1]
0/48	[0.0 ; 7.4]
1/48	[0.4 ; 10.9]
2/48	[1.2 ; 14.0]

10 STATISTICAL ANALYSIS

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

10.1 Baseline demographics

Participants' baseline characteristics will be summarized using descriptive statistics.

10.2 Safety Analyses

Reactogenicity: The number and percentage of subjects experiencing each type of reactogenicity sign or symptom will be tabulated by severity. For a given sign or symptom, each subject's reactogenicity will be counted once under the maximum severity for all assessments. Onset date is the first date when symptom grade is mild or higher. If the resolution form with resolution date filled is submitted for a given symptom, then report the resolution date on this form. But if there is no resolution form nor any date in the resolution form then the resolution date is computed as the first date with grade "None" after "the maximum severity grade" and stays grade "None" for the remainder of the reporting period.

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

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 Study Product Administration Frequencies
- Overall Protocol Status
- Maximum Local and Systemic Reactogenicity Summaries
- Adverse Experiences by Body System and Severity – By Decreasing Frequency
- Adverse Experiences by Preferred Term and Severity – By Decreasing Frequency – Includes Severe, Life-threatening or Fatal Experiences Only
- Adverse Experiences by Preferred Term and Severity – By Decreasing Frequency – Includes Experiences of All Severities
- Adverse Experiences by Preferred Term and Relationship to Study Product – By Decreasing Frequency – Includes Related Experiences Only
- Adverse Experiences by Preferred Term and Relationship to Study Product – By Decreasing Frequency – Includes Experiences of Any Relationship
- Expedited Adverse Experiences (EAEs) Reported to the Regulatory Support Center (RSC)/Serious Adverse Events (SAEs)

Additional tables included in the FSR for Safety:

- AESIs listing
- MAAEs listing
- Pregnancies listing
- HIV Acquisition Results from Lab and Reported by Site
- Study Product Administration Errors
- End of Study Diagnostic ELISA Testing Results
- Local Lab Value Summary Statistics
- Local Laboratory Values Meeting Grade 1 AE Criteria or Above

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 Experiences (EAEs) /Serious Adverse Events (SAEs)
- Adverse Experiences of Special Interest (AESIs)
- Medically Attended Adverse Events (MAAEs)
- Severe, Life-Threatening, or Fatal Adverse Experiences
- Adverse Experiences with Relationship to Study Product
- HIV Acquisition Results from Lab and Reported by Site
- Study Product Administration Errors

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 ALT, Creatinine, Hemoglobin, Platelet Count, WBC, Neutrophil Count, Lymphocyte Count at baseline, visit 3,5, 7, 9, and 11.
  Baseline lab safety values are from screen visit. If there are multiple screen visit, visit date that is closet to 1st vaccination will be used.

12 REFERENCES

1) Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat 1998;52:119-26.