Protocol HVTN 124

Protocol Version 1

A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A, B, C, A/E) / *gag* (C) DNA and gp120 (A, B, C, A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1-uninfected adult participants

1/25/2021
SAP version 1.6

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Protocol Statisticians: Shuying Sue Li, PhD

Approval Signature Page

HVTN 124

Statistical Analysis Plan

A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent *env* (A, B, C, A/E) / *gag* (C) DNA and gp120 (A, B, C, A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1-uninfected adult participants

I have read this Statistical Analysis Plan and approve its contents.

Sue Li

Shuying Sue Li, PhD
Lead Protocol Statistician
HVTN, FHCRC

1/25/2021

Date

If applicable, include other signatures:

Hua Zheng

Hua Zheng, MS
Statistical Research Associate
HVTN, FHCRC

1/25/2021

Date

SAP Modification History

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

Date: 21 Aug 2018
SAP version: Version *1.0*
Modifications: First draft concerning only the analysis of safety endpoints.

Date: 06 Nov. 2019
SAP version: Version *1.1*
Modifications: this version includes the analysis of Nab data.

Date: 02 Jan. 2020
SAP version: Version *1.2*
Modifications: this version includes the analysis of ICS data.

Date: 06 Feb. 2020
SAP version: Version *1.3*
Modifications: this version includes the analysis of ADCC data.
Date: 15 Sep. 2020
SAP version: Version *1.4*
Modifications: this version modified sec 10.3 Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) adding the comparisons of ADCC measured between the Lucifer and GTL assays.

Date: 19 Oct. 2020

SAP version: Version 1.5

Modifications: this version includes the analysis of BAMA data.

General approach in the immunogenicity analysis section was missed in earlier versions and added in this version.

Date: 25 Jan. 2021

SAP version: Version 1.6

Modifications: this version modified the tests used to compare biding antibody titers between treatment arms within each timepoint or between time-points within each treatment arm in BAMA data analysis section.

Table of Contents

1 OVERVIEW

The following describes the Statistical Analysis Plan (SAP) for the analysis of data from HVTN 124 for Safety Monitoring Board (SMB) reports, the Final Study Report (FSR) for Safety, Protocol Team (PT) reports for immunogenicity data, and the FSR for Immunogenicity. As detailed in SCHARP SOP-0013, Revision 5 (effective date: August 15, 2016), this SAP is required prior to the first analysis and must be approved by the lead protocol statistician. SMB reporting begins shortly after enrollment opens, and subsequent revisions are expected to describe analysis of immunogenicity data. The SAP will be reviewed prior to the first SMB report and before the final analysis with all major revisions of the plan archived.

2 PROTOCOL SUMMARY

Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of polyvalent env (A, B, C, A/E) / gag (C) DNA and gp120 (A,B,C,A/E) protein/GLA-SE HIV-1 vaccines (PDPHV-201401) as a prime-boost regimen or co-administered in repeated doses, in healthy, HIV-1-uninfected adult participants

Primary objectives

To evaluate the safety and tolerability of *env* (A, B, C, A/E) / *gag* (C)-expressing DNA plasmids and recombinant gp120 (A,B,C,A/E) protein/GLA-SE adjuvant, given individually, in combination, or as a prime-boost series in healthy HIV-1 uninfected adults

Study products, doses, and routes of administration

- DNA vaccine: *env* (A, B, C, A/E) / *gag* (C) DNA plasmids. A total dose of 2 mg of the 5 DNA plasmids (0.4 mg each) will be given as a 0.8 ml injection IM into the deltoid.
- Protein vaccine: Recombinant gp120 (A, B, C, A/E) proteins/GLA-SE adjuvant. GLA-SE is a stable oil-in-water emulsion (SE) containing glucopyranosyl Lipid A (GLA), a synthetic analog of monophosphoryl Lipid A (MPL®). A total dose of 400 microgram (mcg) of the 4 recombinant proteins (100 mcg each) and 5 mcg of GLA-SE will be given as a 1ml injection IM into the deltoid.
- Placebo for DNA vaccine: Sodium Chloride for Injection, USP 0.9% given as a 0.8 ml injection IM in to the deltoid.
- Placebo for protein vaccine: Sodium Chloride for Injection, USP 0.9% given as a 1 ml injection IM into the deltoid.

Schema

Part A	**Group**	**N**	**Month 0 (Day 0)**	**Month 2 (Day 56)**
	1	10	Protein/ GLA-SE	Protein/ GLA-SE
		2	Placebo	Placebo
		12		

Part B	**Group**	**N**	**Month 0 (Day 0)**	**Month 1 (Day 28)**	**Month 3 (Day 84)**	**Month 6 (Day 168)**	**Month 8 (Day 224)**

	2	21	DNA + Placebo	DNA + Placebo	DNA + Placebo	Protein/ GLA-SE + Placebo	Protein/ GLA-SE + Placebo
		3	Placebo + Placebo	Placebo + Placebo	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo
	3	21	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE	DNA+ Protein/ GLA-SE
		3	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo	Placebo+ Placebo
		48					
Total		60 (52/8)					

Notes

DNA/placebo and protein/GLA-SE and placebo study products will be given in separate arms, such that participants in Part B will receive 1 injection in each arm at each vaccination timepoint (unless medically contraindicated).

Enrollment will proceed in stages to optimize safety. Groups 2 and 3 will enroll simultaneously.

3 OBJECTIVES AND ENDPOINTS

3.1 Primary Objectives and Endpoints

Primary objectives:

- To evaluate the safety and tolerability of *env* (A, B, C, A/E) / *gag* (C)-expressing DNA plasmids and recombinant gp120 (A,B,C,A/E) protein/GLA-SE adjuvant, given individually, in combination, or as a prime-boost series in healthy HIV-1 uninfected adults

Primary endpoints:

- Frequency and severity of local injection site (including DTH) and systemic reactogenicity signs and symptoms, Adverse Events (AD) categorized by MedDRA system organ class, MedDRA preferred term, severity, and assessed relationship to study products; detailed description of all AEs meeting DAIDS criteria for expedited reporting by treatment arm throughout the trial
- The distribution of values of safety laboratory measures: white blood cells, neutrophils, lymphocytes, hemoglobin, alkaline phosphatase, platelets, ALT, AST, and creatinine at baseline and at follow-up visits post vaccination
- Number of participants with early discontinuation of vaccinations and reason for discontinuation

3.2 Secondary Objectives and Endpoints

Secondary objective 1:

- To evaluate the serum IgG binding antibody responses to Env proteins elicited by each vaccination regimen

Secondary endpoint 1:

- Magnitude and breadth of serum HIV-1 Env-specific IgG responses assessed by Binding Antibody Multiplex Assay two weeks after the last vaccination

Secondary objective 2:

- To evaluate the serum neutralizing antibody responses elicited by each vaccination regimen in Part B

Secondary endpoint 2:

- Serum neutralizing antibody responses against Tier 1A, Tier 1B, and selected Tier 2 viruses, assessed by TZM-bl assay, two weeks after the last vaccination in Part B

Secondary objective 3:

- To evaluate each vaccination regimen with specific antibody assays to measure correlates of risk identified in the RV144 trial

Secondary endpoint 3:

- Breadth of gp70-V1V2 IgG and gp120 IgA, assessed by Binding Antibody Multiplex Assay, and ADCC activities against HIV-1 subtypes A, B, C and A/E two weeks after the last vaccination in Part B

Secondary objective 4:

- To evaluate HIV-specific T-cell responses elicited by each vaccination regimen

Secondary endpoint 4:

- Frequency, magnitudes and quality of HIV-1 specific CD4+ and CD8+ T-cell responses as measured by ICS two weeks after the last vaccination

3.3 Exploratory Objectives and Endpoints

Exploratory objective 1:

- To evaluate serum IgG3 binding antibody responses to Env proteins 2 weeks after the third, fourth and fifth vaccinations and six months after the fifth vaccination in Part B

Exploratory objective 2:

- To evaluate HIV-1 Env-specific B-cell responses elicited after the last vaccination in Part B

Exploratory objective 3:

- To determine the frequency of circulating Tfh and plasmablasts in response to each vaccination regimen

Exploratory objective 4:

- To access the elicitation of mucosal immune responses in saliva, semen (men), cervicovaginal fluid (women), and rectal fluid

Exploratory objective 5:

- To access whether the diversity of gut microbiome correlates with vaccine responses using optionally provided stool specimens

Exploratory objective 6:

- To assess the effects of BMI on the safety and immunogenicity of the vaccine regimens

Exploratory objective 7:

- To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm

4 COHORT DEFINITION

Participants

60 healthy, HIV-1-uninfected volunteers aged 18 to 50 years; 52 vaccinees, 8 placebo recipients

Design

Multicenter, randomized, placebo-controlled, double-blinded trial

Duration per participant

- **Part A:** 8 months of scheduled clinic visits (main study) followed by a health contact 12 months after the last vaccination
- **Part B:** 14 months of scheduled clinic visits (main study) followed by a health contact 12 months after the last vaccination

Estimated total study duration

32 months (includes enrollment, planned safety holds, follow-up, and health contact)

5 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.

6 RANDOMIZATION

The randomization will be done in blocks to ensure balance across arms in Part A and in each Group in Part B. The randomization will be stratified by gender.

7 BLINDING

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm assignments (eg, vaccine or placebo). Study product assignments are accessible to those HVTN CRS pharmacists, DAIDS protocol pharmacists and contract monitors, and SDMC staff who are required to know this information in order to ensure proper trial conduct. Any discussion of study product assignment between pharmacy staff and any other HVTN CRS staff is prohibited. The HVTN SMB members also are unblinded to treatment assignment in order to conduct review of trial safety.

Part A will be unblinded independently of Part B. Unblinding need not await completion of health contacts, which are not considered part of the "main study".

When a participant leaves the trial prior to study completion, the participant will be told he or she must wait until the all participants in that part of the study (A or B) are unblinded to learn his or her treatment assignment.

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN 124 PSRT should be consulted before emergency unblinding occurs.

8 STATISTICAL ANALYSIS

All data from HVTN124 enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan (SAP) will address how to analyze the participant's safety data. Analyses are modified intent-to-treat in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

Analyses for primary endpoints will be performed using SAS and R. All other descriptive and inferential statistical analyses will be performed using SAS, StatXact or R statistical software.

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).

8.1 Analysis Variables

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

8.2 Baseline Comparability

Treatment arms will be compared for baseline participant characteristics using descriptive statistics.

8.3 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.

8.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition, to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms will be calculated. Wilcoxon rank sum tests will be used to test for differences in severity between arms.

8.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment arm the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing arms is not planned since interpretation of differences must rely heavily upon clinical judgment.

A listing of SAEs reported to the DAIDS Regulatory Support Center (RSC) Safety Office will provide details of the events including severity, relationship to study product, time between onset and last vaccination, and number of vaccinations received. A separate listing will do the same for AEs of special interest (AESI). AESI for this protocol include but are not limited to autoimmune disorders.

8.3.3 Local laboratory values

Box plots of local laboratory values will be generated for baseline values and for values measured during the course of the study by treatment arm and visit. Each box plot will show the first quartile, the median, and the third quartile. Outliers (values outside the box plot) will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

For each local laboratory measure, summary statistics will also be presented by treatment arm and timepoint, as well as changes from baseline for postenrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the Division of AIDS Table for Grading the Severity of Adults and Pediatric Adverse Events will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

8.3.4 Reasons for vaccination discontinuation and early study termination

The number and percentage of participants who discontinue vaccination and who terminate the study early will be tabulated by reason and treatment arm.

8.4 Immunogenicity Analysis

8.4.1 General approach

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 post infection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks 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% confidence interval estimates calculated using the score test method (52). Because of the small numbers of control participants in each group, no adjustment will be made to the vaccine arm estimates for the false positive rates in the control arms. Barnard or Fisher's exact tests, as specified in the SAP, will be used to compare the response rates of any 2 vaccine arms, with a significant difference declared if the 2-sided p-value is ≤ 0.05. In general Barnard's is preferred since under most circumstances it is more powerful than Fisher's (54).

In addition to response rate estimates for each timepoint, the probability of observing at least 1 positive response by a given timepoint and the probability of observing more than 1 positive response by a given timepoint will be estimated, with corresponding confidence intervals, for each vaccine arm using maximum likelihood-based methods (55).

For quantitative assay data (eg, IgG binding Ab response from BAMA or percentage of positive cells from the ICS assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be used for graphical display of all of the study arms. Typically the results will be shown for each vaccine arm and for the set of placebo arms pooled into one group.

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.

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, ICS). If treatment arm differences for these assays are best summarized by a mixture model, then either Lachenbruch's test statistic (56) or an alternative two-part test (57) (as defined in the SAP) will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal response distributions. Lachenbruch's test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

More sophisticated analyses employing repeated measures methodology (for example, linear mixed models or marginal mean models fit by 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 $p \leq 0.05$.

Based upon previous HVTN trials, missing 10-15% 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 standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing

data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data are missing for an endpoint (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 (58) will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE (59) methods, which are valid under MAR. All of the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

8.5 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 unblinded analyses by treatment assignment require careful consideration and should be made available on a need to know basis only.

8.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 124 PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

8.5.2 Immunogenicity

An unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after at least one of the primary immunogenicity endpoints of the same class (humoral, cell-mediated, innate or mucosal) or, if no primary endpoint of the same class, at least one of the primary immunogenicity endpoints reaches the aforementioned threshold. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs,

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.

9 SAFETY TABLES AND FIGURES

9.1 List of Tables

SMB reports and Safety FSRs include the following tables.

- Enrollment Report
- Demographics and Vaccination 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 Events Only
- Adverse Experiences by Preferred Term and Severity – By Decreasing Frequency – Includes Events of All Severities
- Adverse Experiences by Preferred Term and Severity – By Decreasing Frequency – Includes Related Events Only
- Adverse Experiences by Preferred Term and Relationship to Study Product – By Decreasing Frequency – Includes Events of Any Relationship
- Expedited Adverse Experiences (EAEs) Reported to the Regulatory Support Center (RSC)
- Pregnancy Listing
- AEs of Special Interest (AESI)
- Summary of Adherence to Pause Rules
- Summary of Immediate/Prompt PSRT AE Reviews

9.2 Participant Listings

The following listings of participant-level data are included in the SMB reports.

- Discontinuations
- Pregnancies
- Severe or Life-Threatening Local and Systemic Reactogenicities
- Moderate or Severe Erythema and Induration
- Expedited Adverse Experiences (EAEs)
- Adverse Experiences of Special Interest (AESIs)
- Severe, Life-Threatening, or Fatal Adverse Experiences
- Adverse Experiences with Relationship to Study Product (Grade 2 or higher)
- HIV Infection Results from Lab and Reported by Site
- Study Product Administration Errors

9.3 List of Graphs

- Maximum Local Reactogenicities
- Maximum Systemic Reactogenicities
- Boxplots for Alkaline Phosphatase, AST, ALT, Creatinine, WBC, Hemoglobin, Platelets, Lymphocyte Count, and Neutrophil Count.

10 IMMUNOGENICITY TABLES AND FIGURES, BY ASSAY

10.1 Binding Antibody Multiplex Assay (BAMA)

Serum HIV-1-specific IgA and IgG antibody binding responses will be measured at a 1:10 dilution for IgA and 1:50 dilution for IgG, respectively, against various antigens on a Bio-Plex instrument (Bio-Rad) using a standardized custom HIV-1 Luminex assay (Tomaras and Yates et al., J Virology 2008). The readout is background-subtracted mean fluorescence intensity (MFI), where background refers to a plate-specific background measure. Net MFI is MFI minus Blank, where 'Blank' refers to a sample-specific background measure.
BAMA testing will be performed on all participant serum samples collected from Part A, study Group 1 at visits 2 (Month 0) and 5 (Month 2.5, 2 weeks post the 2nd vaccination) and from Part B: study Groups 2 and 3 at visits 12 (Month 0) and 22 (Month 8.5, 2 weeks post the 5th last vaccination). Specimens from other timepoints as well as other HIV antigens and antibody isotypes may be assayed based on the results of the initial assay. If sufficient immunogenicity is observed for the primary analysis antigens, additional assays will be performed using the exploratory antigens.

The following table lists the antigens for the primary analysis.

Panel	Antigen	Clade	Isolate
Vaccine Recombinant Protein	gp120	A	IgG/IgA
	gp120	B	IgG/IgA
	gp120	C	IgG/IgA
	gp120	AE	IgG/IgA
gp120 Breadth Panel	51802_D11gp120.avi/293F	A1	IgG
	BORI_D11gp120.avi/293F	B	IgG
	TT31P.2792_D11gp120.avi/293F	B	IgG
	B.6249_D11gp120/293F	B	IgG
	A244 D11gp120_avi	CRF01-AE	IgG
	254088_D11gp120.avi/293F	CRF01-AE	IgG
	CNE20_D11gp120.avi/293F	CRF07-BC	IgG
	BJOX002_D11gp120.avi293F	CRF07-BC	IgG
gp70 V1V2 Breadth Panel	gp70-191084_B7 V1V2	A	IgG
	gp70_B.CaseA_V1_V2	B	IgG
	gp70-RHPA4259.7V1V2	B	IgG
	gp70-62357.14 V1V2	B	IgG
	gp70-700010058 V1V2	B	IgG
	gp70-TT31P.2F10.2792 V1V2	B	IgG
	gp70-BF1266_431a_V1V2	C	IgG
	gp70-7060101641 V1V2	C	IgG
	gp70-96ZM61.01 V1V2	C	IgG
	gp70-0014282.42 V1V2	C	IgG
	gp70-CAP210.2.00.E 8 V1V2	C	IgG

	gp70-TV.21 V1V2	C	IgG
	gp70-Ce1086_B2 V1V2	C	IgG
	gp70-CM244.ec1 V1V2	CRF01_AE	IgG
	gp70-C2101.c01_V1V2	CRF01_AE	IgG
	gp70-BJOX002000.03.2 V1V2	CRF07-BC	IgG
Consensus gp140/gp120	Con S gp140 CFI	M	IgG/IgA
	Con 6 gp120/B	M	IgG/IgA
	1086_DCgp120.avi/293F	C	IgG/IgA
	gp41		IgG/IgA
	P24	B	IgG/IgA
	A1.con.env03 140 CF	A	IgG/IgA
	00MSA 4076 gp140	A	IgG/IgA

The following table lists the antigens for exploratory analysis if sufficient immunogenicity responses are observed (for Part B only).

Panel	Antigen	Clade	Isolate
Extended Clade C gp120 Panel	1394C9_G1.D11gp120.avi	C	IgG
	1428_D11gp120.avi/293F	C	IgG
	1641A7_ D11gp120.avi/293F	C	IgG
	CAP210_ D11gp120.avi/293F	C	IgG
	CAP45_D11gp120.avi/293F	C	IgG
	Ce0042_ D11gp120.avi/293F	C	IgG
	CH505TF_ D7gp120.avi/293F	C	IgG
	Du156_ D11gp120.avi/293F	C	IgG
	96XM651. D11gp120.avi	C	IgG
	TV1c8_D11gp120.avi/293F	C	IgG
Extended Clade C V1V2 Panel	gp70-CAP45.2.00.G3 V1V2	C	IgG
	gp70-1294C9G1_V1V2	C	IgG
	gp70-1012.11.TC21.3257 V1V2	C	IgG
	gp70-1051.12.C22 V1V2	C	IgG
	gp70-Ce1176 V1V2	C	IgG
	gp70-Ce704010042_2ES V1V2	C	IgG
	gp70-ConcC V1V2	C	IgG
	gp70-DU156.12.V1V2	C	IgG

Several criteria are used to determine if data from an assay are acceptable and can be statistically analyzed. The blood draw date must be within the allowable visit window as determined by the protocol. Second, if the blank bead negative control exceeds 5,000 MFI, the sample will be repeated. If the repeat value exceeds 5,000 MFI, the sample will be excluded from analysis due to high background.
Samples from post-enrollment visits are declared to have a positive response if they meet three conditions:

1. The net MFI values ≥ antigen-specific cutoff (based on the 95th percentile of the baseline net MFI values that are greater than/equal to 100 but less than 6,500). ,If the cutoff is greater than 5,000 net MFI, then the antigen will be excluded from analysis.
2. The net MFI values are greater than 3 times the baseline net MFI values.
3. The MFI values are greater than 3 times the baseline MFI values.

For each of gp120 breadth panel and gp70 V1V2 breadth panel, magnitude-breadth (MB) and AUC-MB will be calculated for each participant by isotype and post-vaccination timepoint.

Contingent upon these results, samples may be titrated to calculate antibody titers (EC50/AUC), in which case geometric mean titers will be compared.

The treatment arm of receiving 2 protein/GLA-SE vaccines is labelled as T1, the treatment arm of receiving 3 DNA prime vaccinations and 2 DNA+Protein/GLA-SE boost vaccinations in Group 2 is labelled as T2, and the treatment arm of receiving 5 DNA+Protein/GLA-SE vaccinations in Group 3 is labelled as T3. The placebo arm in Part A is labelled as P_A. The placebos in Groups 2 and 3 in Part B are pooled and the treatment arm is labelled as P.

The binding antibody response rates will be compared between treatment arms within each timepoint using Barnard exact test. The response magnitudes, AUC-MB will be compared between treatment arms within each timepoint using Wilcoxon rank sum test. The comparisons of response rates between timepoints within each of treatment arms will be performed using McNemar's test if binding antibody responses will be measured at multiple timepoints. Similarly, the comparisons of binding antibody response magnitudes, AUC-MB between timepoints within each of treatment arms will be compared using Wilcoxon signed rank test.

If antibody titers (EC50/AUC) data are available, two sample t-test will be used to compare log-titers (EC50/AUC) between two treatment arms within each timepoint and paired t-test will be used to compare log-titers between timepoints within each of treatment arms.

The following tables and figures are generated separately for Part A and Part B unless are specified otherwise.

10.1.1 List of Tables

- The binding antibody positive response rates by isotype, antigen, visit and treatment arm
- Summary statistics for magnitudes among the positive responders by isotype, antigen, visit and treatment arm
- Summary statistics for AUC-MB by isotype, breadth panel, visit, and treatment arm
- The comparisons of response rates between T2 and T3 by isotype, antigen, and visit
- The comparisons of response magnitudes between T2 and T3 by isotype, antigen, and visit
- Lists of the positive responders and the magnitudes for those participants
- The comparisons of AUC-MB between T2 and T3 by isotype, breadth panel, and visit
- Geometric mean and 95% CI of titers (EC50/AUC) by isotype, antigen, visit, and treatment arm. 95% CI will be calculated based on a normal approximation for log-transformed titers.
- The comparisons of titers (EC50/AUC) between T2 and T3 by isotype, antigen and visit.
- The comparisons of AUC-MB of titers (EC50/AUC) between T2 and T3 by isotype, breadth panel, and visit

10.1.2 List of Figures

- Boxplots of binding antibody magnitudes by isotype, antigen, visit and treatment arm
- Spaghetti plots of binding antibody magnitudes over time by isotype, antigen, visit and treatment arm
- Magnitude-breadth (MB) curves of binding antibody magnitudes and breadth for a panel of antigens (gp120 breadth panel and gp70 V1V2 breadth panel) by isotype, visit and treatment arm
- Boxplots of binding antibody titers (EC50/AUC) by isotype, antigen, visit and treatment arm
- MB curves of binding antibody titers and breadth for a panel of antigens (gp120 breadth panel and gp70 V1V2 breadth panel) by isotype, visit and treatment arm

10.2 Neutralizing antibody (NAb)

Neutralizing antibodies (NAb) against tier 1A, tier 1B and tier 2 strains of HIV-1 will be measured in TZM-bl cells as a function of reductions in Tat-induced luciferase (Luc) reporter gene expression after a single round of infection with molecularly cloned, Env-pseudotyped viruses. TZM-bl (also called JC57BL-13) is a Hela cell clone that was engineered to express CD4 and CCR5 and to contain integrated reporter genes for firefly luciferase and E. coli β-galactosidase under control of an HIV-1 long terminal repeat (LTR). The cells are highly permissive to infection by most strains of HIV, including primary HIV-1 isolates and molecularly cloned Env-pseudotyped viruses. DEAE-dextran is used in the medium during neutralization assays to enhance infectivity. Expression of the reporter genes is induced in trans by viral Tat protein soon after infection. Luciferase activity is quantified by luminescence and is directly proportional to the number of infectious virus particles present in the initial inoculum. The assay is performed in 96-well culture plates for high throughput capacity. Use of a clonal cell population provides enhanced precision and uniformity. The assay has been formally optimized and validated for single-round infection with either uncloned or molecularly cloned Env-pseudotyped viruses produced by transfection in 293T cells.

Assays will be performed using cryopreserved serum sample starting at a specific dilution determined by the lab PI. Titers will be defined as the serum dilution that reduced relative luminescence units (RLU) by 50% and 80% relative to the RLU in the virus control wells after subtraction of background RLU in control wells (ID50 and ID80). Neutralization ID50 and ID80 titers will be measured against MW965.26 (tier 1A vaccine strain), the tier 2 vaccine strains JR-FL and 92UG037.1, and the heterologous tier 1B strains Bx08.16, Q23.17, 6525.3, ZM197M-PB7, and 1056-10.TA11.1826. The global panel and/or clade-specific panels may be used to assess tier 2 neutralization on a selected subset of participants.

A response is considered positive if the neutralization titer is above a pre-specified cutoff. Magnitude of response will be measured by the natural log of the ID50 and ID80 titer. An aggregate measure of response will be calculated as the area-under-the-magnitude-breadth curve (AUC-MB), which is equivalent to the mean log titer across all viruses in tier 1B and tier 2. If a titer is left censored, half the left censor limit will be used as the titer value.

Neutralization titers will be measured on the primary immunogenicity samples at visit 22 (at week 34, 2 weeks post the 5th vaccination) from all 48 Part B participants. Specimens from other time points may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity time point. Those time points may include week 0 (visit 12 - baseline), week 14 (visit 17 – 2 weeks post 3rd vaccination), week 26

(visit 18 – 2 weeks post 4th vaccination), and/or week 61 (visit 24 – 6 months post 5th vaccination).

Positive response rates will be compared between treatment groups within each time point using Barnard exact test. Titers and AUC-MBs will be compared between treatment groups within each time point using Wilcoxon rank sum test. Positive response rates, titers, and AUC-MBs between time points within treatment group will be also compared using McNemar's tests and Wilcoxon signed rank test, respectively, if neutralizations will be measured at multiple time points.

10.2.1 List of Tables

- The NAb positive response rates by cell type, isolate, visit and group.
- Lists of the positive responders and the titer for those participants.
- Summary statistics for NAb titers among the positive responders.
- The comparisons of response rates between groups by cell type, isolate, and visit.
- The comparisons of titers between groups by cell type, isolate, and visit.
- The comparison of AUC-MB between groups by visit

10.2.2 List of Figures

- Boxplots of neutralizing antibody titers by cell type, isolate, visit, and group.
- Spaghetti plots of neutralizing antibody titer over time by cell type, isolate, and group
- Reverse CDFs of neutralizing antibody titers by cell type, visit and group
- Magnitude-breadth (MB) plots of titer and breadth for a panel of viruses by cell type, visit, and group

10.3 Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

The GranToxiLux ADCC (GTL-ADCC) assay will be used to test reactivity against HIV-env protein coated cells (% Granzyme readout). Cells coated with either gp120 protein A, gp120 protein B, gp120 protein AE, or Clade C TV1 gp120 individually will be tested in a 96 well plate for the Grantoxilux (GTL assay). Participant sera in addition to control sera will be incubated with these HIV proteins and ADCC detected through the use of a Granzyme B substrate (GTL).

Cells infected with HIV-1 IMC TV1, WITO and CM235, individually, will be tested in a 96 well plate for the Luciferase ADCC assay. Participants sera in addition to controls were incubated with the IMC-infected cells and ADCC detected through the use of Viviren luminescence.

The following evaluation criteria will be employed for this assay:

i) GTL: The data from the assay is quantitative. The frequency of positive responses (% responders) peak ADCC, and area under the curve (AUC) will be tabulated to assess differences in ADCC response between the treatment groups.

ii) Luciferase: The data from the assay is semiquantitative. The frequency of positive responses (% responders) peak of specific killing and AUC will be tabulated and compared between the treatment groups.

Positivity criteria are as follows:

i) GTL: peak % response ≥ 8%

ii) Luciferase: peak % response ≥10%, after background subtraction of baseline response and positivity at one of the first two dilutions.

The sera samples at v12 (day 0) and v22 (day 238) from the 48 participants in Part B will be assayed for ADCC responses. Based on the responses at v22, assays may be performed on these participants samples at v17 (day 98), v19 (day 182), and v24 (day 425).

Positive response rates will be compared between treatment groups within each time point using Barnard exact test. Peak of ADCC and AUC ADCC will be compared between treatment groups within each time point using Wilcoxon rank sum test. Positive response rates, peak ADCC, and AUC ADCC between time points within treatment group will be also compared using McNemar's tests and Wilcoxon signed rank test, respectively, if neutralizations will be measured at multiple time points.
Magnitude-Breadth (MB) of peak ADCC and of AUC ADCC will be calculated and displayed for each of two ADCC assays. AUC-MB of peak and AUC ADCC will be compared between the treatment groups within each time point using Wilcoxon rank sum test and between time points within each treatment group using Wilcoxon signed rank test.
In addition, we will evaluate the correlation of AUC-MB of peak ADCC at each time point measured by the Luciferase and the GTL assays. We will also evaluated the correlation of AUC-MB of AUC ADCC at each time point measured by the Luciferase and the GTL assays.

10.3.1 List of Tables

- Response rate of peak net percent Granzyme B activity by treatment group, protein, and visit
- Summary statistics (i.e., min, mean, median, max) of peak net percent Granzyme B activity among all participants by treatment group, protein, and visit
- Summary statistics (i.e., min, mean, median, max) of peak net percent Granzyme B activity among positive responders by treatment group, protein, and visit
- Summary statistics (i.e., min, mean, median, max) of AUC peak net percent Granzyme B activity among all participants by treatment group, protein, and visit
- Summary statistics (i.e., min, mean, median, max) of AUC peak net percent Granzyme B activity among positive responders by treatment group, protein, and visit
- Listing of positive peak net percent Granzyme B
- Response rate comparison of peak net percent Granzyme B activity at v22 (possibly v17, v19, v24) between T2 and T3 by protein
- Response magnitude comparison of peak net percent Granzyme B activity at v22 (possibly v17, v19, v24) between T2 and T3 by protein
- Comparison of AUC peak net percent Granzyme B activity at v22 (possibly v17, v19, v24) between T2 and T3 by protein
- Response rate of peak of specific killing by treatment group, protein, and visit
- Summary statistics (i.e., min, mean, median, max) of peak of specific killing among all participants by treatment group, protein, and visit
- Summary statistics (i.e., min, mean, median, max) of peak of specific killing among positive responders by treatment group, protein, and visit

- Summary statistics (i.e., min, mean, median, max) of AUC peak of specific killing among all participants by treatment group, protein, and visit
- Summary statistics (i.e., min, mean, median, max) of AUC peak of specific killing among positive responders by treatment group, protein, and visit
- Listing of positive peak of specific killing
- Response rate comparison of peak of specific killing at v22 (possibly v17, v19, v24) between T2 and T3 by protein
- Response magnitude comparison of peak of specific killing at v22 (possibly v17, v19, v24) between T2 and T3 by protein
- Comparison of AUC of specific killing at v22 (possibly v17, v19, v24) between T2 and T3 by protein
- Comparison of AUC-MB of peak ADCC at v22 (possibly v17, v19, v24) between T2 and T3
- Comparison of AUC-MB of AUC ADCC at v22 (possibly v17, v19, v24) between T2 and T3
-

10.3.2 List of Graphs

- Boxplots of Peak Percent Granzyme B Activity by protein and treatment arm
- Boxplots of AUC Percent Granzyme B Activity among positive responders according to the peak activity criterion by protein and treatment group
- Boxplots of Peak of specific killing by protein and treatment group
- Boxplots of AUC specific killing among positive responders according to the peak activity criterion by protein and treatment group
- MB plot of Peak ADCC by treatment group, visit, and assay type
- MB plot of AUC ADCC by treatment group, visit, and assay type
- Scatterplot and regression line of AUC-MB of peak ADCC measured by the Luciferase vs AUC-MB of peak ADCC measured by the GTL for each time point (excluding placebos and color-coding for the treatment group T2 and T3)
- Scatterplot and regression line of AUC-MB of AUC ADCC measured by the Luciferase vs AUC-MB of AUC ADCC measured by the GTL (excluding placebos and color-coding for the treatment group T2 and T3)

10.4 Intracellular Cytokine Staining (ICS)

Immunogenicity will be assessed by ICS using PTE_G Peptide Pools (Env-1-PTEG-SEQ, Env-2-PTEG-SEQ, Gag-1-PTEG-SEQ, and Gag-2-PTEG-SEQ) covering Gag and Env. The 17-color ICS staining panel [CD3 BUV737, CD4 BUV395, CD8 BV650, CD14 BV510, CD56 BV570, CXCR5 PE-Dazzle594, PD-1 (CD279) BV605, ICOS (CD278) BV711, CD45RA APC H7 , CCR7 BV786, IFN-γ V450, TNF-α FITC, IL-2 PE, IL-4 PerCP-Cy5.5, IL-17a PE-Cy7, CD154 APC, Granzyme B Alx700] will be used. ICS will be assayed on the samples from all 48 Part B

participants at primary immunogenicity time point (visit 22, two weeks post the 5th vaccination). ICS may also be assayed at secondary immunogenicity time points (e.g. 2 weeks post the 3rd vaccination and 6 months post the last vaccination). Response of CD4+/CD8+ T cells measured by ICS for IFN-γ and/or IL-2 to any HIV PTE peptide pools (Env-1-PTEG-SEQ, Env-2-PTEG-SEQ, Gag-1-PTEG-SEQ, and Gag-2-PTEG-SEQ) covering all genes or individual genes (especially Env and Gag) are primary immunogenicity endpoints. To be specific, the response of CD4+/CD8+ T cells to Env is defined as a sum response to the peptides, Env-1-PTEG-SEQ and Env-2-PTEG-SEQ, the response to Gag is defined as a sum response to Gag-1-PTEG-SEQ and Gag-2-PTEG-SEQ; and the response to any protein is defined as the sum of the response to Env and the response to Gag. Fisher exact test criteria will be used for positivity calls before assay on all samples are complete.

The COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets) statistical framework (Lin, et al. 2015) may also be used to perform joint modelling of multiple T-cell subsets of different cytokine combinations. The functionality score (FS) and the polyfunctionality score (PFS) may be used to summarize the multi-parameter ICS responses.

10.4.1 List of Tables

- Response rate table by T-cell subset, HIV protein (Any, Env, Gag), visit, treatment arm for cells expressing either IL-2 and/or IFN-γ using Fisher's exact test criteria.
- Listing of positive responders for cells expressing either IL-2 and/or IFN-γ based on the criteria used for the response rate table(s).
- Summary statistics (i.e., min, mean, median, max) among responders for T-cell subset, HIV protein, visit, and treatment arm.
- Response rate comparison between T2 and T3 by T-cell subset, HIV protein, and visit using Barnard exact test.
- Response magnitude comparison between T2 and T3 by T-cell subset, HIV protein, and visit using Wilcoxon rank sum test.
- Summary statistics (i.e., min, mean, median, max) of FS and PFS for T-cell subset, HIV protein, visit, and treatment arm.
- FS and PFS comparison between T2 and T3 by T-cell subset, HIV protein and visit using Wilcoxon rank sum test.

10.4.2 List of Graphs

- Bar-chart of response rate by T-cell subset, HIV protein, visit, and treatment arm using Fisher exact test.
- Boxplots of background-adjusted IFN-γ and/or IL-2 response magnitude by T-cell subset, HIV protein, visit, and treatment arm using Fisher exact test
- Boxplots of background-adjusted marginal cytokine response magnitude by T-cell subset, HIV protein, visit, and treatment arm using Fisher exact test
- Boxplots of FS/PFS by T-cell subset, HIV protein, visit, and treatment arm.

11 REFERENCES

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2. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. Nat Biotechnol. 2015;33(6):610-6.