Janssen Research & Development

Statistical Analysis Plan

A Multi-center, Randomized, Double-blind, Placebo-controlled Phase 3 Efficacy Study of a Heterologous Prime/Boost Vaccine Regimen of Ad26.Mos4.HIV and Adjuvanted Clade C gp140 and Mosaic gp140 to Prevent HIV-1 Infection Among Cis-gender Men and Transgender Individual who Have Sex with Cis-gender Men and/or Transgender Individuals

Protocol VAC89220HPX3002 / HVTN 706; Phase 3

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Compliance: The study described in this report was performed according to the principles of Good Clinical Practice (GCP).

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AMENDMENT HISTORY

Version 1.0	13 August 2019	Original version
Version 1.1	26 March 2021	Removal of interim analysis throughout document, References to CTP replaced by text from CTP (Section 1), Section 5.4.2 (Analysis Methods) removed and reference to 5.3.2 (Analysis Methods) added, added option of TAF monitoring (Section 3.5 Monitoring for the use of PrEP), References added
Version 1.2	31 August 2023	This version was created after unblinding of the study due to DSMB stopping rules for non-efficacy. Unblinding at study level took place on 18Jan2023. Adverse events of special interest added in secondary safety endpoint (section 1.1). Quality Tolerance Limits added (Section 3.8). The mITT-3 analysis set has been added (Section 2.2). A VISP analysis set has been added (Section 2.2.3.2.). The censoring for the efficacy analysis is specified (Section 5.3.2). Other exploratory assays (e.g. BAMA and ICS) might be considered (Section 6.3.1.1).
Version 1.3	4 March 2024	References added to reference list.

ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
CI	Confidence Interval
CRF	Case Report Form
CSR	Clinical Study Report
DSMB	Data Safety Monitoring Board
DPS	Data Presentation Specifications
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked Immunospot
GMC	Geometric Mean Antibody Concentration
FAS	Full Analysis Set
FDA	Food And Drug Administration
FTC	Emtricitabine
ICH	International Conference on Harmonization
ITT	Intent-To-Treat
ICS	Intracellular Cytokine Staining
IU/ml	International Units per Milliliter
IVRS	Interactive Voice Response System
LLOQ	Lower Limit of Quantification
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
NA	Not Applicable
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic(s)
PP	Per Protocol
QTL	Quality Tolerance Limit
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
TAF	Tenofovir Alafenamide
TDF	Tenofovir Disoproxil Fumarate
VISP	Vaccine-Induced Seropositivity

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1. INTRODUCTION

This Statistical Analysis Plan (SAP) is restricted to the definitions, algorithms, data handling conventions that will be used and the analyses that are planned per protocol for the final analysis of the study. Mock tables and programming details of the statistical output to be generated are presented in the Data Presentation Specifications (DPS) document. The DSMB analyses and monitoring rules are described in a separate DSMB SAP (EDMS-ERI-187581907). The immunogenicity correlates analysis will be described in a separate Correlates SAP.

1.1. Objectives and Endpoints

Objectives		Endpoints	
Primary			
1	To evaluate the VE of a heterologous vaccine regimen utilizing Ad26.Mos4.HIV and aluminum phosphate-adjuvanted Clade C gp140 and Mosaic gp140 for the prevention of HIV-1 infection in HIV-1 seronegative cis-gender men and transgender individuals having sex with cis-gender men and/or transgender individuals.	1	Confirmed HIV-1 infections diagnosed between Month 7 and Month x (with 24≤x≤30) visits in the PP population.
Secondary			
1	To evaluate the safety and reactogenicity of a heterologous vaccine regimen utilizing Ad26.Mos4.HIV and aluminum phosphate-adjuvanted Clade C gp140 and Mosaic gp140 for the prevention of HIV-1 infection in HIV-1 seronegative cis-gender men and transgender individuals having sex with cis-gender men and/or transgender individuals.	1	• Reactogenicity: Solicited administration site and systemic adverse events for 7 days after each vaccination • Unsolicited adverse events for 28 days after each vaccination • Adverse events of special interest (AESIs) for 6 months after the last vaccination • MAAEs for the entire duration of the study • Serious adverse events for the entire duration of the study • Discontinuations from the study or vaccination due to adverse events
2	To evaluate VE at other timepoints and in other analysis populations.	2	Confirmed HIV-1 infections over different time intervals (eg, VE[0-x months], VE[13-x months]) and in different populations (eg, mITT, mITT-2, mITT3, FIS).
3	To evaluate VE by and adjusting for potential (baseline) covariates.	4	Potential confounders include but are not limited to: demographic characteristics, baseline Ad26 seropositivity status and titer, sexual risk behavior, and pre-exposure prophylaxis (PrEP) use.
Exploratory			
1	To evaluate whether VE differs by phenotypic characteristics of HIV, such as neutralization sensitivity, and whether there is evidence of vaccine-induced immune pressure on the viral phenotype.	1	Confirmed HIV-1 infection diagnosed after Day 1 through Month 30 and inferred transmitted viral isolate(s) phenotype(s) from HIV-1–infected mITT participants at the earliest available post-infection timepoint, and possible subsequent visits.

2	To evaluate whether VE differs by genotypic characteristics of HIV, such as signature site mutations, and whether there is evidence of vaccine-induced immune pressure on the viral sequences.	2	Confirmed HIV-1 infection diagnosed after Day 1 through Month 30 and inferred transmitted viral sequence(s) genotype(s) from HIV-1–infected mITT participants at the earliest available post-infection timepoint, and possible subsequent visits, using sieve analysis methods.
3	To evaluate vaccine effects on virologic and immunologic outcomes among participants that become HIV-1–infected during the study, accounting for ARV use.	3	HIV-1 viral load and $CD4^+$ count over a 6-month period after diagnosis. The frequency and magnitude of HIV-1 cellular (participants at sites with access to sponsor approved PBMC processing facilities) and humoral immune responses in participants that become HIV-1–infected during the study.
4	To evaluate immune correlate(s) of risk of HIV-1 infection and/or correlates of VE.	4	Magnitude and/or frequency of immune responses to vaccination in HIV-1 infected vaccine recipients (cases) relative to a subset of HIV-1 uninfected vaccine recipients (controls) and placebo cases and controls, as relevant. The association of immune response(s) that are identified as being associated with VE in Study VAC89220HPX2008/HVTN 705 (further referred to as HPX2008/HVTN 705), in HIV-1 infected vaccine recipients (cases) relative to a subset of HIV-1 uninfected vaccine recipients (controls) and placebo cases and controls, as relevant.
5	To evaluate the occurrence of VISP following vaccination with heterologous vaccine regimen utilizing Ad26.Mos4.HIV and aluminum phosphate-adjuvanted Clade C gp140 and Mosaic gp140.	5	The frequency of confirmed VISP, determined utilizing a pre-specified diagnostic algorithm to distinguish HIV- 1 infection from VISP (refer to the Study-Specific Procedures Binder for more information) at different time points following vaccination.
6	To describe PROs.	6	Social impact and vaccine regimen acceptance, sexual activity, absenteeism and PrEP use collected through questionnaires completed by study participants.
7	To describe HCRU over the study period.	7	Collection of MAAEs, level of absenteeism and follow-up of HIV infections throughout the study.
8	To evaluate the immune responses elicited by the vaccine regimen.	8	The frequency and magnitude of HIV-1-specific cellular and humoral immune responses.

1.2. Trial Design

This is a multi-center, randomized, parallel-group, placebo-controlled, double-blind, Phase 3 study to demonstrate efficacy of a heterologous prophylactic HIV-1 vaccine regimen consisting of Ad26.Mos4.HIV and a combination of aluminum phosphate-adjuvanted Clade C gp140 and Mosaic gp140. Safety, reactogenicity and immunogenicity will also be evaluated. The study population will include healthy adults considered to be at increased risk of acquiring HIV-1 infection. A target of 3,800 participants, consisting of HIV-1-uninfected cis-gender men and transgender individuals having sex with cis-gender men and/or

transgender individuals, aged ≥18 to ≤60 years, will be randomized in a 1:1 ratio to the study vaccine or placebo. Randomization will be stratified by site. Sample size re-assessment may be performed based on blinded study data or external study data (eg, Phase 2b Study HPX2008/HVTN 705). The target number of HIV-1 infections may be re-assessed based on external study data (eg, Phase 2b Study HPX2008/HVTN 705) only.

Participants will receive intramuscular (IM) doses of study vaccine or placebo at four time points as indicated in the table below.

Table: Vaccination Schedule

Group	N	Month 0	Month 3	Month 6	Month 12
1	1,900	Ad26.Mos4.HIV	Ad26.Mos4.HIV	Ad26.Mos4.HIV + Clade C gp140, Mosaic gp140, adjuvanted	Ad26.Mos4.HIV + Clade C gp140, Mosaic gp140, adjuvanted
2	1,900	Placebo	Placebo	Placebo + Placebo	Placebo + Placebo

Total dose of Ad26.Mos4.HIV is $5x10^{10}$ viral particles (vp)/0.5 mL injection.
Clade C gp140, Mosaic gp140, adjuvanted: adjuvanted protein formulation with a dosage strength of 80 mcg Clade C protein, 75 mcg Mosaic protein and 425 mcg aluminum (as aluminum phosphate adjuvant). Note: previously the dose of Clade C gp140 and/or Mosaic gp140 was reported as mcg of glycoprotein: 125 mcg Clade C gp140 and 125 mcg Mosaic gp140 glycoprotein correspond with 80 mcg and 75 mcg of protein, respectively.

The study comprises of a screening period of 45 days, a 12-month vaccination period and a follow-up period of at least 18 months after the fourth vaccination (until Month 30) in participants who remain HIV-1 negative or up to 6 months after diagnosis of HIV-1 infection in participants who become HIV-1 infected. Participants who completed their Month 30 visit will be followed for HIV infection, MAAEs and serious adverse events until the end of the study (ie, when the last participant completed the Month 30 visit or discontinued earlier). At the end of the study, participants may be offered the possibility to enter a long-term follow-up phase or program (to collect, amongst others, additional durability data).

After vaccination, participants will remain under observation at the study site for at least 30 minutes for presence of any acute reactions. In addition, participants will record solicited signs and symptoms in a diary for 7 days post-vaccination. Unsolicited adverse events will be recorded for all participants until 28 days after each preceding vaccination. Serious adverse events, MAAEs, and adverse events leading to discontinuation will be collected for all participants until the end of the study.

An HIV test will be performed approximately every 3 months. Upon discretion of the investigator, additional HIV tests may be performed during unscheduled visits; participants should refrain from performing any HIV testing outside of the study protocol. Blood samples will be collected at specific visits for determination of humoral immune responses (all participants) and for determination of cellular immune responses (participants at sites with access to sponsor approved PBMC processing facilities). At specific clinic visits, participants will complete Participant Reported Outcomes (PRO) questionnaires, including a social impact questionnaire, a sexual activity questionnaire, a questionnaire on the use of PrEP and questions with regard to the level of absenteeism and the vaccine regimen acceptance.

If a participant becomes HIV-1 infected during the study (confirmed HIV test), the participant will remain in the study but no further scheduled vaccinations will be administered. The participant will be followed-up until approximately 6 months after the diagnosis (see the Time and Events Schedule for Participants Who

Become HIV-1 Infected in the CTP) and will be referred to a local clinic for medical treatment and follow-up on their HIV-1 infection as soon as possible after the diagnosis of the infection.

The sponsor and its partners are committed to ensuring that all study participants receive access to the highest standard of prevention, which may include, but is not limited to, HIV testing, risk reduction counseling, provision of male condoms and lubricants, access to management of sexually transmitted infections (STIs), and appropriate referrals for PrEP and post-exposure prophylaxis (PEP) according to national and/or local guidelines. Note: potential participants choosing to use PrEP will not be eligible for participation in the study as, due to the high effectiveness of PrEP, these individuals are not considered to be at increased risk of HIV acquisition. However, once enrolled in the study and having received their first vaccination, a participant who changes his/her mind regarding PrEP use is permitted to take PrEP according to the site PrEP plan, and will continue to receive further vaccinations. In case of PrEP use during the study, safety monitoring for PrEP will be the responsibility of the prescribing physician. HIV testing should be performed within the study to avoid unblinding due to VISP elicited by the vaccine. Participants should refrain from HIV testing outside of the study protocol. As part of the study protocol, blood samples will be collected for ARV detection in dried blood spot and stored at pre-specified sample collection days for assessment of quantitative concentrations of tenofovir diphosphate. Additional ARV detection can be done on stored blood samples if required as per the Study Specific Procedures Binder. The use of PrEP and adherence to PrEP (if applicable) will be monitored by means of a questionnaire which will be completed by all participants approximately every 3 months.

The NIAID HIV vaccine Data and Safety Monitoring Board (further referred to as DSMB) will serve as an independent DSMB for this study and will monitor data on an ongoing basis to ensure the continuing safety of the participants and will formally monitor the efficacy endpoint.

1.3. Statistical Hypothesis for Trial Objectives

The primary analysis of VE will evaluate the number of HIV-1 infections in the vaccine group compared to the number of HIV-1 infections in the placebo group between Month 7 and Month x (with $24 \leq x \leq 30$) in the PP population. A description of how x will be determined can be found in Section 1.4 below. The null hypothesis H0:VE(7-x months) ≤20% will be tested against the alternative hypothesis H1:VE(7-x months) >20%. Vaccine efficacy is defined as 1-cumulative incidence ratio (vaccine versus placebo) between Month 7 and Month x after first vaccination, and the cumulative incidence in each group will be estimated using the Nelson-Aalen estimator for the cumulative hazard function. Wald confidence intervals will first be constructed for the log-cumulative incidence ratio, and then, mapped into a confidence interval for VE. If the lower bound of the 95% CI for VE(7-x months) is >20% (equivalently, the 1-sided p-value for testing H0: VE[7-x months] ≤20% is below 0.025), H0 will be rejected in favor of H1.

1.4. Sample Size Justification

This study is designed to test the primary hypothesis of VE in the PP population:

H0: VE(7-x months) ≤20% versus H1: VE(7-x months) >20%, with $24 \leq x \leq 30$ (See CTP Section 11.5.1.1).

If the lower bound of the 95% CI for VE(7-x months) is >20% at the primary analysis, the corresponding H0 will be rejected.

The sample size calculations are based on the power of a 1-sided 0.025-level Wald test for comparing cumulative incidences of HIV-1 infection by the Month x visit ($24 \leq x \leq 30$) between randomized groups, in the presence of the sequential monitoring of VE. Power is computed based on simulating 10,000 efficacy trials using the R package seqDesign (Juraska, 2015; Gilbert et al., 2011) under the following assumptions:

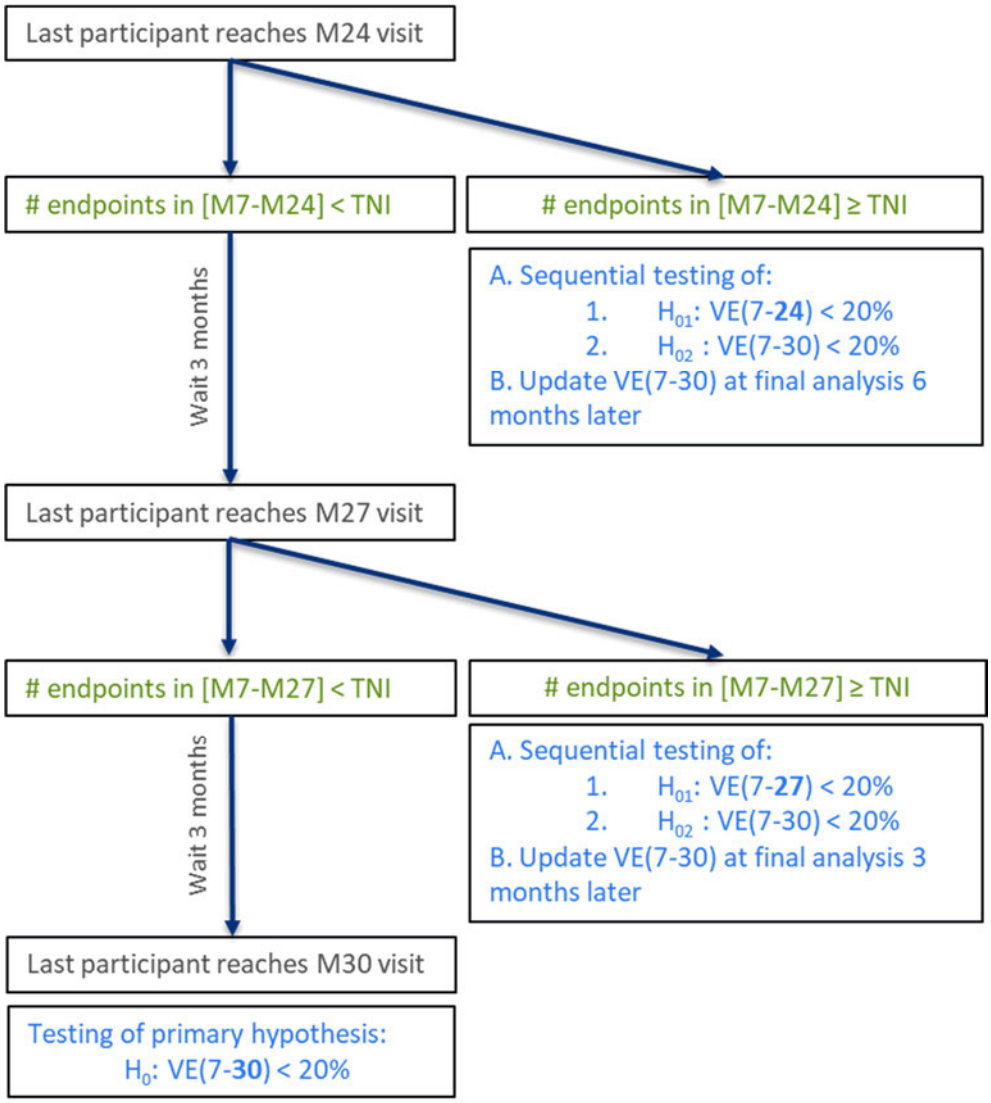
- 10% annual dropout incidence in both groups;
- 5% of participants with at least one missed vaccination (excluded from PP population);
- VE=20% in the first 7 months after first vaccination;
- VE=65% from Month 7 onwards;
- 12-month uniform accrual with halved accrual during the first 3 months;
- Visits approximately every 3 months for HIV-1 diagnostic tests;
- 2% annual HIV-1 incidence in the placebo group;

Under these assumptions, a study of 3,800 participants (randomized 1,900:1,900 to vaccine:placebo) is expected to yield a total of 78 HIV-1 infections in the PP population between the Month 7 and Month 30 visits, considered to be the Target Number of Infections needed to achieve approximately 90% power for rejecting the primary H0, under an alternative VE of 65% and with a one-sided error rate of 2.5%.

The timing of unblinding and conducting the primary analysis and the definition of the primary efficacy endpoint will be determined by when the TNI is reached within a given follow-up period x ($24 \leq x \leq 30$) for each participant. In practice, when the last participant has reached his/her Month 24 visit (or discontinued earlier), it will be assessed whether the TNI has been obtained within the period of Month 7-Month 24. If at that time the number of HIV-1 infections between Month 7 and Month 24 is greater than or equal to the TNI, the primary analysis may be conducted using VE(7-24 months) as the primary efficacy endpoint. Only if this primary H0 is rejected, VE(7-30 months) will be tested as secondary hypothesis, using a fixed sequence hypothesis testing strategy, and using all available durability data between Month 24 and Month 30. If the number of infections between Month 7 and Month 24 is less than the TNI, the study will continue for 3 months until the last participant has reached their Month 27 visit. The same procedure is repeated as described above, using all available HIV-1 infections between Month 7 and Month 27 and with VE(7-27 months) as the primary efficacy endpoint. If the number of infections between Month 7 and Month 27 is less than the TNI, the study will continue for 3 months until the last participant has reached their final study visit at Month 30. At that point, the primary analysis will be conducted regardless of whether the TNI has been reached, using VE(7-30 months) as only efficacy endpoint.

The statistical strategy is schematically presented in Figure 1.

Figure 1: Primary Efficacy Testing – Schematic Overview



H0: null hypothesis; M: month; TNI: Target Number of Infections; VE: vaccine efficacy

Note that investigators and participants will remain blinded to their treatment assignment, at least until the last participant has reached the Month 30 visit or discontinued earlier.

Both the study's sample size as well as the TNI may be adjusted during the study, but prior to unblinding, to ensure sufficient power at the time of the primary analysis. Whereas adjustment of the sample size may be based on internal (but sponsor-blinded) study data (through monitoring of the pooled HIV-1 incidence) as well as on external study data (eg, Phase 2b HPX2008/HVTN 705), any adjustment of the TNI will solely be driven by data external to this study (eg, Phase 2b HPX2008/HVTN 705).

To cope with the dynamic environment of the development, approval and uptake of other prophylactic methods, such as PrEP (which would result in lower than expected annual incidence rates and thus lower study power), an estimated annual incidence rate in the control group of 2% was used to size this study.

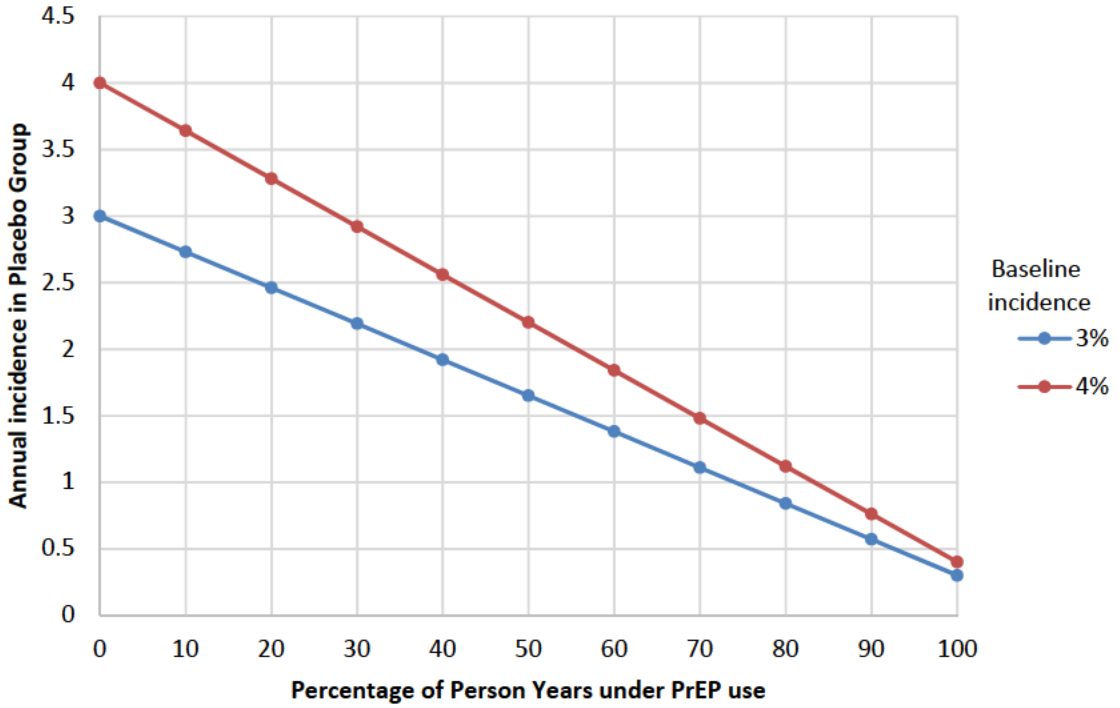
Under the study assumptions described above, the study will also have approximately 80% power for the secondary efficacy endpoint VE(0-x months), assessing VE in the mITT population and accounting for all HIV-1 infections starting from Month 0 (Day 1).

Rationale for the HIV-1 Incidence Assumptions

This study will recruit a large sample of highest-risk MSM and transgender individuals who have sex with men from many regions. In 2011, 62% of estimated new HIV diagnoses in the United States were attributed to male-to-male sexual contact; 39% of these MSM were black or African American (Cooley et al., 2014). Lifetime risk was 1 in 68 for males and 1 in 253 for females. Lifetime risk for men was 1 in 22 for blacks, 1 in 51 for Hispanic/Latinos, and 1 in 140 for whites. By risk group, the highest risk was among MSM (1 in 6) and the lowest was among male heterosexuals (1 in 524). Most of the states with the highest lifetime risk were in the South (Hess et al., 2017). Recent data from clinical studies show placebo incidence rates in high risk MSM of 6.6% in the Ipergay study (France, Canada; Molina et al., 2015), and 3.9% in the iPrEX study for high risk MSM (USA, Brazil, Peru, Ecuador, South Africa and Thailand, Grant et al., 2014). HVTN 505 yielded annual incidence rates of 2.3% in circumcised MSM and TG (USA; Hammer et al., 2013). To help identify the study participants, pre-trial assessments and data will be collected regarding incidence in potential sites.

The assumption of 2% annual HIV-1 incidence in the placebo group accommodates background PrEP use by assuming incidence is decreased by the person-years at risk during which PrEP is used, the efficacy of PrEP during PrEP use, and the adherence to PrEP use. Figure 2 shows the impact of assumed PrEP use and adherence on annual HIV-1 incidence in the placebo group when the initial incidence (in the absence of PrEP) is 3% or 4% and the efficacy of PrEP is assumed to be 90%.

Figure 2: Impact of Assumed PrEP use and Adherence on Annual HIV-1 Incidence in the Placebo Group



HIV-1: human immunodeficiency virus type 1; PrEP: pre-exposure prophylaxis

The assumption of 3% annual incidence in the absence of PrEP, about 35% of person-years at risk during PrEP use, and 90% PrEP efficacy during use results in the assumed 2% annual placebo incidence rate.

A total sample size of 3,800 participants, as based on the efficacy endpoints, will also provide enough power for assessing safety of the vaccine regimen. The probability of observing at least one adverse event occurring at a rate of 1/100 is >99.9% with approximately 1,900 vaccinees. The probability of observing at least 1 adverse event occurring at a rate of 1/1,000 is 85% with approximately 1,900 participants. If no event is observed for a specific (serious) adverse event, then the Bayesian posterior probability that the adverse event rate is below 1/1,000 equals 95% for 1,900 participants and would provide us with 95% confidence that the true incidence is no more than 0.16%.

1.5. Randomization and Blinding

Procedures for Randomization and Stratification

Participants will be randomly assigned to 1 of 2 treatment groups in a 1:1 ratio based on a computer-generated randomization schedule prepared before the study by or under the supervision of the sponsor. The randomization will be balanced by using permuted blocks and will be stratified by study site. Based on this randomization code, the study vaccine/placebo will be packaged and labeled for each participant.

Central randomization will be implemented in this study. The interactive web response system (IWRS) will assign a unique treatment code, which will dictate the treatment assignment and matching study vaccine/placebo vial for the participant. The requestor must use his or her own user identification and personal identification number when contacting the IWRS and will then give the relevant participant details to uniquely identify the participant.

Blinding

The study participants, study-site personnel (except for those with primary responsibility for study vaccine preparation and dispensing), and investigator will be blinded to study vaccine allocation until the end of the study (see Section 10.1). The sponsor and its partners will be blinded to study vaccine allocation until the primary analysis, when the last participant has reached their Month x visit (with $24 \leq x \leq 30$).

The pharmacist with primary responsibility for vaccine preparation (see CTP Section 14.3) will not be blinded to the study vaccine. In order to preserve blinding, he/she will place an overlay on the syringes. Administration of study vaccine to the participants will be performed by a blinded qualified healthcare provider from the study site.

The investigator will not be provided with randomization codes during the study. The codes will be maintained within the IWRS, which has the functionality to allow the investigator to break the blind for an individual participant.

Data that may potentially unblind the treatment assignment will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized. This can include making special provisions, such as segregating the data in question from view by the investigators, clinical team, or others as appropriate until the time of database lock and unblinding. Participants who discontinue vaccination early will be reminded that no HIV testing should be performed outside the study protocol to avoid unblinding.

Under normal circumstances, the blind should not be broken by the investigator until the end of the study and the electronic data capture (eDC) database is finalized unless it is essential for the timely management of the participant. In this case, the investigator may determine the identity of the treatment by contacting the IWRS. While the responsibility to break the code in emergency situations resides solely with the investigator, it is recommended that the investigator contacts the HPX3002/HVTN 706 safety review team if possible to discuss the particular situation, before breaking the blind. Telephone contact with the sponsor or its partners will be available 24 hours per day, 7 days per week. In the event the blind is broken, the sponsor and its partners must be informed as soon as possible. The date and reason for the unblinding must be documented by the IWRS, in the appropriate section of the case report form (CRF), and in the source document. The documentation received from the IWRS indicating the code break must be retained with the participant's source documents in a secure manner.

Participants who have had their treatment assignment unblinded due to safety reasons should continue to return for safety and immunogenicity evaluations and efficacy (see CTP Section 10.2), but will be withdrawn from further study vaccine administration.

2. GENERAL ANALYSIS DEFINITIONS

2.1. Study phases

A baseline (or reference) value will be defined as the value of the last available assessment prior to the first vaccination on Day 1.

The safety analysis will present all results by phase.

Study day or relative day is defined as follows:

Study Day = visit date - date of Day 1 + 1; if visit date ≥ date of Day 1 (date of first vaccination).

Study Day = visit date – date of Day 1; if visit date < date of Day 1 (date of first vaccination).

2.1.1. Phase definitions

The phases and periods in the study will be constructed as follows:

Table 1: Phase and Period Definitions

Phase	Phase number	Period	Period #	Interval	
				From	To
Screening	1			00:00 of the date of signing the informed consent form[a]	One minute prior to Dose 1 on Day 1
Regimen	2	Post-Dose 1	1	Date and time of first vaccination (Day 1)	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 on 28 days after the first vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to post-dose 2
Follow-Up 1	3			One minute after Post-Dose 1 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 at the Date of last visit d) One minute prior to post dose 2
Regimen	2	Post-Dose 2	2	Date and time of second vaccination (Day 85)	Minimum of: a) 23:59 at the date of last contact (for early discontinuation)

Phase	Phase number	Period	Period #	Interval	
				From	**To**
					b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 on Day 28 after the second vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to post-dose 3
Follow-Up 2	4			1 minute after end of Post-Dose 2 period	Minimum of: a) 23:59 at the Date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 at the Date of last visit d) One minute prior to post-dose 3
Regimen	2	Post-Dose 3	3	Minimum of Date and Time of the two Dose 3 Injections (Day 169)	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 on Day 28 after the second vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to post-dose 4
Follow-Up 3	5			1 minute after end of Post-Dose 3 period	Minimum of: a) 23:59 at the Date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 at the Date of last visit d) One minute prior to post-dose 4
Regimen	2	Post-Dose 4	4	Minimum of Date and Time of the two Dose 4 Injections (Day 337)	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 on Day 28 after the second vaccination (23:59 of day of vaccination + 28 days)

Phase	Phase number	Period	Period #	Interval	
				From	To
Follow-Up 4	6			1 minute after end of Post-Dose 4 period	Minimum of: a) 23:59 at the Date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of an interim analysis[c] c) 23:59 at the Date of last visit

NOTE:
[a] The start time of screening phase is 00:00. In case an earlier date is available (e.g. for lab or vital signs then use the very first date in order to include all data)
[b] In case a dose is not administered, the observations end up in the previous Follow-Up phase
[c] For interim analyses a cut-off date will be identified: all data included in the analysis will go up to the cut-off date, any data referring to a later time point will not be included; ongoing events will be included and duration will be up to the cut-off date.

The periods/phases will be used primarily for safety. The post-dose periods (and the regimen phase) are considered active periods/phase, and the screening and follow-up phases are considered non-active phases for the evaluation of safety parameters. Efficacy endpoints will be calculated as described in Section 5.3.

For descriptive statistics over time, assessments (regardless of the investigated parameter) will be allocated to an analysis visit based on the visit number as captured in the database.

For participants who missed one or more doses but continued their planned visit schedule, the measurements after a planned but not administered dose will not be included in graphs and tables showing descriptive statistics over time. Those measurements will be shown in listings, but it will be indicated that these are not used in the analysis. Moreover, the vaccinations done after a missed vaccination will not be shifted to the previous vaccination but will be considered "as planned": if for example the 2nd dose is missed, the 3rd dose vaccination will still be reported as post-dose 3.

Unless stated otherwise all analysis outputs will be shown by vaccine regimen (Vaccine vs Placebo).

2.1.2. Pooling Algorithm for Analysis Centers

All safety and efficacy analyses will be pooled over all centers. Some analyses will be shown by region and/or country.

2.2. Analysis Sets

In the analyses the as-treated principle will be applied.

2.2.1. Full Analysis (FAS) Set

The full analysis set will include all randomized participants who receive at least one vaccine administration. This will be the primary population for the safety analysis.

2.2.2. Efficacy Analysis Set

2.2.2.1. Per Protocol Population (PP)

The per protocol efficacy population will include all participants in the FAS population who have a negative HIV test 4 weeks post 3rd vaccination visit (i.e. at the Month 7 Visit) and who received all planned vaccinations at the first three vaccination visits within the respective visit windows. Participants with major protocol deviations linked to incorrect product administration will be excluded from the PP population. This will be the primary population for the efficacy analysis.

If participants miss their Month 7 Visit, and have a negative test in the first subsequent visit where an HIV-1 test is performed, these participants will remain part of the PP for the efficacy endpoints. If the HIV-1 test is positive at the first subsequent visit, participants are excluded from the PP for the efficacy endpoints. Handling rules of these participants for the immunogenicity correlates analysis will be described in a separate Correlates SAP.

2.2.2.2. Modified Intent-to-Treat (mITT) population

The modified intent to treat efficacy population will include participants in the FAS who are HIV-1 uninfected at the date of the first vaccination. This will be a secondary analysis population for the efficacy analysis.

2.2.2.3. Modified Intent-to-Treat-2 (mITT-2) Population

The mITT-2 will include participants in the FAS who have a negative HIV test 4 weeks post 3rd vaccination visit (ie, at the Month 7 Visit). This will be a secondary analysis population for the efficacy analysis.

If participants miss their Month 7 Visit, and have a negative test in the first subsequent visit where an HIV-1 test is performed, these participants will remain part of the mITT-2 for the efficacy endpoints. If the HIV-1 test is positive at the first subsequent visit, participants are excluded from the mITT-2 for the efficacy endpoints.

2.2.2.4. Modified Intent-to-Treat-3 (mITT-3) Population

The mITT-3 will include participants in the FAS who have a negative HIV test 4 weeks post 3rd vaccination visit (ie, at the Month 7 Visit) and who received all planned vaccinations at the first three vaccination visits regardless of the fact if the vaccinations were within the visit windows. Participants with major protocol deviations linked to incorrect product administration will be excluded from the mITT-3 population. This will be a secondary analysis population for the efficacy analysis.

If participants miss their Month 7 Visit, and have a negative test in the first subsequent visit where an HIV-1 test is performed, these participants will remain part of the mITT-3 for the efficacy endpoints. If the HIV-1 test is positive at the first subsequent visit, participants are excluded from the mITT-3 for the efficacy endpoints.

2.2.2.5. Full Immunization Set (FIS)

The FIS will include participants in the FAS who are HIV-1 uninfected 4 weeks after the 4th vaccination visit (ie, at the Month 13 Visit) and who receive all planned vaccinations within the respective visit windows. This will be a secondary analysis population for the efficacy analysis.

If participants miss their Month 13 Visit, and have a negative test in the first participants visit where an HIV-1 test is performed, these participants will remain part of the FIS for the efficacy endpoints. If the HIV-1 test is positive at the first subsequent visit, participants are excluded from the FIS for the efficacy endpoints. Handling rules of these participants for the immunogenicity correlates analysis will be described in a separate Correlates SAP.

2.2.3. Immunogenicity Analysis Set

2.2.3.1. At risk Immunogenicity Cohort (IC-at risk)

The IC-at risk will include participants in the FAS who are selected for measurement of immune response endpoints at the primary immunogenicity timepoints and who are HIV-1 uninfected 4 weeks after the 3rd vaccination visit (ie, at the Month 7 Visit).

2.2.3.2. Others Analysis Sets

The analysis sets described above can also be used for immunogenicity analysis purposes. The full immunogenicity analyses will be described in a separate Correlates SAP.

For the VISP analyses, a VISP analysis set is defined. Participants are in the VISP set if they received at least one active vaccination and did not become infected during the whole course of the trial.

2.2.4. Definition of Subgroups

The following subgroups maybe be investigated for the most relevant efficacy outputs:

- Region (Europe, Latin America, USA)
- Age (18-20, 21-34, 35-44, ≥45)
- Country
- P(r)EP use
- Baseline Ad26 status (when available)

3. SUBJECT INFORMATION

Participants information will be shown for the Full Analysis (FAS) set.

3.1. Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.
Table 2 below presents a list of the demographic variables that will be summarized by vaccine regimen and overall for the FAS. Demographics will also be summarized by region and/or country using the FAS.

Table 2: Demographic Variables

Continuous Variables:	**Summary Type**
Age ([years])	Descriptive statistics (N, mean, standard deviation [SD], median and range [minimum and maximum]).
Weight (kg)	
Height (cm)	
Body Mass Index (BMI) (kg/m^2)	
Categorical Variables	
Age ([18-20 years, 21-34 years, 35-44 years, and ≥ 45 years])	Frequency distribution with the number and percentage of participants in each category.
Sex at birth	
Gender at screening	
Country	
Race[a]	
Ethnicity	
BMI ([underweight <18.5 kg/m2, normal 18.5-<25 kg/m2, overweight 25-<30 kg/m2, obese ≥30 kg/m2])	
Baseline Risk Behavior, as identified by the attributes at Day 1	
Baseline STI testing results	
Other relevant factors	

[a]If multiple race categories are indicated, the Race is recorded as 'Multiple'

3.2. Disposition Information

The number and percentage of participants screened, participants in the FAS, participants vaccinated and not randomized, participants randomized and not vaccinated and discontinued participants (study discontinuation and vaccination discontinuation) with the reason of discontinuation will be tabulated per vaccine group and overall.

Also the number of participants and percentage per phase will be tabulated.

For the screen failures a tabulation will be made stating the frequency of all I/E criteria.

3.3. Treatment Compliance

Adherence to vaccinations will be tabulated by vaccine regimen and overall, both for individual vaccinations as well as cumulatively over all vaccinations (received all vaccinations, missed 1 vaccination, missed 2 vaccinations, missed more than 2 vaccinations).

3.4. Social Impact Data

Data from the Social Impact Questionnaire will be tabulated by vaccine regimen with emphasis on social impact events.

3.5. Sexual Activity Questionnaire

Data from the sexual activity questionnaire will be summarized by vaccine regimen.

3.6. Use of PrEP

Data from the questionnaire on the use of PrEP will be summarized by vaccine regimen.

3.7. Health Economics Aspects (HCRU)

Health Care Resource Utilization will be summarized based on data collected through MAAEs and follow-up of HIV infections, and complemented by description of absenteeism.

3.8. Protocol Deviations and Quality Tolerance Limits (QTL)

Major protocol deviations will be summarized by vaccine regimen.

In general, the following list of major protocol deviations categories may have the potential to impact participants' rights, safety or well-being, or the integrity and/or result of the clinical study:

- Developed withdrawal criteria but not withdrawn
- Entered but did not satisfy criteria
- Received a disallowed concomitant treatment
- Received wrong vaccination or incorrect dose

Participants with major protocol deviations will be identified prior to database lock and the participants with major protocol deviations will be summarized by category. Any Protocol deviations related to the COVID-19 pandemic will be labelled as COVID-19 related and will be shown in tables and listing.

Quality Tolerance Limit parameters and thresholds are defined and will be monitored in this study. QTL parameters will be summarized. More details are described in the Integrated Analytical Risk-Based Monitoring (iARBM) Plan.

3.9. Concomitant Medications

3.9.1. P(r)EP

3.9.1.1. Dried Blood Spots

The use of oral FTC/TDF as P(r)EP will be tabulated by vaccine regimen.

The prevalence of oral FTC/TDF use will be reported both as any *detectable use* and as *effective use*. More specifically, estimated percentages of persons with detectable FTC/TDF use and effective FTC/TDF use will be reported (See Table 2).

Inferred effective use will be measured as follow. The lower quartile of simulated TFV-DP levels in DBS at 4 doses per week is 719 fmol/punch (Castillo-Mancilla *et al*., 2013) and 700 fmol/punch will be used as the cut-off to define effective PrEP use based on the lower quartile cited above.

An example of PrEP use report statistics is shown in Table 3.

Table 3: Detectable and Effective PrEP/PEP Use, Based on Assayed Dried Blood Spots (DBS)

	Regimen
Time	
Number of specimens collected (assayed)	xxx (xxx)
Proportion with detectible TFV-DP level*100% (95% CI)	xx.x% (xx.x%, xx.x%)
Mean TFV-DP level (95% CI)	xx.x% (xx.x%, xx.x%)
Min-Max	xx.x%- xx.x%
Proportion >700 fmol/punch TFV-DP level*100% (95% CI)	xx.x% (xx.x%, xx.x%)
Mean TFV-DP level (95% CI)	xx.x% (xx.x%, xx.x%)
Min-Max	xx.x%-xx.x%

In addition to TDF, the DSMB might also start monitoring the use of tenofovir alafenamide (TAF) or any other antiretrovirals used as PrEP, when deemed relevant for the trial. TAF is the prodrug of TDF, and requires lower doses to attain the same exposure. Similar outputs as for TDF will be produced for the TAF assessment.

3.9.1.2. Participant Reported P(r)EP Use

Additional to the DBS data on PrEP use, participant-reported data on PrEP use (through the PrEP Questionnaire and reporting in the concomitant medication) will be summarized descriptively. These will provide information on reported PrEP or PEP use, dosing, average pill count per week, and the mode of obtaining PrEP.

3.9.2. Other Concomitant Medications

The analysis of concomitant therapies will be done using the WHO drug coded terms.

There will be special attention to any systemic use of analgesics/antipyretics, started during 8 days following each vaccination (00:00 of day of vaccination + 7 days). Following CMCLASCD (ATC/DD codes) will be used for this: N02A (OPIOIDS) and N02B (OTHER ANALGESICS AND ANTIPYRETICS), M01A (ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS, NON-STEROIDS) and M01B (ANTIINFLAMMATORY/ANTIRHEUMATIC AGENTS IN COMBINATION).[1]The classes will be added in a footnote in all related tables and listings.

Based on their start and stop date, concomitant therapies will be reported in each applicable phase. If a concomitant therapy record misses components of its start and/or stop dates (day and/or month and/or year):
-In case of partial start or stop dates, the concomitant therapy records will be allocated to periods using the available partial information, without imputations. If, for example, only month and year are available, these will be compared to the month and the year of the periods, and the concomitant therapy record will be allocated to the period(s) where these date parts match. This rule may lead to assignment to multiple periods.
-In case of a completely missing start date, the concomitant therapy will be considered as having started before the trial.
-In case of a completely missing end date, the concomitant therapy will be considered as ongoing at the end of the trial.

4. SAFETY

The safety and tolerability endpoints are:

- Unsolicited AEs during the 28 days following each vaccination
- Solicited local and systemic AEs (reactogenicity), collected daily from the day of vaccination for 7 days post-vaccination (day of vaccination and the subsequent 7 days). The investigator assessment of the solicited AEs is used in the safety analysis.
- Serious Adverse Events (SAEs) and Medically Attended Adverse Events (MAAEs) during the entire course of the study.

The safety and tolerability analysis will be performed on the FAS set. Specifically, the analysis for solicited adverse events will be done on those participants in the FAS set for whom reactogenicity assessments are available in the database (either via on-site assessments or via the diary pages of the CRF).

A 'post any dose' period (= regimen phase in section 2.1.1) will be added, that summarizes the safety after all administered doses of the vaccine. Each participant is counted only once. Whenever a participant has the same event after more than one dose, it will be counted only once (and in case of showing attributes, the worst corresponding attribute will be shown) in the post-any dose period. The denominator will be the number of participants that received the considered dose.

4.1. Analysis of Adverse Events

Number and percentage of participants with at least one particular AE (unsolicited/solicited) will be tabulated. Unsolicited AEs will be summarized by System Organ Class and Preferred Term. Solicited AEs will be summarized by class (administration site, systemic) and preferred term.

For solicited AEs following tables will be provided: summary, by worst severity grade, grade 3 or above, related (systemic only), time to onset (in days) and duration (in days) for most frequent events and body temperature. Note: Duration is defined as number of days from the start of the event until resolution of the event. The time to first onset is defined as (date of first onset – reference date + 1). The reference date is the start date of the vaccination period.

For unsolicited AEs following tables will be provided: summary table (including SAE, fatal outcome, AESI and discontinuation), all events, most frequent, grade 3 or above, permanent stop of vaccine, related, SAE and AESI.

Listings and/or participant narratives will be provided as appropriate, for those participants who die, discontinue study vaccinations due to an AE, or experience a severe or serious AE.

4.2. Phase allocation of Adverse Events

Solicited events are always allocated to the respective Post Dose period.

Step 1: Allocation of events to the periods:

Adverse events in the SDTM database are allocated to periods based on their start date/time. If the start date/time of an event falls between (or on) the start and stop date/time of a period, the AE is attributed to that period (treatment-emergent principle).

- In case of partial start or stop dates (i.e. time and/or day and/or month and/or year missing), the events are allocated to the periods using the available partial information on start and end date; no imputation will be done. If, for instance, the AE start date only month and year are available, these data are compared to the month and year information of the periods. This rule may lead to multiplication of the event as a consequence of its assignment to multiple periods.

- In case of a completely missing end date, the date is imputed by the cut-off date of the analysis for participants still ongoing in the study, and by the end date of the last period for participants who discontinued or completed the trial. In case of a completely missing start date, the event is allocated to the first active treatment phase (post dose 1 period), except if the end date of the AE falls before the start of the first active treatment phase (post dose 1 period).

Step 2: Combination of events:

Overlapping/consecutive events are defined as events of the same participant with the same preferred term which have at least 1 day overlap or for which the start date of an event is 1 day after the end date of the preceding event. Overlapping/consecutive events may be combined into one AE or not, according to the following rules:

1) If overlapping/consecutive events start in one of the following periods - Screening or post dose extension (i.e. non-active periods) - followed by an AE in - post-dose period (active period) - they are allocated to their respective periods and are considered as separate events.

2) In case overlapping/consecutive events start within a single period, they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.

3) In case overlapping/consecutive events start in both an active period followed by a nonactive period, they are allocated to the active period only and are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, treatment period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.

4) In case an active period is followed by another active period, and the overlapping/consecutive events start in both periods, they are allocated to their respective period and are considered as separate AEs. The same rule applies for 2 non-active periods.

Remarks:

1. Events can only be combined into one and the same AE if their start and stop dates are known.

2. In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.

3. Time is not considered when determining overlap of events.

4.3. Missing Data

Missing data will not be imputed. Participants who do not report an event will be considered as participants without an event. An AE with a missing severity or relationship will be considered as an AE reported, but will be considered as not reported for the severity or relationship. For example, an AE with missing severity will be considered as an AE reported for the analysis of any grade, but will be considered as not reported for the analysis of grade 3 or above.

5. EFFICACY

5.1. General principles

The primary efficacy analysis will be performed when all participants have reached the Month x (with 24≤x≤30) visit or discontinued earlier and the TNI has been reached in the study. Refer to Figure 1 in Section 1.4 for more information on the timing and strategy of statistical testing.

5.2. Analysis Specifications

5.2.1. Level of Significance

Unless otherwise stated, all confidence intervals presented will be two-sided at the $(1-2\alpha)\times 100\%$ level with $\alpha = 0.025$. The multiple testing strategy is applied by means of fixed sequence testing and is illustrated in Figure 1.

5.2.2. Data Handling Rules

The time between enrollment and the date of HIV-1 infection diagnosis is evaluated for all participants. Enrollment is defined as the date of the first vaccination. Participants becoming HIV infected or dropping out before their Month 7 visit or not having received the first 3 vaccinations within the per protocol specified time window will be excluded from the primary PP analysis. The failure times of participants without HIV-1 infection will be right-censored at the date of the last HIV-1 negative test or at the month 30 visit, whichever occurs earlier. In all interim analyses of VE (eg. for DSMB), participants who have not experienced the primary endpoint will be right-censored at the time of their last HIV-1 negative test.

Dropouts will be right-censored at the time of their last HIV-1 negative test.

5.3. Primary Efficacy Endpoint(s)

5.3.1. Definition

The primary efficacy endpoint consists of confirmed HIV-1 infections diagnosed between Month 7 and Month x (with $24 \leq x \leq 30$) visits in the per-protocol (PP) population.
We define the primary vaccine efficacy parameter, i.e. VE(7–x), as one minus the probability of the primary efficacy endpoint between month 7 and month x for the vaccine group divided by the probability of the primary efficacy endpoint between month 7 and month x for the placebo group times 100 percent. The Month 7 to Month x vaccine and placebo incidences in the primary VE definition are estimated using cumulative incidences of HIV-1 infection between Months 7 and x obtained from the Nelson-Aalen cumulative hazard function estimator, where:

$$\widehat{VE} = \left(1 - \frac{\tilde{H}_{Vx}(t)}{\tilde{H}_{Pbo}(t)}\right) \times 100\%,$$

with $\widetilde{H(t)}$. the Nelson Aalen estimator of the cumulative incidence in the Vaccine or Placebo arm at time t (see e.g. Klein and Moeschlberger, 1997, p. 83-96). Let

$$\tilde{H}(t) = \sum_{t_i \leq t} \frac{d_i}{Y_i}, \qquad \text{with} \qquad \tilde{\sigma}_H^2(t) = \sum_{t_i \leq t} \frac{d_i}{Y_i^2},$$

where Y_i is the number of individuals at risk at time t_i and d_i is the number of HIV1-infections at time t_i .

5.3.2. Analysis Methods

VE(7-x) is the target parameter for the primary analysis of overall VE. The primary analysis tests

the null hypothesis H_0: $VE(7-x) \leq 20\%$ versus

the alternative hypothesis H_1: $VE(7-x) > 20\%$

using a 1-sided 0.025 α-level Wald test of the difference of log cumulative hazard functions at month x for the vaccine group and the control group. See further details in Appendix 1. When the primary analysis is performed at month x (between 24 and 30), the failure times of participants with last HIV-1 negative test at or after the month x visit are right-censored at the right edge of the month x visit window. For participants with last HIV-1 negative test prior to the month x visit and without diagnosis of the HIV-1 infection primary endpoint at or before the month x visit, their failure times are right-censored at the date of the last HIV-negative test.

In addition, to assess potential time-effects of vaccine efficacy, the Nelson-Aalen method will be used to plot the estimated cumulative incidence rates over time for the vaccine and placebo groups. This method will be used to estimate cumulative vaccine efficacy over time, defined as above in 5.3.1, with the method of Parzen, Wei and Ying applied to construct pointwise and simultaneous $(1-2\alpha) \times 100\%$ CIs. In addition, the fluctuations of the instantaneous VE will be visualized graphically (Gilbert, Wei, Kosorok, & Clemens, 2002).

Cox proportional hazards model will also be used for estimating VE(7-x months), measured by one minus the hazard ratio (vaccine versus placebo) and testing whether the VE(7-x months) is > or ≤20%. For the Cox proportional hazards model, the same censoring will be applied as for the cumulative incidence model used for the primary analysis.

5.4. Secondary Vaccine Efficacy Analyses

5.4.1. mITT-2

As a key secondary objective, the VE beyond Month 7 will be evaluated in all participants, regardless of whether they received the first three vaccinations according to the protocol-specified schedule (mITT-2). The secondary endpoint consists of confirmed HIV-1 infections diagnosed between Month 7 and Month x (with $24 \leq x \leq 30$) and vaccine efficacy will be defined as one minus the probability of the secondary efficacy endpoint between month 7 and month x for the vaccine group divided by the probability of the secondary efficacy endpoint between month 7 and month x for the placebo group times 100 percent. The Month 7 to Month x vaccine and placebo incidences in the secondary VE definition are estimated using cumulative incidences of HIV-1 infection between Months 7 and x obtained from the Nelson-Aalen cumulative hazard function estimator, where:

$$\widehat{VE} = \left(1 - \frac{\tilde{H}_{Vx}(t)}{\tilde{H}_{Pbo}(t)}\right) \times 100\%,$$

with $\widehat{H(t)}$. the Nelson Aalen estimator of the cumulative incidence in the Vaccine or Placebo arm at time t (see e.g. Klein and Moeschlberger, 1997, p. 83-96). Let

$$\tilde{H}(t) = \sum_{t_i \le t} \frac{d_i}{Y_i}, \qquad \text{with} \qquad \tilde{\sigma}_H^2(t) = \sum_{t_i \le t} \frac{d_i}{Y_i^2},$$

where Y_i is the number of individuals at risk at time t_i and d_i is the number of HIV1-infections at time t_i .

For the analysis of the secondary endpoint in the mITT-2 population, all analysis methods described in Section 5.3.2 will be applied.

5.4.2. Other vaccine efficacy analyses

5.4.2.1. Time intervals

Other secondary objectives will evaluate the VE over different time intervals at least VE[0-x months] and VE[13-x months]), but will not anymore be assessed by 6-monthly time intervals.

5.4.2.2. Analysis Sets

VE in different analysis sets (eg, mITT, mITT3, FIS), using similar methods as described in Section 5.4 will also be evaluated.

5.4.2.3. Subgroup and Covariate-adjusted Analyses

Subgroup and covariate-adjusted analyses will be performed. These subgroups/covariates may include PrEP use (based on the different PrEP use data sources), baseline Ad26 seropositivity status and titers, presence of risk factors for HIV infection, and other baseline demographic factors (age strata/region/country).

If there is substantial PrEP use detected over the course of the study (eg prevalence at any time above 20%), then a Cox model with PrEP detectability as a time-varying covariate may be used to assess different vaccine efficacy by time-varying subgroup and for each subgroup separately. More specifically, the primary analysis may be repeated where only mITT infection endpoints with no evidence of PrEP use at the time of HIV-1 diagnosis and at the time of the earliest evidence of infection will be included in the analysis. FTC/TDF concentrations (see Section 3.6.1) will be used to determine eligibility for this analysis. A participant is eligible if their concentration is below the lower limit of detection as defined above at the diagnosis visit and at the visit with earliest evidence of HIV-1 infection (if different from the diagnosis visit). Since the ARVs are only detectable in plasma for roughly 14 days after a single dose (Patterson et al. 2011) and some participants may have become infected before the 14-day window, with this approach we are not assured that all those included in the analysis were not using prophylactic ARVs at the time of infection. Therefore an additional analysis may be conducted that addresses this issue by also excluding participants

from the HIV-1 acquisition analysis if they self-report ARV use in the last 30 days at either the diagnosis visit or the last visit prior to diagnosis.

5.4.2.4. Bayesian analysis HPX2008

An exploratory analysis that uses Bayesian methods may be conducted to evaluate VE. The prior to be used for this analysis will be a robust, meta-analytic one based on pre-specified weighting of the external Phase 2b data (study HPX2008/HVTN 705) as informative prior and a non-informative prior. Details of pre-specified weights and priors will be specified in the DPS before doing the analysis after unblinding of HPX3002.

5.4.2.5. Correlates

If VE is established, an attempt will be made to identify correlates of increased risk of HIV-1 infection, correlates of VE, and best individual-level classifiers of HIV infection, and to assess mediators of vaccine efficacy. To this extent, appropriate statistical methodology will be applied or developed, and will be described in a (separate) SAP for correlate identification. A pre-specified case-control study of immune responses elicited by vaccination in active vaccine recipients may be performed to identify vaccine-induced correlates of protection and/or risk. Both single parameter and combinations of multiple immunologic parameter models will be explored. The presence of time-dependent correlates of protection may be examined in addition to single time point immune responses, including the peak immune response after the primary vaccination series, or the time point most closely preceding the diagnosis of HIV-1 infection. To further ascribe immune correlates of protection and/or specifically of VE, placebo case and control samples may also be included in a secondary stage of analysis.

6. IMMUNOGENICITY ANALYSIS

This section refers to the general analysis of immunogenicity and does not include details on the correlates analysis (separate SAP).

The analysis will be done on a subset of the complete set of participants who were randomized and vaccinated and for whom immunogenicity data are available, excluding participant samples with major protocol deviations expecting to impact the immunogenicity outcomes. Samples obtained after missed doses, incomplete doses or samples obtained after HIV infection will be excluded from the analysis.

6.1. Parameters

The following humoral and cellular immunogenicity may be assessed by immune responses against the insert or relevant antigens from isolated HIV-1 strains using (conditional that data are available): ELISA (total IgG titers), BAMA (breadth and magnitude of binding antibody responses), IFN-γ ELISPOT (T-cells producing producing IFN-γ) and/or ICS (T-cells expressing cytokines on a 28-color flow cytometric panel). Additional cellular and/or humoral immunogenicity analyses might be considered. Immunogenicity against the vector will be explored using an adenovirus 26 neutralization assay to assess neutralizing antibody responses against the vector.

6.2. Handling of Missing and/or Unquantifiable Immune Response Data

Missing immune response data will not be imputed.

Values below the limit of quantification or limit of detection will be imputed. Details per assay will be in the DPS.

6.3. Immune Response Analysis

No formal hypothesis on immunogenicity will be tested.

A selection of tables and figures may be produced by subgroup. Subgroups may include country, Ad26 baseline serostatus, P(r)EP use, age, BMI and race.

6.3.1. Immunogenicity against the insert

6.3.1.1. Humoral assays

For **ELISA** assays (when available) following results may be calculated: N, geometric mean[§] and corresponding 95% CI of the actual values and fold increases from baseline will be tabulated and graphically presented. *[§]calculate the mean and corresponding 95%CI of the log^x transformed values, back-transform this mean [i.e. x^mean] and CI [i.e. x^CI].*

Actual values and fold changes from baseline are tabulated and shown as dot plots with dots for participant values, and the corresponding geometric mean and 95% CI per time point for each assay. In addition, GMT plots over time, combining the regimens in one graph (without individual participant dots) will also be created.

In the graphs, original values will be displayed on the $\log^{10}$ scale.

For **BAMA** (when available) following results will be calculated for binding antibody responses to each individual antigen and for the breadth score: N, median response and corresponding quartile range, min and max will be tabulated and graphically presented. Cumulative number of responses across the IgG/antigen classes may be tabulated.

Breadth scores are calculated from antigen-level log10 truncated Net MFI, where before applying the log10 transformation. Magnitude-breadth (MB) plots for the BAMA assay will be provided to explore the magnitude and breadth of each individual serum sample assayed. MB curves will show, for each possible MFI threshold, the fraction of antigens with MFI greater than this threshold. Weights will be applied to take into account the correlation among the antigens (more details will be specified in the DPS). The group-specific curve obtained as the average MB across all subjects in that group will be displayed. The AUC-MB is calculated as the average of the MFI over the panel of antigens.

6.3.1.2. Cellular assays

For **ICS** (when available) the following results will be calculated: for CD4+ and CD8+ and for each antigen (combined and separated pools): N, median, quartile range, min, max, percentage of responders and corresponding 95% CI will be tabulated for each available cytokine background adjusted percentage. Graphical presentations will be provided displaying dots for the subject values and including the median and the percentage of responders. In the graphs the actual values will be shown and the LLOQ cut-off will be visualized, the values below LLOQ will be visualized with the value imputed as LLOQ/2. In the graphs, original values will be displayed on the log10 scale.

For **ELISpot** (when available) following results will be calculated: N, median, quartiles and range of the actual values will be tabulated and graphically presented.

Tables with the corresponding descriptive statistics will be provided.

Participants profiles of the actual values over time will be graphically presented.

Actual values are shown as box plots with dots for participant values, and the corresponding median and interquartile range per time point for each assay. In addition, box plots over time, combining the regimens in one graph (without individual participant dots) will also be created. For the graphs, original values will be displayed on the $\log^{10}$ scale.

6.3.2. Immunogenicity against the vector

For the **Ad26 vector neutralization assay** the following statistics will be calculated for the seropositivity endpoint: N, % seropositivity, geometric mean and corresponding 95% CI of the actual values.

Participants profiles of the actual values over time will be graphically presented.

Actual values and fold changes (from baseline) are tabulated and shown as dot plots with dots for participant values, and the corresponding geometric mean and 95% CI per time point for each assay.

Participants profiles of the assays against the insert will be repeated, highlighting participants with pre-existing immunity at baseline against the vectors.

Scatterplots with the seropositivity in the Ad26 vector neutralization assay versus the assays evaluating the vaccine response will be provided for the most important time points. In these scatter-plots the actual values will be shown, even if they are below the LLOQ.

REFERENCES

Castillo-Mancilla J, Zheng JH, Rower J et al. Tenofovir, Emtricitabine, and Tenofovir Diphosphate in Dried Blood Spots for Determining Recent and Cumulative Drug Exposure. Aids Research and Human Retroviruses. 2013; 29(2): 384-390. https://doi.org/10.1089/aid.2012.0089

Cooley LA, Oster AM, Rose CE et al. Increases in HIV testing among men who have sex with men--National HIV Behavioral Surveillance System, 20 U.S. Metropolitan Statistical Areas, 2008 and 2011. PLoS One. 2014 Sep 2;9(9):e104162.

Gilbert P, Wei L, Kosorok M, Clemens J. Simultaneous inferences on the contrast of two hazard functions with censored observations. Biometrics. 2002 Dec;58(4):773-80. doi: 10.1111/j.0006-341x.2002.00773.x.

Gilbert PB, Grove D, Gabriel E, et al. A Sequential Phase 2b Trial Design for Evaluating Vaccine Efficacy and Immune Correlates for Multiple HIV Vaccine Regimens. Stat Commun in Infect Dis, 2011;3(1). https://doi.org/10.2202/1948-4690.1037.

Grant RM, Anderson PL, McMahan V et al. An observational study of preexposure prophylaxis uptake, sexual practices, and HIV incidence among men and transgender women who have sex with men. Lancet Infect Dis 2014;14(9):820-829.

Hammer SM, Sobieszczyk ME, Janes H et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med. 2013;369(22): 2083-2092.

Hess KL, Hu X, Lansky A et al. Lifetime risk of a diagnosis of HIV infection in the United States. Ann Epidemiol. 2017 Apr;27(4):238-243. doi: 10.1016/j.annepidem.2017.02.003. Epub 2017 Feb 21.

Juraska M. Package “seqDesign.” 2015.

Klein, J.P. and Moeschberger, S. (1997) Survival Analysis Techniques for Censored and Truncated Data. Springer, Berlin.

Molina JM, Capitant C, Spire B et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. N Engl J Med. 2015 Dec 3;373(23):2237-46.

Patterson KB, Prince HA, Kraft E, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. Science Translational Medicine. 2011;3(112):112re4. doi:10.1126/scitranslmed.3003174

Parzen M, Wei L, Ying Z. Simultaneous confidence intervals for the difference of two survival functions. Scandinavian Journal of Statistics. 1997; 24(3):309-314. https://doi.org/10.1111/1467-9469.t01-1-00065.

APPENDIX 1: ADDITIONAL STATISTICAL DETAILS

Analysis primary endpoint

95% Wald confidence interval

Consider $\widetilde{H}_.$ the Nelson Aalen estimator of the cumulative incidence in the Vaccine or Placebo arm (see e.g. Klein and Moeschberger, 2003, p. 83-96):

$$\widetilde{H}(t) = \sum_{t_i \le t} \frac{d_i}{Y_i}, \qquad \text{with} \qquad \tilde{\sigma}_H^2(t) = \sum_{t_i \le t} \frac{d_i}{Y_i^2},$$

where Y_i is the number of individuals at risk at time t_i and d_i is the number of HIV1-infections at time t_i.

Let $\widetilde{H}_{Ratio}(t) = \ln(\frac{\widetilde{H}_{Vx}(t)}{\widetilde{H}_{Pbo}(t)})$, i.e. natural logarithm of the ratio of the cumulative incidences of the vaccine arm over the placebo arm, with approximate variance: $\sigma^2_{\mathrm{H_{Ratio}}}(\mathrm{t}) = \mathrm{var}\left(\mathrm{Ln}\left(\frac{\widetilde{H}_{Vx}(t)}{H_{Pbo}(t)}\right)\right) \approx \frac{\tilde{\sigma}^2_{H\,Pbo}(t)}{\widetilde{H}_{Pbo}(t)^2} + \frac{\tilde{\sigma}^2_{H\,Vx}(t)}{\widetilde{H}_{Vx}(t)^2}$ resulting in $(1-2\alpha)\times 100\%$ CIs for the VE:

$$[1 - \exp(\widetilde{\mathrm{H}}_{\mathrm{Ratio}}(t) + z_{1-\alpha} \times \sqrt{\sigma^2_{\widetilde{\mathrm{H}}_{\mathrm{Ratio}}}(\mathrm{t})});$$

$$1 - \exp(\widetilde{\mathrm{H}}_{\mathrm{Ratio}}(t) - z_{1-\alpha} \times \sqrt{\sigma^2_{\widetilde{\mathrm{H}}_{\mathrm{Ratio}}}(\mathrm{t})}\,)],$$

With $z_{1-\alpha}$ the $(1-\alpha)\times\ 100\%^{\text{th}}$ quantile of the standard normal distribution.