Janssen Vaccines & Prevention B.V.

Statistical Analysis Plan

A Phase 1/2a Study to Evaluate the Safety/Tolerability and Immunogenicity of Homologous Ad26 Mosaic Vector Vaccine Regimens or Ad26 Mosaic and MVA Mosaic Heterologous Vector Vaccine Regimens, with High-Dose, Low-Dose or no Clade C gp140Protein Plus Adjuvant for HIV Prevention

Protocol HIV-V-A004 (IPCAVD009); Phase 1/2a

JNJ-55471468, JNJ-55471494, JNJ-55471520, JNJ-55471533, JNJ-55471572, JNJ55471585

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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

V1.0 15 May 2018	First version
V2.0 22 April 2022	Updated version to include long term extension data up to week 336

ABBREVIATIONS

ADCP	antibody dependent cellular phagocytosis
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
BAMA	binding antibody multiplex assay
BIDMC	Beth Israel Deaconess Medical Center
BMI	body mass index
CRF	case report form
CTP	clinical trial protocol
DAIDS	Division of AIDS
DP	drug product
dTA	data transfer agreement
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ELISPOT	Enzyme-Linked ImmunoSpot
ENV	envelope
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
HD	high dose
HIV	human immunodeficiency virus
ICS	intracellular cytokine staining
IG	immunogenicity
LD	low dose
LLOQ	lower limit of quantification
LOD	limit of detection
LTE	long term extension
SAE	serious adverse event
ULN	upper limit of normal
VISP	vaccine induced seropositivity
VISR	vaccine induced seroreactivity
VRC	Vaccine Research Center
WBC	white blood cell

DEFINITION OF TERMS

<table>
<tr><td>Randomization</td><td>1:1:1:1:1:1:1:1 ratio</td></tr>
<tr><td>Study vaccine</td><td>- Ad26.Mos.HIV (Ad26.Mos.1.Env + Ad26.Mos1.Gag-Pol + Ad26.Mos2.Gag-Pol): Total dose is $5x10^{10}$ viral particles (vp) per 0.5 mL injection
- MVA-Mosaic (MVA-Mosaic1 + MVA-Mosaic2): Total dose is 10^8 plaque-forming units (pfu) per 0.5 mL injection
- gp140 DP:
  - <u>Low-dose:</u> gp140 DP with 50 mcg total protein, mixed with aluminum phosphate adjuvant (0.425 mg aluminum) at the pharmacy, per 0.5 mL injection
  - <u>High-dose:</u> gp140 DP with 250 mcg total protein, mixed with aluminum phosphate adjuvant (0.425 mg aluminum) at the pharmacy, per 0.5 mL injection
- Placebo: 0.9% saline, 0.5 mL injection</td></tr>
<tr><td>Vaccination compliance</td><td>Each vaccine administered (if applicable) according to planned schedule</td></tr>
<tr><td>Vaccine regimen</td><td>
<table>
<tr><th>Group</th><th>Planned N</th><th>Day 1, Week 12</th><th>Week 24, Week 48</th></tr>
<tr><td>1</td><td>50</td><td>Ad26.Mos.HIV</td><td>Ad26.Mos.HIV + gp140 (250mcg+adjuvant)</td></tr>
<tr><td>2</td><td>50</td><td>Ad26.Mos.HIV</td><td>Ad26.Mos.HIV + gp140 (50mcg+adjuvant)</td></tr>
<tr><td>3</td><td>50</td><td>Ad26.Mos.HIV</td><td>Ad26.Mos.HIV + Placebo</td></tr>
<tr><td>4</td><td>50</td><td>Ad26.Mos.HIV</td><td>MVA-Mosaic + gp140 (250mcg+adjuvant)</td></tr>
<tr><td>5</td><td>50</td><td>Ad26.Mos.HIV</td><td>MVA-Mosaic + gp140 (50mcg+adjuvant)</td></tr>
<tr><td>6</td><td>50</td><td>Ad26.Mos.HIV</td><td>MVA-Mosaic + Placebo</td></tr>
<tr><td>7</td><td>50</td><td>Ad26.Mos.HIV</td><td>gp140 (250mcg+adjuvant) + Placebo</td></tr>
<tr><td>8</td><td>50</td><td>Placebo</td><td>Placebo + Placebo</td></tr>
</table>
</td></tr>
</table>

1. INTRODUCTION

This statistical analysis plan (SAP) was initially written to describe the analyses of the main part of the study (up to Week 96). This updated version covers also the reporting of data collected till the end of the long term extension (LTE) which lasted up to Week 336 only for group 1 and 2 (final analysis at end of study). At the time this SAP was written the Week 96 statistical analysis was already performed and will not be rerun. The below described analyses will be performed on fully unblinded data.

1.1. Trial Objectives

See CTP, Section 2.1.

1.2. Trial Design

See CTP, Section 3.1.

1.3. Statistical Hypotheses for Trial Objectives

No formal statistical hypothesis will be tested.

1.4. Sample Size Justification

See CTP, Section 11.2.

1.5. Randomization and Blinding

See CTP, Section 5.

2. GENERAL ANALYSIS DEFINITIONS

A baseline (or reference) value will be defined as the value of the last available assessment performed prior to the first dose (active vaccine or placebo).

2.1. Analysis Windows

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

Table 3: Phase and Period Definitions

Phase	Phase number	Period	Period number	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 Dose 1 (Day 1)	Minimum of: a) Maximum (28 days after first vaccination at 23.59, scheduled visit 4 weeks after first vaccination at 23:59) b) 23:59 at the date of last contact (for early discontinuation)
Follow-Up 1	3			1 minute after end of Post-Dose 1 period	Minimum of: a) One minute prior to date and time of the next vaccination b) 23:59 at the date of last contact (for early discontinuation)
Regimen	2	Post-Dose 2	2	Date and time of Dose 2 (Day 85)	Minimum of: a) Maximum (28 days after second vaccination at 23.59, scheduled visit 4 weeks after second vaccination at 23:59)

Phase	Phase number	Period	Period number	Interval	
				From	To
					b) 23:59 at the date of last contact (for early discontinuation) c) One minute prior to post-dose 3
Follow-Up 2	4			1 minute after end of Post-Dose 2 period	Minimum of: a) One minute prior to date and time of the next vaccination b) 23:59 at the date of last contact (for early discontinuation)
Regimen	2	Post-Dose 3	3	Minimum of Date and Time of the two Dose 3 Injections (Day 169)	Minimum of: a) Maximum (28 days after third vaccination at 23.59, scheduled visit 4 weeks after third vaccination at 23:59) b) 23:59 at the date of last contact (for early discontinuation)
Follow-Up 3	5			1 minute after end of Post-Dose 3 period	Minimum of: a) One minute prior to date and time of the next vaccination b) 23:59 at the date of database cut-off[c] in case of interim c) 23:59 at the date of last contact (for early discontinuation)
Regimen	2	Post-Dose 4	4	Minimum of Date and Time of the two Dose 4 Injections (Day 337)	Minimum of: a) Maximum (28 days after fourth vaccination at 23.59, scheduled visit 4 weeks after fourth vaccination at 23:59) b) 23:59 at the date of database cut-off[c] in case of interim c) 23:59 at the date of last contact (for early discontinuation)
Follow-Up 4	6			1 minute after end of Post-Dose 4 period	Minimum of: a) Last available visit for completers[c] b) 23:59 at the date of last contact (for early discontinuation)[c]

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 This timepoint is intended to be at the end of the main study i e Week 96, cut-off used is 29AUG2017 LTE data will be reported separately

For the immunogenicity analyses no phases will be constructed.

The periods/phases will be used primarily for safety and concomitant medication allocation. The post-dose periods (and the regimen phase) are considered active periods/phase, the screening and follow-up phases are considered non-active phases. There will be no phase defined for the LTE part of the study.

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 subjects that received a first dose, but did not receive a 2nd dose while still continuing their planned visit schedule, the measurements after the planned but not administered 2nd 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 one will be not be shifted to the previous one but will be considered as planned: in the example above if the 2nd is missed, the 3rd vaccination will still be reported as post-dose 3.

2.2. Pooling Algorithm for Analysis Centers

Not planned, subgroups analyses by region are described in section 2.4.

2.3. Analysis Sets

2.3.1. Full Analysis Set

The Full Analysis Set (FA) will consist of all subjects who were randomized and who received at least one dose of study vaccine. This will be the primary population for all analyses (except immunogenicity, see below) described in this document.

2.3.2. Per-Protocol Immunogenicity Analysis Set

The per protocol immunogenicity (PPI) population consist of all subjects who have received at least the first three vaccinations, according to the protocol-specified vaccination schedule (+/- 2 weeks), have at least one measured post-dose blood sample collected and were not diagnosed with HIV during the study. Samples taken after Week 48 from subjects in the PPI population who missed the 4th vaccination or did not receive the 4th vaccination in the protocol-specified time window (+/- 2 weeks) will be excluded from the analysis.

The analysis of the immune responses will be performed on the per protocol immunogenicity population.

2.3.1. LTE Analysis Set

The Long Term Extension (LTE) population consists of all subjects in the Per-Protocol Immunogenicity Analysis Set, randomized in group 1 or 2 who had at least one visits in the long term extension part of the study.

2.4. Definition of Subgroups

The following subgroups will be investigated for safety (AE, Lab):

- Sex (Female, Male)
- Race (Caucasian, Black or African American, Asian, Other[a])
- Region (East Africa, South Africa, Thailand, USA)
- BMI [<25; 25-<30; ≥30]
-

The following subgroups will be investigated for immunogenicity:

- Region (East Africa, South Africa, Thailand, USA)
- AD26 VNA at baseline (<LLOQ, >=LLOQ)

The following subgroups will be investigated for the social impact questionnaire:

- Region (East Africa, South Africa, Thailand, USA)
- Sex (Female, Male)

[a] Subgroup “Other” should contain all remaining categories: Other, Multiple, American Indian or Alaska Native, etc.

3. INTERIM ANALYSIS AND DATA REVIEW COMMITTEE

A Data Monitoring Committee (DMC) was planned to specifically review the safety data at three specific time points to ensure safety of the subjects:

- Review blinded safety data (2 weeks of follow up) after 30% of subjects have received their first injection to decide whether further dosing can continue;
- Review unblinded safety data (2 weeks of follow up) after 10% of subjects have received their third injection to decide whether further dosing can continue;
- Review unblinded safety data (2 weeks of follow up) after 30% of subjects have received their third injection to decide whether further dosing can continue.

These analyses are described in a separate document DMC Charter and DMC SAP.

4. SUBJECT INFORMATION

Subject information will be analyzed based on the FA analysis set unless otherwise specified.

Continuous variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean (mean), 95% confidence interval (CI) for the mean, standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum and maximum. The minimum and maximum will be presented to the same number of decimal places as the original data. The mean and median will be rounded to one more decimal place than the original data, while the SD, SE and 95% CI to two more decimal places.

4.1. Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.

- Sex[a] (Female/Male)
- Age (years)
- Race
- Ethnicity
- Region
- Country
- Height (cm)
- Weight (kg)
- BMI (kg/m^2), calculated from the recording of baseline height and weight.

4.2. Disposition Information

Number and percentage of subjects that were 1) screened, 2) screening failures, 3) subjects meeting eligibility criteria 4) randomized and vaccinated, 5) randomized not vaccinated and 6) vaccinated but not randomized will be tabulated.

Number and percentage of subjects that completed and those who discontinued together with the reason(s) for discontinuation will be tabulated. This will be done for completion/vaccine discontinuation from further vaccination and from the trial at Week 96 , at the end of LTE. Number and percentage of subjects at each planned LTE visit will also be tabulated.

4.3. Concomitant Medications

The analysis of concomitant medications will be using the WHO drug dictionary as provided in the clinical database.

[a] At screening

Based on their start and stop date, concomitant therapies will be reported in each period during which they were applied.
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.
- 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/cut-off date.

Concomitant therapies will be tabulated. There will be special attention to Acetaminophen, NSAIDS or Antihistamines to identify medication that can mask local or systemic solicited events in the 8 days after the vaccinations and to Glucocorticosteroids for possible influence on immunogenicity results. Glucocorticosteroids will be flagged in the overall CM listing.After Week 96 only specific CM were to be collected (CTP Section 8) thus the reporting for the LTE will be limited to those as required by CTP.

4.4. Exposure

Number and percentage of subjects by number of received vaccinations will be tabulated.
Tabulation of vaccine administered within and outside the protocol-specified time windows (-1/+3 weeks of the planned vaccination and additional 2 weeks) will be provided as well.

4.5. Protocol Deviations

All major protocol deviations will be listed.

4.6. Medical History

Medical history data will be listed.

5. SAFETY

The safety and tolerability endpoints are:

- Unsolicited AEs 28 days following each vaccination
- Solicited local and systemic AEs (reactogenicity), collected daily from day of vaccination for 7 days post-vaccination (day of vaccination and the subsequent 7 days).

The safety and tolerability analysis will be performed on the FA set. Specifically, the analysis for solicited adverse events will be done on those subjects in the FA set for whom reactogenicity assessments are available in the database (either via on-site assessments or via the diary pages of the CRF).
Safety data will be tabulated per period by regimen. Only the active periods will be shown in the tables (AE, Lab, VS and ECG), except for SAEs and HIV infections. In addition, for a selection of tables (AE, Lab), tabulations per subject by vaccine and per dose by vaccine will be provided. During the LTE only for SAEs and HIV infections will be collected.

In the ‘per subject by vaccine’ analysis, the safety will be presented per periods (post-dose 1, 2, 3, 4) by vaccine rather than by regimen. A ‘post any dose’ period (= regimen phase in section 2.1) will be added, that summarizes the safety after all administered doses of a vaccine. The post-any-dose period allows determining how many subjects reported events after administration of a certain vaccine regardless the number of doses. Each subject is counted only once. In case a subject has the same event after more than one dose, it is counted only once (and in case of showing attributes, the worst corresponding attribute is shown) in the post-any dose period. The denominator is the number of subjects that received the considered vaccine in the considered period.

In the ‘per dose by vaccine’ analysis, the safety will also be presented by vaccine. This table allows determining the incidence of events per administered dose of a certain vaccine. For example, if a subject has the same event at least

once after dose 1 and at least once after dose 2 of the same vaccine, it is counted 2 times (so the numerator is the sum of post-dose 1, 2, 3 and 4 events in the "per subject by vaccine" table). In case of showing attributes, the worst corresponding attribute of each period are shown. The denominator is the total number of doses administered of the considered vaccine over all subjects.

At the 3th and 4th vaccination timepoint two vaccines should be administered at two different injection sites (left/right deltoid). The local solicited events should be linked to the medication injected in that specific injection site. However for some subjects the link between local AE and injection site might be unavailable, so the AE will be attributed to both medications received by the subject (e.g. if a subject receiving Ad26 + gp140 HD with AE "Erythema": Erythema will be counted for Ad26 and for gp140 HD). Systemic solicited and unsolicited AE will be reported under both the administered medications.

5.1. Adverse Events

The analysis of AEs will be based on the MedDRA coded terms as provided in the clinical database.

5.1.1. Definitions

Emerging adverse events will be defined as any AE starting or worsening on or after the start of the first vaccination on Day 1.

Solicited AEs are precisely defined events (local and systemic) that subjects are specifically asked about and which are noted by subjects in the memory aid. Unsolicited AEs are all AEs that a subject experienced, but were not specifically asked about.

Both systemic solicited and unsolicited AEs will be considered to be related to the use of the study vaccine if the attribution is possibly, probably or very likely. An AE will be considered not related with the use of the study vaccine if the attribution is not related or doubtful.
Relationship of all AEs (systemic solicited and unsolicited) to the study vaccine will be assessed by the investigator.
Local solicited AEs will always be considered as related to the injection.

The severity of the AEs will be classified as Mild, Moderate, Severe, Life-threatening or Fatal.
The grading of the adverse events will occur according to the DAIDS grading list (see CTP appendix 1).
Period allocation of AEs is defined in Section 5.1.3.

5.1.2. Analysis of Adverse Events

AEs (unsolicited and solicited AEs) will be tabulated by presenting the number and percentage of subjects having at least one of the observed AEs.
The unsolicited AEs will be summarized by System Organ Class and Preferred Term. The solicited AEs will be summarized by class (local, systemic) and solicited term. For solicited as well as unsolicited AEs, tables focusing on severity, relatedness and both grade 3 severity and relatedness to vaccine will be created. SAEs, AEs with fatal outcome and AEs leading to permanent stop will also be tabulated. Separate summary tables for the solicited and the unsolicited events will be produced.
Events of interest (confirmed HIV infection) will be tabulated.

For the solicited local and systemic AEs, the duration and time to first onset of the events may also be summarized. If a subject experiences more than one occurrence of a solicited event, the maximum duration of the events will be used. The time to first onset is defined as (date of first onset – reference date + 1). The reference date is the date of the respective vaccination.

AEs summary tables for solicited and unsolicited AEs, tabulation of AEs by worst severity for the solicited AEs and overall incidence tabulation will be provided by subgroups (section 2.4) per period by regimen.

5.1.3. Period Allocation of Adverse Events

The analysis of AEs consists of two steps: first, AEs are allocated to periods; second, overlapping/consecutive AEs within a period are combined.
Unless otherwise specified, only emerging AEs are presented and are allocated to the periods as described below.
Solicited AEs will always be considered as emergent, and will be allocated to the respective post-dose x periods.
In the below text active periods refer to the post-dose x (x=1,2,3,4) and the regimen period. The post-dose 1 period (and the regimen period) are considered as first active period.

STEP 1: allocation of events to the periods
Adverse events present in the SDTM database are allocated to periods based on their start date. If the start date of an event falls between (or on) the start and stop date of a period, the AE is attributed to that period.
Incomplete dates (i.e. *day and/or month and/or year missing*):

- In case of partial start or stop dates, the events are allocated to the periods using the available partial information on start and end date, no imputation is done. If, for instance, for the AE start date only month and year is available, these data are compared with the month and year info 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 subjects still ongoing in the study, and by the end date of the last period for subjects who discontinued or completed the trial.

STEP 2: combining events
Overlapping/consecutive events are defined as events in the same subject with the same preferred term which have at least 1 day in 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) In case a non-active period (e.g. Screening) is followed by an active period, and the overlapping/consecutive events start in both periods, they are allocated to their respective period 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 that contribute to this AE are retained as individual records in the ADAM database but are assigned the same onset, period, and total duration.
3) In case an active period is followed by a non-active period (e.g. Follow-Up), and the overlapping/consecutive events start in both periods, they are allocated to the active period and are considered as one and the same AE. The individual events that contribute to this AE are retained as individual records in the ADAM database but are assigned the same duration, onset and active period.
4) In case an active period is followed by an 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 complete.
2. In case the completely missing end date is imputed (for phase allocation), this date is also considered as a complete date.
3. Time during the day is not considered when determining overlap of events.
4. Solicited AEs will never be attributed to the Screening Phase

5.1.4. Handling of Missing Data for Adverse Events

Missing data will not be imputed. If the severity is missing for a solicited AE, then it is considered as unknown and not taken into account for the analysis.

5.1.5. Solicited Local (Injection Site) Reactions

The analysis of local solicited adverse events will include:
1. Pain
2. Erythema
3. Induration

4. Swelling
5. Itching
6. Warmth

5.1.6. Solicited Systemic Adverse Events

The analysis of systemic solicited adverse events will include:

1. Fever (defined as body temperature of 38.0°C or higher)
2. Headache
3. Fatigue
4. Myalgia
5. Nausea
6. Vomiting
7. Arthralgia
8. General Itching
9. Chills
10. Rash

5.2. Clinical Laboratory Tests

The data will be summarized by the type of laboratory test.

In case a laboratory test result is *censored* (no numeric value is available, but only a verbatim term) then a numeric value will be imputed by a value preceding or exceeding the cut-off value with one unit. (<x: subtract 1 unit from x, >x: add 1 unit to x)

Toxicity grades will be determined according to the DAIDS Grading list (See also Appendix 1). In case no toxicity grades are defined for a test, the abnormalities (above/below normal range) will be used. In determining toxicity grades, the following rules are applied:

- worst grades/abnormalities are determined over the whole observational period for each trial phase separately, including all post-baseline measurements of that phase.
- The abnormalities 'abnormally low' and 'abnormally high' are considered equally important, i.e. if a subject has as well an abnormally low as an abnormally high value post-baseline, both abnormalities are shown in the tables. (This means that the sum of the percentages can be more than 100%)
- Note: as the grading scale for some parameters in the grading table has some gaps (zones where no toxicity grade definition exists), laboratory results falling in these zones will be allocated to the adjacent worst-case grade.
- If a lab value falls within the grading as specified in the grading table but also within the (local) lab normal limits, the value is considered as normal

Definition emergent: An abnormality (toxicity grade or abnormality based on normal ranges) will be considered emergent in a particular phase if it is worse than the baseline (baseline = prior very first vaccination). If the baseline is missing, the abnormality is always considered as emergent. A shift from 'abnormally low' at baseline to 'abnormally high' post baseline (or vice versa) is also emergent.

5.2.1. Analysis methodology

Laboratory data will be analyzed based on the full analysis set. Unless specified otherwise, percentages are calculated versus the number of subjects in the analysis set with non-missing data for the parameter, period (if applicable) and treatment group under evaluation.

Tabulations of the worst graded abnormalities per period following vaccination will be provided. Grade 3 and 4 toxicities developed following vaccination will be listed. Tabulations of the worst emerging abnormalities (below/ above) will be performed for tests that have no grading.

5.3. Vital Signs and ECG

5.3.1. Definitions

Vital sign and ECG measurements will be performed at the time points indicated in the Time and Events Schedule (see CTP). Following parameters will be summarized:

- Temperature (°C)
- Blood Pressure: systolic/diastolic (mmHg)
- Pulse rate (bpm)
- PR (ms)
- QTc (ms)

For definition of emergent refer to section 5.2.

5.3.2. Analysis methodology

Any clinically relevant vital signs and ECG abnormalities occurring from signing of the ICF onwards must be recorded on the adverse event page of the CRF and are analyzed as AEs. Tabulations of the parameters per period following vaccination will be provided. All findings will be listed by subject and by time point. Tabulations of the worst emerging abnormalities will be performed for parameters if applicable (see grading on Appendix 1).

Listings of all the ECG results and ECG abnormalities reported by the ECG reader will be provided.

Physical examination data will only be listed.

6. IMMUNOGENICITY

6.1. Analysis specifications

The analysis of immunogenicity will be done on the PPI analysis set (Section 2.3).

6.2. Immune Response Parameters

Humoral and cellular immune responses as measured by the following immune assays will be analyzed:

Humoral response
- Env ELISA IgG-t gp140
- ELISA IgG 1-4
- TZM-BL
- ADCP gp140
- BAMA
- AD26 VNA

Cellular response
- ELISpot
- ICS

6.3. Immune Response Analysis

No formal hypothesis on immunogenicity will be tested.

Humoral assays

For all the humoral assays the immune response values will be $\log_{10}$-transformed before any further handling. The log10-transformed values will be used throughout the analysis. In the graphs, original values will be displayed on the log10 scale.

For each assay and at each time point geometric mean of actual values, geometric mean increases and percentage of responders, all with corresponding 95%Cis will be provided in the tables. The definition of responders is defined in

section 6.4. for each assay. Graphical presentations will be provided displaying dots for the subject values and including the geometric mean and the percentage of responders. If available, baseline values will be summarized pooling the 8 groups and will be displayed on the left of each graph. Week 28 and Week 52 timepoints will be plotted next to each other for each group, a line will link the geometric mean (or the median) of the 2 different time points within the same group.
In the graphs the actual values will be shown and the LLOQ cut-off will be visualized, the values below LLOQ or below LOD will be visualized with the value imputed as described in section 6.3.
Vaccine durability will be shown by linear plots of the GM/median for each group.

Reverse distribution curves of the actual values will be provided for selected assays.
For BAMA the median will be reported instead of the geometric mean. Cumulative number of responses across the IgG/antigen classes will be tabulated.
Magnitude-breadth (MB) plots for the BAMA assay will be provided at each timepoint to explore the magnitude (MFI of IgG3 and of IgG breadth binding antibodies) and breadth (number antigens at a given MFI) 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.
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.

Cellular response

For Elispot and for each peptide: n, median, quartile range, min, max of actual values, percentage of responders above a predefined threshold (see section 6.4) and corresponding 95% CI will be tabulated.
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 described in section 6.3.
Reverse distribution curves of the actual values will be provided.
In the graphs, original values will be displayed on the $\log_{10}$ scale.

ICS
1) T cells: CD4+, CD8+
Antigen: Combined pools and separated pools
Cytokine counts: Marginal, Boolean AND (co-expression) counts, Boolean OR (and/or)

For CD4+ and CD8+ and for each antigen (combined and separated pools): n, median, quartile range, min, max, percentage of responders (see section 6.4) and corresponding 95% CI will be tabulated for each available cytokine count listed below.

Cytokine (ISSCAT)	**Description**
CD154+	Marginal
GzB+	Marginal
ICOS+	Marginal
IFNg+	Marginal
IL2+	Marginal
IL17a+	Marginal
IL4+	Marginal
TNFa+	Marginal
IFNg+/IL2+	Boolean AND (co-expression)
IFNg+/IL2+/TNFa+	Boolean AND (co-expression)
IFNg+/TNFa+	Boolean AND (co-expression)
IL2+/TNFa+	Boolean AND (co-expression)
IFNg+ or IL2+	Boolean OR (and/or)
IFNg+ or IL2+ or TNFa+	Boolean OR (and/or)

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 $\log_{10}$ scale.

The technical details for the calculation of the cytokine background adjusted percentages to be displayed in the above described outputs will be outlined in the DPS.

Additional timepoints between and tests within one of the above listed assays may be investigated for exploratory purposes. These data and data from timepoints belonging to the long term extension part of the study will be analyzed using the approaches described above.

6.4. Handling of Missing and/or Invalid Immune Response Data

Analysis will be carried out on the available data, no imputation will be done for missing samples.
BAMA and ICS unreliable values should be excluded from the analysis: those records will be flagged in the SDTM dataset.
LLOQs by assay/test are listed in Table 1. Values below LLOQ or LOD will be handled as follows:

- Calculation of Geomean (humoral response) and median (cellular response):
  - values < LLOQ are imputed =LLOQ/2
  - values = LOD are not imputed unless no LLOQ is available. In that case LOD will handled similarly to LLOQ (see ADCP)

- Calculation of fold increases from baseline:
  - values <LLOQ are imputed =LLOQ
  - values =LOD are imputed = LLOQ when LLOQ is available

6.5. Immune response assays: details

The details for each immune response assay are listed in following table. Final values of LLOQ/LOD might deviate from the table below, the dTA approved by the lab will be taken as source.

Assay	Test	Lab	Time Points	LLOQ	LOD	Unit	Responder definition (R)	Fold increase (FI) calculation
ELISA	AD26 VNA	Janssen Vaccines and Prevention	Wk0	17	NA	IC90	>LLOQ	NA
Env ELISA IgG-t gp140	Clade A (92UG037.1) Clade B (1990a) Clade C (Con C) Clade C (C97ZA.012)* Mos1	Janssen Vaccines and Prevention	Wk0 Wk 28 Wk 52 Wk78 Wk96	625 156.25 625 156.25 78.125	0	EU/ml	1) if baseline <LLOQ or missing, R>LLOQ 2) if baseline >=LLOQ, R=3-fold increase from baseline	1) if baseline >LLOQ, FI=Value post-baseline/Value wk0 2) if baseline<LLOQ, FI=value post-baseline/LLOQ
Env ELISA IgG1-4	Clade C (C97ZA.012) IgG1 Clade C (C97ZA.012) IgG2 Clade C (C97ZA.012) IgG3 Clade C (C97ZA.012) IgG4	BIDMC	Wk0 Wk 28 Wk 52 Wk78 Wk96	12.3 28.7 12.4 13.2	4	EC50		
Env ADCP gp140	Clade A (92UG037.1) Clade B (1990a) Clade C (Con C) Clade C (C97ZA.012)* Mos1	Ragon	Wk0 Wk 16 (n=72) Wk 28 Wk 52 Wk78 Wk96	NA	5.16 6.43 6.49 4.32 4.28	PS	1) if baseline <LOD, R>LOD 2) if baseline >=LOD, R=3-fold increase from baseline	1) if baseline >LOD, FI=Value post-baseline/Value wk0 2) if baseline<LOD, FI=value post-baseline/LOD
TZM-bl	<u>Tier1</u> Clade C (MW965.26)	Duke U	Wk28 Wk52	20	NA	ID50	>LLOQ	NA
	<u>Tier 2</u> Clade A (398F1) n Clade AC (246F3) n Ab Clade AE (CNE55) nAb Clade AE (CNE8) nAb Clade B (TRO11) nAb Clade B (X2278) nAb Clade BC (BJOX2000) nAb Clade BC (CH119) nAb		Wk28 (n=72)	10				

Assay	Test	Lab	Time Points	LLOQ	LOD	Unit	Responder definition (R)	Fold increase (FI) calculation
	Clade C (Ce1176) nAb Clade C (25710) nAb Clade C (Ce0217) nAb Clade G (X1632) nAb							
BAMA IgG3	HIV ENV Con S gp140 CFI (Clade M) HIV ENV Con 6 gp120/B (Clade M) HIV ENV gp41 HIV ENV 1086C_D7gp120 (Clade C) HIV ENV 1086C gp140 (Clade C) HIV ENV 1086C_V1V2 (Clade C) HIV ENV gp70_B.CaseA_V1V2 (Clade B)	Duke U	Wk0 Wk 28 Wk 52 Wk78 Wk96	100[a]	NA	MFI	BAMA interpretation= 1	1) if baseline >LLOQ, FI=Value post baseline/Value wk0 2) if baseline<LLOQ, FI=value post baseline/LLOQ
BAMA IgGA	Con S gp140 CFI Con 6 gp120/B gp41 1086C_D7gp120.avi/293F A1.con.env03 140 CF 00MSA 4076 gp140		Wk0 Wk 28 Wk 52 Wk78					
BAMA IgG1	Con S gp140 CFI Con 6 gp120/B gp41 1086C_D7gp120.avi/293F 1086C gp140C_avi C.1086C_V1_V2 Tags gp70_B.CaseA_V1_V2							

[a] This is not LLOQ but positivity cutoff as provided by the lab applied and is based on the 3 response call criteria which include the 95% of baseline responses per antigen

Assay	Test	Lab	Time Points	LLOQ	LOD	Unit	Responder definition (R)	Fold increase (FI) calculation
BAMA Breadth	HIV ENV gp120 Clade A (51802) IgG-t Ab Clade AE (254008) IgG-t Ab Clade AE (A244) IgG-t Ab Clade B (B.6240) IgG-t Ab Clade B (BORI) IgG-t Ab Clade B (TT31P) IgG-t Ab Clade BC (CNE20) IgG-t Ab Clade BC(BJOX002) IgG-t Ab Clade C(1086C_D7) IgG-t Ab B Clade M (Con 6) IgG-t Ab HIV ENV gp140 Clade B (SC42261) IgG-t Ab Clade C (CH505TF) IgG-t Ab C Clade A (9004S) IgG-t Ab C Clade B (RHPA) IgG-t Ab C Clade B (WITO)IgG-t Ab C Clade C (1086C) IgG-t Ab C Clade C (BF1266) IgG-t Ab CF Clade AE (conAE) IgG-t Ab CFI Clade M(Con S) IgG-t Ab HIV ENV gp41 IgG-t Ab HIV ENV gp70 Clade A (191084)IgG-t Ab Clade AE (C2101) IgG-t Ab Clade AE (CM244) IgG-t Ab Clade B (62357.14) IgG-t Ab Clade B (CaseA) IgG-t Ab Clade B (RHPA4259) IgG-t Ab Clade B (TT31P) IgG-t Ab Clade B(700010058) IgG-t Ab Clade BC (BJOX) IgG-t Ab Clade C (96ZM651) IgG-t Ab Clade C (BF1266) IgG-t Ab		Wk0 Wk 28 Wk 52 Wk78 Wk96					

Assay	Test	Lab	Time Points	LLOQ	LOD	Unit	Responder definition (R)		Fold increase (FI) calculation
	Clade C (CAP210) IgG-t Ab Clade C(Ce1086) IgG-t Ab Clade C(TV1.21)IgG-t Ab Clade C (001428)IgG-t Ab Clade C (7060101641)IgG-t Ab								
BAMA IgG2	HIV ENV gp41 IgG2 Ab HIV ENV gp70 Clade C (Ce1086) IgG2 Ab HIV ENV gp70 Clade B (CaseA) IgG2 Ab HIV ENV gp140CFI Clade M (Con S) IgG2 Ab HIV ENV gp140 Clade C (1086C) IgG2 Ab HIV ENV gp120/B Clade M (Con 6) IgG2 Ab HIV ENV gp120 Clade C (1086C_D7) IgG2 Ab		Wk0 Wk 28						
BAMA IgG4	HIV ENV gp70 Clade C (Ce1086) IgG4 Ab HIV ENV gp70 Clade B (CaseA) IgG4 Ab HIV ENV gp41 IgG4 Ab HIV ENV gp140CFI Clade M (Con S) IgG4 Ab HIV ENV gp140 Clade C (1086C) IgG4 Ab HIV ENV gp120/B Clade M (Con 6) IgG4 Ab HIV ENV gp120 Clade C (1086C_D7) IgG4 Ab		Wk0 Wk 28						
ELISpot	Env peptide pool Mos1 Pol peptide pool Mos1 Gag peptide pool Mos1 Env peptide pool Mos2	BIDMC	Wk0 Wk26 Wk 50 Wk78	55	0	SFC/10^6 PBMC	1) if baseline <threshold, R>threshold 2) if baseline	73[b] 112 87 70	NA

Assay	Test	Lab	Time Points	LLOQ	LOD	Unit	Responder definition (R)		Fold increase (FI) calculation
	Pol peptide pool Mos2 Gag peptide pool Mos2 Env peptide pool PTE[a] (Env peptide pool 1 PTE, Env peptide pool 2 PTE, Env peptide pool 3 PTE) Pol peptide pool PTE Gag peptide pool PTE		Wk96				>=threshold, R=3-fold increase from baseline	63 55 100 105 181	
ICS (CD4+, CD8+)	<u>Combined pools</u> HIV Gag pep pool (Mos1) HIV Pol pep pool (Mos1) HIV ENV pep pool (Mos1) HIV ENV pep pool clade C (C97ZA.012) HIV ENV pep pool (Clinical PTE) <u>Separated pools</u> HIV Pol RT pep pool (Mos1)* HIV Pol RNAseInt pep pool 1 (Mos1) HIV ENV gp120 pep pool 1 (Mos1)* HIV ENV gp41 pep pool 1 (Mos1) HIV ENV gp120 pep pool clade C (C97ZA.012) HIV ENV gp41 pep pool clade C (C97ZA.012) HIV ENV pep pool 1 (Clinical PTE)[a]	FHCRC	Wk26 Wk 50 Wk78 Wk96	NA	NA	% of CD4+/CD8+ T cells	ICS interpretation=1		NA

[a] ENV pep pool (PTE) will be provided split in pool 1,2,3: for the purpose of the Week 28 PA they will be reported only as sum of the 3: HIV ENV pep pool. The ADAM should however contain also the 3 separated pools.

[b] Threshold for ELISpot test is based on the 95 percentile from the baseline values of about 350 subjects on that test in the HIV-V-A004 study

Assay	Test	Lab	Time Points	LLOQ	LOD	Unit	Responder definition (R)	Fold increase (FI) calculation
	HIV ENV pep pool 2 (Clinical PTE)[a] HIV ENV pep pool 3 (Clinical PTE)[a]							

NA: not applicable: PS: phagocytic score; SFC: spot forming cells, PBMC: peripheral blood mononuclear cell, MFI: median fluorescent intensities
* Clade C (C97ZA.012) for Elisa and ADCP, ENV gp120 Mos1 and Pol RT Mos1 for ICS will be available for group 1 at additional intermediate timepoints: exploratory scatterplots with timepoint on the x-axis will be provided.

[a] ENV pep pool 1,2,3 (PTE Clinical): calculation of the pure cytokine count cannot be obtained from the combined HIV ENV pep pool (only Boolean OR data will be available) so it will be derived from the 3 separated pools.

7. VISP

VISP data was collected throughout the study outside of the EDC database. An overview of the results (Reactive, Indeterminate, Not Reactive) of the HIV Ag/Ab Combo 4th generation assays will be provided by visit and by brand name.

8. SOCIAL IMPACT QUESTIONNAIRE

Data from the Vaccine Research Center (VRC) Social Impact Questionnaire will be listed and summarized using descriptive statistics.

APPENDIX 1: LABORATORY, VITAL SIGNS AND ECG ABNORMALITY GRADINGS

LABORATORY[a]				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
HEMATOLOGY				
Absolute Neutrophil Count (ANC)[c]	1.000 x 10^9 – 1.300 x 10^9/L	0.750 x 10^9 – 0.999 x 10^9/L	0.500 x 10^9 – 0.749 x 10^9/L	<0.500 x 10^9/L
Hemoglobin (Hgb) (HIV NEGATIVE ONLY)	10.0 – 10.9 g/dL or Any decrease[b] 2.5 – 3.4 g/dL	9.0 – 9.9 g/dL or Any decrease[b] 3.5 – 4.4 g/dL	7.0 – 8.9 g/dL or Any decrease[b] ≥ 4.5 g/dL	<7.0 g/dL
Platelets, decreased	100.000 x 10^9 – 124.999 x 10^9/L	50.000 x 10^9 – 99.999 x 10^9/L	25.000 x 10^9 – 49.999 x 10^9/L	<25.000 x 10^9/L
WBC, decreased	2.000 x 10^9 – 2.500 x 10^9/L	1.500 x 10^9 – 1.999 x 10^9/L	1.000 x 10^9 – 1.499 x 10^9/L	<1.000 x 10^9/L
CHEMISTRIES				
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	>10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	>10.0 x ULN
Creatinine	1.1 – 1.3 x ULN	1.4 – 1.8 x ULN	1.9 – 3.4 x ULN	≥ 3.5 x ULN
URINALYSIS				
Hematuria	6-10 RBV/HPF	>10 RBV/HPF	Gross, with or without clots or with RBC casts	Transfusion indicated
Proteinuria, random collection	1+	2-3+	4+	NA

[a] This list is restricted to the laboratory parameters collected in this study for which a DAIDS grading is available. Only ranges applicable for adults are reported. For a complete list refer to the CTP appendix 1

[b] The decrease is a decrease from baseline

c If ANC is not available the values will be derived by from the ratio Neutrophils/Leucocytes

VITAL SIGNS AND ECG					
PARAMETER	**UNIT**	**GRADE 1**	**GRADE 2**	**GRADE 3**	**GRADE 4**
Temperature[b]	°C	≥38.0 - ≤38.4	≥38.5 - ≤38.9	≥39.0 - ≤40.0	>40.0
Hypertension[b]					
Systolic Blood Pressure	mmHg	≥140 - ≤159	≥160 - ≤179	≥180	NA
Diastolic Blood Pressure	mmHg	≥90 - ≤99	≥100 - ≤109	≥110	NA
Hypotension	mmHg	NA	NA	NA	NA
Pulse rate					
Tachycardia	bpm	≥101-≤115	≥116 - ≤130	>130	NA
Bradycardia	bpm	≥50 - ≤54	≥45 - ≤49	<45	NA
QTc[ab]	msec	≥450 - ≤ 470 or increase ≤30 from baseline	>470 - ≤ 490 or increase >30 and ≤50 from baseline	>490 or increase >50 from baseline	NA
PR[b]	msec	≥210 - ≤ 250	>250	NA	NA

[a] According to Bazett's or Fridericia's formula

[b] DAIDS grading, see also CTP appendix 1