Janssen Vaccines & Prevention B.V.

Statistical Analysis Plan

A randomized, parallel-group, placebo-controlled, double-blind Phase 1/2a study in healthy HIV uninfected adults to assess the safety/tolerability and immunogenicity of 2 different prime/boost regimens; priming with trivalent Ad26.Mos.HIV and boosting with trivalent Ad26.Mos.HIV and Clade C gp140 plus adjuvant OR priming with tetravalent Ad26.Mos4.HIV and boosting with tetravalent Ad26.Mos4.HIV and Clade C gp140 plus adjuvant

Protocol VAC89220HPX2004; Phase 1/2a

JNJ-55471468, JNJ-55471494, JNJ-55471520, JNJ-55471585, JNJ-64219324

*Janssen Vaccines & Prevention B.V. is a Janssen pharmaceutical company of Johnson & Johnson.

Status:	Approved - V1.1
Date:	2 May 2022
Prepared by:	Janssen Infectious Diseases BVBA
Document No.:	EDMS-ERI-175658987

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

TABLE OF CONTENTS

TABLE OF CONTENTS **2**

AMENDMENT HISTORY **4**

ABBREVIATIONS **4**

DEFINITION OF TERMS **5**

1. INTRODUCTION **6**
1.1. Trial Objectives 6
1.2. Trial Design 6
1.3. Statistical Hypotheses for Trial Objectives 6
1.4. Sample Size Justification 6
1.5. Randomization and Blinding 6

2. GENERAL ANALYSIS DEFINITIONS **6**
2.1. Analysis Windows 6
2.2. Pooling Algorithm for Analysis Centers 8
2.3. Analysis Sets 8
2.3.1. Full Analysis Set 8
2.3.2. Per-Protocol Immunogenicity Analysis Set 8
2.3.3. LTE Analysis Set 9
2.4. Definition of Subgroups 9

3. INTERIM ANALYSIS AND DATA REVIEW COMMITTEE **9**

4. SUBJECT INFORMATION **9**
4.1. Demographics and Baseline Characteristics 10
4.2. Disposition Information 10
4.3. Concomitant Medications 10
4.4. Exposure 11
4.5. Protocol Deviations 11
4.6. Medical History 11

5. SAFETY **11**
5.1. Adverse Events 12
5.1.1. Definitions 12
5.1.2. Analysis of Adverse Events 12
5.1.3. Transforming On-site Assessments and Diaries of Solicited Adverse Events into an Analysis Format 13
5.1.4. Handling of Missing Data for Adverse Events 15
5.1.5. Solicited Local (Injection Site) Reactions 15
5.1.6. Solicited Systemic Adverse Events 15
5.2. Clinical Laboratory Tests 16
5.2.1. Analysis methodology 16
5.3. Vital Signs 17
5.3.1. Definitions 17
5.3.2. Analysis methodology 17

6. IMMUNOGENICITY **17**
6.1. Analysis specifications 17
6.2. Immune Response Parameters 17
6.3. Immune Response Analysis 18
6.4. Handling of Missing and/or Invalid Immune Response Data 20
6.5. Immune response assays: details 22

7. SOCIAL IMPACT QUESTIONNAIRE 26

APPENDIX 1: LABORATORY, VITAL SIGNS AND ABNORMALITY GRADINGS 27

AMENDMENT HISTORY

V1.0 19 March 2019	First version
V2.0 2 May 2022	Updated version to include long term extension data up to week 264

ABBREVIATIONS

ADCP	antibody dependent cellular phagocytosis
AE	adverse event
BAMA	binding antibody multiplex assay
BIDMC	Beth Israel Deaconess Medical Center
BMI	body mass index
CI	confidence interval
CRF	case report form
CTP	clinical trial protocol
DAIDS	Division of AIDS
DP	drug product
DPS	data presentation specification
ELISA	enzyme-linked immunosorbent assay
ELISPOT	Enzyme-Linked ImmunoSpot
ENV	Envelope
FHCRC	Fred Hutchinson Cancer Research Center
GM	geometric mean
GMR	geometric mean ratio
HIV	human immunodeficiency virus
ICS	intracellular cytokine staining
IG	immunogenicity
LLOQ	lower limit of quantification
LOD	limit of detection
LTE	long term extension
PReP	Pre-Exposure Prophylaxis
SAE	serious adverse event
SDTM	study data tabulation model
ULN	upper limit of normal
VRC	Vaccine Research Center
RBC	red blood cell
WBC	white blood cell

DEFINITION OF TERMS

<table>
<tr><td>Randomization</td><td>5:1:10:2 ratio</td></tr>
<tr><td>Study vaccine</td><td>- Ad26.Mos4.HIV (Ad26.Mos.1.Env + Ad26.Mos.2.Env +Ad26.Mos1.Gag-Pol + Ad26.Mos2.Gag-Pol): Total dose is $5x10^{10}$ viral particles (vp) per 0.5 mL injection
- 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
- gp140 DP: 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>1A</td><td>55</td><td>Ad26.Mos.HIV</td><td>Ad26.Mos.HIV + gp140</td></tr>
<tr><td>1B</td><td>11</td><td>Placebo</td><td>Placebo + Placebo</td></tr>
<tr><td>2A</td><td>110</td><td>Ad26.Mos4.HIV</td><td>Ad26.Mos4.HIV + gp140</td></tr>
<tr><td>2B</td><td>22</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 72). This updated version covers also the reporting of data collected till the end of the long-term extension (LTE) which lasted up to Week 264 (final analysis at end of study) for the group 2A receiving the full vaccination regimen 'Ad26.Mos4.HIV/Ad26.Mos4.HIV + gp140'. At the time this SAP was written the Week 72 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)

Table 3: Phase and Period Definitions

Phase	Phase number	Period	Period number	Interval	
				From	To
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) 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 the 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 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 72 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 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 country 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.

2.3.3. LTE Analysis Set

The Long Term Extension (LTE) population consists of all subjects in the Per-Protocol Immunogenicity Analysis Set, randomized in group 2A receiving the full vaccination regimen 'Ad26.Mos4.HIV/Ad26.Mos4.HIV + gp140' who had at least one visit in the long term extension part of the study.

2.4. Definition of Subgroups

The following subgroups will be investigated for the most relevant immunogenicity assays (more details in section 6.3):

- Country (Rwanda, USA)
- Ad26 VNA at baseline (<LLOQ, >=LLOQ)[a]
- Sex (Female, Male)
- Race (Caucasian, Black or African American, Asian, Other[b])
- BMI [<18.5; 18.5-<25; 25-<30; ≥30][a]

3. INTERIM ANALYSIS AND DATA REVIEW COMMITTEE

A Data Review Committee (DRC) 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 15% of subjects had received their first injection to decide whether further dosing can continue;
- Review blinded safety data (2 weeks of follow up) after 30% of subjects had received their first injection to decide whether further dosing can continue;
- Review blinded safety data (2 weeks of follow up) after 30% of subjects had received their third injection to decide whether further dosing can continue.

These analyses are described in a separate DRC Charter.

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.

[a] Baseline Ad26 VNA and BMI will also investigated as continuous variables.
[b] Subgroup "Other" should contain all remaining categories: Other, Multiple, American Indian or Alaska Native, etc.

4.1. Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.

- Sex (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 but not randomized, 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 72 and 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.

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 systemic glucocorticosteroids during the whole study duration for possible influence on the immunogenicity results. Systemic glucocorticosteroids will be flagged in the overall CM listing. Subjects taking PrEP during the study will be listed. After Week 72 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), except for SAEs. In addition, for a selection of tables (AE, Lab), tabulations per subject by vaccine and per dose by vaccine will be provided.

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

Solicited AEs shown in the tables are extracted from the diary pages of the CRF. For unsolicited AEs, only the AEs within the 28-day period following each vaccination will be presented in the safety tables except for SAE and AESI (AE of special interest), which will be captured and tabulated in the outputs covering the whole study period. All other collected unsolicited adverse events will be presented through listings.

Solicited local AEs will be by definition considered as related to the study vaccine.

The severity of the AEs will be classified as grade 1 to 4. Solicited events that are graded less than grade 1, are not considered as AE. In case no grades are available, the grading of the solicited events will occur according to the grading list in attachment 1 of the CTP.

5.1.2. Analysis of Adverse Events

Number and percentage of subjects 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 (local, systemic) and preferred term.

For solicited AEs following tables will be provided: summary, by worst severity grade, grade 3, 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, permanent stop of vaccine, related, SAE and AESI.

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

5.1.3. Transforming On-site Assessments and Diaries of Solicited Adverse Events into an Analysis Format

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

When creating the analysis dataset for solicited AEs (dataset ADAESOL), solicited AEs (recorded by day) need to be converted into the format of unsolicited AEs (recorded by event). For this purpose, the start date of the AE will be considered as the date of first occurrence of the solicited AE (both local and systemic). If on subsequent day(s), the same grade is reported, the last reported date is used as the end date of the AE. A new record is created in case the grade of the event changes. If there is a time gap of at least 1 day between 2 (or more) occurrences of the particular solicited AE, then the second (and/or next) occurrence will be considered as a new AE. In case no data is reported for a day, this is analyzed as no event reported. If the on-site assessment differs in grade or relatedness with a corresponding day (eg, day of vaccination) of diary data, the on-site assessment should be recorded as a separate record in the database. The following example shows how the solicited AE should be converted into a format of unsolicited AEs:

Data from the Subject Diary

Subject: 0001

Solicited systemic AE: Headache

	On-site Assessment	**Diary Data**							
Solicited AE	Day 1 01Jan16	Day 1 01Jan16	Day 2 02Jan16	Day 3 03Jan16	Day 4 04Jan16	Day 5 05Jan16	Day 6 06Jan16	Day 7 07Jan16	Day 8 08Jan16
Grade	2	1	1	0	3	3	1	0	0
Relatedness	Doubtful	Probable							
Serious		N							
Outcome		Recovered/Resolved							

The data should be converted and stored in the AE dataset as follows:

Subject No.	**AE**	**Start Date**	**Stop Date**	**Severity**	**Relatedness**	**Serious**	**Outcome**	**AEID**
0001	Headache	01JAN16	01JAN16	2	Doubtful			1

0001	Headache	01JAN16	02JAN16	1	Probable	N	Recovered/Resolved	1
0001	Headache	04JAN16	05JAN16	3	Probable	N	Recovered/Resolved	1
0001	Headache	06JAN16	06JAN16	1	Probable	N	Recovered/Resolved	1

If a solicited AE ends after Day 8:

- The stop date of the event is the "Date of last day of symptom" as recorded in the eCRF and the "maximum severity" after Day 8 as recorded in the CRF. A separate record is created for this, in case this severity deviates from the previous record.

Note: To complete the start and end-date based on diary data, the date will be calculated based on the actual day that the AE occurred relative to vaccination and not on the reported date. For example, if the vaccination is on 01-JAN-2016, and the AE starts on Day 3, the start date will be set to the 03-JAN-2016, independent of the reported actual date.

For the calculation of duration, the first and last day is used, irrespective of whether interruptions occurred in between by missing reporting days or Grade 0 events. In the above example, the 4 records contribute to the same AE, therefore AEID is set to the same value and the duration of the AE is set to 6 for all records.

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 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: Combination of events:

Overlapping/consecutive events are defined as events of the same subject 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 non-active 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.

5.1.4. Handling of Missing Data for Adverse Events

Missing data will not be imputed. Subjects who do not report an event will be considered as subjects without an event. The analysis of the solicited AEs will include only documented safety data (i.e. in case severity is missing it is not considered an event).

5.1.5. Solicited Local (Injection Site) Reactions

The analysis of local solicited adverse events will include:

1. Pain/Tenderness
2. Erythema
3. Induration/Swelling

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. Chills

5.2. Clinical Laboratory Tests

Any clinically relevant lab value measured from signing of the ICF onwards must be recorded on the adverse event page of the CRF and are analyzed as AE. 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

5.3.1. Definitions

Vital sign 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)

For definition of emergent refer to section 5.2.

5.3.2. Analysis methodology

Any clinically relevant vital signs 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).

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 however at week 28 the differences between the 2 active groups (Ad26.Mos4.HIV + gp140/ Ad26.Mos.HIV + gp140) will be explored by means of a GMR including 95%CI and by a 2-sample t -test on the log data if those are normally distributed or by a Wilcoxon rank sum test otherwise. Those analyses will be performed on the following assays:

- ELISA: Clade C (C97ZA.012), Mos1
- ADCP: Clade C (C97ZA.012), Mos1
- ELISpot: Env peptide pool PTE, Gag peptide pool PTE, Pol peptide pool PTE

Humoral response

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. Also, mean fold change from highest post-baseline value to week 72 will be reported. 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 all the groups and will be displayed on the left of each graph. Data from post-baseline timepoints will be plotted next to each other for each group, a symbol will identify the geometric mean (or the median) of each group and timepoint.

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.

Graphs by subgroups listed in section 2.4 will be done for Elisa Clade C (C97ZA.012), ADCP Clade C (C97ZA.012).

Durability will be shown as a line plots displaying the GM/median for each vaccine regimen.

Magnitude-breadth (MB) plot for the ELISA assay will be provided at each timepoint to explore the magnitude and breadth of each individual serum sample assayed (antibody titers of 5 Clades). MB curves will show, for each possible titer threshold, the proportion of antibody titers 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 titers over the panel of antigens.

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 week 28 to explore the magnitude (MFI of IgG3 and of IgG breath binding antibodies) 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.

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 LLOQ/2.

In the graphs, original values will be displayed on the $\log_{10}$ scale. Graphs by subgroups listed in section 2.4 will be done for ELISpot Env peptide pool PTE, Gag peptide pool PTE and Pol peptide pool PTE.

ICS

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 background adjusted percentages 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)

Cytokine (ISSCAT)	Description
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 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 section 6.5. Values below LLOQ or LOD will be handled as follows:

- Calculation of Geomean (humoral response except ADCP) and median (cellular response):
  - values < LLOQ are imputed =LLOQ/2
  - values = LOD are imputed =LLOQ/2

  For ADCP only:
  - values < LOD are imputed =LOD/2

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

  For ADCP only:
  - values < LOD are imputed =LOD

Values above the upper limit of quantification (ULOQ) will be handled as follows:

- Calculation of geomean and median:
  - Values>ULOQ are imputed=ULOQ.
- Calculation of fold increases from baseline:
  - Values >ULOQ are imputed with ULOQ

6.5. Immune response assays: details

The details for each immune response assay are listed in following table.

Assay	Test	Lab	LLOQ[a]	LOD[a]	ULOQ[a]	Unit	Responder definition (R)	Fold increase (FI) calculation
VNA	Ad26 VNA	Janssen Vaccines and Prevention	17	NA	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	625 156.25 625 156.25 78.125	0	1600000 800000 1600000 400000 400000	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 IgG1,3	Clade C (C97ZA.012) IgG1 Clade C (C97ZA.012) IgG3	BIDMC	12.3 12.4	4	NA	EC50		
Env ADCP gp140	Clade A (92UG037.1) Clade B (1990a) Clade C (Con C) Clade C (C97ZA.012) Mos1	Ragon	NA	5.16 6.43 6.49 4.32 4.28	NA	PS	1) if baseline <LOD or missing, 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	Clade C (MW965.26) Clade C (C97ZA.012)	Duke U	20 20	NA	NA	ID50	>LLOQ	NA

[a] In case final LLOQ and ULOQ values present in the dataset differ from those listed in this table, those provided from the lab should be used.

Assay	Test	Lab	LLOQ[a]	LOD[a]	ULOQ[a]	Unit	Responder definition (R)	Fold increase (FI) calculation
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.Case A_V1V2 (Clade B)	Duke U	100[a]	NA	NA	MFI	BAMA interpretation flag = 1	1) if baseline >LLOQ, FI=Value post baseline/Value wk0 2) if baseline<LLOQ, FI=value post baseline/LLOQ
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							

[a] This is not LLOQ but "positive" threshold as provided by the lab

Assay	Test	Lab	LLOQ[a]	LOD[a]	ULOQ[a]	Unit	Responder definition (R)		Fold increase (FI) calculation
	CFI Clade M(Con S) IgG-t Ab <u>HIV ENV gp41</u> IgG-t Ab <u>HIV ENV gp70</u> 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 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								
ELISpot	Env peptide pool Mos1 Pol peptide pool Mos1 Gag peptide pool Mos1 Env peptide pool Mos2 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)	BIDMC	55	0	NA	SFC/10^6 PBMC	1) if baseline <threshold or missing, R>threshold 2) if baseline >=threshold, R=3-fold increase from baseline	73[b] 112 87 70 63 55 100	NA

[a] ENV pep pool (PTE) will be provided split in pool 1,2,3: for the purpose of the statistical analysis 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	LLOQ[a]	LOD[a]	ULOQ[a]	Unit	Responder definition (R)	Fold increase (FI) calculation
	Pol peptide pool PTE Gag peptide pool PTE						105 181	
ICS (CD4+, CD8+)	<u>Combined pools</u> HIV Gag pep pool HIV Pol pep pool (Mos1) HIV Env pep pool (Mos1) HIV Env pep pool clade C (ZA) HIV Env pep pool (Mos1 ZA) HIV Env Pol Gag pep pool <u>Separated pools</u> HIV Pol RT pep pool (Mos1) HIV Gag 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 (ZA) HIV Env gp41 pep pool clade C (ZA)	FHCRC	NA	NA	NA	% of CD4+/CD8+ T cells	ICS interpretation flag=1	NA

NA: not applicable: PS: phagocytic score; SFC: spot forming cells, PBMC: peripheral blood mononuclear cell, MFI: median fluorescent intensities

7. 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 ABNORMALITY GRADINGS

LABORATORY[a]				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
HEMATOLOGY				
Absolute Neutrophil Count (ANC) (cells/L)[c]	800 x 10^9 – 1.000 x 10^9	0.600 x 10^9 – 0.799 x 10^9	0.400 x 10^9 – 0.599 x 10^9	<0.400 x 10^9
Hemoglobin (Hgb) (HIV NEGATIVE ONLY) Male Female	 10.0 – 10.9 g/dL 9.5 – 10.4 g/dL	 9.0 – 9.9 g/dL 9.4 – 8.5 g/dL	 7.0 – 8.9 g/dL 8.4 – 6.5 g/dL	 <7.0 g/dL <6.5 g/dL
Platelets, decreased[b]	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[b]	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	6-9 RBC per high power field	≥10 RBC per high power field	Gross, with or without clots or with RBC casts	Transfusion indicated
Proteinuria, random collection	1+	2+	3+ or higher	NA

[a] This list is restricted to the laboratory parameters collected in this study for which a DAIDS 2.0 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[a]					
PARAMETER	**UNIT**	**GRADE 1**	**GRADE 2**	**GRADE 3**	**GRADE 4**
Temperature	°C	≥38.0 - ≤38.5	≥38.6 - ≤39.2	≥39.3 - ≤39.9	≥40.0
Hypertension					
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

[a] DAIDS 2.0 grading, see also CTP appendix 1