Janssen Vaccines & Prevention B.V.*

Statistical Analysis Plan

A Combined Phase 1/2a, Exploratory Study of a Therapeutic Vaccine Using an Adenovirus Type 26 Vector Prime and Modified Vaccinia Ankara Boost Combination With Mosaic Inserts in HIV-1 Infected Adults who Initiated Antiretroviral Treatment During Acute HIV Infection

Protocol VAC89220HTX1001; Phase 1/2a

Ad26.Mos.HIV/MVA-Mosaic

*Janssen Vaccines & Prevention B.V. (formerly known as Crucell Holland B.V.) is a Janssen pharmaceutical company of Johnson & Johnson and is hereafter referred to as the sponsor of the study.

Status: Approved
Date: 17 October 2018
Prepared by: Janssen Vaccines & Prevention B.V.
Document No.: EDMS-ERI-166858471

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

TABLE OF CONTENTS

AMENDMENT HISTORY

SAP Version	Approval Date
Original SAP	17 July 2018
Final SAP	17 Oct 2018

The statistical analysis plan (SAP) from the interim analysis for cohort criteria for proceeding to ARV ATI phase is available in ERIS (EDMS-ERI-150930582, V1.0).

The statistical analysis plan (SAP) from the final analysis is available in ERIS (EDMS-ERI-150930582, V2.0).

1. INTRODUCTION

This SAP is applicable to the final analysis defined in the VAC89220HTX1001 protocol (EDMS-ERI-90101185, V 17.0). The final analysis will be performed once all subjects have completed their final study visit or discontinued earlier. Interim analyses may be performed prior to the final analysis. These interim analyses would occur in a group-unblinded manner, but no subject-level unblinding would occur. The final analysis will be unblinded on subject-level.

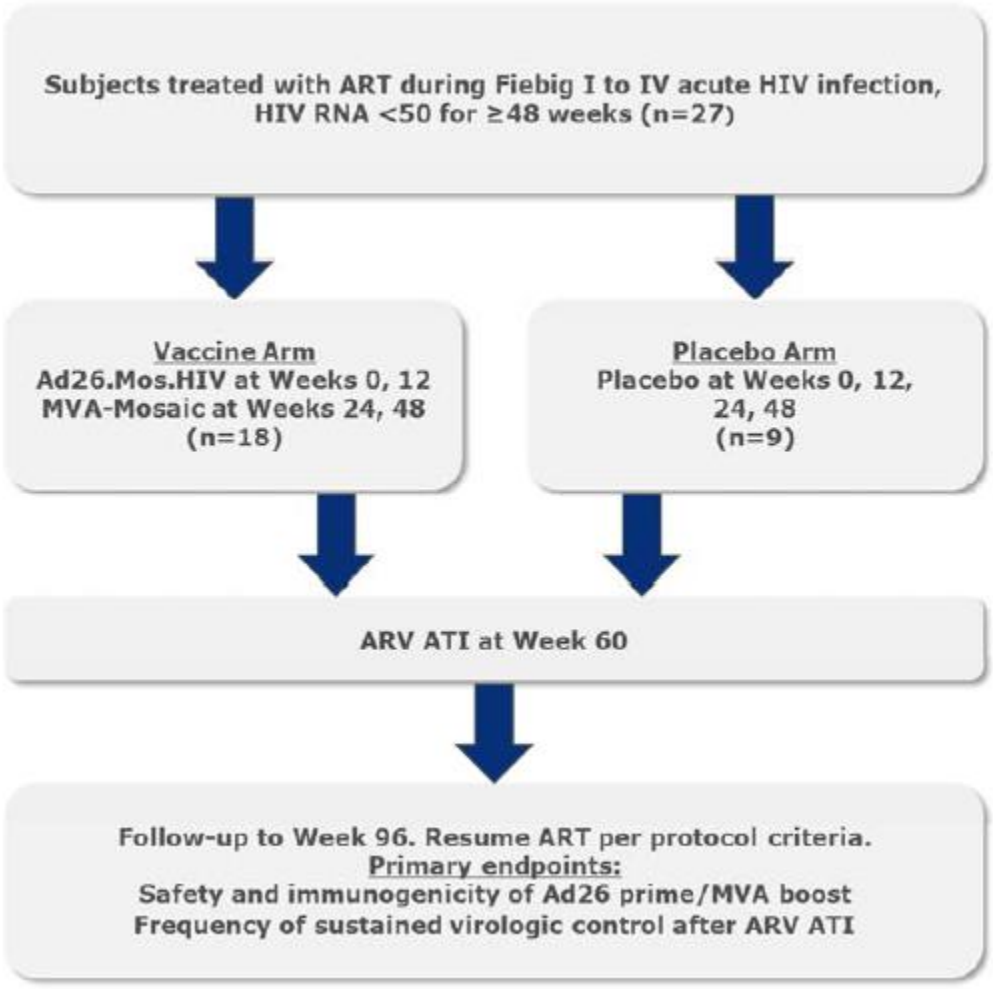
Data from subject 37 will be excluded (EC decision: “Subject to be removed from the project. Subject’s data shall not be used for analysis. This is due to the fact that the subject is not eligible to participate since the beginning of the trial). This subject will be excluded from the safety, efficacy and immunogenicity analysis.

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

See CTP, Section 2.2.

1.4. Sample Size Justification

See CTP, Section 9.4.

1.5. Randomization and Blinding

See CTP, Section 3.3 & 3.4.

1.6. Changes to planned analyses

NAP

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. Listings will be shown per phase and time point.

Immunogenicity results will be presented per scheduled time point as appropriate.

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

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 stage 2 (and the regimen phase) are considered active periods/phase, the screening and follow-up phases are considered non-active phases.

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.

2.1.1.1. Stage 1

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

Phase	Phase number	Period	Period number	Interval	
				From	**To**
					b) 23:59 at the date of last contact (for early discontinuation)
Regimen	2	Post-Dose 2	2	Date and time of Dose 2	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)
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 Dose 3	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 Dose 4	Minimum of: a) Maximum (28 days after fourth vaccination at 23.59, scheduled visit 2 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) 23:59 of the date prior to ART interruption b) 23:59 at the date of last contact (for early discontinuation)

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 safety 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.

2.1.1.2. Stage 2

Phase	Phase number	Period	Period number	Interval	
				From	To
Stage 2	7	ATI	5	00:00 of the date of ART interruption	Minimum of: a) Last available visit for completers (no ART resumption) b) One minute prior to ART resumption. If no time available: 23:59 of the date prior to ART resumption
Stage 2	7	ART resumption	6	Date time of ART resumption. If no time available: 00:00 of the date of ART resumption	Minimum of: a) Last available visit for completers b) 23:59 at the date of last contact (for early discontinuation)

2.2. Pooling Algorithm for Analysis Centers

NAP

2.3. Analysis Sets

Vaccination assignment will follow the as treated principle.

2.3.1. Full Analysis Set (FAS)

The full analysis set will include all randomized subjects with at least one vaccine administration documented. Subject 37 as indicated above will be excluded from this analysis set.

2.3.2. Immunogenicity Analysis Set

2.3.2.1. Immunogenicity Population (IP)

All immunogenicity analyses will be performed on subjects that were randomized and received at least 3 vaccinations. For the unblinded final analysis only: vaccinations should be given within the protocol-specified windows +/- 7 days for whom immunogenicity data are available excluding subjects with major protocol deviations expecting to impact the immunogenicity outcomes.

In addition, all samples obtained after missed doses will be excluded from the analysis (eg if a subject in group 1 received their first 3 vaccinations, but not the last, samples pertaining to the first 3 vaccinations can still be used, but not for the 4^{th} vaccination. Similarly, if a subject received their first two and their last vaccination but missed the third vaccination then samples up and till the second vaccination will be used, later samples will be excluded).

2.3.3. Efficacy Analysis Set

2.3.3.1. Efficacy Population (EP)

The primary efficacy population will consist of all subjects who undergo ARV ATI at Week 60 (Stage 2), regardless of the time or outcome of treatment interruption. Subject 37 as indicated above will be excluded from this analysis set.

2.3.3.2. Per Protocol Efficacy Population (PPE)

The per protocol efficacy population will include all randomized and fully vaccinated subjects for whom efficacy data concerning endpoint measures are available excluding subjects with major protocol deviations expecting to impact the efficacy outcomes.

If there is no difference in subjects between the EP and PPE population, all the outputs will state the analysis is done on the EP population.

2.4. Definition of Subgroups

Three subgroups based on their time to rebound will be made. The cut-off values are to be chosen such that 33.3% of all vaccinated subjects from the active arm fall in the first subgroup (1st tertile), 33.3% fall in the second subgroup (2nd tertile), 33.3% fall in the third subgroup (3rd tertile). These groups will be shown in a different color in the graphs. Analogously, tertiles subgroups will be computed in the placebo arm. Here the cut-off values are to be chosen such that 33.3% of all vaccinated subjects from the placebo arm fall in the first subgroup (1st tertile), 33.3% fall in the second subgroup (2nd tertile), 33.3% fall in the third subgroup (3rd tertile).

3. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE REVIEW

The final analysis will be performed once all subjects have completed their final study visit or discontinued earlier. Interim analyses may be performed prior to the final analysis. These analyses would occur in a group-unblinded manner, but no subject-level unblinding would occur. One interim analysis is performed on W26 ICS data to evaluate the subjects entering in ATI. One interim analysis is performed on immunogenicity data only up to W50. The final analysis will evaluate safety, immunogenicity and efficacy.

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, where available.

- Sex[a] (Female/Male)
- Age (years)
- Race
- Ethnicity

[a] At screening

- Region
- Country
- Height (cm)
- Weight (kg)
- BMI (kg/m^2), calculated from the recording of baseline height and weight

4.2. Disease Baseline Characteristics

The following disease baseline characteristics will be summarized, where available:

- HIV RNA
- Date of HIV diagnosis
- ART history
- Fiebig Stage (I, II, III or IV)
- CD4 Cell Count
- CD8 Cell Count
- Hepatitis B/C at screening
- VDRL at screening
- TPHA at screening

4.3. Disposition Information

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

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

4.4. Treatment Compliance

The number of missed vaccinations will be tabulated.

4.5. Protocol Deviations

Major protocol deviations will be summarized.

4.6. 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 Glucocorticosteroids during the whole study duration for possible influence on immunogenicity results. Glucocorticosteroids will be flagged in the overall CM listing.

ART will be listed before and after ATI separately for each subject.

5. SAFETY

Safety analyses will be performed on the FAS. Continuous variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean (mean), 95% CI for the mean (if applicable, large studies), standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum and maximum. Frequencies and percentages (one decimal place) will be generated for categorical variables. No formal comparisons between groups will be provided.

Safety data will be analysed by vaccine regimens as designed per protocol (per regimen analysis). In case of multiple vaccinations, data will be presented by phase as well as over the entire regimen. For stage 2 safety data will be shown by phase and period, where applicable (no solicited adverse events). Denominator for the percentages is the number of subjects in the considered population and phase for a certain regimen (incidence per 100 subjects/phase).

5.1. Adverse Events (AE)

5.1.1. Definitions

Solicited AEs shown in the tables are extracted from the diary pages/ on-site assessment pages of the CRF (systemic solicited events: fever, fatigue, headache, myalgia, arthralgia, chills, nausea, vomiting, rash, general itching and local solicited events: erythema, warmth, itching, swelling,

induration and pain/tenderness). For unsolicited AEs, only the AEs within the 28-day period following each vaccination will be presented in the safety tables except for SAEs, 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. For stage 2, AEs will be shown by phase and period, where applicable.

Solicited local AEs will be by definition considered as related to the study vaccine. Solicited events are always allocated to the respective Post Dose period.

The severity of the AEs will be classified as grade 1 to 4, using the grades in SDTM. 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 be based on the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.1 dated July 2017 found on the website: http://rsc.tech-res.com/docs/default-source/safety/daidsgradingcorrecetedv21.pdf?sfvrsn=6.
Solicited events which meet the AE criteria are reported in the AE domain. Separated analysis datasets should be created for unsolicited AEs and solicited AEs.

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, and discontinuation), all events, most frequent, at least grade 3, permanent stop of vaccine, related and SAE.

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 related grade 3 or 4 event or SAE.

5.1.3. Phase allocation of Unsolicited Adverse Events

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. Missing Data

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).

5.2. Laboratory, ECG and Vital Signs

For laboratory safety parameters, vital signs and ECG (if applicable) only abnormalities emerging after vaccination will be tabulated by worst abnormality grade using the table in attachment 1. QT interval abnormalities will be defined only for corrected QT values (QTcF or QTcB) if available (no recalculation from QT values will be performed). Abnormalities on the changes from reference, if available, for QT interval will be categorized in '<30ms', '[30; 60]ms' and '>60ms'. Increases of less than 30ms will not be considered as an abnormal increase.

A listing of subjects with fever according to the DAIDS grading table will be provided.

An abnormality (toxicity grades according to the DAIDS (website: http://rsc.tech-res.com/docs/default-source/safety/daidsgradingcorrecetedv21.pdf?sfvrsn=6) or abnormality based on normal ranges) will be considered as emerging in a particular period if it is worse than the baseline value. If the baseline is missing, the abnormality is always considered emerging. A shift from 'abnormally low' at baseline to 'abnormally high' post baseline (or vice versa) is also emerging. 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 exceeding the cut-off value with one unit. (<x: subtract 1 unit from x, >x: add 1 unit to x; <3.45 is imputed with 3.44).
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 period separately, including all post-baseline measurements of that period.

- 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 by 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.

For the grades, no distinction will be made between test results of samples obtained under fasting and under non-fasting conditions: in case limits under fasting and non-fasting conditions differ, the limits of the conditions (fasting/non-fasting) of scheduled visits as planned in the CTP will always be used, also for samples obtained under a different condition (e.g. samples of withdrawal visits).

6. IMMUNOGENICITY ANALYSIS

The analysis of immunogenicity will be done on the IP set. Also, in addition to the general graphs, graphs with the following specifications will be made. Three subgroups based on their time to rebound will be made. The cut-off values are to be chosen such that 33.3% of all vaccinated subjects from the active arm fall in the first subgroup (1st tertile), 33.3% fall in the second subgroup (2nd tertile), 33.3% fall in the third subgroup (3rd tertile). These groups will be shown in a different color in the graphs. Analogously, tertiles subgroups will be conducted in the placebo arm. Here the cut-off values are to be chosen such that 33.3% of all vaccinated subjects from the placebo arm fall in the first subgroup (1st tertile), 33.3% fall in the second subgroup (2nd tertile), 33.3% fall in the third subgroup (3rd tertile). Subjects who are still undetectable on the W84 visit for immunogenicity will be shown with a different symbol.

The timing of the rebound is defined as follows:

A) Using the first time of multiple consecutive determinations of HIV-1 RNA above 1000 copies/ml at least 1 week apart for the real rebounders.
B) Using the last determination of HIV-1 RNA above 1000 copies/ml before restart of ART for the others.

In addition, subgroups will be created based on AD26 baseline seropositivity.

6.1. Parameters

The following humoral and cellular immune responses are measured by immunogenicity against the insert.

Humoral response
- Env ELISA IgG-t gp140 (Clade A (92UG037.1); Clade B (1990a); Clade C (Con C); Clade C (C97ZA.012); Mos1)
- ELISA IgG 1-4 (Clade C (C97ZA.012) IgG1; Clade C (C97ZA.012) IgG2; Clade C (C97ZA.012) IgG3; Clade C (C97ZA.012) IgG4)
- ADCC

Cellular response
- ELISpot
- ELISpot breadth
- ICS

The following humoral responses are measured by immunogenicity against the vector:

- Ad 26 VNA

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

Missing immune response data will not be imputed.

Values below the lower limit of quantification (LLOQ) or limit of detection (LOD) will be handled as follows:

- Calculation of geomean and median:
  - values<LLOQ are imputed=LLOQ/2.
- Calculation of fold increases from baseline:
  - values <LLOQ are imputed with LLOQ.
- Note: for ADCP the LOD will be considered and the same rules apply using LOD as reference.

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.

6.3. Immune Response Analysis

No formal hypothesis on immunogenicity will be tested.

6.3.1. Immunogenicity against the insert:

6.3.1.1. Humoral assays

For the **humoral** assays following results will be calculated and tabulated: N, geometric mean[§] and corresponding 95% CI, fold increases from baseline and responder rates. *[§]calculate the mean and corresponding 95%CI of the log[10] transformed values, back-transform this mean [i.e. 10^mean] and CI [i.e. 10^CI].*

For all the humoral assays the immune response values will be log10-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.

Graphical presentations will be provided displaying dots for the subject values and including the N, geometric mean and the percentage of responders. Baseline values will be summarized by pooling the groups and will be displayed on the left of each graph. In the graphs the actual values will be shown and the LLOQ cut-off will be visualized as a horizontal dotted line together with the reference 'LLOQ'. The values below LLOQ will be visualized with the value imputed as described in section 6.4 (column LLOQ or LOD).

In general a sample is considered positive, if the value is above the assay LLOQ. A response will be defined if the sample interpretation is negative at baseline and positive post-baseline, or, if sample interpretation is positive at both time points and there is a >x-fold increase from baseline (x-fold increase on the original scale). The definition of responders is defined in section 6.4. for each assay.

6.3.1.2. Cellular assays

For **cellular** assays following results will be calculated: N, median, quartile range, and min-max range of the actual values together with responders will be tabulated and graphically presented.

Tables with the corresponding descriptive statistics will be provided.

Actual values are shown as plots with dots for subject values, and the corresponding median and interquartile range per time point for each assay. At the bottom the N, median value and percentage of responders will be shown. For Elispot, subject values post-baseline will be defined as responder when the value is above threshold and threshold is defined as the 95th percentile of all baseline data calculated per peptide pool (reference A004 data). The definition of responders is defined in section 6.4.

For the graphs, original values will be displayed on the $\log^{10}$ scale. In the graphs the actual values will be shown and the P95 cut-off will be visualized as a horizontal dotted line together with the reference 'P95=value for each peptide pool. The values below LLOQ will be visualized with the value imputed as described in section 6.2.

6.3.2. Immunogenicity against the vector:

For **VNA** following statistics will be calculated: N, geometric mean (see above for the calculation) and corresponding 95% CI of the actual values.

Actual values are tabulated and shown as dot plots with dots for subject values, and the corresponding geometric mean and 95% CI at baseline.

6.4. Immunogenicity Assays

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

Assay	Test	Lab	Time Points	LLOQ	LOD	Treshold	ULOQ	Unit	Responder definition (R)	Fold increase (FI) calculation
Env ELISA IgG-t gp140	Clade A (92UG037.1) Clade B (1990a) Clade C (Con C) Clade C (C97ZA.012) Mos1	Janssen Vaccines &Prevention	Wk 0, 4, 16, 26, 50, 60, 84, 96	625 156.25 625 156.25 78.125	0			EU/ml	1) if baseline <LLOQ, 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 50	12.3 28.7 12.4 13.2	4			EC50		
ADCC	HIV ENV gp140 Mos1 Fun Ab	Schuetz (MHRP)	Wk0 Wk 50	NAP			NAP	%		
Ad26 VNA	Ad26 Vector Neutralization	Janssen Vaccines& Prevention	Wk0	17	NAP			IC90	>LLOQ	NAP
ELISpot	HIV IFNg ENV pep pool (Mos1) HIV IFNg Pol pep pool (Mos1) HIV IFNg Gag pep pool (Mos1) HIV IFNg ENV pep pool (Mos2) HIV IFNg Pol pep pool (Mos2) HIV IFNg Gag pep pool (Mos2) Env peptide pool PTE (Env peptide pool 1 PTE, Env peptide pool 2 PTE, Env peptide pool 3 PTE) HIV IFNg ENV1 pep pool (Clinical PTE) HIV IFNg ENV2 pep pool (Clinical PTE) HIV IFNg ENV3 pep pool (Clinical PTE) HIV IFNg Pol pep pool (Clinical PTE) HIV IFNg Gag pep pool (Clinical PTE) HIV IFNg ENV1 pep subpool (Mos1) HIV IFNg ENV2 pep subpool (Mos1) HIV IFNg ENV3 pep subpool (Mos1) HIV IFNg ENV4 pep subpool (Mos1) HIV IFNg ENV5 pep subpool (Mos1) HIV IFNg ENV6 pep subpool (Mos1)	BIDMC	Wk0 Wk26 Wk 50	 55 55 55 55	0	73[a] 112 87 70 63 55 100 105 181		SFC/10^6 PBMC	1) if baseline<threshold, R>threshold. 2) if baseline >= threshold, R=3-fold increase from baseline. (reference A004) For the subpools: Responder: >55	NAP

	HIV IFNg ENV7 pep subpool (Mos1) HIV IFNg ENV8 pep subpool (Mos1) HIV IFNg ENV9 pep subpool (Mos1) HIV IFNg ENV10 pep subpool (Mos1) HIV IFNg ENV11 pep subpool (Mos1) HIV IFNg ENV12 pep subpool (Mos1) HIV IFNg ENV13 pep subpool (Mos1) HIV IFNg ENV14 pep subpool (Mos1) HIV IFNg ENV15 pep subpool (Mos1) HIV IFNg ENV16 pep subpool (Mos1) HIV IFNg ENV17 pep subpool (Mos1) HIV IFNg Gag1 pep subpool (Mos1) HIV IFNg Gag2 pep subpool (Mos1) HIV IFNg Gag3 pep subpool (Mos1) HIV IFNg Gag4 pep subpool (Mos1) HIV IFNg Gag5 pep subpool (Mos1) HIV IFNg Gag6 pep subpool (Mos1) HIV IFNg Gag7 pep subpool (Mos1) HIV IFNg Gag8 pep subpool (Mos1) HIV IFNg Gag9 pep subpool (Mos1) HIV IFNg Gag10 pep subpool (Mos1) HIV IFNg Gag11 pep subpool (Mos1) HIV IFNg Gag12 pep subpool (Mos1) HIV IFNg Pol1 pep subpool (Mos1) HIV IFNg Pol2 pep subpool (Mos1) HIV IFNg Pol3 pep subpool (Mos1) HIV IFNg Pol4 pep subpool (Mos1) HIV IFNg Pol5 pep subpool (Mos1) HIV IFNg Pol6 pep subpool (Mos1) HIV IFNg Pol7 pep subpool (Mos1) HIV IFNg Pol8 pep subpool (Mos1) HIV IFNg Pol9 pep subpool (Mos1) HIV IFNg Pol10 pep subpool (Mos1) HIV IFNg Pol11 pep subpool (Mos1) HIV IFNg Pol12 pep subpool (Mos1) HIV IFNg Pol13 pep subpool (Mos1) HIV IFNg Pol14 pep subpool (Mos1) HIV IFNg Pol15 pep subpool (Mos1) HIV IFNg Pol16 pep subpool (Mos1) HIV IFNg Pol17 pep subpool (Mos1) HIV IFNg Pol18 pep subpool (Mos1) HIV IFNg Pol19 pep subpool (Mos1) HIV IFNg Pol20 pep subpool (Mos1) HIV IFNg Pol21 pep subpool (Mos1) HIV IFNg ENV1 pep subpool (PTE) HIV IFNg ENV2 pep subpool (PTE) HIV IFNg ENV3 pep subpool (PTE) HIV IFNg ENV4 pep subpool (PTE) HIV IFNg ENV5 pep subpool (PTE) HIV IFNg ENV6 pep subpool (PTE)									

	HIV IFNg ENV7 pep subpool (PTE) HIV IFNg ENV8 pep subpool (PTE) HIV IFNg ENV9 pep subpool (PTE) HIV IFNg ENV10 pep subpool (PTE) HIV IFNg ENV11 pep subpool (PTE) HIV IFNg ENV12 pep subpool (PTE) HIV IFNg ENV13 pep subpool (PTE) HIV IFNg ENV14 pep subpool (PTE) HIV IFNg ENV15 pep subpool (PTE) HIV IFNg ENV16 pep subpool (PTE) HIV IFNg ENV17 pep subpool (PTE) HIV IFNg Gag1 pep subpool (PTE) HIV IFNg Gag2 pep subpool (PTE) HIV IFNg Gag3 pep subpool (PTE) HIV IFNg Gag4 pep subpool (PTE) HIV IFNg Gag5 pep subpool (PTE) HIV IFNg Gag6 pep subpool (PTE) HIV IFNg Gag7 pep subpool (PTE) HIV IFNg Gag8 pep subpool (PTE) HIV IFNg Gag9 pep subpool (PTE) HIV IFNg Gag10 pep subpool (PTE) HIV IFNg Gag11 pep subpool (PTE) HIV IFNg Gag12 pep subpool (PTE) HIV IFNg Pol1 pep subpool (PTE) HIV IFNg Pol2 pep subpool (PTE) HIV IFNg Pol3 pep subpool (PTE) HIV IFNg Pol4 pep subpool (PTE) HIV IFNg Pol5 pep subpool (PTE) HIV IFNg Pol6 pep subpool (PTE) HIV IFNg Pol7 pep subpool (PTE) HIV IFNg Pol8 pep subpool (PTE) HIV IFNg Pol9 pep subpool (PTE) HIV IFNg Pol10 pep subpool (PTE) HIV IFNg Pol11 pep subpool (PTE) HIV IFNg Pol12 pep subpool (PTE) HIV IFNg Pol13 pep subpool (PTE) HIV IFNg Pol14 pep subpool (PTE) HIV IFNg Pol15 pep subpool (PTE) HIV IFNg Pol16 pep subpool (PTE) HIV IFNg Pol17 pep subpool (PTE) HIV IFNg Pol18 pep subpool (PTE) HIV IFNg Pol19 pep subpool (PTE) HIV IFNg Pol20 pep subpool (PTE) HIV IFNg Pol21 pep subpool (PTE) HIV IFNg ENV1 pep subpool (Mos2) HIV IFNg ENV2 pep subpool (Mos2) HIV IFNg ENV3 pep subpool (Mos2) HIV IFNg ENV4 pep subpool (Mos2) HIV IFNg ENV5 pep subpool (Mos2) HIV IFNg ENV6 pep subpool (Mos2)									

	HIV IFNg ENV7 pep subpool (Mos2) HIV IFNg ENV8 pep subpool (Mos2) HIV IFNg ENV9 pep subpool (Mos2) HIV IFNg ENV10 pep subpool (Mos2) HIV IFNg ENV11 pep subpool (Mos2) HIV IFNg ENV12 pep subpool (Mos2) HIV IFNg ENV13 pep subpool (Mos2) HIV IFNg ENV14 pep subpool (Mos2) HIV IFNg ENV15 pep subpool (Mos2) HIV IFNg ENV16 pep subpool (Mos2) HIV IFNg ENV17 pep subpool (Mos2) HIV IFNg Gag1 pep subpool (Mos2) HIV IFNg Gag2 pep subpool (Mos2) HIV IFNg Gag3 pep subpool (Mos2) HIV IFNg Gag4 pep subpool (Mos2) HIV IFNg Gag5 pep subpool (Mos2) HIV IFNg Gag6 pep subpool (Mos2) HIV IFNg Gag7 pep subpool (Mos2) HIV IFNg Gag8 pep subpool (Mos2) HIV IFNg Gag9 pep subpool (Mos2) HIV IFNg Gag10 pep subpool (Mos2) HIV IFNg Gag11 pep subpool (Mos2) HIV IFNg Gag12 pep subpool (Mos2) HIV IFNg Pol1 pep subpool (Mos2) HIV IFNg Pol2 pep subpool (Mos2) HIV IFNg Pol3 pep subpool (Mos2) HIV IFNg Pol4 pep subpool (Mos2) HIV IFNg Pol5 pep subpool (Mos2) HIV IFNg Pol6 pep subpool (Mos2) HIV IFNg Pol7 pep subpool (Mos2) HIV IFNg Pol8 pep subpool (Mos2) HIV IFNg Pol9 pep subpool (Mos2) HIV IFNg Pol10 pep subpool (Mos2) HIV IFNg Pol11 pep subpool (Mos2) HIV IFNg Pol12 pep subpool (Mos2) HIV IFNg Pol13 pep subpool (Mos2) HIV IFNg Pol14 pep subpool (Mos2) HIV IFNg Pol15 pep subpool (Mos2) HIV IFNg Pol16 pep subpool (Mos2) HIV IFNg Pol17 pep subpool (Mos2) HIV IFNg Pol18 pep subpool (Mos2) HIV IFNg Pol19 pep subpool (Mos2) HIV IFNg Pol20 pep subpool (Mos2) HIV IFNg Pol21 pep subpool (Mos2)									
ICS	HIV ENV1 pep pool (Mos1) HIV ENV2 pep pool (Mos1) HIV Pol1 pep pool (Mos1) HIV Pol2 pep pool (Mos1) HIV Gag pep pool (Mos1)	Schuetz (MHRP)	W0 W26 W50 W60 W84 W96	CD4 for IFNg %:.03 CD8 for IFNg %:.09				% of CD4+/CD8+ T cells expressing IFNg	Only for IFNg: 1)Baseline <LLOQ: if post baseline values ≥LLOQ	NAP

	CD4+INFg+ CD8+INFg+ CD4+IL2+ CD8+IL2+ CD4+TNFa+ CD8+TNFa+ CD4+IL2-IFNg-TNFa+ CD4+IL2-IFNg+TNFa- CD4+IL2-IFNg+TNFa+ CD4+IL2+IFNg-TNFa- CD4+IL2+IFNg-TNFa+ CD4+IL2+IFNg+TNFa- CD4+IL2+IFNg+TNFa+ CD8+IL2-IFNg-TNFa+ CD8+IL2-IFNg+TNFa- CD8+IL2-IFNg+TNFa+ CD8+IL2+IFNg-TNFa- CD8+IL2+IFNg-TNFa+ CD8+IL2+IFNg+TNFa- CD8+IL2+IFNg+TNFa+ CD4+CD154+ CD4+CD154+INFg+ CD4+CD154+IL2+ CD4+CD154+TNFa+ CD8+CD107a+ CD8+CD107a+INFg+ CD8+CD107a+IL2+ CD8+CD107a+TNFa+ CD8+CD107a+GrzB+ CD4+CD154+GrzB+ CD4+GrzB+ CD4+IL4+ CD4+IL4+IL2+ CD4+IL4+INFg+ CD4+IL4+IL2-INFg+ CD4+IL4+IL2+INFg-								2)Baseline >=LLOQ : if post baseline values are 2 times the baseline value	

a: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

7. EFFICACY ANALYSIS

The frequency and duration of sustained viremic control (> 24 weeks of plasma HIV RNA <50 copies/ml) after ATI will be tabulated. In addition this will be repeated for HIV RNA < 1000 copies/ml).

A tabulation will be provided of the restart of ART. Median (min, max, quartiles) time to ART restart will also be tabulated.

A Kaplan-Meier plot of the time to ART restart after ATI together with a log-rank test will be provided comparing the placebo and the vaccine group.

Individual subject profiles of plasma HIV RNA copies/ml will be graphically shown together with relevant data (eg start of ART reinitiating and immunogenicity assay results (titer, %), biomarkers of HIV reservoir (HIV total DNA), acute retroviral syndrome, CD4/CD8 cell counts) aligning timepoints.

For the total HIV DNA the frequency of subjects that are undetectable at each timepoint will be tabulated and graphically shown.

If corresponding data is available the following analyses will be performed:

A tabulation will be made on any viral resistance that occurred after ATI.

A listing with subjects experiencing acute retroviral syndrome post ARV ATI will be provided.

The molecular sequence sieve effects of vaccine therapy on breakthrough rebound viremia before and after cessation of therapy will be evaluated.

The peripheral blood mononuclear cells (PBMC) phenotype, pattern of soluble factors and immune functional responses before and after ARV ATI in both the vaccine and placebo arms and compared to historical untreated acute infection cases in RV217 will be evaluated.

7.1. Efficacy Assays

LBTESTCD	LBTEST	LBDESCR	LBCAT	LBSPEC	LBMETHOD	LBSCAT	LBORRESU	LBLLOQ	LBLOD	TESTTYPE	LBNAM	POST PROCESSING COMMENTS
HIVDNA	HIV DNA	PBMC HIV DNA	CHEMISTRY	PERIPHERAL BLOOD MONONUCLEAR CELL	QUANTITATIVE REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION		copies/10E6 cells	x	x	CONTINUOUS	x	
HIVVLD	HIV Viral Load	Quantitative HIV RNA	CHEMISTRY		COBAS TAQMAN		Copies/mL			CONTINUOUS		