Janssen Vaccines & Prevention B.V.*

Statistical Analysis Plan

A Staged Phase 3 Study, Including a Double-Blinded Controlled Stage to Evaluate the Safety and Immunogenicity of Ad26.ZEBOV and MVA-BN-Filo as Candidate Prophylactic Vaccines for Ebola

Protocol VAC52150EBL3001; Phase 3

VAC52150 (Ad26.ZEBOV/MVA-BN-Filo [MVA-mBN226B])

*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.

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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

SAP Version 1.0 was approved on May 13th, 2015, when amendment 1 of the protocol was applicable.

SAP Version 2.0 was approved on March 15th, 2016, when amendment 3 of the protocol was applicable. The major change in the SAP reflects that stage 3 is no longer applicable.

SAP Version 3.0 was approved on May 6th, 2017, when amendment 5 of the protocol was applicable. The major changes are collection of Immediate Reportable Events (IREs), a third vaccination in stage 1 and extension of the FU period in stage 2 adults for 2 years.

SAP Version 4.0 was approved on March 21, 2018, when amendment 5 of the protocol was applicable. Only minor changes and clarifications were implemented before the analysis of the healthy adults of Stage 1 and 2.

SAP Version 5.0 was approved on June 22, 2018, when amendment 6 of the protocol was applicable. The overall rationale for this amendment**:** The purpose for this amendment is to align all the statistical analyses of Phases 2 and 3 Ebola studies and to address a few remarks from the Food and Drug Administration (FDA). The changes made together with the rationale of each change are as follows:

Rationale: Third dose for subjects in Stage 1 added. Windowing of immunogenicity samples adapted (in relation to late boost samples).
2.1. Analysis Visit Windows and Periods

Rationale: An exploratory crossreactivity analysis against SUDV/MARV for the enzyme-linked immunosorbent assay (ELISA) and the Virus Neutralization Assay (VNA) has been added, which was not mentioned in the clinical trial protocol. This is also documented as a change in the planned analysis.
6.3. Immunogenicity against SUDV/MAV GP

Rationale: Text is added to clarify that dot plots will be generated for both ELISA and VNA.
6.2.1.3 Analysis Methods

Rationale: Text is added to clarify that summary tabulations of SAEs, AEs with fatal outcome, AEs leading permanent discontinuation from boost vaccination, Grade 3 AEs and IREs will be presented by System Organ Class (SOC) and Preferred Term (PT).
7.1.3. Analysis Methods

Rationale: It is clarified that imputation of missing end dates of ongoing AEs will only be used to derive the duration of the events. Nevertheless, all missing AE end dates will be kept as unknown in the analysis dataset and listings.
Attachment 1: PERIOD ALLOCATION OF ADVERSE EVENTS

Rationale: It is clarified that summary tabulations of solicited AEs will count each event once (i.e., assigning the highest grade and the relatedness that most implicates the vaccine) within an analysis period and that there will not be multiple listings of the same AE on the same day.

Attachment 4: TRANSFORMING ON-SITE ASSESSMENTS AND DIARIES ASSESSMENTS OF SOLICITED ADVERSE EVENTS INTO AN ANALYSIS FORMAT

ABBREVIATIONS

AE	adverse event
aMLV	amphotropic murine leukemia virus
BMI	body mass index
CDC	Center for Disease Control and Prevention
CI	confidence interval
CRF	case report form
CSR	Clinical Study Report
IDMC	Independent Data Monitoring Committee
DPS	Data Programming Specifications
EBOV GP	Ebola virus (formerly known as Zaire ebolavirus) Mayinga glycoprotein
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EU/mL	ELISA units/mL
FAS	Full Analysis Set
FDA	Food and Drug Administration
ICH	International Council on Harmonization
IRE	Immediate Reportable Events
ITT	Intent-to-Treat
IVRS/IWRS	Interactive Voice/Web Response System
LLOQ	Lower limit of quantification
MARV GP	Marburg virus Musoke glycoprotein
MedDRA	Medical Dictionary for Regulatory Activities
NSAIDs	non-steroidal anti-inflammatory drugs
PI	principal investigator
PP	Per Protocol
PRNT	plaque reduction neutralization test
SUDV GP	Sudan virus glycoprotein
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SE	standard error
VNA	virus neutralization assay
WHO	World Health Organization

1 INTRODUCTION

This is the statistical analysis plan (SAP) for the Phase 3 VAC52150EBL3001 study of the Ebola project. This SAP will be applicable to the study results in Stage 1 and 2 and is based on Amendment 6 to the clinical protocol, approved 20 June 2018.

In this SAP, prime and boost vaccines will be used interchangeably as Dose 1 and Dose 2 vaccines, respectively. The programming specifications are described in the Data Presentation Specifications (DPS) document.

1.1 Trial Objectives

The primary objective of Stage 1 is:

- To evaluate the safety of a heterologous prime-boost regimen utilizing Ad26.ZEBOV and MVA-BN-Filo given at a 56-day interval.

The secondary objective of Stage 1 is:

- To assess humoral immune responses to a heterologous prime-boost regimen utilizing Ad26.ZEBOV and MVA-BN-Filo as measured by an enzyme-linked immunosorbent assay (ELISA) at 21 days post-boost vaccination.
- To assess the safety and tolerability of a third vaccination using Ad26.ZEBOV administered at least 2 years post prime.

The exploratory objectives of Stage 1 are:

- To assess humoral immune responses to a heterologous prime-boost regimen utilizing Ad26.ZEBOV and MVA-BN-Filo as measured by ELISA at other relevant time points.
- To assess neutralizing antibody responses directed against EBOV GP induced by the heterologous prime-boost regimen as measured by a virus neutralization assay (VNA) depending on sample and assay availability.
- To assess antibody responses directed against the Ad26 and/or MVA vector as measured by VNA, and/or plaque reduction neutralization test (PRNT) depending on sample and assay availability.
- To assess humoral immune responses to a heterologous prime-boost regimen utilizing Ad26.ZEBOV and MVA-BN-Filo as measured by ELISA following a third vaccination using Ad26.ZEBOV at 2 years post prime.
- To assess crossreactivity against SUDV/MARV for the enzyme-linked immunosorbent assay (ELISA) and the Virus Neutralization Assay (VNA).

The primary objective of Stage 2 is:

- To evaluate the safety of a heterologous prime-boost regimen utilizing Ad26.ZEBOV and MVA-BN-Filo given at a 56-day interval compared to an active control vaccine.

The secondary objective of Stage 2 is:

- To assess humoral immune responses to a heterologous prime-boost regimen utilizing Ad26.ZEBOV and MVA-BN-Filo as measured by an ELISA at 21 days post-boost vaccination.

The exploratory objectives of Stage 2 are:

- To assess humoral immune responses to a heterologous prime-boost regimen utilizing Ad26.ZEBOV and MVA-BN-Filo as measured by ELISA at other relevant time points.
- To assess neutralizing antibody responses directed against EBOV GP induced by the heterologous prime-boost regimen as measured by VNA depending on sample and assay availability.
- To assess antibody responses directed against the Ad26 and/or MVA vector as measured by VNA, and/or PRNT depending on sample and assay availability.
- To assess crossreactivity against SUDV/MARV for the enzyme-linked immunosorbent assay (ELISA) and the Virus Neutralization Assay (VNA).

1.2 Trial Design

This is a staged Phase 3 study with an open-label uncontrolled stage (Stage 1) and a double-blinded controlled stage (Stage 2) to evaluate the immunogenicity and safety of a heterologous prime-boost regimen where Ad26.ZEBOV at a dose of $5x10^{10}$ vp will be used to prime a filovirus-specific immune response and MVA-BN-Filo at a dose of $1x10^{8}$ Inf U will be used to boost the immune response 56 days later. In Stage 1, a third vaccination using Ad26.ZEBOV will be administered at least 2 years post prime to subjects who consent to this.

The study will be conducted as follows:

- Stage 1: The study will commence with vaccination of a group of approximately 40 adult subjects aged 18 years or older. The objective of this initial stage of the study is to evaluate the safety and immunogenicity of the prime-boost regimen in the adult Sierra Leonean population.
- Stage 2: Approximately 976 subjects aged 1 year or older will be individually randomized in a 3:1 ratio to receive the Ad26.ZEBOV/MVA-BN-Filo prime-boost regimen or an active control vaccine and placebo. The aim is to enroll approximately 400 adults (aged 18 years or older) and approximately 576 children aged ≥1 year (with about 192 children in each of the 3 age groups [i.e., 12 to 17 years, 4 to 11 years, and 1 to 3 years, inclusive]). Enrollment will be staggered, starting with the eldest age group. The decision to proceed to the next age group will be based on an evaluation by the Independent Data Monitoring Committee (IDMC). Randomization will be stratified by age group.

1.3 Statistical Hypotheses for Trial Objectives

As this study is designed to provide descriptive information regarding safety and immunogenicity without formal treatment comparisons, no formal statistical hypothesis testing is planned.

1.4 Sample Size Justification

See section 11.3 of the protocol [(1)].

1.5 Randomization and Blinding

Randomization

There will be no randomization in Stage 1. However, assignment for the third vaccination of subjects enrolled in Stage 1 will be done by Interactive Voice/Web Response System (IVRS/IWRS).

The randomization through IVRS/IWRS in Stage 2 will be 3:1 (Ad26.ZEBOV/MVA-BN-Filo: active control) and will be stratified by age group.

Blinding

Blinding procedures are not applicable for Stage 1.

In Stage 2, study-site personnel (except for those with primary responsibility for study vaccine preparation and dispensing), sponsor personnel and subjects will be blinded to the study vaccine allocation until all subjects have completed the Day 360 post-prime visit (children and adolescents) or the 2-year post-prime visit (adults) or were discontinued earlier.

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

If interim analyses are performed, the randomization codes and, if required, the translation of randomization codes into treatment and control groups, will be disclosed to those authorized and only for those subjects included in the interim analysis. In case of an interim analysis, the randomization codes for adults (≥18 years) and for children (≥1 to 17 years) will be disclosed separately.

For further details on blinding see protocol section 5.

2 GENERAL ANALYSIS DEFINITIONS

Data from Stage 1 will be summarized separately. Data from Stage 2 will be shown by randomization group, and by age group. A baseline (or reference) value will be defined as the value of the last available assessment performed prior to the prime vaccination on Day 1.

Unless otherwise specified, the statistical analysis will present all results by period, except for subject information sections. For immunogenicity, vital signs, physical examination and lab parameters, data will be presented per time point as appropriate.

Summary statistics of the study data will be presented by vaccine regimen. Assessments over time are reported for each period or visit (see Section 2.1).

- Categorical variables will be summarized with a frequency table showing counts and percentages.

- Continuous variables will be summarized using the following statistics, as appropriate: number of observations, geometric mean, arithmetic mean (mean), 95% confidence interval (CI) for the mean, standard deviation (SD), standard error (SE), median, quartiles (Q1 and Q3), minimum and maximum.

- Binomial variables will be summarized using the following statistics: number of observations, percentages and Exact Clopper-Pearson 95% confidence interval (CI).

Unless otherwise specified, listings will only be generated for the primary and final analyses, as appropriate.

2.1 Analysis Windows and Analysis Periods

For descriptive statistics over time, assessments (regardless of the investigated parameter) will be allocated to an analysis visit based on the actual date, visit slotting will be done as specified below.

The number of days in the phase (relday) will be defined as:

relday = visit day – reference day+1 for visits on or after the reference day,
relday = visit day – reference day for visits before the reference,

where the reference day equals the date of the vaccination. For some timepoints the second (or third) vaccination will be used as the reference day (see Table 1 below).

All visits will be allocated to the following time points, based on the relday. A per protocol time interval is defined for immunogenicity analysis only. The windows are defined as per protocol.

Table 1: Visit Windows – Stage 1 and 2

Analysis timepoint	**Target Day**	**Time interval PP (days)**	**Time interval FAS (days)**	**Comments**
Day 1 (Baseline) ^	1	≤1	≤1	
Day 8 (7 days Post-dose 1)°	8	NA	[2 – 18]	
Day 29 (28 days Post-dose 1)°	29	[22 – 36]	[19 – 43]	Only applies to stage 2
Day 57 (56 days Post-dose 1)#°	57	[50 – 64]	[44 – 71]	
Late Boost#			≥72	Only applies to stage 2
Day 64 (7 days Post-dose 2)	8*	NA	[2-11]*	
Day 78 (21 days Post-dose 2)	22*	[15-29]*	[12-45]*	
Day 156 (155 days Post-dose 1)	156	[149-163]	[126-186]	Only applies to stage 1
Day 237 (180 days Post-dose 2)	180*	[150-210]*	[136-270]*	Only applies to stage 2
Day 361 (360 days Post-dose 1)	360	[330-390]	[300-450]	
Day 541 (540 days Post-dose 1)	540	[510-570]	[451-630]	
Day 721 (720 days Post-dose 1)	720	[690-750]	[631-810]	Only applicable for stage 1 if no 3rd vaccination took place
Day 901 (900 days Post-dose 1)	900	[870-930]	[811-990]	
Day 1081 (1080 days post-dose 1)	1080	[1050-1110]	[991-1170]	

(Table continues on next page)

° : Only if before or on the day of dose 2.

(Table continued from previous page)

Visit windows which only apply to stage 1 subjects who received a third vaccination				
Day 721 (720 days Post-dose 1)^	720	[720-810]	[631-810]	
Day 725 (4 days Post-dose 3)	5	[5 – 5]**	[2-6]**	
Day 728 (7 days Post-dose 3)	8**	[8 – 8]**	[7-11]**	
Day 742 (21 days Post-dose 3)	22**	[15-29]**	[12-45]**	
Day 901 (180 days post-dose 3)	180**	[150-210]**	[46-270]**	
Day 1081 (360 days post-dose 3)	360**	[330-390]**	≥271**	

NA: Not applicable, no immunogenicity assessment
*: Reference days are calculated relative to second vaccination (Boost).
**: Reference days are calculated relative to third vaccination (Boost).
^: For safety assessments, the date and time of the baseline assessment should fall before the date and time of first vaccination.
#: For immunogenicity, in case the day 57 Post prime/24 months post prime is used as a reference, the assessment should fall on the same date as the second vaccination. Similarly, for subjects that have consented to receive the third vaccination, in case the day 721 (720 days post prime) is used as a reference, the assessment should fall on the same date as the third vaccination. If an immunogenicity sample is taken on the day of late boost, this sample will also be used as a reference in the FAS analysis only, as late boost subjects are excluded from the per protocol analysis set.

For immunogenicity assessments or in case time is missing, the date of the baseline assessment should fall before or on the date of first vaccination.

Immune response measurements obtained after a missed second or a missed third dose will not be included in tables or graphs. They will however be included in listings, and marked as “not used in the analysis”.

Only the analysis time points and assays that are in scope for a specific statistical analysis (e.g. Interim Analysis, Final Analysis) are to be considered when assigning assessments to analysis time points. If two assessments fall within the same interval, the one closest to the target day will be used for the descriptive statistics/tabulations per time point and graphics to have only one evaluation per subject per analysis time point. If distances of both assessments to the target day are equal, the measurement with the latest date will be used. Only planned analysis timepoints are shown in tabulations and graphics.

All assignments will be made in chronological order. Once an assessment is assigned to an analysis window, it will no longer be used for a later window.

For adverse events, laboratory abnormalities, vital signs abnormalities and concomitant therapies, analysis periods will be constructed as follows:

Table 2: Period Definitions

Analysis period		**Interval**	
		From	**To**
Screening		00:00 of the date of signing the informed consent form	One minute prior to Dose 1 on Day 1
Post-dose 1		Date and time of Dose 1	Minimum of: a) 23:59 on the date of last contact (for early study discontinuations) b) 23:59 on the date of database cut-off in case of interim analysis c) 23:59 at relative Day 29 post-dose 1 d) One minute prior to Dose 2
Post-dose 1 Follow-up (FU)		One minute after the end of post-dose 1 period	Minimum of: a) 23:59 on the date of last contact (for early study discontinuations) b) 23:59 on the date of database cut-off in case of interim analysis c) One minute prior to Dose 2
Post-dose 2		Date and time of Dose 2	Minimum of: a) 23:59 on the date of database cut-off b) 23:59 on the date of last contact (for early study discontinuations) c) 23:59 at relative Day 29 post-dose 2
Post Dose 2 Follow-up (FU)		One minute after the end of post-dose 2 period	For Stage 1 Subjects who consented to receive the 3rd dose: Minimum of: a) 23:59 on the date of last contact (for early study discontinuations) b) 23:59 on the date of database cut-off in case of interim analysis c) One minute prior to Dose 3 For all other subjects: Minimum of a) 23:59 on the date of database cut-off b) 23:59 on the date of last contact (for completion or early study discontinuations)
Post-dose 3*		Date and time of Dose 3	Minimum of: a) 23:59 on the date of last contact (for early study discontinuations) b) 23:59 on the date of database cut-off in case of interim analysis c) 23:59 at relative Day 29 post-dose 3
Post-dose 3 Follow-up (FU)*		One minute after the end of post-dose 3 period	Minimum of a) 23:59 on the date of database cut-off b) 23:59 on the date of last contact (for completion or early study discontinuations)

*: only for subjects in Stage 1 that consented to receive the third dose.

Remarks:

- Subjects who did not receive Dose 2/Dose 3 will not have a Post-dose 2/dose 3 period.

2.2 Pooling of data

Results will be pooled over sites.

2.3 Analysis Sets

2.3.1 Full Analysis Set

The Full Analysis Set (FAS) which will include all subjects who received at least one study vaccination in stage 1 or stage 2. Data will be shown by treatment group (as treated).

"As treated" should be interpreted as follows: subjects who receive only the prime vaccine (AD26.ZEBOV or Pbo) will be included in the respective treatment groups AD26-MVA or Pbo-Pbo. Subjects who receive a regimen that is not planned for in the study (for example, Ad26.ZEBOV prime/Ad26.ZEBOV boost or MVA-BN-Filo prime/MVA-BN-Filo boost or MVA-BN-Filo prime only) will be excluded from summary analyses (ie, tables and graphs) and listed separately.

The immuno analysis on the full analysis set (including those subjects who received a late boost vaccination because of the study pause) will be included as sensitivity analysis to investigate the impact on the immune response.

All safety analyses will be performed on the full analysis set.

2.3.2 Per Protocol Analysis set

The per protocol analysis set includes all randomized and vaccinated subjects, who received both the prime and boost vaccinations (administered within the protocol-defined window), have at least 1 post-vaccination (i.e., after the date of vaccination) evaluable immunogenicity sample, and have no major protocol violations influencing the immune response. Similarly, the per protocol analysis set for subjects in Stage 1 that have consented to receive a third boost, includes all randomized and vaccinated subjects, who received the prime and both boost vaccinations (administered within the protocol-defined visit window), have at least 1 post-vaccination (i.e., after the date of vaccination) evaluable immunogenicity sample, and have no major protocol violations influencing the immune response. The primary immunogenicity analysis will be performed on the per protocol analysis set.

2.4 Definition of Subgroups

Data from Stage 2 will be shown by age group (1-3 years, 4-11 years, 12-17 years, and 18 years or more).

For immunogenicity analysis on the FAS, subgroup analysis will be done for subjects having received or not having received the delayed boost. Details for which tables this will be applied will be given in the DPS.

3 CHANGES TO THE PLANNED ANALYSIS

The safety analysis set which is referenced in the protocol is replaced by the Full Analysis Set, but has the same definition. The Full Analysis Set will be used for analysis on subject information and safety.

The per protocol analysis set is redefined to exclude subjects whose boost vaccination falls outside the protocol-defined window.

To assess the cross-reactivity of the vaccine-induced immune responses against SUDV/MARV GP, exploratory analyses has been added in this SAP. Quantification of antibodies binding to SUDV/MARV GP using as measured by ELISA(ELISA units/mL (EU/mL)), as well as serum titers of neutralization antibody reactivity against SUDV/MARV GP using VNA [IC50 titer]) will be analyzed

4 INTERIM ANALYSIS AND DATA MONITORING COMMITTEE REVIEW

Interim analyses may be performed during the study. Their purpose is to support future program-related decisions and to fulfill regulatory requirements in a timely manner.

An IDMC will be established to monitor safety data on an ongoing basis to ensure the continuing safety of the subjects enrolled in this study.

For more details refer to Section 11.7 of the protocol [(1)].

5 SUBJECT INFORMATION

Subject information will be analyzed based on the FAS.

5.1 Demographics and Baseline Characteristics

Demographic characteristics and screening/baseline characteristics (e.g., physical examination, medical history) will be tabulated and summarized with descriptive statistics for the vaccinated group.

The following demographic and baseline characteristics will be summarized.

- Sex (Female/Male)
- Age (years)
- Age category (years) matching the cohorts
- Race
- Ethnicity
- Height (cm)
- Weight (kg)
- BMI (kg/m^2), calculated from the recording of baseline height and weight for adults and adolescents (subjects aged >11 years).
- Weight-for-Length for children 1 to 2 years of age based on WHO growth charts

- Weight-for-Age for children aged 2-11 years based on Center for Disease Control and Prevention (CDC) growth charts
- The charts are available from the link below: https://www.cdc.gov/growthcharts/who_charts.htm#The%20WHO%20Growth%20Charts

4.1.3. Description of the procedure to determine the Weight-for-Length percentile

Reference materials can be found on this link for boys: https://www.cdc.gov/growthcharts/who/boys_weight_head_circumference.htm, and on this link for girls: https://www.cdc.gov/growthcharts/who/girls_weight_head_circumference.htm.

First round the length of the subject to the nearest half centimeter. Using the appropriate chart (boys vs. girls), determine the values of L, M, and S to use. Use the following formula to calculate the z-score:

$$z = \frac{\left(\left(\frac{weight}{M}\right)^{L} - 1\right)}{(S \times L)}$$

Using the Standard Normal distribution, determine the percentile this z-value corresponds to.

Example:
Sex: female, length (rounded to nearest half centimeter): 51 cm, weight: 3.45kg. From the WHO chart, L = -0.3833, M = 3.5636, S = 0.09076. The calculated z-score is:

$$z = \frac{\left(\left(\frac{3.45}{3.5636}\right)^{-0.3833} - 1\right)}{(0.09076 \times -0.3833)} = -0.35918$$

This z-score corresponds to the 36th percentile (rounded). This can be found using the Sas CDF function (for this example: CDF('NORMAL',-0.35918)), or with the R pnorm function (for this example, pnorm(-0.35918)).

4.1.4. Description of the procedure to determine the Weight-for-Age percentile

Reference materials (in Excel format) can be found on this link for boys and girls: https://www.cdc.gov/growthcharts/data/zscore/wtage.xls. Consult https://www.cdc.gov/growthcharts/percentile_data_files.htm for more information on the data contained in the Excel file and instructions on how to calculate the exact percentile.

First determine the age point to use. Age at the date of the corresponding weight assessment is to be used. Age is listed at the half month point for the entire month; for example, 1.5 months represents 1.0-1.99 months or 1.0 month up to but not including 2.0 months of age. Using the appropriate chart (boys vs. girls), determine the values of L, M, and S to use. The remainder of the procedure is identical to the one described for the WHO.

5.2 Disposition Information

The number and percentage of subjects screened, randomized (stage 2), vaccinated and included in each analysis set will be tabulated.

Number and percentage of subjects in the full analysis set that completed the study and those who were ongoing or discontinued together with the reason(s) for discontinuation will be tabulated. This will be done for completion/discontinuation from vaccination and from the study.

5.3 Protocol Deviations

All major protocol deviations will be tabulated by the deviation category. Major protocol deviations that may have an influence on immune response will be flagged in a listing.

5.4 Medical History

Medical history information will be listed.

5.5 Concomitant Medications

The analysis of concomitant therapies will be done using the World Health Organization (WHO) drug coded terms as provided in the clinical database.

Based on their start and stop date, concomitant therapies will be reported in each analysis period during which they were applied. For missing or partial start and/or stop dates (time and/or day and/or month and/or year) the following allocation rules will be applied:

- 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.

Concomitant therapies will be tabulated per period (including the follow-up periods). A listing of all concomitant therapies will be provided. All therapies taken within 30 days prior to signing of the informed consent form will also be listed.

There will be special attention to analgesics/antipyretics such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin, administered until day 8 post-vaccination (including the day of vaccination) following each vaccination.

Remarks:

In addition to the date information, time information is considered to allocate concomitant therapies to periods, if available.

6 IMMUNOGENICITY

The primary analysis of immunogenicity will be done on the per protocol analysis set (as defined in Section 2.3).

6.1 Immunogenicity against EBOV GP

6.1.1 Humoral Immune Response

6.1.1.1 Parameters

Humoral immune responses, as measured by the following assay, will be analyzed.

- **Binding antibody responses using Filovirus Animal Nonclinical Group (FANG) enzyme-linked immunosorbent assay (ELISA):** Quantification of antibodies binding to EBOV GP using ELISA units/mL (EU/mL).

For ELISA antibody response with more than one result per sample (per subject and per time point), the antibody response will be taken as the median of the results of that sample.

In addition, the following will be defined for ELISA (EU/mL):

- **Sample interpretation:** a sample will be considered positive, if the value is above the assay-specific lower limit of quantification (LLOQ).
- **Responder**:
  - If sample interpretation is negative at baseline but positive post-baseline and the post-baseline value is greater than 2.5 x LLOQ; OR
  - If sample interpretation is positive at both baseline and post-baseline and there is a greater than 2.5-fold increase from baseline (2.5-fold increase on the original scale).

Humoral immune responses, as measured by the following assay, will also be analyzed.

- **Neutralization antibody responses using virus neutralization assay (VNA):** Serum titer of neutralizing antibody reactivity against EBOV GP (inhibitory concentration [IC_{50} titer])

In addition, the following will be defined for VNA parameters:

- **Sample interpretation**: a sample will be considered positive, if the value is above the assay-specific lower limit of quantification (LLOQ) and if the value is greater than 3x the IC_{50} titer from amphotropic murine leukemia virus [aMLV] otherwise the sample is considered negative.
- **Responder**:
  - If sample interpretation is negative at baseline but positive post-baseline and the post-baseline value is greater than 2 x LLOQ; OR
  - If sample interpretation is positive at both baseline and post-baseline and there is a greater than 2.0-fold increase from baseline (2.0-fold increase on the original scale).

6.1.1.2 Handling of Missing/Invalid Data

ELISA:
For ELISA binding antibody responses, values below the LLOQ will be imputed with LLOQ/2 EU/mL.

For the calculation of the fold increases, values of binding antibody responses below the LLOQ will be imputed with LLOQ EU/mL. The LLOQ is provided in the database.

VNA:
If the EBOV IC_{50} titer value is <3x the aMLV IC_{50} titer, it will be imputed with half of the assay-specific LLOQ for the calculation of geometric means.

For the calculation of the fold increases, values of neutralizing antibody responses less than 3x aMLV will be imputed with the assay-specific LLOQ.

Note: If the aMLV IC_{50} titer (negative control) is a censored value, i.e., <40, the aMLV IC_{50} titer will be imputed with 40.

6.2 Immunogenicity against Ad26 and/or MVA vector

For immunogenicity against the Ad26, a vector specific reference value will be defined as follows: the reference value of the considered vector is the value closest or at the date of administration of that vector.

6.2.1 Humoral Immune Response

6.2.1.1 Parameters

Humoral immune responses of the various regimens tested, as measured by the following assays, will be analyzed.

- **Ad26 virus neutralization assay (VNA):** titers of neutralizing antibodies to the Ad26 vector (unit: IC_{90} titer)
- **MVA plaque reducing neutralization test (PRNT):** Serum titers of neutralizing antibodies to the MVA vector (unit: IC_{50} titer)

The following will also be defined for Ad26 VNA and MVA PRNT titers:

- **Sample interpretation:** a sample will be considered positive, if the value is above the corresponding assay LLOQ.

These immune response values will be $\log_{10}$-transformed before any further handling. Results will be presented on the $\log_{10}$ scale, geometric mean titers and geometric mean increases with corresponding 95% CIs will be provided in the tables.

6.2.1.2 Handling of Missing/Invalid Data

For all outcomes (Ad26 VNA, MVA PRNT), values below the corresponding LLOQ will be imputed with half of the LLOQ (LLOQ/2).

6.3 Immunogenicity against SUDV/MARV GP

6.3.1 Humoral Immune Response

6.3.1.1 Parameters

Humoral immune responses, as measured by the following assay, will be analyzed.

- Binding antibody responses using enzyme-linked immunosorbent assay (ELISA): Quantification of antibodies binding to SUDV/MARV GP using ELISA units/mL (EU/mL).
- Neutralization antibody reactivity against SUDV/MARV GP using VNA (IC50 titer)

6.4 Analysis

Summary statistics of actual values (on the $\log_{10}$ scale) will be presented for all immunogenicity parameters at each time point. Geometric mean and fold increase (from Pre-dose 1, Pre-dose 2 and Pre-dose 3, if applicable) with corresponding 95% CI will be calculated for ELISA antibody responses (EU/mL) and VNA (IC_{50} titer).

Humoral immune responses will be graphically represented using regimen profile plots for both ELISA and VNA outcomes. If appropriate, additional graphical representations (on a log10-scale) will be provided for ELISA and VNA using dot plots (with distinction between responders and non-responders), by vaccination schedule.

Responders and sample interpretation over time will be summarized, for ELISA (EU/mL) and VNA (IC_{50} titer) parameters.

Correlations between the different assays (ELISA, VNA) and correlation between antibodies binding to EBOV GP and antibodies binding to SUDV/MARV GP using ELISA (units: EU/mL) will be investigated.

Separately for each of the assays (ELISA, VNA), a linear mixed model with the unstructured variance-covariance and an unstructured mean (i.e., including time as a categorical variable) will be used to estimate the geometric mean concentrations and the associated 95% CI at these timepoints.

Positive sample interpretation (for Ad26 VNA and MVA PRNT) will be summarized at baseline together with corresponding 95% exact Clopper-Pearson CIs.

7 SAFETY

No formal statistical testing of safety data is planned. Safety data will be performed on the full analysis set. There will be two sets of safety tables:

- one set for the stage 1 and stage 2 adult safety data (shown by stage and treatment group [as treated]),

- and one set for the stage 2 adolescents and children safety data (shown by age group and treatment group [as treated]). The age groups are: 12-17 years, 4-11 years, and 1-3 years. Of note, an "All subjects" column will also be present in the adolescents and children tables, which will pool the data of the 1-17 year-old subjects.

Safety data will be analyzed descriptively for subjects receiving vaccination (including 95% confidence intervals, if applicable).

Baseline for all safety parameters will be defined as the last value before the first dose.

The safety and tolerability data include the following:

- Solicited local and systemic adverse events (reactogenicity) until 7 days post vaccination.
- Unsolicited adverse events, from provision of informed consent onwards until 56 days after the boost vaccination in Stage 1 and then again from the day of the third vaccination until 28 days after the third vaccination, and from provision of informed consent onwards until 28 days after the prime vaccination in Stage 2 and then again until 28 days after the boost vaccination.
- Serious adverse events (SAEs) and immediate reportable events (IREs) until the end of the study.

7.1 Adverse Events

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

For Stage 1 and 2, the following summaries are planned:

All reported adverse events (solicited local, solicited systemic, and unsolicited) after the first vaccination will be attributed to analysis periods as defined in Attachment 1. For each adverse event, the number and percentage of subjects who experience at least one occurrence of the given event will be summarized. Summaries, listings, datasets and/or subject narratives may be provided as appropriate, for those subjects who die, discontinue study vaccinations due to an adverse event, or experience a severe or serious adverse event.

7.1.1 Definitions

Solicited AEs are precisely defined events (local and systemic) that subjects are specifically asked about and which are noted by subjects in the diary. All other AEs are considered unsolicited. Please refer to CTP[1], Section 9.3.2 for further details.

Causality

Solicited local AEs will be considered as related to the study vaccine. Unsolicited and solicited systemic 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. Please refer to CTP [(1)], Section 12.1.2 for further details.

Severity

The severity of the AEs is classified as mild, moderate or severe. Please refer to CTP [(1)], Section 12.1.3 for further details. Solicited events that are graded less than mild, are not considered as AE.

For induration/swelling both the diameter and grading as reported by the investigator are reported in the eCRF. Diameter will be used to derive the toxicity grading. The highest grading should be used when both the diameter grading and the investigator-reported grading are available

7.1.1.1 Solicited Local (Injection Site) Reactions

The analysis of solicited local AEs will include:

- Pain/Tenderness
- Erythema
- Swelling/Induration
- Itching

7.1.1.2 Solicited Systemic Adverse Events

The analysis of systemic solicited adverse events will include:

for preverbal children/infants:

- fever
- vomiting
- reduced activity, somnolence, fatigue
- irritability/fussiness/crying/screaming
- loss of appetite

for young children, adolescents, and adults:

- Chills
- Headache
- Fatigue/Malaise
- Myalgia
- Nausea/Vomiting
- Arthralgia
- Fever (defined as body temperature of 38°C or higher)

7.1.1.3 Immediate Reportable Events

The following list of neuroinflammatory disorders are categorized as IREs, and should be reported to the sponsor within 24 hours of becoming aware of the event.

- Cranial nerve disorders, including paralyses/paresis (e.g., Bell's palsy)
- Optic neuritis
- Multiple sclerosis

- Transverse myelitis
- Guillain-Barré syndrome, including Miller Fisher syndrome, Bickerstaff's encephalitis and other variants
- Acute disseminated encephalomyelitis, including site specific variants: e.g., non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis
- Myasthenia gravis and Lambert-Eaton myasthenic syndrome
- Immune-mediated peripheral neuropathies and plexopathies (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy)
- Narcolepsy
- Isolated paresthesia of >7 days duration

7.1.2 Imputation

Missing data will not be imputed. If relationship of AEs to the study vaccine could not be derived (i.e., missing or unknown), it will be considered as unknown, for analysis purposes. Solicited local AEs will be considered as related with the use of the study vaccine. Missing relationship will not be imputed.

Period allocation of AEs is defined in Attachment 1

7.1.3 Analysis

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 (SOC) and Preferred Term (PT). The solicited AEs will be summarized by class (local, systemic) and preferred term. For solicited as well as unsolicited AEs, tables focusing on severity and relatedness to vaccine will be created.

SAEs, AEs with fatal outcome, AEs leading to permanent discontinuation of the study vaccine, Grade 3 AEs and IREs will be listed. Tables, summarizing all those parameters will also be created. Tables will be presented after each vaccination and by regimen. Summary tabulations by SOC and PT for each of the category of events (i.e., SAEs, AEs with fatal outcome, AEs leading to permanent discontinuation from boost vaccination, Grade 3 AEs and IRE's) will also be generated for the entire reporting period.

For the solicited local and systemic AEs, the duration and time to first onset of the events will 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 start date of each vaccination period. Duration and time to onset of AEs will be expressed in days.

Summaries, listings, datasets and/or subject narratives may be provided as appropriate, for those subjects who die, discontinue study vaccinations due to an AE, or experience a severe or SAE.

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

A listing of all SAEs during the study period will be presented. A listing of all IREs (if flagged in the database) will be provided as well.

7.2 Clinical Laboratory Tests

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)

Toxicity grades will be determined according to the scales in Attachment 2 and 3. For adults and adolescents, the FDA toxicity grading scale will be used, for children, the DMID grading scale will be used. 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:

- Highest grades/worst abnormalities are determined over the entire period (e.g., Post-dose 1, Post-dose 2 or Post-dose 3) separately, including all post-baseline measurements of the corresponding period.
- The abnormalities "abnormally low" and "abnormally high" are considered equally important and both abnormalities are shown in the tables. (This means that the sum of the percentages can exceed 100%).
- If a laboratory value falls within the grading as specified in the grading table but also within the local laboratory normal limits, the value is considered as normal or Grade 0.
- Laboratory results falling between the grading scales will be allocated to the adjacent worst-case grade (because the scale for some parameters in the grading table is not continuous as there may be zones where toxicity grade definitions do not exist).

Definition emerging abnormalities following vaccination: An abnormality (toxicity grade 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.

7.2.1 Analysis methodology

Laboratory data will be analyzed based on the full analysis set. The data will be summarized by the type of laboratory test.

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 vaccination group under evaluation.

Tabulations of the highest emerging graded abnormalities per period and overall will be provided. Special attention will be given to subjects who develop grade 3 toxicities. For tests that have no grading, tabulations of the worst emerging abnormalities (below/ above), will be performed.

7.3 Vital Signs and Physical Examination Findings

Vital sign abnormalities will be determined in accordance with the DMID Vital Signs Toxicity Grading (attachment 2.3.) for adults and adolescents. The worst abnormalities (following vaccination) of vital signs will be tabulated (i.e., showing number and percentage) and listed.

For children, vital signs will be summarized and listed without grading. This summary will be presented per time point and showing the mean, standard deviation, median, Q1, Q3, minimum and maximum. Post-baseline assessments will be allocated to an analysis visit based on the eCRF visit; no visit slotting will be done. Scheduled visits will be used whenever available. If only unscheduled visits are available for a time point, the one closest to the scheduled visit will be used (in case of equidistance, the latest assessment will be selected). The baseline is the last available assessment prior to first vaccination.

Focus will be on the abnormalities that occur during the Post-dose 1, Post-dose 2 and Post-dose 3 periods, as well as the Regimen phase. The Post-dose 3 period is only applicable to subjects from Stage 1 that have consented to receive a third dose.

It is important to note that a full physical examination is only conducted at screening. At other visits, a brief, symptom-directed examination (including body length/height and weight in children aged 12 months up to and including 24 months at the time of the prime and the boost vaccination) will be performed based on any clinically relevant issues, clinically relevant symptoms and medical history. Therefore, only a listing of subjects with worst physical examination findings (i.e., abnormalities) following vaccination will be provided.

REFERENCES

1. Clinical Protocol VAC52150EBL3001: Amendment 5. A Staged Phase 3 Study, Including a Double-Blinded Controlled Stage to Evaluate the Safety and Immunogenicity of Ad26.ZEBOV and MVA-BN-Filo as Candidate Prophylactic Vaccines for Ebola (4 May 2017)

ATTACHMENTS

1 PERIOD ALLOCATION OF ADVERSE EVENTS

Solicited AEs are always allocated to the Post Dose 1, Post Dose 2, or Post Dose 3 period, and the regimen period. Unsolicited AEs are allocated to the different periods according to the following rules:

Step 1: Allocation of events to the periods:

Adverse events present 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).

Incomplete dates (i.e. time and/or 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 will be done. If, for instance, for 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 because of its assignment to multiple periods (see below example).
- In case of a completely missing end date (i.e., only for the calculation of duration):
  - In case the AE is not flagged as ongoing, the end date is considered as unknown, therefore the date will remain missing
  - In case the AE is flagged as ongoing 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.

  Examples:
  Screening period: start date: 14JUN2016 - stop date: 28JUN2016
  Post-dose 1 period: start date: 28JUN2016 - stop date: 19JUL2016

  1) Adverse event: start date: JUN2016- stop date: 15JUL2016
  As the start date only has information about month and year, only this information will be used from the periods (i.e. assuming any day of Jun is possible) and therefore the AE will be assigned to the Screening Period as well as to the Post-dose 1 Period.

  2) Adverse event: start date: JUL2016- stop date 14JUL2016
  As the AE starts after the Screening Period and after the start of the Post-dose 1 period, it is only assigned to the Post-dose 1 period.

  Remarks:
  - In addition to the date information, time information is considered to allocate AEs to periods, if available.
  - The imputation of missing end dates of ongoing AEs will only be used to derive the duration of the event (i.e., to give an indication of the minimum duration). The imputed end dates will not be shown in the data listings.

Step 2: Combination of events:
Overlapping/consecutive events are defined as events in 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 1 FU (i.e. non-active periods) - followed by an AE in - Post-dose 1 or Post-dose 2 (active periods) - 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.
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.
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 known.
2. In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.
3. Time is not considered when determining overlap of events.
4. Solicited AEs will never be attributed to the Screening Period

Examples:
Screening period: start date: 14JUN2016 - stop date: 28JUN2016
Post-dose 1 period: start date: 28JUN2016 - stop date: 26JUL2016
Post-dose 1 FU period: start date: 27JUL2016 - stop date: 15AUG2016

Example for the above scenario 1
AE1: start date: 20JUN2016- stop date: 10JUL2016
AE2: start date: 08JUL2016- stop date: 18JUL2016
AE1 will be attributed to the screening period and AE2 to the Post-dose 1 period.

Example for the above scenario 3
AE1: start date: 18JUL2016- stop date: 28JUL2016
AE2: start date: 28JUL2016- stop date: 08AUG2016
As AE1 starts in the active period (Post-dose 1) and overlaps with AE2 which starts in a non-active period (Post-dose 1 FU), this AE is considered as a single AE in the AE analysis starting on 18JUL2016 and ending on 08AUG2016 and is attributed to the post-dose 1 period.

2 TOXICITY TABLES FOR USE IN TRIALS ENROLLING HEALTHY ADULTS

The abbreviations used in the following tables are:

ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; AV block: atrioventricular block; bpm: beats per minute; CK: creatine kinase; FEV_1: forced expiratory volume in 1 second; g: gram; HI: high; HPF: high power field; INR: international normalized ratio; IV: intravenous; LO: low; mEq: milliequivalent; mm Hg: millimeter of mercury; N: not graded; PT: prothrombin time; PTT: partial thromboplastin time; QTc: QT-interval corrected for heart rate; QTcB: Bazett's corrected QT interval; QTcF: Fridericia's corrected QT interval; RBC: red blood cell; Rx: therapy; ULN: upper limit of normal

2.1 CLINICAL ADVERSE EVENTS IN HEALTHY ADULTS

Grading scale used for clinical adverse events is adapted from the Division of Microbiology and Infectious Diseases (DMID) Toxicity Tables (2014). For adverse events not included in the tables below, refer to the severity criteria guidelines in Section 12.1.3 of the CTP.

Cardiovascular	**Grade 1**	**Grade 2**	**Grade 3**
Arrhythmia		Asymptomatic, transient signs, no Rx required	Recurrent/persistent; symptomatic Rx required
Hemorrhage, blood loss	Estimated blood loss ≤100 mL	Estimated blood loss >100 mL, no transfusion required	Transfusion required
QTcF (Fridericia's correction) * or QTcB (Bazett's correction)	Asymptomatic, QTc interval 450-479 ms, *OR* Increase in interval <30 ms above baseline	Asymptomatic, QTc interval 480-499 ms, *OR* Increase in interval 30-50 ms above baseline	Asymptomatic, QTc interval ≥500 ms, *OR* Increase in interval ≥60 ms above baseline
PR interval (prolonged)	PR interval 0.21-0.25 s	PR interval >0.25 s	Type II 2nd degree AV block *OR* Ventricular pause >3.0 s
Respiratory	**Grade 1**	**Grade 2**	**Grade 3**
Cough	Transient; no treatment	Persistent cough	Interferes with daily activities
Bronchospasm, acute	Transient; no treatment; FEV_1 71%-80% of peak flow	Requires treatment; normalizes with bronchodilator; FEV_1 60%-70% (of peak flow)	No normalization with bronchodilator; FEV_1 <60% of peak flow
Dyspnea	Does not interfere with usual and social activities	Interferes with usual and social activities, no treatment	Prevents daily and usual social activity or requires treatment
Gastrointestinal	**Grade 1**	**Grade 2**	**Grade 3**
Diarrhea	2-3 loose or watery stools or <400 g/24 hours	4-5 loose or watery stools or 400-800 g/24 hours	6 or more loose or watery stools or >800 g/24 hours or requires IV hydration

* Inclusion dependent upon protocol requirements.

Reactogenicity	Grade 1	Grade 2	Grade 3
Local reactions			
Pain/tenderness at injection site	Aware of symptoms but easily tolerated; does not interfere with activity; discomfort only to touch	Notable symptoms; required modification in activity or use of medications; discomfort with movement	Incapacitating symptoms; inability to do work or usual activities; significant discomfort at rest
Erythema/redness[†]	2.5-5 cm	5.1-10 cm	>10 cm
Induration/swelling[‡]	2.5-5 cm and does not interfere with activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity
Itching at the injection site	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work or cancellation of social activities
Systemic reactions			
Allergic reaction	Pruritus without rash	Localized urticaria	Generalized urticaria; angioedema or anaphylaxis
Headache	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work or cancellation of social activities
Fatigue/malaise	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work or cancellation of social activities
Myalgia	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work or cancellation of social activities

[†] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[‡] Induration/swelling should be evaluated and graded using the functional scale by the investigator during diary review as well as the actual measurement documented by the subject in the diary.

Reactogenicity (continued)	**Grade 1**	**Grade 2**	**Grade 3**
Arthralgia	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work or cancellation of social activities
Chills	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work or cancellation of social activities

2.2 LABORATORY TOXICITY GRADING

Grading scale used for lab assessments is based on 'FDA's Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials', but grade 3 and 4 are pooled below, consistent with the 3 scale toxicity grading used throughout the protocol. If a laboratory value falls within the grading as specified below but also within the local laboratory normal limits, the value is considered as normal. For hemoglobin only, the change from reference is used for the grading. The FDA table does not include toxicity grading for hematocrit, RBC counts or INR.

Blood, Serum, or Plasma Chemistries[§]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Sodium (mEq/L or mmol/L)	132-134	130-131	≤129
	144-145	146-147	≥148
Potassium (mEq/L or mmol/L)	3.5-3.6	3.3-3.4	≤3.2
	5.1-5.2	5.3-5.4	≥5.5
Glucose (mg/dL)	65-69	55-64	≤54
	100-110[**]	111-125	>125
	110-125[††]	126-200	>200
Blood urea nitrogen	23-26 (mg/dL)	27-31 (mg/dL)	>31 (mg/dL)
Creatinine	1.5-1.7 (mg/dL)	1.8-2.0 (mg/dL)	>2.0 (mg/dL)
Calcium (mg/dL)	8.0-8.4	7.5-7.9	<7.5
	10.5-11.0	11.1-11.5	>11.5
Magnesium (mg/dL)	1.3-1.5	1.1-1.2	<1.1
Phosphorus (mg/dL)	2.3-2.5	2.0-2.2	<2.0
CK (mg/dL)	1.25-1.5 x ULN	1.6-3.0 x ULN	≥3.1 x ULN
Albumin (g/dL)	2.8-3.1	2.5-2.7	<2.5
Total protein (g/dL)	5.5-6.0	5.0-5.4	<5.0
Alkaline phosphatase (U/L)	1.1-2 x ULN	2.1-3 x ULN	>3 x ULN
AST (U/L)	1.1-2.5 x ULN	2.6-5 x ULN	>5 x ULN
ALT (U/L)	1.1-2.5 x ULN	2.6-5 x ULN	>5 x ULN
Bilirubin, serum total (mg/dL) – when accompanied by any increase in Liver Function Test	1.1–1.25 x ULN	1.2 –1.5 x ULN	>1.5 x ULN
Bilirubin, serum total (mg/dL) – when Liver Function Test is normal	1.1–1.5 x ULN	1.6–2.0 x ULN	>2.0 x ULN
Amylase (U/L)	1.1-1.5 x ULN	1.6-2.0 x ULN	>2.0 x ULN
Lipase (U/L)	1.1-1.5 x ULN	1.6-2.0 x ULN	>2.0 x ULN
Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Hemoglobin (women) change from baseline (g/dL) Hemoglobin (men) change from baseline (g/dL)	Any decrease-1.5	1.6-2.0	>2.0
	Any decrease-1.5	1.6-2.0	>2.0
White blood cell count (cell/mm^3)	10,800-15,000	15,001-20,000	>20,000
	2,500-3,500	1,500-2,499	<1,500
Lymphocytes (cell/mm^3)	750-1,000	500-749	< 500
Neutrophils (cell/mm^3)*	1,500-2,000	1,000-1,499	< 1000
Eosinophils (cell/mm^3)	650-1500	1501-5000	> 5000
Platelets (cell/mm^3)	90,000-99,999	80,000-89,999	<80,000
Coagulation			
PT (seconds)	1.0-1.10 x ULN	1.11-1.20 x ULN	>1.20 x ULN
International Normalized Ratio (INR)[‡‡]	1.1-1.5 x ULN	1.6-2.0 x ULN	>2.0 x ULN
PTT or aPTT (seconds)	1.0-1.2 x ULN	1.21-1.4 x ULN	>1.4 x ULN
Fibrinogen (mg/dL)	400-500	501-600	>600
	150-200	125-149	<125
Urine			

[§] Depending upon the laboratory used, reference ranges, eligibility ranges and grading may be split out by sex and/or age.

[**] Fasting.

[††] Non-fasting.

[‡‡] For INR, the values in the table are based on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, 2009.

Blood, Serum, or Plasma Chemistries§	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Protein (dipstick)	Trace	1+	2+
Glucose (dipstick)	Trace	1+	2+
Blood (microscopic) - red blood cells per high power field (RBC/HPF)	1-10	11-50	>50 and/or gross blood

2.2.1 Ranges to convert FDA scale to SI units

Blood, Serum, or Plasma Chemistries§§	**LO/HI/N** ***	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Glucose (mmol/L)	LO	3.61-3.38	3.05-3.60	≤3.04
	HI †††	5.55-6.11	6.12-6.94	>6.94
	HI ‡‡‡	6.11-6.94	6.95-11.10	>11.10
Blood urea nitrogen (mmol/L)	HI	8.2-9.3	9.4-11.1	>11.1
Creatinine (µmol/L)	HI	133-150	151-177	>177
Calcium (mmol/L)	LO	2.00-2.10	1.87-1.99	<1.87
	HI	2.62-2.74	2.75-2.87	>2.87
Magnesium (mmol/L)	LO	0.53-0.62	0.45-0.52	<0.45
Phosphorus (mmol/L)	LO	0.74-0.81	0.65-0.73	<0.65
Cholesterol (mmol/L)	HI	5.20-5.43	5.44-5.82	>5.82
Coagulation				
Fibrinogen (µmol/L)	HI	11.76-14.70	14.71-17.65	>17.65
	LO	4.41-5.88	3.68-4.40	<3.68

2.3 VITAL SIGNS TOXICITY GRADING

Grading scale used for vital signs is according to DMID Toxicity Tables (2014)

Vital Signs	**Mild (Grade 1)** §§§	**Moderate (Grade 2)**	**Severe (Grade 3)**
Fever (°C) ****	38.0-38.4	38.5-38.9	>38.9
Fever (°F)	100.4-101.1	101.2-102.0	>102.0
Tachycardia	101-115 bpm	116-130 bpm	>130 bpm or ventricular dysrhythmias
Bradycardia	50-54 or 45-50 bpm if baseline <60 bpm	45-49 or 40-44 bpm if baseline <60 bpm	<45 or <40 bpm if baseline <60 bpm
Hypertension (systolic) - mm Hg ††††	141-150	151-160	>160
Hypertension (diastolic) - mm Hg	91-95	96-100	>100
Hypotension (systolic) - mm Hg	85-89	80-84	<80

§§ Depending upon the laboratory used, reference ranges, eligibility ranges and grading may be split out by sex and/or age.
*** Low, High, Not Graded.
††† Fasting.
‡‡‡ Non-fasting.
§§§ If initial bound of grade 1 has gap from reference range or eligibility range, calculations based on the New England Journal of Medicine (NEJM) reference ranges.
**** Applies to all routes.
†††† Assuming subject is awake, resting, and supine; for adverse events, 3 measurements on the same arm with concordant results.

Tachypnea - breaths per minute	23-25	26-30	>30

3 TOXICITY TABLES FOR USE IN TRIALS ENROLLING CHILDREN GREATER THAN 3 MONTHS OF AGE

The abbreviations used in the following tables are:

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CNS: central nervous system; CK: creatine kinase; hpf: high power field; GGT: gamma glutamyl transferase; mEq: milliequivalent; PT: prothrombin time; PTT: partial prothrombin time; ULN: upper limit of normal

3.1 CLINICAL ADVERSE EVENTS

Grading scale used for clinical adverse events is adapted from the DMID Pediatric Toxicity Tables for Children Greater Than 3 Months of Age (2007).

Gastrointestinal	**Grade 1**	**Grade 2**	**Grade 3**
Nausea/vomiting	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work or cancellation of social activities
Diarrhea	Slight change in consistency and/or frequency of stools	Liquid stools	Liquid stools greater than 4x the amount or number normal for the child
Appetite	-	Decreased appetite	Appetite very decreased, no solid food taken
Abdominal Pain	Mild	Moderate; no treatment needed	Moderate; treatment needed
Constipation	Slight change in consistency/frequency of stool	Hard, dry stools with a change in frequency	Abdominal pain
Reactogenicity	**Grade 1**	**Grade 2**	**Grade 3**
Local reactions			
Pain/tenderness at injection site	Aware of symptoms but easily tolerated; does not interfere with activity; discomfort only to touch	Notable symptoms; required modification in activity or use of medications; discomfort with movement	Incapacitating symptoms; inability to do work or usual activities; significant discomfort at rest
Erythema/redness	< 10 mm	10-25 mm	26-50 mm
Induration/swelling	< 10 mm	10-25 mm	26-50 mm
Itching at the injection site	Infrequent, brief episode of scratching, easily distracted from scratching	Frequent, longer episodes of scratching, difficult to distract	Near constant scratching, or scratching during sleep; excoriation of skin
Edema	< 10 mm	10-25 mm	26-50 mm
Rash at the injection site	< 10 mm	10-25 mm	26-50 mm

Systemic reactions			
Allergic reaction	Pruritus without rash	Pruritic rash	Mild urticaria
Reactogenicity (continued)	**Grade 1**	**Grade 2**	**Grade 3**
Headache	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work/school or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work/school or cancellation of social activities
Fatigue/malaise	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work/school or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work/school or cancellation of social activities
Myalgia	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work/school or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work/school or cancellation of social activities
Arthralgia	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work/school or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work/school or cancellation of social activities
Chills	Minimal symptoms; caused minimal or no interference with work, school or self-care activities	Notable symptoms; required modification in activity or use of medications; did not result in loss of work/school or cancellation of social activities	Incapacitating symptoms; required bed rest and/or resulted in loss of work/school or cancellation of social activities
Central Nervous System (CNS)	**Grade 1**	**Grade 2**	**Grade 3**
Generalized CNS Symptoms			Dizziness
Level of activity		Slightly irritable OR slightly subdued	Very irritable OR lethargic
Visual		Blurriness, diplopia, or horizontal nystagmus of <1 hour duration, with spontaneous resolution	More than 1 episode of Grade 2 symptoms per week, or an episode of Grade 2 symptoms lasting more than 1 hour with spontaneous resolution by 4 hours, or vertical nystagmus
Myelopathy		None	None

Peripheral Nervous System	**Grade 1**	**Grade 2**	**Grade 3**
Neuropathy/Lower Motor Neuropathy		Mild transient paresthesia only	Persistent or progressive paresthias, burning sensation in feet, or mild dyesthesia; no weakness; mild to moderate deep tendon reflex changes; no sensory loss
Myopathy or Neuromuscular Junction Impairment	Normal or mild (<2 x ULN) CK elevation	Mild proximal weakness and/or atrophy not affecting gross motor function. Mild myalgias with/without mild CK elevation (<2 x ULN)	Proximal muscle weakness and/or atrophy affecting motor function with/without CK elevation; or severe myalgias with CK>2 x ULN
Other	**Grade 1**	**Grade 2**	**Grade 3**
Fever	38.0-38.4 °C or 100.4-101.1 °F	38.5-40 °C or 101.2-104.0 °F	Greater than 40 °C or 104.0 °F
Cutaneous	Localized rash	Diffuse maculopapular rash	Generalized urticaria
Stomatitis	Mild discomfort	Painful, difficulty swallowing, but able to eat and drink	Painful: unable to swallow solids
Clinical symptom not otherwise specified in this table	No therapy; monitor condition	May require minimal intervention and monitoring	Requires medical care and possible hospitalization
Laboratory values not otherwise specified in this table	Abnormal, but requiring no immediate intervention; monitor	Sufficiently abnormal to require evaluation as to causality and perhaps mild therapeutic intervention, but not of sufficient severity to warrant immediate changes in study vaccine	Sufficiently severe to require evaluation and treatment, including at least temporary suspension of study vaccine

3.2 LABORATORY TOXICITY GRADING

Serum chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Bilirubin (when accompanied by any increase in other liver tests)	1.1-<1.25 x ULN	1.25-<1.5 x ULN	≥1.5 x ULN
Bilirubin (when other liver function tests are in normal range)	1.1-<1.5 x ULN	1.5-<2.0 x ULN	≥2.0 x ULN
AST	1.1-<2.0 x ULN	2.0-<3.0 x ULN	≥3.0 x ULN
ALT	1.1-<2.0 x ULN	2.0-<3.0 x ULN	≥3.0 x ULN
GGT	1.1-<2.0 x ULN	2.0-<3.0 x ULN	≥3.0 x ULN
Pancreatic amylase	1.1-1.4 x ULN	1.5-1.9 x ULN	≥2.0 x ULN
Uric acid	7.5-9.9 mg/dL	10-12.4 mg/dL	≥12.5 mg/dL
CK	See Neuromuscular Toxicity		
Creatinine 3 months < 2 years* of age	0.6-0.8 x ULN	0.9-1.1 x ULN	≥1.2 x ULN
Creatinine 2 – 12 years of age	0.7-1.0 x ULN	1.1-1.6 x ULN	≥1.7 x ULN
Creatinine >12 years of age	1.0-1.7 x ULN	1.8-2.4 x ULN	≥2.5 x ULN
Hypernatremia (mEq/L or mmol/L)	-	<145-149 mEq/L	≥150 mEq/L
Hyponatremia (mEq/L or mmol/L)	-	130-135 mEq/L	≤129 mEq/L
Hyperkalemia (mEq/L or mmol/L)	5.0-5.9 mEq/L	6.0-6.4 mEq/L	≥6.5 mEq/L
Hypokalemia (mEq/L or mmol/L)	3.0-3.5 mEq/L	2.5-2.9 mEq/L	≤2.4 mEq/L
Hypercalcemia	10.5-11.2 mg/dL	11.3-11.9 mg/dL	≥12.0 mg/dL
Hypocalcemia	7.8-8.4 mg/dL	7.0-7.7 mg/dL	≤6.9 mg/dL
Hypomagnesemia	1.2-1.4 mEq/L	0.9-1.1 mEq/L	≤0.8 mEq/L
Hyperglycemia	116-159 mg/dL	160-249 mg/dL	≥250 mg/dL
Hypoglycemia	55-65 mg/dL	40-54 mg/dL	≤39 mg/dL
Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Hemoglobin for children >3 months and <2 years of age	9.0-9.9 mg/dL	7.0-8.9 mg/dL	<7.0 mg/dL
Hemoglobin for children ≥2 years* of age	10.0-10.9 mg/dL	7.0-9.9 mg/dL	<7.0 mg/dL
Absolute neutrophil count	750-1200/mm^3	400-749/mm^3	≤399/mm^3
Platelets	-	50,000-75,000/mm^3	≤49,999/mm^3
PT	1.1-1.2 x ULN	1.3-1.5 x ULN	≥1.6 x ULN
PTT	1.1-1.6 x ULN	1.7-2.3 x ULN	≥2.4 x ULN
Urinalysis	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Proteinuria	1+ or < 150 mg/day	2+ or 150-499 mg/day	3+ or ≥500 mg/day
Hematuria	Microscopic <25 cells/hpf	Microscopic >25 cells/hpf	-

**: Age ranges adapted to avoid gaps in the original table.*

3.2.1 Ranges for children <12 years to convert FDA scale to SI units:

Serum chemistry	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Uric acid	446-589 µmol/L	590-738 µmol/L	>738 µmol/L
Hypercalcemia	2.62-2.79 mmol/L	2.80-2.97 mmol/L	>2.97 mmol/L
Hypocalcemia	1.95-2.10 mmol/L	1.75-1.94 mmol/L	<1.75 mmol/L
Hypomagnesemia	0.60-0.70 mmol/L	0.45-0.59 mmol/L	<0.45 mmol/L
Hyperglycemia	6.44-8.83 mmol/L	8.84-13.82 mmol/L	>13.82 mmol/L
Hypoglycemia	3.05-3.61 mmol/L	2.22-3.04 mmol/L	<2.22 mmol/L

4 TRANSFORMING ON-SITE ASSESSMENTS AND DIARIES ASSESSMENTS OF SOLICITED AES INTO ANALYSIS FORMAT

When creating the analysis dataset for solicited AEs, solicited AEs (recorded by day) need to be converted into the format of unsolicited AEs (recorded by event).

All diary data will be considered, as well as any post-dose on-site assessment (scheduled as well as unscheduled) within 8 days after vaccination. For solicited local AEs for which a diameter is measured, the maximum of diameter derived grade and investigator severity will be used.

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 one day between two (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 (if collected) with the corresponding diary data, only the highest grade and highest level of relatedness per AE will be kept in the analysis database and used in the tables and listings. If relatedness is not collected for an on-site temperature assessment, then relatedness collected on the diary will be used.

The example below 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							

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

Subject No.	**AE**	**Start Date (Char)**	**Stop Date (Char)**	**Severity**	**Relatedness**	**AEID**	**Duration**
0001	Headache	01Jan16	02Jan16	2	Probable	1	6
0001	Headache	04Jan16	05Jan16	3	Probable	1	6
0001	Headache	06Jan16	06Jan16	1	Probable	1	6

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 starting on day 9 is created for this, in case this severity deviates from the previous record.

Of note, to complete the start and end-date based on diary data, the date will be calculated based on the day the AE is reported relative to vaccination and not on the reported date. For example, if the vaccination is on 1st JAN2016, and the AE starts on DAY 3, the start date will be set to the 3rd of January 2016 independent of the reported actual date. For the on-site assessments, the actual date as reported in the database will be used.

For the calculation of duration, the first and last day is used, even in case interruptions occurred in between by missing reporting days or 0 grade. 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.