Janssen Research & Development *

Statistical Analysis Plan

A Randomized, Observer-blind, Placebo-controlled, Phase 2 Study to Evaluate the Safety, Tolerability and Immunogenicity of Three Prime-boost Regimens of the Candidate Prophylactic Vaccines for Ebola Ad26.ZEBOV and MVA-BN-Filo in Healthy Adults in Europe

Protocol VAC52150EBL2001; Phase 2

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

Innovative Medicines Initiative-2 EBOVAC2 Consortium Partners (London School of Hygiene and Tropical Medicine, Institut National de la Santé et de la Recherche Médicale, University of Oxford, Le Centre MURAZ, and Janssen Vaccines & Prevention B.V.)

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* 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

Version	Date	Description
1.0	19 February 2018	Initial version
2.0	30 May 2018	Amendment 1 (this document)

The overall rationale for Amendment 1: The purpose for this amendment is to align all the statistical analyses of Phase 2 and 3 Ebola studies and to address the remarks from the Food and Drug Administration (FDA). The changes made together with the rationale for each change are as follows:

Rationale: The Day 181 analysis window has been replaced with the "≥Day 99" window to align with the sensitivity analysis. The 180 days post-boost analysis window for the immunogenicity analysis set is also updated to ensure that data points in the corresponding window for the per protocol analysis are also included.

2.1. Analysis Visit Windows and Periods

Rationale: Immunogenicity assessments after a planned but not administered dose will only be shown in data listings and not included in tabulations and graphs.

2.3 Analysis Sets

Rationale: A text is added to clarify that dot plots will be generated for both ELISA and VNA.

6.2.1.3 Analysis Methods

Rationale: Definitions for the sample interpretation for cellular data have been updated. The definition of "responder" for the cellular assay has also been updated due to lack of information on the assay characteristics.

6.2.2.1. Parameters

Rationale: A text is added to clarify that 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 tabulations of solicited AEs will count each event once (ie, 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 2: TRANSFORMING ON-SITE ASSESSMENTS AND DIARIES OF

SOLICITED ADVERSE EVENTS INTO AN ANALYSIS FORMAT

Rationale: Minor editorial changes have been made throughout the document.

ABBREVIATIONS

Ad26	adenovirus serotype 26 (vector)
Ad26.ZEBOV	adenovirus serotype 26 expressing the Ebola virus Mayinga glycoprotein
AE	adverse event
aMLV	amphotropic Murine Leukemia Virus
BMI	body mass index
CI	confidence interval
(e)CRF	(electronic) case report form
CTP	clinical trial protocol
DMID	Division of Microbiology and Infectious Diseases
EBOV	Ebola virus
ELISA	enzyme-linked immunosorbent assay
EU	European Union
FANG	Filovirus Animal Nonclinical Group
FDA	Food and Drug Administration
GP	glycoprotein
IC_{50}	50% inhibitory concentration
ICF	informed consent form
ICH	International Council for Harmonisation
ICS	intracellular cytokine staining
IDMC	Independent Data Monitoring Committee
IFN-γ	interferon-γ
IL	interleukin
Inf U	infectious units
IRE	immediate reportable event
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MVA	Modified Vaccinia Ankara
MVA-BN-Filo	Modified Vaccinia Ankara - Bavarian Nordic vector expressing the glycoproteins of Ebola virus, Sudan virus and Marburg virus and the nucleoprotein of Tai Forest virus (formally known as *Côte d'Ivoire ebolavirus*)
NSAID	non-steroidal anti-inflammatory drug
PBMC	peripheral blood mononuclear cells
PT	Preferred Term
SAE	serious adverse event
SAP	statistical analysis plan
SFU	spot-forming units
SOC	System Organ Class
TAFV	Tai Forest virus
TNF-α	tumor necrosis factor-α
VNA	virus neutralization assay
vp	viral particles
WHO	World Health Organization
ZEBOV	Zaire ebolavirus

1. INTRODUCTION

This is the statistical analysis plan (SAP) for the VAC52150EBL2001 study. It describes the final analysis (when all subjects have completed the last study-related visit or discontinued earlier) to be performed.

Note that vaccination was halted in this study following a case of Miller Fisher syndrome after receipt of Modified Vaccinia Ankara Bavarian Nordic vector expressing multiple filovirus proteins (MVA-BN-Filo) or placebo. Therefore, there were delays in scheduled boost vaccinations for some subjects. Due to this delay, an initially planned primary analysis (when all subjects have completed the 6-month post-boost visit or discontinued earlier) was not performed.

1.1. Trial Objectives

Primary Objective

The primary objective is to assess the safety and tolerability of 3 vaccination schedules of adenovirus serotype 26 expressing the Ebola virus Mayinga glycoprotein (Ad26.ZEBOV) and MVA-BN-Filo administered intramuscularly as heterologous prime-boost regimens on Days 1 and 29, Days 1 and 57, or Days 1 and 85.

Secondary Objectives

The secondary objectives are:

- To assess humoral immune responses, as measured by enzyme-linked immunosorbent assay (ELISA), to the Ebola virus (EBOV) glycoprotein (GP) 21 days post boost of 3 vaccination schedules of Ad26.ZEBOV and MVA-BN-Filo administered intramuscularly as heterologous prime-boost regimens on Days 1 and 29, Days 1 and 57, or Days 1 and 85 (Groups 1-3).

- To assess safety and tolerability after Ad26.ZEBOV vaccination administered (at a dose of $5x10^{10}$ viral particles [vp]) intramuscularly on Day 1 (Group 4, vector shedding in France).

In addition, several exploratory objectives were specified in the clinical trial protocol (CTP)[1,2] and will be investigated if corresponding outcome measures are available.

1.2. Trial Design

This was a randomized, observer-blind, placebo-controlled, parallel-group, multicenter, Phase 2 study to evaluate the safety, tolerability and immunogenicity of 3 heterologous prime-boost regimens using Ad26.ZEBOV at a dose of $5x10^{10}$ vp as prime and MVA-BN-Filo at a dose of $1x10^{8}$ infectious units (Inf U, nominal titer) as boost at a 28-, 56- or 84-day interval in healthy adult subjects in Europe (United Kingdom [UK] and France). The 3 prime-boost regimens only differed in the timing of the boost vaccination (ie, 28, 56 or 84 days after prime, respectively referred to as Groups 1, 2 and 3), while the dose of each study vaccine (Ad26.ZEBOV, MVA-BN-Filo or placebo) and the sequence of vaccination were identical. In an additional group (Group 4) in France, Ad26 vector shedding, safety, tolerability and immunogenicity following a single vaccination with Ad26.ZEBOV at a dose of $5x10^{10}$ vp was evaluated.

The subject population consisted of healthy men and women aged between 18 and 65 years (inclusive), who never received a candidate Ebola vaccine before and had no prior exposure to Ebola virus (including travel to West Africa less than 1 month prior to screening) or a diagnosis of Ebola virus disease.

At study entry, subjects were offered the option to enroll into Cohorts I (open-label), II, or III in the UK or into Cohorts II or III, or Group 4 in France. In Cohorts II and III in both countries, core immunogenicity assessments (humoral and cellular assays) were performed. In Cohort II in both countries, additional immunogenicity assessments were done. In Cohort I, plasmablast response kinetics were evaluated for the determination of the optimal sampling time points of the B-cell response as part of the additional immunogenicity assessments in Cohort II in the UK. In the UK, Cohorts I and III started in parallel, while Cohort II in the UK only started when the peak of B-cell response after prime vaccination in Cohort I was identified. In France, Cohorts II and III started in parallel (as there was no Cohort I) and were independent of Cohort I in the UK. In Group 4, immunogenicity assessments (humoral assays) and Ad26 vector shedding assessments were performed. Below is a schematic overview of the study design. For further details, see Section 3 of the CTP[1,2].

Groups 1-3

Study Cohorts	Randomization Ratio (Active:Placebo)	Group 1 N=204	Group 2 N=204	Group 3 N=204	Cohort Total N=612	UK N=321	France N=291
Cohort I	-	10/0	10/0	10/0	**30**	30	-
Cohort II	14:1	84/6	84/6	84/6	**270**	135	135
Cohort III	10:3	80/24	80/24	80/24	**312**	156	156

Group 4

Randomization Ratio (Active:Placebo)	Group 4 N=18	Group Total N=18	UK N=0	France N=18
5:1	15/3	**18**	-	18

Groups 1, 2, and 3: prime on Day 1, followed by boost 28, 56 or 84 days after prime, respectively. Group 4: vaccination on Day 1; N: number of subjects to receive study vaccine (Ad26.ZEBOV, MVA-BN-Filo or placebo)

1.3. Statistical Hypotheses for Trial Objectives

As this study is designed to provide descriptive information regarding safety and immunogenicity without formal vaccination schedule comparisons, no formal statistical hypothesis testing will be performed.

1.4. Sample Size Justification

An originally planned sample size of 612 subjects were to substantially contribute to the overall safety database of the Ad26.ZEBOV and MVA-BN-Filo prime-boost regimens. An additional 18 subjects were planned to be enrolled in Group 4, out of which 15 and 3 subjects were to receive Ad26.ZEBOV and placebo, respectively. Due to the study halt following a case of Miller Fisher syndrome, many subjects did not receive the boost vaccination or had late boost vaccination. Because of this, it will be difficult to evaluate the planned regimens. Therefore, recruitment in the study was stopped and the total sample size reduced to approximately 423 subjects. See Section 11.2 of the CTP[1,2] for further details.

1.5. Randomization and Blinding

Randomization was used to minimize bias in the assignment of subjects to vaccination schedules (groups), to increase the likelihood that known and unknown subject attributes (eg, demographic and baseline characteristics) are evenly balanced across groups, and to enhance the validity of possible statistical comparisons across groups. In addition, randomization was used to minimize bias in the assignment of subjects to study vaccine (active vaccine versus placebo).

Central randomization was implemented in this study. At baseline, subjects were randomly assigned to 1 of 3 groups in a 1:1:1 ratio in Cohorts I, II, and III. Blinding procedure were not applied to Cohort I (as all subjects received Ad26.ZEBOV and MVA-BN-Filo in an open-label fashion). Cohorts II and III were blinded. Within group, subjects were randomly assigned to Ad26.ZEBOV and MVA-BN-Filo, or placebo in a 14:1 and 10:3 ratio in Cohorts II and III, respectively, based on a computer-generated randomization schedule prepared before the start of the study under the supervision of the sponsor. Subjects in Group 4 were randomly assigned (in a blinded fashion) to Ad26.ZEBOV or placebo in a 5:1 ratio. The randomization within each group was balanced by using randomly permuted blocks and was stratified by country in Cohorts II and III. Randomization in each group (for Cohorts II and III) was further stratified by age at randomization (≤50 years, >50 years).

In Cohorts II and III, subjects and study-site personnel were blinded to the study vaccine allocation within groups until the last subject of the study completed the 6-month post-boost visit or discontinued earlier and the clinical database was locked, except for unblinded qualified study-site personnel with primary responsibility for study vaccine preparation and dispensing, and not involved in any other study-related procedure. The study vaccines were administered by a study vaccine administrator (ie, a trained study nurse, medical doctor, or otherwise qualified health care provider who had no other study function).

For Group 4, subjects and study-site personnel were blinded to the study vaccine allocation until the last subject of Group 4 completed the 28-day post-vaccination visit or discontinued earlier and the clinical database was locked, except for unblinded qualified study-site personnel with primary responsibility for study vaccine preparation and dispensing, and not involved in any other study-related procedure.

Data that could potentially unblind the study vaccine assignment (ie, study vaccine preparation/accountability data, immunogenicity data or other specific laboratory data) was handled with special care to ensure that the integrity of the data was maintained and the potential for bias was minimized. This could include making special provisions, such as segregating the data in question from view by the investigators, clinical team, or others as appropriate until the time of database lock and unblinding. The pharmacy and preparation of study vaccines were monitored by an independent study vaccine monitor (ie, an unblinded study vaccine monitor assigned to the study who is responsible for the unblinded interface between the sponsor and the investigational site pharmacy).

2. GENERAL ANALYSIS DEFINITIONS

The type I error rate (α) is set to 0.05 and corresponding 2-sided 95% confidence intervals (CIs) will be calculated wherever applicable. Adjustment of the α (type I error) level due to

multiplicity is not applicable for this study as no planned formal statistical hypothesis testing will be performed.

Analysis and evaluation are defined separately for each parameter later in this document together with the description of rules for handling missing or incomplete data. The analyses will include vaccinated subjects with respect to the actual vaccine administered. The subjects who were vaccinated according to MVA-BN-Filo prime/Ad26.ZEBOV boost or a homologous (ie, Ad26.ZEBOV prime/ Ad26.ZEBOV boost or MVA-BN-Filo prime/MVA-BN-Filo boost) schedule will be excluded from summary analyses (ie, tables and graphs) and listed separately.

In general, the study data will be analyzed as follows:

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

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

Baseline value will be defined as the value of the last available assessment performed prior to the prime vaccination, unless specified otherwise.

For safety assessments, the *baseline value* will be an assessment performed prior or on the date (if only time of assessment is missing) of the prime vaccination. The baseline value for immunogenicity assessments will be an assessment performed before or on the date of the prime vaccination. In case of multiple values, the value closest to the vaccination will be used as the baseline.

Reference value (only for immunogenicity) will be defined as the assessment performed on the date of the boost vaccination. In case of multiple values (on the same day of vaccination), the value closest to the vaccination will be used as the reference value.

V*isit day* will be determined relative to the actual day of vaccination (date of prime vaccination or date of boost vaccination).

Repeated assessments of immunogenicity and safety will be allocated to analysis windows and periods based on Table 1 and Table 2.

2.1. Analysis Visit Windows and Periods

Because subjects do not always adhere to the protocol visit schedule, the following rules will be applied to assign actual visits (immunogenicity) to analysis visits. The analysis visit windows and target days for each visit are displayed in Table 1. The reference day will be generally defined as:

Day of prime (Dose 1) vaccine administration if the actual visit occurs prior to the boost (Dose 2) vaccine administration.

Day of boost vaccine (Dose 2) administration if the actual visit occurs after the boost vaccine administration.

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 visit windows. If a subject has 2 or more assessments within the same interval (analysis visit window), the one closest to the target day will be used for generating tables with descriptive statistics and graphical displays presenting data per time point. If 2 assessments are equidistant from the target day within the same interval, the latest assessment will be used. All assignments will be made in chronological order.

Because the analysis of adverse events (AEs) and laboratory abnormalities will be presented per period (and not per time point), these will be assigned to the analysis period and/or phase based on Table 2.

Table 1: Analysis Visit Windows

Time Interval (Label on Output)	Time Interval PP[a] (Day)	Time Interval IG[b] (Day)	Target Time Point (Day)
Cohorts II and III			
Prior to boost vaccination			
Day 1 (Baseline)	≤ 1	≤ 1	1
Day 15 (14 days Post-dose 1)	[14; 16]	[2; 21]	15
Day 29 (28 days Post-dose 1)	[28; 30]	[22; 42]	29
Day 57 (56 days Post-dose 1)	[56; 58]	[43; 70]	57
Day 85 (84 days Post-dose 1)	[84; 86]	[71; 98]	85
≥Day 99 (≥98 days Post-dose 1)[c]	NA	≥99	-
Day 365 (364 days Post-dose 1)	[335; 395]	≥ 99	365
After boost vaccination			
Day 36 (7 days Post-dose 2)	[7; 9]	[2; 14]	8
Day 64 (7 days Post-dose 2)			
Day 92 (7 days Post-dose 2)			
≥Day 106 (7 days Post-dose 2)	NA		
Day 50 (21 days Post-dose 2)	[19; 25]	[15; 101]	22
Day 78 (21 days Post-dose 2)			
Day 106 (21 days Post-dose 2)			
≥Day 120 (21 days Post-dose 2)	NA		
Day 209 (180 days Post-dose 2)	[166; 196]	[102; 196]	181
Day 237 (180 days Post-dose 2)			
Day 265 (180 days Post-dose 2)			
≥ Day 279 (180 days Post-dose 2)	NA		

Time Interval (Label on Output)	Time Interval PP[a] (Day)	Time Interval IG[b] (Day)	Target Time Point (Day)
Day 365 (364 days Post-dose 1)[d]	[335; 395]	≥ 224	365
Group 4			
Day 1 (Baseline)	≤ 1	≤ 1	1
Day 29 (28 days Post-dose 1)	[28; 30]	[2; 41]	29
Day 57 (56 days Post-dose 1)	[42; 72]	[42; 73]	57
Day 90 (89 days Post-dose 1)	[75; 105]	[74; 135]	90
Day 180 (179 days Post-dose 1)	[150; 210]	≥136	180

[a]The analysis based on the per protocol analysis set will be restricted to data points that fall within this window (ie, protocol-defined window).

[b]The analysis based on the immunogenicity analysis set will be restricted to data points that fall within this window.

[c]This analysis visit is only applicable to the sensitivity analysis of subjects in Group D (ie, ≥99-day interval vaccination schedule) as shown in Table 3 below. For the other analyses (ie, non-sensitivity), this will correspond to the Day 365 analysis visit.

[d]For the windows after Dose 2 administration, only Day 365 window is taken with respect to the day of Dose 1 administration.

NA: Not applicable

Note 1: The analysis windows and target days are based on the relative day (ie, with respect to reference day [day of Dose 1 or Dose 2 administration]). The reference day of Post-dose 1 and Post-dose 2 assessments will be dates of Dose 1 and Dose 2 administration, respectively.

Note 2: Derivation of changes from pre-dose should be performed with respect to the reference value.

Note 3: Immunogenicity sample collection at 180 days post-prime visit was discontinued according to protocol amendment 3 onward. However, this was recorded for some subjects up to protocol amendment 2 or due to the study pause (late boost vaccination) and will fall within the ≥Day 99 window. If the analysis visit window contains the day of actual boost vaccination for this visit, then the assessment closest to the vaccination will be used.

Note 4: The same (ie, with respect to the number of days after boost vaccination) post-boost visits are labeled differently. Day 36, Day 50 and Day 209 apply only to the 28-day interval schedule, Day 64, Day 78 and Day 237 apply only to the 56-day interval schedule. Day 92, Day 106 and Day 265 apply only to the 84-day interval schedule. ≥Day 106, ≥Day 120 and ≥Day 279 apply only to the ≥99-day interval schedule.

Note 5: 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.

Table 2: Analysis Periods

Phase	Period	Interval	
		From	To
Screening		00:00 on the date of signing the informed consent form	One minute prior to Dose 1 administration on Day 1
Regimen*	Post-dose 1	Date and time of Dose 1 administration	Minimum of: a) 23:59 on the date of last contact (for early study discontinuations) b) 23:59 on the date of relative Day 29 Post-dose 1 c) one minute prior to Dose 2 administration
Post-dose 1 FU		One minute after the end of the Post-dose 1 period	Minimum of: a) 23:59 on the date of last contact (for early study discontinuation or completion) b) one minute prior to Dose 2 administration
Regimen*	Post-dose 2	Date and time of Dose 2 administration	Minimum of: a) 23:59 on the date of last contact (for early study discontinuation or completion) b) 23:59 on the date of relative Day 29 Post-dose 2 **Note:** subjects who do not receive Dose 2 will not have a Post-dose 2 period.
Post-dose 2 FU		One minute after the end of the Post-dose 2 period	23:59 on the date of last contact (for early study discontinuation or completion)

Note 1: * Regimen period includes both the Post-dose 1 and Post-dose 2 periods.
Note 2: FU = follow-up.

2.2. Pooling Algorithm for Analysis Centers

This study is conducted at multiple sites in 2 countries (ie, UK and France). The data from the countries and sites will be pooled (matching on cohort and vaccination schedule) for analysis.

2.3. Analysis Sets

Full Analysis Set

The full analysis set includes all subjects who were randomized and received at least 1 dose of study vaccine (Ad26.ZEBOV, MVA-BN-Filo or placebo), regardless of the occurrence of protocol deviations.

Immunogenicity Analysis Set

The immunogenicity analysis set includes all randomized and vaccinated subjects, who have at least 1 post-vaccination (ie, after the date of vaccination) evaluable immunogenicity sample.

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 (ie, 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. The analysis on the immunogenicity 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. For the sensitivity analysis, subjects will also be categorized according to the actual Ad26.ZEBOV prime/MVA-BN-Filo boost interval as shown in Table 3. In all cases for subjects who received Dose 1 (ie, only prime vaccination) but not Dose 2 while still continuing their planned visit schedule, the immune response measurements after the planned but not administered Dose 2 will not be included in graphs and tables showing descriptive statistics. These measurements will however be shown in listings, together with the indication that they are not used in the analysis.

Table 3: Vaccination Schedules for Sensitivity Analysis

Group	Group Label (Ad26.ZEBOV, MVA-BN-Filo)	Window (Days)
A	28-day interval	14-42
B	56-day interval	43-70
C	84-day interval	71-98
D	≥99-day interval	≥99

Note: the day of the prime vaccination is Day 1

2.4. Definition of Subgroups

There are 3 cohorts (each with Groups 1, 2 and 3) and Group 4 (UK: Cohorts I, II, and III; France: Cohorts II, III and Group 4) in this study. The subgroups that will be analyzed are shown in
Table 4.

Table 4: Cohort, Age Group Combinations and Parameters

Study Cohort	Age Group	Analysis Parameters
Cohort I[†]	18-65 years	- Subject information - Safety
Group 4[††]	18-65 years	- Subject information - ELISA (units/mL) - VNA (IC_{50} titers) - Safety
Cohorts II and III	18-65 years	- Subject information - ELISA (units/mL) - VNA (IC_{50} titers) - ICS - Safety

Study Cohort	Age Group	Analysis Parameters
	18-50 years	- ELISA (units/mL)
	51-65 years	

† ELISA (units/mL), VNA (IC_{50} titers) and ICS are not assessed in Cohort I (ie, according to the CTP).
†† ICS is not assessed in Group 4 (ie, according to the CTP).

3. CHANGES TO THE PLANNED ANALYSIS

- Even in the absence of baseline immunogenicity samples, the post-baseline samples are of interest. Therefore, the immunogenicity analysis set is redefined to include subjects without baseline immunogenicity samples, provided that at least 1 post-vaccination evaluable immunogenicity sample is available for the subjects.

- Because different levels of post boost immunogenicity response are expected for different vaccination schedules, the per protocol analysis set is redefined to exclude subjects whose boost vaccination falls outside the protocol-defined window. Similar to the immunogenicity analysis set, the per protocol analysis set is also refined to include subjects without baseline immunogenicity samples.

4. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE REVIEW

There was no interim analysis performed for this study. However, an independent data monitoring committee (IDMC) was instituted prior to the start of the study. The IDMC periodically reviewed safety data to ensure progressive safety of the subjects. Ad-hoc IDMC reviews of the safety data were also requested by the sponsor for any single event or combination of multiple events which were considered to jeopardize the safety of the subjects. See the IDMC charter[4] and the associated SAP[5] for further details.

For all cases of IDMC reviews, the data package and analysis results that could contain any piece of unblinding information were kept in a strictly confidential place, with access for IDMC and the independent statistical support group members only, until unblinding of the study by the sponsor.

5. SUBJECT INFORMATION

Subject information will be analyzed based on the full analysis set. The data will be presented separately for Cohort I and Group 4. For cohorts II and III, the presentation will be based on the pooled data (see

Table 4), unless otherwise specified.

5.1. Disposition Information

The number and percentage of subjects randomized, vaccinated and entered in each analysis period will be tabulated. Subject assignment to vaccine regimen will be provided in a data listing (including the assigned regimen and the actual regimen received).

Furthermore, the number and percentage of subjects in the full analysis set who completed and those who discontinued together with the reason(s) for discontinuation will be tabulated and listed. This will be done for completion/discontinuation from study vaccination and from the trial.

5.2. Protocol Deviations

Subjects with protocol deviations will be identified prior to the database lock. The major protocol deviations will be summarized by the deviations category. A listing of the major protocol deviations will also be generated. The deviations that have the potential to influence immune response will be flagged in the listing.

5.3. Demographics and Baseline Characteristics

The demographic and baseline characteristics will be presented for the pooled Cohorts II and III data. Additionally, the data will be presented separately for each cohort and Group 4. The following demographic characteristics will be summarized.

- Sex (Female/Male)
- Age (years)
- Age group (≤50 years versus >50 years)
- Race
- Ethnicity
- Country (UK versus France)
- Height (cm)
- Weight (kg)
- Body mass index (BMI, kg/m^2), calculated from baseline height and weight

5.4. Prior and Concomitant Medications

The analysis of pre-study and concomitant therapies will be based on the World Health Organization (WHO) drug coded terms as provided in the clinical database. If the coded term for a concomitant medication is missing, then the reported term will be used and flagged in the table. The concomitant therapies will be tabulated per period. Additionally, a listing of all pre-study and concomitant therapies will be provided. There will be special attention to the use of analgesics/antipyretics (such as acetaminophen, non-steroidal anti-inflammatory drugs [NSAIDs] and aspirin) with onset during the first 8 days (start from the day of vaccination) following each vaccination.

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

- In case of partial start or stop dates, the concomitant therapy records will be allocated to analysis 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 analysis 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.

Remark: In addition to the date information, time information is considered to allocate concomitant therapies to periods and/or phases, if available.

6. IMMUNOGENICITY

6.1. Endpoints

Secondary Endpoint:

Binding antibody levels elicited by vaccination using EBOV GP ELISA at 21 days post boost, as measured by ELISA (in ELISA units/mL).

Exploratory Outcomes:

Several exploratory objectives are specified in the CTP[1,2]. Those objectives will be investigated for all corresponding available response outcomes, including the following:

- Humoral immune responses against EBOV GP as measured by:
  - ELISA (ELISA units/mL), at all available timepoints.
  - Neutralizing antibody response in titers that inhibit viral infection by a certain percentage (IC_{50}), at all available timepoints.

- Cellular immune responses against EBOV GP as measured by:
  - Percentage of CD4+ T cells producing interferon (IFN)-γ, tumor necrosis factor-α (TNF-α) and/or interleukin (IL)-2 (using intracellular cytokine staining [ICS]), at all available timepoints.
  - Percentage of CD8+ T cells producing IFN-γ, TNF-α and/or IL-2 (using ICS), at all available timepoints.

6.2. Immunogenicity Against the Insert

6.2.1. Humoral Immune Responses

6.2.1.1. Parameters

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

- **Binding antibody responses using Filovirus Animal Nonclinical Group (FANG) ELISA:** Quantification of antibodies binding to EBOV GP using the ELISA units/mL readout.

In addition, the following will be defined for ELISA (ELISA units/mL) binding antibody responses:

- **Sample interpretation:** A sample will be considered positive, if the value is above the 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×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).

- **Neutralizing antibody responses using virus neutralization assay (VNA):** titers of EBOV GP-specific neutralizing antibodies (unit: 50% inhibitory concentration [IC_{50}]).

For VNA (IC_{50} titer) responses, the following will also be defined:

- **Sample interpretation:** a sample is considered positive if the value is greater than both the assay-specific LLOQ and 3x(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×LLOQ; OR
  - If sample interpretation is positive at both baseline and post-baseline and there is a greater than 2-fold increase from baseline (2-fold increase on the original scale).

6.2.1.2. Data Handling Rules

For ELISA binding antibody responses, values below the LLOQ will be imputed with LLOQ/2.
For the calculation of fold increases, the values below LLOQ will be imputed with the LLOQ.
For VNA titers, values less than the assay-specific LLOQ or less than 3×aMLV will be imputed with half of the assay-specific LLOQ. For the calculation of fold increases, values that are less than the assay-specific LLOQ or less than 3×aMLV will be imputed with the assay-specific LLOQ.

Remark: If an aMLV titer (negative control) is a censored value (ie, <**40**), then it will be imputed with **40** before proceeding with further computations.

6.2.1.3. Analysis Methods

- The available humoral immune responses (immunogenicity against EBOV GP) will be evaluated for cohorts II and III. The pooled cohorts II and III data (ie, ELISA) will also be presented by age group. The Group 4 data will be presented separately. See

Table 4 for more details.

Summary statistics (ie, geometric mean and corresponding 95% CIs) will be calculated and presented for ELISA binding antibody responses (ELISA units/mL) and VNA (IC_{50} titer) at each time point.

Regimen profiles of the geometric mean concentrations with 95% CIs will also be presented. In addition, graphs of the reverse cumulative distributions (ie, percentage of subjects versus the magnitude of the antibody response levels) will be provided for the following time points, if available:

- Baseline and pre-boost
- Baseline and 21 days post boost
- Baseline and 180 days post boost
- Baseline and Day 365 post prime

For both ELISA (ELISA units/mL) and VNA (IC_{50} titer), additional graphical representations (on a log_{10}-scale) will be provided using dot plots (with distinction between positive and negative sample interpretations), by vaccination schedule.

Responder rates and positive sample interpretation will be summarized (ie, showing number, percentage and the exact 95% Clopper-Pearson CI) for ELISA (ELISA units/mL) antibody responses and VNA (IC_{50} titers) per time point.

6.2.2. Cellular Immune Responses

6.2.2.1. Parameters

Cellular immune responses, as measured by the following assay, will be analyzed:

Intracellular cytokine staining (ICS): The following responses are measured:

- CD4+: IL-2, IFN-γ, and/or TNF-α responses to EBOV GP.
- CD8+: IL-2, IFN-γ and/or TNF-α responses to EBOV GP.

In addition, the following will be defined for the ICS:

- **Sample interpretation:** The sample positivity (interpretation) will be determined for each of the two peptide pools separately. If a sample is considered positive for at least one of the peptide pools the sample is considered positive. If a sample is positive for either IFN-γ or IL-2, it is considered positive. A sample is considered positive when the EBOV peptide pool stimulated readout is greater than 3-fold the mock (unstimulated) readout and the mock-subtracted value is greater than the threshold (LLOQ).
- **Responder:** This will be defined based on the assay characteristics. A false positivity criterion of less than 10% for naïve samples (from non-vaccinated subjects) will be applied.

6.2.2.2. Data Handling Rules

For the ICS, values below the LLOQ will be imputed with half of the LLOQ (LLOQ/2). For the calculation of fold increases, values below the LLOQ will be imputed with the LLOQ.

6.2.2.3. Analysis Methods

The cellular immune responses will be evaluated based on the Cohort II data (see

Table 4).

Summary statistics (ie, median, quartiles [Q1, Q3]) will be presented for all the continuous cellular immunogenicity outcomes at each time point. Also, the median fold increases (from Pre-dose 1 and Pre-dose 2) with the corresponding quartiles (Q1 and Q3) will be presented.

Graphical representations (on a $\log_{10}$-scale) will be provided using dot plots (with distinction between responders and non-responders), by vaccination schedule. Also, regimen profiles of the vaccinations schedules showing the median and the quartiles (Q1 and Q3) will be presented. Responder and positive sample interpretation rates will be summarized (ie, showing number, percentage and the corresponding exact 95% Clopper-Pearson CIs) per timepoint.

The proportions of EBOV GP-specific CD4+ and CD8+ T cells (ie, cells producing at least 1 of the 3 investigated cytokines) will also be tabulated per timepoint and shown in a pie chart. The magnitude of each cytokine subset will be shown in a bar chart. Both pie chart and bar chart will be restricted to responders.

7. SAFETY

The safety and tolerability data include the following:

- AEs collected from signing of the informed consent form (ICF) onwards until the 42-day post-boost visit.
- Serious adverse events (SAEs) and immediate reportable events (IREs) from signing of the ICF onwards until the end of the study.
- Solicited local and systemic AEs (reactogenicity) recorded during the first 8 days (including the day of the vaccination) following each vaccination.

The safety and tolerability data will be summarized based on the full analysis set. The analysis will be based on the actual dose (ie, Dose 1 and Dose 2 for the prime and boost vaccinations, respectively) that the subjects received. For example, if Dose 2 is not administered to a subject, then that subject will not be included in the analysis of AEs in the Post-dose 2 period. Focus will be on safety signals detected during the Post-dose 1 and Post-dose 2 periods, as well as the Regimen phase.

- The safety data will be evaluated based on the pooled (matching on vaccination schedules) cohorts II and III. The data will be presented separately for Cohort I and Group 4. See

Table 4 for an overview.

7.1. Adverse Events

The analysis of AEs is based on the medical dictionary for regulatory activities (MedDRA) coded terms as provided in the clinical database. All reported AEs (solicited local, solicited systemic, and unsolicited) during the vaccination periods (Post-dose 1, Post-dose 2 and Regimen Phase) (ie, AEs following vaccination and AEs that have worsened since baseline) will be included in the analysis. Listings of AEs will include all reported AEs.

It is important to note that the AEs include any occurrence that is new in onset or aggravated in severity, toxicity grade or frequency from the baseline condition, or clinically relevant abnormal results of diagnostic procedures, including clinically relevant laboratory test abnormalities.

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. Refer to Sections 9.2 and 12.1.1 of the CTP[1] for further details.

Solicited Local (Injection Site) Reactions

The analysis of local solicited AEs will include:

- Pain/Tenderness
- Erythema
- Induration/Swelling
- Itching at injection site

Solicited Systemic Adverse Events

The analysis of systemic solicited AEs will include:

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

Serious Adverse Events

SAEs will be collected from signing of the ICF until the end of the study. An SAE based on the International Council for Harmonization (ICH) and the European Union (EU) Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is medically important*.

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

Immediate Reportable Events

The following list of neuroinflammatory disorders are categorized as IRE, and should be reported to the sponsor within 24 hours of after becoming aware of the event using the IRE Form. Relevant data from the IRE form will be captured in the clinical database.

- Cranial nerve disorders, including paralyses/paresis (eg, 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: eg, 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

Causality

The solicited local AEs will be considered as related to the study vaccine, by definition. The unsolicited and solicited systemic AEs will be considered as related to the use of the study vaccine if the attribution is possible, probable 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. Refer to Section 12.1.2 of the CTP[1], for further details.

Severity Criteria

The severity of the AEs is classified by the investigator as mild, moderate or severe using the Division of Microbiology and Infectious Diseases (DMID) Toxicity Table for Use in Trials Enrolling Healthy Adults (Attachment 3).

Solicited events that are graded less than mild, are not considered AEs. For some solicited events (eg, induration/swelling), the diameter and grading (as reported by the investigator [ie, functional grade]) are collected in the electronic case report form (eCRF). The diameter will be used to derive the toxicity grading. The worst grade should be used when both the diameter derived

grade and the investigator-reported grade are available. If either the diameter-derived or the investigator-reported grade is available, then this should be used.

7.1.2. Data Handling Rules

Missing data will not be imputed. If the severity is missing for a solicited AE, then it will not be considered as an AE. If relationship of AEs to the study vaccine could not be derived (ie, missing or unknown), it will be considered as unknown, for analysis purposes. Local solicited AEs will be considered as related with the use of the study vaccine, by default.

Solicited events will always be allocated to the analysis period (Section 2.1). For analysis purpose, the AEs will be allocated to periods and/or phases as described in Attachment 1.

7.1.3. Analysis Methods

In general, the AEs following vaccination will be summarized (ie, tables of descriptive statistics) by vaccination schedule and presented per period/phase. The presentation will be according to the cohorts as shown in
Table 4. Similar summary tables will also be provided pooled by vaccine (dose).

Furthermore, unsolicited AEs will be summarized (showing number and percentage) by System Organ Class (SOC) and Preferred Term (PT). Solicited AEs (recorded by day) will be converted into the analysis format of unsolicited AEs (recorded by event), as detailed in Attachment 2. These solicited AEs will be summarized by class (local, systemic) and Preferred Term. For solicited as well as unsolicited AEs, tables focusing on severity will be created. Focus will also be on the relationship (to the study vaccine) of the solicited systemic and unsolicited AEs.

The SAEs, AEs with fatal outcome, AEs leading to permanent discontinuation from boost vaccine administration, Grade 3 AEs and IREs will also be listed. A table summarizing all those parameters will further be created and presented per analysis period and vaccination schedule. Summary tabulations by SOC and PT for each of the category of events (ie, SAEs, AEs with fatal outcome, AEs leading to permanent discontinuation from boost vaccination, Grade 3 AEs and immediate reportable events) will also be generated on the entire reporting period and presented by vaccination schedule. Subject narratives will be generated for these events, except Grade 3 AEs. For Grade 3 AEs, the narratives will only be generated for those AEs that are considered related to study vaccination.

For the most frequent (at least 10% of subjects in any vaccination schedule) 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 1 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 (ie, Post-dose 1 or Post-dose 2). Duration and time to onset of AEs will be expressed in days.

7.2. Clinical Laboratory Tests

This section concerns the clinical laboratory test data. The analysis of the laboratory assessments will be based on the Food and Drug Administration (FDA)'s Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (Attachments 3). In case no toxicity grades are defined for a test, the abnormalities above or below the normal range will be used.

It is important to note that any abnormal laboratory value that represents a clinically relevant increase in toxicity grade post study vaccination is also recorded on the AE page of the eCRF and will be analyzed as AEs.

7.2.1. Definitions

In determining toxicity grades, the following rules will be applied:

- Worst grades/abnormalities are determined over the entire period (eg, Post-dose 1 or Post-dose 2) 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).

An abnormality (toxicity grade or abnormality based on normal ranges) will be considered as following vaccination in a period and/or phase if it is worse than the corresponding baseline record. If the baseline value is missing, the abnormality is always considered as following vaccination. A shift from "abnormally low" at baseline to "abnormally high" post-baseline (or vice versa) is also considered as an abnormality following vaccination.

7.2.2. Data Handling Rules

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:

- For integer x:
  - If the value is $<x$ then impute with x-1.
  - If the value is $>x$ then impute with x+1.
- For mantissa (decimal part) x:
  - If the value is $<y.x$ then impute with $y.x$-0.1 (if x has 1 decimal place of precision).
  - If the value is $>y.x$ then impute with $y.x$+0.1 (if x has 1 decimal place of precision).

Remark: The value added or subtracted from the mantissa should follow from its mantissa.

Example:

If value is <5 or >10 impute with 4 and 11 respectively. If value is <5.3 or >10.7 then impute with 5.2 and 10.8 respectively. If value is <5.32 or >10.73 then impute with 5.31 and 10.74 respectively.

7.2.3. Analysis Methods

Laboratory abnormalities will be determined in accordance with the toxicity grading tables (Attachments 3), and in accordance with the normal ranges of the clinical laboratory. The worst abnormalities following vaccination will be summarized (ie, showing number and percentage) by regimen and presented per period/phase, with special attention to Grade 3 toxicities. Focus will be on clinical abnormalities that occur during Post-dose 1 and Post-dose 2 periods, as well as the Regimen Phase. Similar tables for worst abnormalities will be provided pooled by vaccine (dose). A listing will also be provided for subjects with any abnormal laboratory findings following vaccination.

7.3. Vital Signs and Physical Examination Findings

Vital sign abnormalities will be determined in accordance with the DMID Vital Signs Toxicity Grading (Attachment 3). Because vital signs are only assessed at screening and pre-vaccination (ie, pre-prime and pre-boost), descriptive statistics will not be generated for them. Instead, a listing will be generated for subjects with any abnormality.

It is important to note that a full physical examination was only conducted at screening. At other visits, only abbreviated, symptom-directed examinations are performed per the investigator's discretion. Therefore, only a listing of subjects with worst physical examination findings (ie, abnormalities) following vaccination will be provided.

Also, any clinically relevant vital signs or physical examination abnormalities occurring from signing of the ICF onwards until 42-day post-boost vaccination was recorded on the AE page of the eCRF and will be analyzed as AE.

7.4. Electrocardiogram

Note that a single, 12-lead ECG was performed at screening, pre-boost and 7 days post-boost, and interpreted locally. Additional ECG monitoring could be done at other time points during the study, only if clinically indicated based on signs and symptoms. Therefore, only a listing of subjects with an electrocardiogram abnormality following vaccination will be generated.

REFERENCES

1. Clinical Protocol VAC52150EBL2001 Amendment 5: A Randomized, Observer-blind, Placebo-controlled, Phase 2 Study to Evaluate the Safety, Tolerability and Immunogenicity of Three Prime-boost Regimens of the Candidate Prophylactic Vaccines for Ebola Ad26.ZEBOV and MVA-BN-Filo in Healthy Adults in Europe. - Janssen Vaccines & Prevention B.V. (April 2017).
2. Clinical Protocol VAC52150EBL2001 Amendment 5 Country-specific for France: A Randomized, Observer-blind, Placebo-controlled, Phase 2 Study to Evaluate the Safety, Tolerability and Immunogenicity of Three Prime-boost Regimens of the Candidate Prophylactic Vaccines for Ebola Ad26.ZEBOV and MVA-BN-Filo in Healthy Adults in Europe. Janssen Vaccines & Prevention B.V. (April 2017).
3. Horton H, Thomas EP, Stucky JA, et al. Optimization and Validation of an 8-Color Intracellular Cytokine Staining (ICS) Assay to Quantify Antigen-Specific T Cells Induced by Vaccination. J Immunol Methods. 2007;323(1) 39–54.
4. VAC52150EBL2001-Independent Data Monitoring Committee Charter. Janssen Vaccines & Prevention B.V. (September 2016)
5. VAC52150EBL2001-Independent Data Monitoring Committee Statistical Analysis Plan. Janssen Vaccines & Prevention B.V. (July 2015)

ATTACHMENTS

1. PERIOD ALLOCATION OF ADVERSE EVENTS

Solicited events will always be allocated to the Post-dose 1 or Post-dose 2 period, as appropriate.

Unsolicited AEs will be allocated to the different periods per the following rules:

***Step 1*: Allocation of unsolicited events to the periods/phases:**

The AEs present in the database are allocated to periods/phases 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/phase, the AE is attributed to that period/phase (ie, AEs following vaccination).

Incomplete dates (ie, time and/or day and/or month and/or year missing):

1) In case of partial start or stop dates, the events are allocated to the periods/phases 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/phases. This rule may lead to multiplication of the event because of its assignment to multiple periods/phases (see below example).
2) In case of a completely missing start date, the event will be allocated to the appropriate period/phase (eg, Post-dose 1 or Post-dose 2) and consequently the Regimen period; except if the end date of the AE falls before the start of the Post-dose 1 or Post-dose 2 period.

3) In case of a completely missing end date (only for the calculation of duration):
    - 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/phase for subjects who discontinued or completed the trial.
    - In case the AE is not flagged as ongoing, the end date is considered as unknown, and the date will remain missing.

Examples:

Screening Phase: 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/phases (ie, assuming any day of Jun is possible) and therefore the AE will be assigned to the Screening Phase 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 Phase 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 (ie, 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 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 a non-active phase (Screening or any of Post-dose FU phases) followed by an AE in an active (Post-dose 1 or Post-dose 2) period, they are allocated to their respective periods/phases and are considered as separate events.
2) In case overlapping/consecutive events start within a single period/phase, they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the Analysis Data Model (ADaM) database but are assigned the same onset, period/phase, and total duration.
3) In case overlapping/consecutive events start in an active period followed by a non-active phase, they are allocated only to the active period 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, period, and total duration.
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 phases.
    - Remarks:
        1. Time is not considered when determining overlap of events.
        2. Events can only be combined into one and the same AE if their start and stop dates are known.
        3. In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.

    Examples:
    Screening phase: start date: 14JUN2016 - stop date: 28JUN2016
    Post-dose 1 period: start date: 28JUN2016 - stop date: 26JUL2016
    Post-dose 1 FU phase: 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 Phase 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 phase (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. TRANSFORMING ON-SITE ASSESSMENTS AND DIARIES OF SOLICITED ADVERSE EVENTS INTO AN 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 (including the day of vaccination) 8 days after vaccination. For solicited local AEs for which a diameter is measured, the maximum of diameter derived grade and investigator severity (if available) 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 same type of the 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 the relatedness that most implicates the vaccine per AE will be kept in the analysis database and used in the tables and listings. The following example shows how the solicited AE should be converted into a format of unsolicited AEs:

Data from the Subject Diary

Subject: PPD

Solicited systemic AE: Headache

	On-site Assessment	Diary Data							
Solicited AE	Day 1 PPD	Day 1 PPD	Day 2 PPD	Day 3 PPD	Day 4 PPD	Day 5 PPD	Day 6 PPD	Day 7 PPD	Day 8 PPD
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
PPD	Headache	PPD	PPD	2	Probable	1
PPD	Headache	PPD	PPD	3	Probable	1
PPD	Headache	PPD	PPD	1	Probable	1

If a solicited AE ends after Day 8:

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

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

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

It is important to note that the occurrence of solicited injection site reaction and/or solicited systemic adverse events considered to be related to the study vaccine and persisting for at least 3 days will result in a study pause. However, the above rule for calculating duration of AEs may incorrectly indicate that the pausing rule is met. Therefore, a listing of subjects with Grade 3 (severe) AEs will be generated with an indication that interruption between AE start and AE end are not considered in the calculation of the duration of the AEs.

3. 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

CLINICAL ADVERSE EVENTS

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[1].

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)[a] 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 30-60 ms above baseline[b]	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**
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	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
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[c]	2.5-5 cm	5.1-10 cm	>10 cm
Induration/swelling[d]	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

[a] Inclusion dependent upon protocol requirements.

[b] The Grade 2 increase in interval is changed from 30-50 ms to 30-60 ms since the original DMID Toxicity Tables (2014) did not cover the increase in interval between 50 and 60 ms.

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

[d] Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

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
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

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[a]	**LO/HI/N** [b]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Sodium (mEq/L or mmol/L)	LO	132-134	130-131	≤129

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

[b] Low, High, Not Graded.

	HI	144-145	146-147	≥148
Potassium (mEq/L or mmol/L)	LO	3.5-3.6	3.3-3.4	≤3.2
	HI	5.1-5.2	5.3-5.4	≥5.5
Glucose (mg/dL)	LO	65-69	55-64	≤54
	HI [a]	100-110	111-125	>125
	HI [b]	110-125	126-200	>200
Blood urea nitrogen (mg/dL)	HI	23-26	27-31	>31
Creatinine (mg/dL)	HI	1.5-1.7	1.8-2.0	>2.0
Calcium (mg/dL)	LO	8.0-8.4	7.5-7.9	<7.5
	HI	10.5-11.0	11.1-11.5	>11.5
Magnesium (mg/dL)	LO	1.3-1.5	1.1-1.2	<1.1
Phosphorus (mg/dL)	LO	2.3-2.5	2.0-2.2	<2.0
CK (mg/dL)	N	1.25-1.5 x ULN	1.6-3.0 x ULN	≥3.1 x ULN
Albumin (g/dL)	LO	2.8-3.1	2.5-2.7	<2.5
Total protein (g/dL)	LO	5.5-6.0	5.0-5.4	<5.0
Alkaline phosphatase (U/L)	N	1.1-2 x ULN	2.1-3 x ULN	>3 x ULN
AST (U/L)	HI	1.1-2.5 x ULN	2.6-5 x ULN	>5 x ULN
ALT (U/L)	HI	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)	N	1.1-1.5 x ULN	1.6-2.0 x ULN	>2.0 x ULN
Lipase (U/L)	N	1.1-1.5 x ULN	1.6-2.0 x ULN	>2.0 x ULN

Hematology	**LO/HI/N [c]**	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Hemoglobin (women) change from baseline (g/dL)	LO	Any decrease-1.5	1.6-2.0	>2.0
Hemoglobin (men) change from baseline (g/dL)	LO	Any decrease-1.5	1.6-2.0	>2.0
White blood cell count (cell/mm^3)	HI	10,800-15,000	15,001-20,000	>20,000
	LO	2,500-3,500	1,500-2,499	<1,500
Lymphocytes (cell/mm^3)	LO	750-1,000	500-749	< 500
Neutrophils (cell/mm^3)	LO	1,500-2,000	1,000-1,499	< 1000
Eosinophils (cell/mm^3)	HI	650-1500	1501-5000	> 5000
Platelets (cell/mm^3)	LO	125,000-140,000	100,000-124,999	<100,000
Coagulation				
PT (seconds)	HI	1.0-1.10 x ULN	1.11-1.20 x ULN	>1.20 x ULN
International Normalized Ratio (INR)[d]	HI	1.1-1.5 x ULN	1.6-2.0 x ULN	>2.0 x ULN
PTT or aPTT (seconds)	HI	1.0-1.2 x ULN	1.21-1.4 x ULN	>1.4 x ULN
Fibrinogen (mg/dL)	HI	400-500	501-600	>600

[a] Fasting.
[b] Non-fasting.
[c] Low, High, Not Graded.
[d] 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.

	LO	150-200	125-149	<125
Urine				
Protein (dipstick)	HI	Trace	1+	2+
Glucose (dipstick)	HI	Trace	1+	2+
Blood (microscopic) - red blood cells per high power field (RBC/HPF)	HI	1-10	11-50	>50 and/or gross blood

RANGES TO CONVERT FDA SCALE (mg/dL) TO SI UNITS

Blood, Serum, or Plasma Chemistries[a]	**LO/HI/N** [b]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Glucose (mmol/L)	LO	3.61-3.38	3.05-3.60	≤3.04
	HI [c]	5.55-6.11	6.12-6.94	>6.94
	HI [d]	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

VITAL SIGNS TOXICITY GRADING

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

Vital Signs	**LO/HI/N** [e]	**Mild (Grade 1)** [f]	**Moderate (Grade 2)**	**Severe (Grade 3)**
Fever (°C)[g]	HI	38.0-38.4	38.5-38.9	>38.9
Fever (°F)	HI	100.4-101.1	101.2-102.0	>102.0
Tachycardia	HI	101-115 bpm	116-130 bpm	>130 bpm or ventricular dysrhythmias
Bradycardia	LO	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[h]	HI	141-150	151-160	>160

[a] Depending upon the laboratory used, reference ranges, eligibility ranges and grading may be split out by sex and/or age.
[b] Low, High, Not Graded.
[c] Fasting.
[d] Non-fasting.
[e] Low, High, Not Graded.
[f] 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.
[g] Axillary temperature. A protocol should select either °C or °F for inclusion.
[h] Assuming subject is awake, resting, and supine; for adverse events, 3 measurements on the same arm with concordant results.

Hypertension (diastolic) - mm Hg	HI	91-95	96-100	>100
Hypotension (systolic) - mm Hg	LO	85-89	80-84	<80
Tachypnea - breaths per minute	HI	23-25	26-30	>30