Janssen Vaccines & Prevention B.V.*

Statistical Analysis Plan
(Interim and Final Analyses)

A Randomized, Observer-blind, Placebo-controlled, Phase 2 Study to Evaluate the Safety, Tolerability and Immunogenicity of Different Prime-boost Regimens of the Candidate Prophylactic Vaccines for Ebola Ad26.ZEBOV and MVA-BN-Filo in Healthy Adults, Including Elderly Subjects, HIV-infected Subjects, and Healthy Children in Two Age Strata in Africa

Protocol VAC52150EBL2002; Phase 2

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.)

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

Version	Date	Description
1.0	19 March 2018	Initial version
2.0	25 June 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 281" analysis window has been added to align with the sensitivity analysis of subjects whose 2nd vaccination was administered late. 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. The per protocol analysis set is updated to incorporate the 3rd vaccination in the substudy. Also, a sensitivity analysis will be performed to contrast the immunogenicity profile of subjects in the substudy who received the 3rd vaccination within 30 days of Day 365 visit versus those who received it after 30 days of Day 365 visit. Information was added to clarify.

2.3. Analysis Sets

Rationale: Details are added on the methodology for calculating the weight-for-age percentiles of children aged 4 to 11 years.

5.3. Demographics and Baseline Characteristics

Rationale: Use of antiretroviral medications may influence the VNA readout (ie, high background) and the observed titers may not represent the actual VNA titers in HIV-infected subjects. One possible explanation for these observations is that antiretroviral medications may interfere with pseudovirion expressions. It is therefore clarified that only qualitative analysis of the observed VNA titers will be performed for the HIV-infected subjects (Cohort 2a).

6.2.1.1 Parameters

6.2.1.3 Analysis Methods

Rationale: Dot plots will be generated for both ELISA and VNA. Information was added to clarify.

6.2.1.3. Analysis Methods

Rationale: Definitions for the sample interpretation and responder for cellular assays have been updated to include more details.

6.2.2.1. Parameters

Attachment 6: ICS CALCULATION

Rationale: It is clarified 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; and that 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 (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
CDC	Centers for Disease Control and Prevention
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
ELISpot	enzyme-linked immunospot
EU	European Union
FANG	Filovirus Animal Nonclinical Group
FDA	Food and Drug Administration
FU	follow-up
GP	glycoprotein
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
IC_{50}	50% inhibitory concentration
IC_{90}	90% 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
SAE	serious adverse event
SAP	statistical analysis plan
SFU	spot-forming units
TNF-α	tumor necrosis factor-α
VNA	virus neutralization assay
vp	viral particles
WHO	World Health Organization
ZEBOV	Zaire ebolavirus

1. INTRODUCTION

This statistical analysis plan (SAP) describes the analyses that are planned for study VAC52150EBL2002. It is applicable to the interim and final analyses outlined in Section 4.

1.1. Trial Objectives

Primary Objective

The primary objective was to assess the safety and tolerability of different vaccination schedules of adenovirus serotype 26 expressing the Ebola virus Mayinga glycoprotein (Ad26.ZEBOV) and Modified Vaccinia Ankara Bavarian Nordic vector expressing multiple filovirus proteins (MVA-BN-Filo) administered intramuscularly as heterologous prime-boost regimens on Days 1 and 29, Days 1 and 57, or Days 1 and 85 in healthy adults, including elderly subjects, and on Days 1 and 29 or Days 1 and 57 in human immunodeficiency virus (HIV)-infected subjects and healthy children in 2 age strata.

Secondary Objectives

The secondary objectives were:

- To assess humoral immune responses, as measured by enzyme-linked immunosorbent assay (ELISA), to the Ebola virus (EBOV) glycoprotein (GP) at 21 days post boost of different 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 in healthy adults, including elderly subjects, and on Days 1 and 29 or Days 1 and 57 in HIV-infected subjects and healthy children in 2 age strata.
- To assess the safety and tolerability of a third vaccination with Ad26.ZEBOV administered at least 1 year post prime in a subset of approximately 90 healthy adults, including elderly subjects.

Exploratory Objectives

Several exploratory objectives were specified in the protocol. Those objectives will be investigated depending on the availability of the corresponding response outcomes. See the clinical trial protocol (CTP)[1] for the complete list of the exploratory objectives.

1.2. Statistical Hypotheses for Trial Objectives

No formal statistical hypothesis testing was planned for this study. This is because the primary purpose of the study was to provide descriptive information regarding safety and immunogenicity of the heterologous prime-boost regimens on Days 1 and 29, Days 1 and 57, or Days 1 and 85 in healthy adults, including elderly subjects, and on Days 1 and 29 or Days 1 and 57 in HIV-infected subjects and healthy children in 2 age strata.

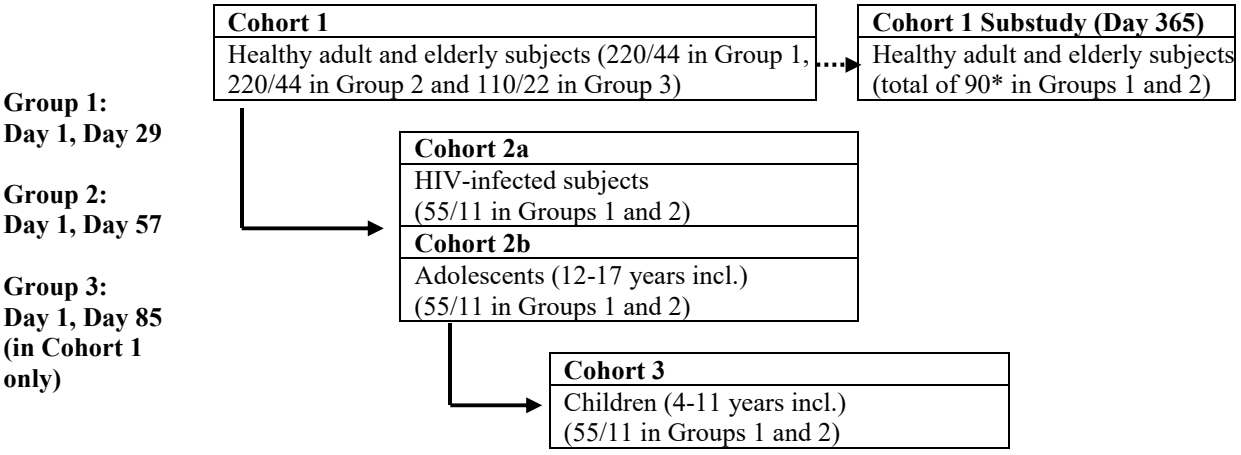
1.3. Trial Design

This was a randomized, observer-blind, placebo-controlled, parallel-group, multicenter, Phase 2 study in Africa to evaluate the safety, tolerability and immunogenicity of different heterologous prime-boost regimens using Ad26.ZEBOV at a dose of $5x10^{10}$ viral particles (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 and elderly subjects (aged 18 to 70 years). The same prime-boost schedules, except for the 84-day interval, were evaluated in HIV-infected subjects (aged 18 to 50 years) and healthy children in 2 age strata (aged 4 to 11 years, and 12 to 17 years). At selected sites in Cohort 1 (Groups 1 and 2), a third vaccination with Ad26.ZEBOV was administered at least 1 year post prime to subjects who consented to this (Cohort 1 substudy). Subjects who received a late boost vaccination or did not receive the boost vaccination at all due to a study pause were not included in the Cohort 1 substudy (Figure 1). See Section 3 of the CTP[1] for further details.

In this study, it was planned to enroll approximately 1,056 subjects, with 660 healthy adult and elderly subjects (aged 18 to 70 years, Cohort 1), 132 HIV-infected adult subjects (aged 18 to 50 years, Cohort 2a), 132 healthy adolescents (aged 12 to 17 years, Cohort 2b) and 132 healthy children (aged 4 to 11 years, Cohort 3). See Figure 1 for a schematic overview of the distribution of the subjects. Eligible subjects were those who had never received a candidate Ebola vaccine and had no prior exposure to EBOV (including travel to epidemic Ebola areas less than 1 month prior to screening) or a diagnosis of EBOV disease. See the CTP[1] for further details.

Figure 1: Schematic Overview of the Study



Number/number: number of subjects per group randomized to Ad26.ZEBOV and MVA-BN-Filo/placebo.

Note 1: Cohorts 2a and 2b: started when 25% of subjects from Cohort 1 had reached the 7-day post-prime visit
Note 2: Cohort 3: started when 50% of subjects from Cohort 2b had reached the 7-day post-prime visit
* Subjects who received Ad26.ZEBOV and MVA-BN-Filo received Ad26.ZEBOV as third vaccination at least 1 year post prime. Subjects who received placebo received placebo as third vaccination at least 1 year post prime.

1.4. Sample Size Justification

An overall planned sample size of approximately 1,056 subjects was to include 880 subjects who were to receive active prime-boost vaccination and 176 subjects who were to receive placebo. The data obtained in these subjects will substantially contribute to the overall safety database of the Ebola program. See Section 11.2 of the CTP[1] for details on the sample size justification.

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).

Based on the subject's demographics, subjects were enrolled in one of the cohorts at study entry. Central randomization was implemented in this study. In Cohort 1, subjects were enrolled in parallel and randomized in a 1:1:1 ratio to Groups 1, 2 and 3 at baseline until a target of approximately 132 subjects was included in Group 3. Afterwards, randomization in this cohort proceeded in a 1:1 ratio to Groups 1 and 2. In Cohorts 2a, 2b, and 3, subjects were enrolled in parallel and randomized in a 1:1 ratio to Groups 1 and 2 at baseline. Subjects were randomly assigned to groups within cohorts (stratified by peripheral blood mononuclear cells [PBMC] sampling capability of the selected sites), and within groups and age strata randomly assigned to Ad26.ZEBOV and MVA-BN-Filo, or placebo in a 5:1 ratio, based on a computer-generated randomization schedule prepared before the study by or under the supervision of the sponsor. The randomization within each group was balanced by using randomly permuted blocks. Within Cohort 1, there was stratification by age (adults ≥18 to ≤50 years of age versus elderly >50 years of age). In the substudy, subjects who received Ad26.ZEBOV and MVA-BN-Filo received Ad26.ZEBOV as third vaccination. Subjects who received placebo received placebo as third vaccination.

Within each cohort, study-site personnel (except for those with primary responsibility for study vaccine preparation and dispensing) and subjects were blinded to the study vaccine allocation until the last subject in that cohort had completed the study. The sponsor personnel were blinded to study vaccine allocation within groups until the last subject in that cohort had completed the 6-month postboost visit or discontinued earlier. Refer to Section 5 of the CTP[1] for further details.

Data that could potentially unblind the study vaccine assignment (ie, study vaccine preparation/accountability data, immunogenicity data or other specific laboratory data) were handled with special care to ensure that the integrity of the data was maintained and the potential for bias was minimized. This included 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 was monitored by an independent study vaccine monitor.

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.

The analysis is defined separately for each parameter later in this document together with the description of rules for handling missing or incomplete data. The analysis will include vaccinated subjects with respect to the actual vaccine administered. The subjects who received only Ad26.ZEBOV prime vaccination will be included in accordance with the group to which they were randomization. On the other hand, any subject who was vaccinated according to MVA-BN-Filo prime only, MVA-BN-Filo prime/Ad26.ZEBOV boost or a homologous schedule (ie, Ad26.ZEBOV prime/Ad26.ZEBOV boost or MVA-BN-Filo prime/MVA-BN-Filo boost) will be excluded from summary 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% 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 (ie, date of Doses 1, 2 or 3).

Repeated assessments of immunogenicity and safety will be allocated to analysis windows and periods based on

Table 1 and Table 2, as described below.

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 assessments) to analysis visits. The analysis visit windows and target days for each visit are displayed in
Table 1. The reference day will be defined as:

- Day of first (Dose 1) vaccination if the actual visit occurs prior to the second (Dose 2) vaccination.
- Day of second (Dose 2) vaccination if the actual visit occurs after the second vaccination but prior to the third (Dose 3: only for subjects in the substudy) vaccination.

- Day of third (Dose 3: only for subjects in the substudy) vaccination if the actual visit occurs after the third vaccination.

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 (except CD4+ cell counts in HIV-infected subjects) 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. For CD4+ cell counts, the electronic case report form (eCRF) visit schedules will be used for the post baseline assessments. If only unscheduled visits are present for a time point, then the one closest to the scheduled visit will be used. If distances of multiple assessments to the scheduled visit are equal, the measurement with the latest date will be used. Similar to the CD4+ cell counts, the eCRF visits will also be used to present vital signs data of Cohort 3 subjects per time point.

Table 1: Analysis Visit Windows

Time Interval (Label on Output)	Time Interval PP[a] (Day)	Time Interval IG[b] (Day)	Target Time Point (Day)
Prior to Second (Dose 2) Vaccination			
Day 1 (Baseline)	≤ 1	≤ 1	1
Day 15 (14 days Post-dose 1)	[13; 17]	[2; 21]	15
Day 29 (28 days Post-dose 1)	[26; 32]	[22; 42]	29
Day 57 (56 days Post-dose 1)	[54; 60]	[43; 70]	57
Day 85 (84 days Post-dose 1)	[82; 88]	[71; 98]	85
Day 141(140 days Post-dose 1)	NA	[99; 168]	-
Day 197 (196 days Post-dose 1)	NA	[169; 224]	-
Day 253 (252 days Post-dose 1)	NA	[225; 280]	-
≥Day 281 (≥280 days Post-dose 1)[d]	NA	≥281	-
Day 365 (364 days Post-dose 1)	[335; 395]	≥281	365
After Second (Dose 2) Vaccination but Prior to Third (Dose 3) Vaccination			
Day 36 (7 days Post-dose 2)	[6; 10]	[2; 15]	8
Day 64 (7 days Post-dose 2)			
Day 92 (7 days Post-dose 2)			
Day 148 (7 days Post-dose 2)	NA		
Day 204 (7 days Post-dose 2)			
Day 260 (7 days Post-dose 2)			
≥Day 289 (7 days Post-dose 2)			
Day 50 (21 days Post-dose 2)	[19; 25]	[16; 101]	22

Time Interval (Label on Output)	Time Interval PP[a] (Day)	Time Interval IG[b] (Day)	Target Time Point (Day)
Day 78 (21 days Post-dose 2)			
Day 106 (21 days Post-dose 2)			
Day 162 (21 days Post-dose 2)	NA		
Day 218 (21 days Post-dose 2)			
Day 274 (21 days Post-dose 2)			
≥Day 303 (21 days Post-dose 2)			
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 321 (180 days Post-dose 2)	NA		
Day 377 (180 days Post-dose 2)			
Day 433 (180 days Post-dose 2)			
≥Day 462 (180 days Post-dose 2)			
Day 365 (364 days Post-dose 1)[e]	[335; 395]	≥222	365
Day 365 (Pre-dose 3)[f]	[335; 395]	≥365	-
After Third (Dose 3) Vaccination			
Day 369 (4 days Post-dose 3)	[4; 6]	[2; 6]	5
Day 372 (7 days Post-dose 3)	[6; 10]	[7; 15]	8
Day 386 (21 days Post-dose 3	[19; 25]	[16; 101]	22
Day 545 (180 days Post-dose 3)	[166; 196]	[102; 273]	181
Day 730 (365 days Post-dose 3)	[335; 395]	≥274	365

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

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

[c] This analysis visit (Day 15) applies only to subjects in Groups 2 and 3 of Cohort 1.

[d] This analysis visit is only applicable to the sensitivity analysis of subjects in Group G (ie, ≥281-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.

[e] For the windows after Dose 2 administration, only Day 365 (364 days Post-dose 1) window is taken with respect to the day of Dose 1 administration.

[f] This analysis visit applies only to the subjects in the substudy (ie, Groups 1 and 2 subjects in Cohort 1 who received 3rd vaccination). For this visit, the assessment closest (but prior) to the vaccination should be used. If this visit coincides with the Day 365 (Day 365 Post-dose 1) visit, then the assessment should be assigned to both visits.

NA: Not applicable.

Note 1: The analysis windows and target days are based on the relative day (ie, with respect to the reference day [day of Dose 1, Dose 2 or Dose 3 administration]). The counting restarts from the day of a vaccination (ie, clock reset).

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

Time Interval (Label on Output)	Time Interval PP[a] (Day)	Time Interval IG[b] (Day)	Target Time Point (Day)

Note 3: Immunogenicity sample collection at Day 141, Day 197, Day 253 and Day 281 visits were not planned according to the protocol. However, these visits were recorded for some Cohort 1 subjects due to the study pause (ie, late second [Dose 2] vaccination). If the analysis visit window contains the day of actual boost vaccination for these visits, then the assessment closest to the vaccination will be used.

Note 4: The same (ie, with respect to the number days since 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 to the 56-day interval schedule. Day 92, Day 106 and Day 265 apply only to the 84-day interval schedule. Day 148, Day 162 and Day 321 apply only to the 140-day interval schedule. Day 204, Day 218 and Day 377 apply only to the 196-day interval schedule. Day 260, Day 274 and Day 433 apply only to the 252-day interval schedule. ≥Day 289, ≥Day 303 and ≥Day 462 apply only to the ≥281-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 d) 23:59 on the date of database cut-off (in case of interim analysis)
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 c) 23:59 on the date of database cut-off (in case of interim analysis)
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 c) 23:59 on the date of database cut-off (in case of interim analysis) **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	Minimum of: a) 23:59 on the date of database cut-off (in case of interim analysis) b) 23:59 on the date of last contact (for early study discontinuation or completion)
Regimen*	Post-dose 3	Date and time of Dose 3 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 3 c) 23:59 on the date of database cut-off (in case of interim analysis) **Note:** subjects who do not receive Dose 3 will not have a Post-dose 3 period.

Post-dose 3 FU		One minute after the end of the Post-dose 3 period	Minimum of: a) 23:59 on the date of database cut-off (in case of interim analysis) b) 23:59 on the date of last contact (for early study discontinuation or completion)

Note 1: * Regimen period includes both the Post-dose 1, Post-dose 2 and Post-dose 3 periods.
Note 2: FU = follow-up.

2.2. Pooling Algorithm for Analysis Centers

There are multiple sites in this study and data from the various sites will be pooled 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 had at least 1 post-vaccination (ie, after the date of vaccination) evaluable immunogenicity sample.

Per Protocol Analysis Set

The per protocol analysis set for the main study includes all randomized and vaccinated subjects, who received both the prime and boost vaccinations (administered within the protocol-defined visit 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. Similarly, the per protocol analysis set for the substudy includes all subjects in the per protocol set for the main study who received the 3rd vaccination and have at least 1 post 3rd 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. Additionally, the analysis will be repeated on the immunogenicity analysis set. For subjects who received Dose 1 (ie, only prime vaccination) but not Dose 2 (or Dose 3) while still continuing their planned visit schedule, the immune response measurements after the planned (ie, upper limit of the analysis visit window that covers the target day for the boost vaccination) 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.

It is important to note that vaccination was halted in the Ebola program following a case of Miller Fisher syndrome after receipt of MVA-BN-Filo or placebo. Therefore, there were delays

in scheduled boost vaccinations for some subjects in Cohort 1. A sensitivity analysis will be performed on Cohort 1 to investigate the impact of late boost vaccination on the immune response. For this sensitivity analysis, subjects will be categorized according to the actual Ad26.ZEBOV prime/MVA-BN-Filo boost interval as shown in Table 3.

Further, some subjects in the substudy received the 3rd vaccination within 30 days after Day 365 (post-prime) whereas others received it beyond 30 days after Day 365 (post-prime). Therefore, another sensitivity analysis will be performed to investigate the robustness of receiving the 3rd vaccination within 30 days after Day 365 (post-prime) contrasted with receiving 3rd vaccination beyond 30 days after Day 365 (post-prime).

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	140-day interval	[99; 168]
E	196-day interval	[169; 224]
F	252-day interval	[225; 280]
G	≥281-day interval	≥281

Note: The day of the prime vaccination is Day 1.

2.4. Definition of Subgroups

There are 4 cohorts in this study. Subject information and safety data will be analyzed per cohort and vaccination schedule. The immunogenicity data will be presented per cohort (as shown in Table 4) and vaccination schedule. Only for Cohort 1, ELISA [units/mL] will also be presented by age stratum (aged ≤50 years and aged >50 years).

Table 4: Cohort, Age Group Combinations and Parameters

Cohort	Age Group	Analysis Parameters
1	≥18 years	- ELISA (units/mL) - VNA (IC_{50} titers) - ELISpot - ICS - Ad26 VNA
	≤50 years	ELISA (units/mL)
	>50 years	
2a	18-50 years	- ELISA (units/mL) - VNA (IC_{50} titers) - ELISpot - ICS - Ad26 VNA
2b	12-17 years	- ELISA (units/mL)

		- VNA (IC_{50} titers) - ELISpot - ICS - Ad26 VNA
3	4-11 years	- ELISA (units/mL) - VNA (IC_{50} titers) - ELISpot - ICS - Ad26 VNA

Ad26: adenovirus serotype 26 (vector); ELISA: enzyme-linked immunosorbent assay
ELISpot: enzyme-linked immunospot; IC_{50}: 50% inhibitory concentration; ICS: intracellular cytokine staining; VNA: virus neutralization assay;

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

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. The progression to a next cohort in the study was based on the favorable IDMC review of safety data. See the IDMC charter[3] and the associated SAP[4] for further details. For all cases, the data package and analysis results that contained 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.

The current SAP is applicable to the interim and final analyses. In each cohort, after completion of the 6-month post-boost visit by all subjects, an interim analysis will be conducted on safety and selected immunogenicity data and the cohort will be unblinded to the sponsor. Nevertheless, study-site personnel, subjects and sponsor personnel involved in subject level data review will remain blinded until the last subject in that cohort has completed the study. For the Cohort 1 substudy (ie, subjects who received the third vaccination), an additional interim analysis may be performed when all those subjects have completed the 6-month post-third vaccination visit, or discontinued earlier. Note that planned interim analyses may be combined with a subsequent analysis, if deemed necessary (eg, when the dates of 2 planned analyses are very close). On the other hand, additional interim analyses may also be performed during the study for regulatory purposes or for informing future vaccine-related decisions in a timely manner. The final analysis will be performed when all subjects have completed the last study-related visit or discontinued earlier (including subjects participating in the Cohort 1 substudy).

5. SUBJECT INFORMATION

Subject information will be analyzed based on the full analysis set. In general, the data will be presented by cohort and vaccination schedule

5.1. Disposition Information

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

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 deviation 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 following demographic characteristics will be summarized.

- Sex (Female/Male)
- Age (years)
- Age group (≤50 years versus >50 years), only for healthy adult and elderly subjects (Cohort 1)
- Race
- Ethnicity
- Height (cm)
- Weight (kg)
- Body mass index (BMI, kg/m^2), calculated from baseline height and weight, only for subjects aged >11 years
- Weight-for-age percentile at baseline, only for children aged 4 to 11 years using Centers for Disease Control and Prevention (CDC) Clinical Growth Chart[5]

To obtain the weight-for-age percentile for children aged 4 to 11 years, the z-score (z) will first be determined as:

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

Where L, M and S denote the power in the Box-Cox transformation, median and generalized coefficient of variation, respectively. The L, M and S are the values from the appropriate CDC table[6] corresponding to the age in months of the child. Note that age is listed in the table at the half month point for the entire month. For instance, 30.5 months represents 30.0-30.99 months (or 30.0 month up to but not including 31.0 months of age). The percentile corresponding to the calculated z-score will then be obtained based on the Standard Normal distribution.

For example, to obtain the weight-for-age z-score of a 4-year-old male who weighs 16.5 kg, the L, M and S values from the appropriate (WTAGE: CDC Clinical Growth Chart Percentile Data File[6]) table are L=-0.915241589, M=16.31676727, and S=0.11995532. Using the above formula, the calculated z-score for this child is 0.093. This z-score corresponds to the 54th percentile.

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 analgesics/antipyretics (such as acetaminophen, non-steroidal anti-inflammatory drugs [NSAIDs] and aspirin) administered during the first 8 days (including the day of the vaccination) following each vaccination. Special attention will also be given to HIV-related concomitant therapies (only for HIV-infected subjects, Cohort 2a).

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.

Exploratory Outcomes:

Several exploratory objectives were specified in the CTP[1]. Those objectives will be investigated depending on the availability of the response outcome. The exploratory response outcomes could include but may not be limited to the following:

- Humoral immune responses against EBOV GP as measured by:
  - ELISA (units/mL), collected at all timepoints (as specified in the CTP[1]).
  - Neutralizing antibody response in titers that inhibit viral infection by a certain percentage (IC_{50}), collected at all timepoints (as specified in the CTP[1]).
- Cellular immune responses against EBOV GP as measured by:
  - Number of interferon (IFN)-γ producing T cells (using ELISpot), collected at baseline, pre-boost, 21 days post boost, 180 days post boost and Day 365 post prime.
  - Percentage of CD4+ T cells producing IFN-γ, tumor necrosis factor-α (TNF-α) and/or interleukin (IL)-2 (using ICS), collected at baseline, pre-boost, 21 days post boost, 180 days post boost and Day 365 post prime.
  - Percentage of CD8+ T cells producing IFN-γ, TNF-α and/or IL-2 (using ICS), collected at baseline, pre-boost, 21 days post boost, 180 days post boost and Day 365 post prime.
- Anti-vector neutralizing antibody responses as measured by Ad26 VNA (serum titers of neutralizing antibodies to Ad26 vector 90% inhibitory concentration [IC_{90}]), collected at baseline.

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 assays, 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 (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 VNA:** titers of EBOV GP-specific neutralizing antibodies (unit: 50% inhibitory concentration [IC_{50}]).

For VNA (IC_{50}) 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).

Important: The pseudovirions used in the pseudo VNA (psVNA) are constructed using an HIV-genomic luciferase vector. During assay validation, a high non-EBOV GP-specific background signal was observed for 1 out of 3 HIV-positive sera tested. Hence, the accuracy of the quantitative measurement of the neutralizing antibody response can be affected in HIV+ serum samples. Therefore, the above responder definition will not be used for the HIV-infected cohort and only a qualitative readout of the psVNA will be reported for the HIV-infected cohort, using the VNA sample interpretation to classify a sample as either negative or positive.

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 below 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 below 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 humoral immune responses (ELISA [units/mL] and VNA [IC_{50} titer]) will be evaluated separately for each cohort. Within each cohort, the data will be presented by vaccination schedule. See Section 2.4 (and Table 4) for details on the subgroup analyses that will be performed.

Except for VNA analysis of the HIV-infected subjects (Cohort 2a), the humoral immune responses of all other cohorts will be analyzed as follows. Summary statistics (ie, geometric means and corresponding 95% CIs) will be calculated and presented for ELISA (units/mL) binding antibody responses and VNA (IC_{50} titers) at each time point. The geometric mean fold increase (from Pre-dose 1, Pre-dose 2 and Pre-dose 3 [only for the Cohort 1 substudy]) with corresponding 95% CI will also be presented per time point for these parameters.
Regimen profiles of the geometric mean concentrations with 95% CIs will be presented. Additional regimen profiles of the geometric mean concentrations with 95% CIs will be generated by pre-existing antibody response against Ad26 vector (ie, pre-existing Ad26 versus no pre-existing Ad26). 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-dose 2
- Baseline and Day 21 Post-dose 2
- Baseline and 180 days Post-dose 2
- Baseline and Day 365 Post-dose 1
- Baseline and 4 days Post-dose 3
- Baseline and 7 days Post-dose 3
- Baseline and 21 days Post-dose 3
- Baseline and Day 180 Post-dose 3
- Baseline and Day 365 Post-dose 3

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. A data listing will also be generated.

For the HIV-infected subjects (Cohort 2a), only positive sample interpretation will be summarized (ie, showing number, percentage and the exact 95% Clopper-Pearson CI) per time point.

6.2.2. Cellular Immune Responses

6.2.2.1. Parameters

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

- **ELISpot:** EBOV GP-specific IFN-γ producing T cells, measured as the number of spot-forming units per million peripheral blood mononuclear cells (SFU/10^6 PBMC).

The following will be defined for ELISpot:

- **Sample interpretation:** Sample positivity (interpretation) will be determined for each of the peptide pools separately. If a sample is considered positive for at least one of the peptide pools, the sample is considered positive. A sample will be 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. The threshold is assay-specific and will be provided by the vendor.
- **Responder:**

- If sample interpretation is negative at baseline but positive post-baseline and the post-baseline value is greater than 2×Threshold; OR
- If sample interpretation is positive at both baseline and post-baseline and there is a greater than 2-fold increase from baseline.

- **ICS:** The following responses will be 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.

For each type of cytokine expressing T cell (CD4+ or CD8+), each peptide pool (GP1 or GP2) and both negative controls, the database contains the total cell count of cytokine expressing T cells and the marginal cytokine expressing T cells subsets (in the text below referred to as "*cytnum*") and the total number of cells (*nsub*). For the computation of the background adjusted percentage for any peptide pool (GP1 and GP2 combined) for the total cytokine response and the "pure" cytokine combinations, see the detailed steps outlined in Attachment 6.

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

- **Sample interpretation:** For each antigen, the total cytokine count of the considered antigen is compared with the total cytokine count in all negative control samples with the Fisher's exact test (Table 5). The test is repeated for both peptide pools (GP1 and GP2) and for each antigen. If the observed p-value for at least 1 peptide pool (GP1 or GP2) is below $10^{-5}/2$ (division by 2 due to Bonferroni correction as this is done for each pool), the sample interpretation is considered positive for that antigen[2].

Table 5: Generic Contingency Table for the Fisher's Exact Test

	GPx (x =1 or 2)	Negative controls
Cells expressing cytokines	$cytnum_x$	*cytnum_neg1*+*cytnum_neg2* (or in case of 1 negative control: *cytnum_neg*)
Cells not expressing cytokines	$nsub_x - cytnum_x$	(*nsub_neg1*+*nsub_neg2*)- (*cytnum_neg1*+*cytnum_neg2*) (or in case of 1 negative control: *nsub_neg*-*cytnum_neg*)

cytnum: marginal cytokine counts; *cytnum neg1*: cell counts of one negative control; *cytnum_neg2*: cell counts for the other negative control; *nsub*: total number of cells for the cytokine assay; *nsub_neg1*: total number of cells for one control assay; *cytnum_neg2*: total number of cells for the other control assay. Note that a given negative control (ie, in case of 2 negative controls) cannot be linked to a specific peptide pool (GP1 nor GP2) because the necessary linking information will not be available in the database.

- **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 in background adjusted total cytokine response.

6.2.2.2. Data Handling Rules

For ELISpot, values below the positivity threshold will be imputed with half of the threshold. For ICS, values below the LLOQ will be imputed with half of the LLOQ (LLOQ/2).

For the calculation of fold increases, ELISpot values below the positivity threshold will be imputed with the threshold; and ICS values below the LLOQ will be imputed with the LLOQ.

6.2.2.3. Analysis Methods

The cellular immune responses will be evaluated separately for each cohort of the study. Within each cohort, the data will be presented by vaccination schedule. The cellular data will not be presented by age stratum. See Table 4 for details.

Summary statistics (ie, median, quartiles [Q1, Q3]) will be presented for all the continuous cellular immunogenicity outcomes (ie, ELISpot and ICS) at each time point. Also, the median fold increases (from Pre-dose 1, Pre-dose 2 and Pre-dose 3 [only for the Cohort 1 substudy]) with the corresponding quartiles (Q1 and Q3) will be presented per time point for these parameters.

Graphical representations (on a $\log_{10}$-scale) will be provided using dot plots, by vaccination schedule. Also, regimen profiles of the medians and the quartiles (Q1 and Q3) will be presented. Another regimen profile (medians and quartiles) will be generated by pre-existing antibody response against Ad26 vector (ie, pre-existing Ad26 versus no pre-existing Ad26). Responder and positive sample interpretation rates will be summarized (ie, showing number, percentage and the corresponding exact 95% Clopper-Pearson CIs) per timepoint. A data listing will also be generated.

For ICS, 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.

6.3. Immunogenicity Against the Vector

6.3.1. Humoral Immune Responses

For immunogenicity against the Ad26 vector, a vector-specific reference value will be defined as the value closest but prior to the administration of the vector.

6.3.1.1. Parameters

Immune responses, as measured by the Ad26 VNA (IC_{90} titers) will be analyzed. The following will also be defined for Ad26 VNA titers:

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

6.3.1.2. Data Handling Rules

For the outcome (ie, Ad26 VNA), values below the LLOQ will be imputed with half of the LLOQ (LLOQ/2).

6.3.1.3. Analysis Methods

The humoral immune responses (ie, immunogenicity against Ad26 vector) will be evaluated separately for each cohort (Table 4). Within each cohort, the data will be presented by vaccination schedule.

The geometric means and corresponding 95% CIs will be calculated. Positive sample interpretation will be summarized (ie, showing number, percentage and the exact 95% Clopper-Pearson CI). A data listing will also be generated.

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-last vaccination (excluding third vaccination) visit.
- AEs collected from the day of the third vaccination onwards until 28 days thereafter (note: events that started before the third vaccination but are still present at the time of third vaccination will also be recorded).
- 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, Dose 2 and Dose 3 for the first, second and third vaccination, 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, Post-dose 2 and Post-dose 3 periods, as well as the Regimen phase.

In general, safety data will be evaluated separately for each cohort. Within each cohort, the data will be presented by vaccination schedule.

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 Post-dose 3) (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 Section 9.3 and Section 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

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 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). For the pediatric, the severity of AEs is classified using the DMID Pediatric Toxicity Tables for Children Greater Than 3 Months of Age (Attachment 4).

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 or 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 definition.

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. 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 study vaccination, 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. 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 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, Post-dose 2 or Post-dose 3). 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 (Attachment 3 and Attachment 5) and the DMID Pediatric Toxicity Tables for Children Greater Than 3 Months of Age (Attachment 4) as appropriate. 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, 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).

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 (except CD4+ cell counts in HIV-infected subjects) will be determined in accordance with the toxicity grading tables (Attachment 3 and Attachment 4), 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, Post-dose 2 and Post-dose 3 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. For CD4+ cell counts in HIV-infected subjects, a listing will also be generated.

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). For Cohort 3, the vital signs will be summarized and listed. The summary will be presented per time point and showing the mean, standard deviation, median, Q1, Q3, minimum and maximum. For the other cohorts (ie, Cohorts 1, 2a and 2b), only a listing of subjects with vital sign abnormalities will be provided because the assessments are only done at screening and pre-vaccination (ie, prior to Doses 1 and 2).

It is important to note that a full physical examination is 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 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-second vaccination and again from third vaccination (only for subjects in Cohort 1 substudy) until 28 days thereafter is recorded on the AE page of the eCRF and will be analyzed as AE.

7.4. Electrocardiogram

Note that a single, 12-lead electrocardiogram was performed at screening for subjects ≥18 years (Cohorts 1 and 2a) and interpreted locally. Additional electrocardiogram 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 VAC52150EBL2002 Amendment 3: A Randomized, Observer-blind, Placebo-controlled, Phase 2 Study to Evaluate the Safety, Tolerability and Immunogenicity of Different Prime-boost Regimens of the Candidate Prophylactic Vaccines for Ebola Ad26.ZEBOV and MVA-BN-Filo in Healthy Adults, Including Elderly Subjects, HIV-infected Subjects, and Healthy Children in Two Age Strata in Africa. Walter Reed Army Institute of Research and Janssen Vaccines & Prevention B.V. (May 2017).
2. 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.
3. VAC52150EBL2002-Independent Data Monitoring Committee Charter. Janssen Vaccines & Prevention B.V. (September 2016).
4. VAC52150EBL2002-Independent Data Monitoring Committee Statistical Analysis Plan Amendment 1. Crucell Holland B.V. (September 2015).
5. Centers for Disease Control and Prevention. Clinical Growth Chart. Available at:

   htttp://www.cdc.gov/growthcharts/clinical_charts.htm. Accessed 10 March 2015.
6. Centers for Disease Control and Prevention Clinical Growth Chart Percentile Data File. Available at: https://www.cdc.gov/growthcharts/percentile_data_files.htm. Accessed 28 May 2018.

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 (ie, 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 calculation of duration), 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 relatedness assessment indicating the highest relatedness to study vaccination 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: 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**
0001	Headache	01Jan16	02Jan16	2	Probable	1
0001	Headache	04Jan16	05Jan16	3	Probable	1
0001	Headache	06Jan16	06Jan16	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	1.26 –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	>1.4 x ULN

[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.

			ULN	
Fibrinogen (mg/dL)	HI	400-500	501-600	>600
	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)	N	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

[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.

VITAL SIGNS TOXICITY GRADING

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

Vital Signs	LO/HI/N [a]	Mild (Grade 1) [b]	Moderate (Grade 2)	Severe (Grade 3)
Fever (°C)[c]	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[d]	HI	141-150	151-160	>160
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

[a] Low, High, Not Graded.

[b] 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.

[c] Applies to all temperature routes.

[d] Assuming subject is awake, resting, and supine; for adverse events, 3 measurements on the same arm with concordant results.

4. 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 glutamyltransferase; mEq: milliequivalent; PT: prothrombin time; PTT: partial prothrombin time; ULN: upper limit of normal

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). For adverse events not included in the tables below, refer to the severity criteria guidelines in Section 12.1.3 of the CTP[1].

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

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

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 greater than 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 greater than 3 months and less than 2 years of age	9.0-9.9 mg/dL	7.0-8.9 mg/dL	<7.0 mg/dL
Hemoglobin for children greater than 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	-

RANGES TO CONVERT FDA SCALE TO SI UNITS (CHILDREN <12 YEARS)

Serum chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Uric acid	446-589 µmol/L	590-738 µmol/L	>738 µmol/L
Hypernatremia	-	145-149 mmol/L	>149 mmol/L
Hyponatremia	-	130-135 mmol/L	<130 mmol/L
Hyperkalemia	5.0-5.9 mmol /L	6.0-6.4 mmol/L	>6.4 mmol/L
Hypokalemia	3.0-3.5 mmol /L	2.5-2.9 mmol/L	<2.5 mmol/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

5. HEMOGLOBIN CUT-OFF VALUES

Where no institutional normal reference ranges is available for hemoglobin, the following cut-off values are proposed. It is imperative to note that there are no standard accepted normative values for hemoglobin in most African countries and therefore, the following recommendations are based on the review of several published sources and in consultation with the sites involved with the study. In the table below, 'simplified' means that the number of cut-off categories has been reduced to decrease complexity and facilitate understanding about eligibility. Similarly, 'adjusted for safety' means that the references may quote lower average or -2 SD values for hemoglobin but these values are considered to be too low for these subjects.

Group	Value (g/dL)		Reference	Outcome
Adult and HIV+	Male 12.1	Female 9.5	LLN value for local sites in Kenya and Uganda, current values being used in Phase 1	Values kept for consistency
Adolescent 16-18 yrs	Male 12.1	Female 9.5	Robins reference 10.4 g/dL for girls and 12.4 g/dL for boys	Values simplified to correspond to adult cut-offs
Adolescent 11-15 yrs	Male 11.0	Female 9.5	Robins reference 11.0 g/dL for girls and boys	Value adjusted down for boys, no change for girls
Children 6-10 yrs	11.0		Robins reference 10.7 g/dL for girls and boys	Value simplified
Children 2-5 yrs	11.0		Robins reference 10.4 g/dL for girls and boys, LLN value for local lab in Kenya 11.5 g/dL for girls and 14.5 g/dL for boys, Schellenberg reference 8.2-9.3 g/dL average anemic defined as <11.0 g/dL	Value simplified and adjusted for safety to 11.0 g/dL
Children 1-2 yrs	11.0		Schellenberg reference 8.0 g/dL average anemic defined as <11.0 g/dL, DMID toxicity table 11.0 g/dL	Value simplified and adjusted for safety to 11.0 g/dL

Robins EB, Blum S. Hematologic reference values for African American children and adolescents. Am J Hematol. 2007:82(7):611-614.

Schellenberg D, Schellenberg JR, Mushi A, et al. The silent burden of anaemia in Tanzanian children: a community-based study. Bulletin of the World Health Organization 2003;81(8):581-590.

DMID US FDA Guidance Document Division of Microbiology and Infectious Diseases (DMID) Pediatric Toxicity Tables November 2007.

6. ICS CALCULATION

For each type of cytokine expressing T cells (CD4+ or CD8+), each peptide pool (GP1 or GP2) and both negative control, the database will contain:

- Total number of cells (*nsub*)
- Total cell count of cytokine expressing T cells (any of IL-2 or IFN-γ or TNF-α present)
- Marginal cytokine expressing T cells subsets (*cytnum*)

To calculate the background adjusted percentage of any subset (GP1 and GP2 combined) for the total cytokine response and the "*pure*" cytokine combinations (table below), the following steps should be followed:

Cytokine combinations			**Formula for "pure" cytokine counts**
IFN-γ	**IL-2**	**TNF-α**	
+	+	+	IFN-γ+IL-2+TNF-α+
-	+	+	(IL-2+TNF-α+) - (IFN-γ+IL-2+TNF-α+)
+	-	+	(IFN-γ+TNF-α+) - (IFN-γ+IL-2+TNF-α+)
+	+	-	(IFN-γ+IL-2+) - (IFN-γ+IL-2+TNF-α +)
-	-	+	(TNF-α+) - (IFN-γ+TNF-α+) - (IL-2+TNF-α+) + (IFN-γ+IL-2+TNF-α+)
-	+	-	(IL-2+) - (IFN-γ+IL-2+) - (IL-2+TNF-α+) + (IFN-γ+IL-2+TNF-α+)
+	-	-	(IFN-γ+) - (IFN-γ+IL-2+) - (IFN-γ+TNF-α+) + (IFN-γ+IL-2+TNF-α+)

1. Apply the formula from the table above with the marginal cytokine counts (*cytnum*) for each "pure" cytokine combination (*c_cytnum*)

   Example: for IFN-γ- and IL-2+ and TNF-α+:

   $$C\_\text{cytnum} = \text{cytnum}_{(\text{IL-2+TNF}-\alpha+)} - \text{cytnum}_{(\text{IFN}-\gamma+\text{IL-2+/TNF}-\alpha+)} \qquad \text{(Ex1)}$$

   For each cytokine combination apply the formula also for both negative controls (*c_cytnum_neg*).

2. Because we cannot link a specific negative control to a given peptide pool (GP1 or GP2), the average of the negative controls (*c_cytnum_neg*) will be used.

   For each peptide pool (GP1 or GP2), calculate the background adjusted percentages $\left(\text{c\_pctpos}_{\text{adj}_x}\right)$ for each cytokine combination using the following formula:

   For each $\text{GP}x (x = 1 \text{ or } 2)$,

   $$\text{c\_pctpos}_{\text{adj}_x} = \left\{\frac{\text{c\_cytnum}_x}{\text{nsub}_x} - \frac{\text{mean(c\_cytnum\_neg)}}{\text{mean(nsub\_neg)}}\right\} \times 100$$

   Where $nsub_x$ and *nsub_neg* are total number of cells for the cytokine assay and control assay respectively.

Note: If replicate negative control samples are available for a subject at one timepoint, only the ones belonging to the same assay as the antigens should be used in the above formula.

3. If the background adjusted percentage is negative, it should be set to 0. The total background adjusted "*pure*" percentage is the sum of the background adjusted percentages from peptide pools GP1 and GP2. It is expressed as

$$\mathrm{Max}\left(\mathrm{c\_pctpos}_{\mathrm{adj}_1}, 0\right) + \mathrm{Max}\left(\mathrm{c\_pctpos}_{\mathrm{adj}_2}, 0\right)$$

4. The total background adjusted "*pure"* percentages will be used for the pie charts.
5. The background adjusted total cytokine response is determined by Steps 2 and 3 above. They will be graphically displayed on the $\log_{10}$-scale and the original scale.
6. Excluded data points:

   There are 2 reasons **to filter** data:

   - High background: this is only captured on the records" IFN-γ+ or IL2+"
   - Total cell count (nsub) < 5000

In addition, a certain part of the data may be **unreliable**. If for a considered type of cytokine expressing T cells (CD4+ or CD8+), a part of the results from a sample or all the results from a sample are filtered or unreliable, the entire sample will be excluded from the analysis. That is, the background adjusted total cytokine response for the considered subject, T cell sample (CD4+ or CD8+) and time point is missing and the background adjusted percentages of the "pure" cytokine combinations are not displayed.