Janssen Vaccines & Prevention B.V.

Statistical Analysis Plan

A Randomized, Double-blind, Phase 1/2a Study to Evaluate the Safety, Tolerability and Immunogenicity of Ad26.RSV.preF in Adults 18 to 50 Years of Age and RSV-Seropositive Toddlers 12 to 24 Months of Age

Protocol VAC18194RSV2001; Phase 1/2a

VAC18194 (Ad26.RSV.preF)

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

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

ABBREVIATIONS

AE	adverse event
CEC	Clinical Endpoint Committee
CI	confidence interval
CRF	case report form
CSR	Clinical Study Report
DMC	Data Monitoring Committee
DPS	Data Presentation Specifications
IDMC	Independent Data Monitoring Committee
eCRF	electronic case report form
ELISA	Enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
GMC	Geometric mean antibody concentration
FA	Full Analysis Set
FDA	Food and Drug Administration
ICH	International Conference on Harmonization
ITT	Intent-to-Treat
ICS	Intracellular Cytokine Staining
IU/ml	International units per milliliter
IVRS	interactive voice response system
LLOQ	lower limit of quantification
LOCF	last observation carried forward
LRTI	lower respiratory tract infection
MedDRA	Medical Dictionary for Regulatory Activities
NA	Not Applicable
PI	principal investigator
PPI	Per-protocol Immunogenicity
RSV	Respiratory syncytial virus
RTI	Respiratory tract infection
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
TLF	Tables, Listings and Figures

DEFINITION OF TERMS

Active vaccine	Ad26.RSV.preF
Study vaccine	Ad26.RSV.preF or Placebo

1. INTRODUCTION

This is the Statistical Analysis Plan (SAP) applicable for the VAC18194RSV2001 trial. This SAP is applicable for the following analysis:

- **Cohort 0**: primary analysis will be performed when all subjects have reached Day 57 (28 days post-Dose 2) or discontinued earlier. All available safety and immunogenicity data gathered so far will be included in the analysis; unblinded at the group level.

- **Cohort 1:** interim analysis will be performed when 12 subjects have reached Day 8 or discontinued earlier. All available safety of the 12 subjects and all available immunogenicity data gathered up to that point will be included in the analysis; unblinded at the group level. For the safety analysis snapshot data will be used: there will be no formal cleaning for this analysis. Data will be cleaned in an ongoing basis.

- **Cohort 1:** interim analysis will be performed when at least 12 subjects have reached Day 29 or discontinued earlier. All available safety and all immunogenicity data gathered up to that point will be included in the analysis; unblinded at the group level.

- **Cohort 1:** Depending on the outcome of the first 2 interim analysis of Cohort 1 an additional interim analysis may be performed when at least 24 subjects have reached Day 29 (28 days post-Dose 1) or discontinued earlier. All available safety and immunogenicity data gathered so far will be included in the analysis; unblinded at the group level.

- **Cohort 1**: primary analysis will be performed when all subjects have reached Day 57 (28 days post-Dose 2) or discontinued earlier. All available safety and immunogenicity data gathered so far will be included in the analysis; unblinded at the group level.

- **Final analysis of both cohorts**; at the end of the study, 1 year after the first dose; unblinded.

- Additional interim analyses (blinded or unblinded at the group level) may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner, or upon health authority request.

This document contains all information needed for performing a full safety and immunogenicity analysis. Tables, listings and figures (TLF) which need to be generated for each analysis will be described in separate data presentation specifications (DPS) document.

1.1. Trial Objectives

Primary Objective

- To assess the safety and tolerability of an intramuscular regimen of two doses of $1x10^{11}$ viral particles (vp) of Ad26.RSV.preF in adults aged 18 to 50 years.
- To assess the safety and tolerability of an intramuscular regimen of two doses of $5x10^{10}$ vp of Ad26.RSV.preF in RSV-seropositive toddlers aged 12 to 24 months.

Secondary Objectives

- To assess the humoral and cellular immune responses as measured by virus neutralization assay, F-protein binding antibodies (pre-F and post-F) and intracellular cytokine staining (ICS; to assess Th1/Th2 subtyping).

Exploratory Objectives

- To further assess the humoral and cellular immune responses elicited by Ad26.RSV.preF. The assays to be used may include, but are not limited to, RSV strain cross-neutralization, anti-F protein antibody specificity and functionality characterization, adenovirus neutralization assays, molecular antibody characterization (avidity, Fc cell interaction, antibody isotyping), evaluation of the cellular immune response (interferon gamma [IFNγ] enzyme-linked immunospot [ELISpot] assay) and functional and memory immune response (by ICS), and cytokine profiles of RSV F-protein peptide-stimulated peripheral blood mononuclear cell (PBMC) supernatant.
- To assess RSV infection rates in Ad26.RSV.preF and placebo subjects.
- To monitor for severe RSV-lower respiratory tract infection (LRTI) in Cohort 1 (RSV-seropositive toddlers aged 12 to 24 months).
- To assess symptoms of respiratory illness via the respiratory tract infection (RTI) symptoms

1.2. Trial Design

This is a multi-center, randomized, double-blind, Phase 1/2a study, to be conducted in 12 male and female adults aged ≥18 to ≤50 years and 36 male and female RSV-seropositive toddlers aged ≥12 to ≤24 months of age.

Subjects will be enrolled into sequential age cohorts:

- 18 to 50-year-old adults, into **Cohort 0**
- 12 to 24-month-old seropositive toddlers, into **Cohort 1**

In Cohort 0, subjects will be randomized 2:1 to receive either Ad26/Ad26 ($1x10^{11}$ vp in 0.5 mL) or placebo/placebo (saline), and in Cohort 1, subjects will be randomized 2:1 to receive either Ad26/Ad26 ($5x10^{10}$ vp in 0.25 mL) or placebo/placebo. A schematic overview of the study design and groups is depicted in Table 1 .

Table 1: Study Design

Cohort	*Group*	*N*	*Day 1*	*Day 29/Week 4*
ADULTS 18 to 50 years				
0	Group 1	8	Ad26.RSV.preF ($1x10^{11}$ vp)	Ad26.RSV.preF ($1x10^{11}$ vp)
	Group 2	4	Placebo	Placebo
TODDLERS 12 to 24 months: RSV seropositive				
1	Group 3	32	Ad26.RSV.preF ($5x10^{10}$ vp)	Ad26.RSV.preF ($5x10^{10}$ vp)
	Group 4	16	Placebo	Placebo

The study will have three phases: a **screening phase**, an **active phase** and a **safety follow-up phase** through 1 year after the first dose.

The end of the active phase will be when the last subject completes the visit at 6 months after the final

dose. The end of the study will be the last subject's last visit (by telephone) at the end of the safety follow-up phase. For further information related to the design of the study refer to CTP, Section 3.1.

1.3. Statistical Hypotheses for Trial Objectives

The objective of the study is to evaluate whether Ad26.RSV.preF is safe, well-tolerated and immunogenic in adults and RSV-seropositive toddlers. No formal statistical hypothesis is planned. Safety parameters will be summarized descriptively. Geometric mean titers with 95% confidence intervals (CI) for humoral assays and medians with interquartile for cellular assays will be calculated.

1.4. Sample Size Justification

The number of subjects chosen for this study will provide a preliminary safety and immunogenicity assessment. Placebo recipients are included for blinding and safety purposes and will provide additional control specimens for immunogenicity assays.

1.5. Randomization and Blinding

Central randomization will be implemented in this study. Within each cohort, subjects will be randomly assigned to one of the two groups based on a computer-generated randomization schedule prepared before the study by or under the supervision of the sponsor. The randomization will be balanced by using randomly permuted blocks. No stratification will be applied. The interactive web response system (IWRS) will assign a unique code, which will dictate the group assignment for the subject. The requestor must use his or her own user identification and personal identification number when contacting the IWRS, and will then give the relevant subject details to uniquely identify the subject.

The subjects, study-site personnel, and investigator will be blinded to study vaccine allocation throughout the study, except for the pharmacist or qualified staff member with primary responsibility for study vaccine preparation and dispensing. Unblinding will occur at the time of

the first interim analysis. The results will only be available to a selected group of sponsor personnel, excluding personnel involved in data collection and data management. For further information please refer to CTP Section 5.

1.6. Changes to planned analyses

Two addition interim analyses for Cohort 1 have been added, for the purpose of informing future vaccine-related decisions in a timely manner. The first interim analysis will be performed when 12 subjects have reached Day 8, and the second one when at least 12 subjects have reached Day 29. Given that the first interim analysis coincides with the planned IDMC analysis, the IDMC members will receive immediately unblinded at the group level safety results instead of blinded, as specified in the CTP. That way the IDMC and the study team will possess the same information regarding safety. Moreover, the conduct of the planned interim analysis, when at least 24 subjects of Cohort 1 have reached Day 29 (28 days post-Dose 1) or discontinued earlier, will be dependent on the outcome of the previous interim analyses and is at the discretion of the sponsor.

2. GENERAL ANALYSIS DEFINITIONS

2.1. Study phases

A baseline (or reference) value will be defined as the value of the last available assessment prior to the first vaccination on Day 1.

The safety analysis will present all results by period. Immunogenicity results will be presented per scheduled time point as appropriate. Listings will be shown per phase and time point.

Study day or relative day is defined as follows:

Study Day = visit date - date of Day 1 + 1; if visit date > date of Day 1 (date of first vaccination).

Study Day = visit date - date of Day 1; if visit date < date of Day 1 (date of first vaccination).

2.1.1. Phase definitions

The phases in the study will be constructed as follows:

Table 2: Phase Definitions

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form[a]	One minute prior to start of post dose 1 period
Regimen	2	Post-dose 1	1	Date and time of first vaccination (Visit Day 1)	Minimum of: a) One minute prior to date and time of the next vaccination. If second vaccination was not administrated then use maximum (28 days after first vaccination at 23.59, scheduled visit 4 weeks after first vaccination at 23:59) b) 23:59 at the date of last contact (for early discontinuation) c) 23:59 at the date of database cut-off ate in case of interim.
Regimen	2	Post-dose 2	2	Date and time of second vaccination (Visit Day 29)	Minimum of: a) Maximum (28 days after second vaccination at 23.59, scheduled visit 4 weeks after second vaccination at 23:59) a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off date in case of interim.
Follow-up	3			One minute after the last Post-dose period ends	a) 23:59 at the date of last contact. b) 23:59 at the date of database cut-off date in case of interim.

[a] in case an earlier date is available (eg. for lab or vital signs), then use the very first date to include all data

2.2. Pooling Algorithm for Analysis Centers

Data will be pooled across the different centers.

2.3. Analysis Sets

2.3.1. Full Analysis Set (FA)

The **Full Analysis (FA) Set** includes all subjects who were randomized and received at least one dose of study vaccine, regardless of the occurrence of protocol deviations. Vaccination assignment will follow the as-treated principle. All safety analyses will be based on the FA set. As a sensitivity analysis, key immunogenicity tables will also be based on the FA set.

2.3.2. Immunogenicity Analysis Set

2.3.2.1. Per Protocol Immunogenicity Population (PPI)

The **Per-protocol Immunogenicity Set (PPI**) will include all randomized and vaccinated subjects for whom immunogenicity data are available, excluding subject samples with major protocol deviations expecting to impact the immunogenicity outcomes.

In addition, the following samples will not be included in the PPI set:

- If subjects miss one or more doses, but continue the planned visit schedule, samples taken after the planned but missed dose(s) will not be taken into account;
- For subjects who experience a natural RSV infection (based on RT-PCR, or other sources), samples taken after the natural infection are not be taken into account.

The analysis of immunogenicity will be based on the PPI set.

3. DATA MONITORING COMMITTEE REVIEW

An independent data monitoring committee (IDMC) will be established to monitor severe LRTI. The presence of severe LRTI will be determined by the Clinical Endpoint Committee (CEC), a separate committee (see CTP Section 11.9.2). The outcome of the adjudication of the CEC as well as the determination of RSV positivity will be provided to the IDMC.

The IDMC will review 7-day post-dose 1 safety data of the first 12 Cohort 1 subjects and will convene as needed on an ad hoc basis to ensure the continuing safety of the subjects enrolled in this study. The IDMC will also convene to discuss any situation that meets a study vaccination pausing rule. The IDMC will monitor severe RSV-LRTI as detailed in the IDMC charter. A separate SAP for the IDMC analysis will be provided.

4. SUBJECT INFORMATION

Subject information will be shown for the full analysis set.

4.1. Demographics and Baseline Characteristics

Demographic characteristics and screening/baseline characteristics will be tabulated and summarized with descriptive statistics per vaccine regimen and over all subjects.

The following demographic and baseline characteristics will be summarized.

- Sex (Female/Male)
- Age (years)
- Race
- Ethnicity
- Height (cm), only for Cohort 0
- Weight (kg), only for Cohort 0
- BMI (kg/m2), only for Cohort 0
- Growth Percentile, weight by age*, only for Cohort 1
- Growth Percentile, height/length by age *, only for Cohort 1

4.2. Disposition Information

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

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

4.3. Treatment Compliance

The number of missed vaccinations will be tabulated.

4.4. Protocol Deviations

Major protocol deviations will be summarized.

4.5. Concomitant Medications

The analysis of concomitant therapies will be done using the WHO drug coded terms.

There will be special attention to analgesics/antipyretics such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin, administered during 8 days following each vaccination (00:00 of day of vaccination + 7 days.

Based on their start and stop date, concomitant therapies will be reported in each applicable phase. If a concomitant therapy record misses components of its start and/or stop dates (day and/or month and/or year):

- In case of partial start or stop dates, the concomitant therapy records will be allocated to periods using the available partial information, without imputations. If, for example, only month and year are available, these will be compared to the month and the year of the periods, and the concomitant therapy record will be allocated to the period(s) where these date parts match. This rule may lead to assignment to multiple periods.
- 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.

5. SAFETY

Safety analyses will be performed on the FA. Continuous variables will be summarized using the following statistics, as appropriate: number of observations, median, quartiles (Q1 and Q3), minimum and maximum. Frequencies and percentages (one decimal place) will be generated for categorical variables. No formal comparisons between groups will be provided.

Two types of safety tables will be shown. In the by regimen layout, safety data will be analyzed by vaccine regimens as designed per protocol; data will be presented by period as well as over the entire regimen. Denominator for the percentages is the number of subjects in the considered population and period for a certain regimen (incidence per 100 subjects/period). In the second, by vaccine, safety data will be presented by the different vaccines used in the study but by considering every vaccination administered as a separate safety episode. Denominator for the percentages is the number of vaccinations administered of a certain vaccine (rate per 100 vaccinations of a particular vaccine).

5.1. Adverse Events (AE)

5.1.1. Definitions

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

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

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

5.1.2. Analysis of Adverse Events

Number and percentage of subjects with at least one particular AE (unsolicited/solicited) will be tabulated. Unsolicited AEs will be summarized by System Organ Class and Preferred Term. Solicited AEs will be summarized by class (local, systemic) and preferred term.

For solicited AEs following tables will be provided: summary, by worst severity grade, grade 3, related (systemic only), time to onset (in days) and duration (in days) for most frequent events and body temperature. Note: Duration for solicited AEs is defined as number of days from the start of the event until resolution of the event. The time to first onset is defined as (date of first onset – reference date + 1). The reference date is the start date of the period.

For unsolicited AEs following tables will be provided: summary table (including SAE, fatal outcome, AESI and discontinuation), all events, most frequent, grade 3, permanent stop of vaccine, related, SAE.

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

5.1.3. Phase allocation of Unsolicited Adverse Events

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

Solicited AEs from the diary will be added to the ADAM database for unsolicited AEs according to the same principles. This means the same event occurring on different days will be allocated to one row with the start date of the AE being the first date of the event and the end date for the event is the last subsequent day of the event. A change in grade will trigger a new row to be added. The same occurs in case of non-subsequent events (for example grade 1 nausea on day 1, 2 and 3 and also on day 6 and 7, which will be allocated to two rows; the duration of the event is 7 days). For further details related to transforming the on-site assessments and diaries of solicited AEs into analysis format please refer to Attachment 2.

Step 1: Allocation of events to the periods:

Adverse events in the SDTM database are allocated to periods based on their start date/time. If the start date/time of an event falls between (or on) the start and stop date/time of a period, the AE is attributed to that period (treatment-emergent principle).

- In case of partial start or stop dates (i.e. time and/or day and/or month and/or year missing), the events are allocated to the periods using the available partial information on start and end date; no imputation will be done. If, for instance, the AE start date only month and year are available, these data are compared to the month and year information of the periods. This rule may lead to multiplication of the event as a consequence of its assignment to multiple periods.

- In case of a completely missing start date, the event is allocated to the first active treatment phase (post dose 1 period), except if the end date of the AE falls before the start of the first active treatment phase (post dose 1 period).

- In case of a completely missing end date, the date is imputed by the cut-off date of the analysis for subjects still ongoing in the study, and by the end date of the last period for subjects who discontinued or completed the trial.

Step 2: Combination of events:

Overlapping/consecutive events are defined as events of the same subject with the same preferred term which have at least 1 day overlap or for which the start date of an event is 1 day after the end date of the preceding event. Overlapping/consecutive events may be combined into one AE or not, according to the following rules:

1) If overlapping/consecutive events start in one of the following periods - Screening or post dose extension (i.e. non-active periods) followed by an AE in post-dose period (active period) - they are allocated to their respective periods and are considered as separate events.

2) In case overlapping/consecutive events start within a single period, they are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.

3) In case overlapping/consecutive events start in both an active period followed by a non active period, they are allocated to the active period only and are considered as one and the same AE. The individual events which contribute to this AE are retained as individual records in the ADaM database but are assigned the same onset, treatment period, and total duration. All related attributes to the AE/phase/period should also be consistent with the new event.

4) In case an active period is followed by another active period, and the overlapping/consecutive events start in both periods, they are allocated to their respective period and are considered as separate AEs. The same rule applies for 2 non-active periods.

Remarks:

1. Events can only be combined into one and the same AE if their start and stop dates are known.

2. In case the completely missing end date is imputed (for period allocation), this date is also considered as a complete date.

3. Time is not considered when determining overlap of events.

5.1.4. Missing Data

Missing data will not be imputed. Subjects who do not report an event will be considered as subjects without an event. The analysis of the solicited AEs will include only documented safety data.

5.1.5. Solicited Local (Injection Site) Reactions

The analysis of local solicited adverse events, for all subjects will include:

- Erythema
- Induration/swelling
- Pain/tenderness

5.1.6. Solicited Systemic Adverse Events

The analysis of systemic solicited adverse events for adult subjects will include:

- Fatigue
- Headache
- Myalgia
- Arthralgia
- Chills
- Nausea
- Fever (ie, body temperature ≥ 38 °C)

The analysis of systemic solicited adverse events for pediatric subjects will include:

- Loss of appetite
- Vomiting
- Diarrhea
- Decreased activity/lethargy
- irritability/crying
- Fever (ie, body temperature ≥ 38 °C)

5.2. Clinical Laboratory Tests

For laboratory safety parameters, only abnormalities emerging after vaccination will be tabulated by worst abnormality grade using the FDA table in Attachment 1.

An abnormality (toxicity grade or abnormality based on normal ranges) will be considered as emerging in a particular period if it is worse than the baseline value. If the baseline is missing, the abnormality is always considered emerging. A shift from ‘abnormally low’ at baseline to ‘abnormally high’ post baseline (or vice versa) is also emerging. In case a laboratory test result is censored (no numeric value is available, but only a verbatim term) then a numeric value will be imputed by a value exceeding the cut-off value with one unit. (<x: subtract 1 unit from x, >x: add 1 unit to x; <3.45 is imputed with 3.44).

In case no toxicity grades are defined for a test, the abnormalities (above/below normal range) will be used. In determining toxicity grades, the following rules are applied:

- worst grades/abnormalities are determined over the whole observational period for each trial period separately, including all post-baseline measurements of that period.
- The abnormalities 'abnormally low' and 'abnormally high' are considered equally important, i.e. if a subject has as well an abnormally low as an abnormally high value post-baseline, both abnormalities are shown in the tables. (This means that the sum of the percentages can by more than 100%)
- Note: as the grading scale for some parameters in the grading table has some gaps (zones where no toxicity grade definition exists), laboratory results falling in these zones will be allocated to the adjacent worst-case grade.
- If a lab value falls within the grading as specified in the grading table but also within the local lab normal limits, the value is considered as normal.

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

5.3. Vital Signs and Physical Examination Findings

Similarly, only vital signs abnormalities emerging after vaccination will be tabulated by worst abnormality grade.

For cohort 0, heart rate (beats per minutes, bpm), respiratory rate (breaths per minute), systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) will be collected. The respective vital signs abnormalities are defined in Table 3. Moreover, only the vital signs values will be used, no clinical interpretations, therefore, grade 3 and 4 is shown combined as grade 4 always requires clinical interpretation.

Table 3: Vital Signs Toxicity table for Cohort 0

Vital Signs	Grade 1	Grade 2	Grade 3/4
Tachycardia – beats (HR) per minute	101 – 115	116 – 130	>130
Bradycardia – beats (HR) per minute	50 – 54	45 – 49	< 45
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	>155
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	>100
Hypotension (systolic) - mm Hg	85 – 89	80 – 84	< 80
Respiratory Rate - breaths per minute	17-20	21-25	>25

For Cohort 1, heart rate (beats per minutes, bpm) and respiratory rate (breaths per minute) will be collected. Since no vital sign toxicity table is available for toddlers any abnormality will be recorded as an AE, by the investigator. Only respiratory rates will be summarized with descriptive statistics.

For both cohorts, temperature (diary, on site assessments) will be allocated to predefined temperature intervals (from 37.5° C until 40°C, in steps of half degree increments; eg <37.5, 37.5-<38, 38-<38.5, … >40). Tables will show the maximum temperature for both diary and onsite assessments separately. A listing of subjects with fever according to the FDA grading table will also be provided.

Respiratory rates will also be summarized descriptively for both Cohorts.

Any abnormal physical examination result will be documented as AEs, by the investigator.

6. IMMUNOGENICITY ANALYSIS

The analysis of immunogenicity will use the PPI set. The most important tables might be repeated on the FA set as a sensitivity analysis.

6.1. Parameters

The following humoral and cellular immune responses will be measured in the different vaccine regimen tested. However not all assays will be available for all immunogenicity analyses covered by this SAP. Further information on which assays will be analyzed in each one of the analysis, will be included in the corresponding DPS documents.

Immunogenicity against the insert:

Humoral immune response

- RSV neutralization A strain (titers of neutralizing antibodies).
- RSV F protein enzyme-linked immunosorbent assay (ELISA; pre- and/or post-fusion F antibodies).
- RSV neutralization B and/ or a different A strain (titers of neutralizing antibodies), if available.
- Functional and molecular antibody characterization, if available.

Cell-mediated immune response

- Intracellular cytokine staining (ICS, unit: % of subset) or cytokine analysis. Analysis of CD4 and CD8 T-cell subsets and their cytokine expression patterns will be determined by flow cytometry after RSV F-protein peptide stimulation (including, but not limited to CD4/CD8, interleukin-2 [IL-2], IFNγ, TNFα and Th1/Th2 subtyping).
  **Cytokine analysis for Th1/Th2 profiling will be done in cases where no ICS data can be generated due to insufficient number of PBMCs for ICS assay (see exploratory endpoints below for description)*

- ELISpot IFNγ assay (units: SFU/10^6 PBMC). An ELISpot assay is used to quantify the amount of peripheral blood mononuclear cells (PBMCs) able to produce IFNγ upon RSV F-protein peptide stimulation, if available.

- PBMC secreted cytokines and ELISpot IFNγ are exploratory endpoints and will only be analyzed, if available.

- Cytokine analysis, Cytokine profiles of (in vitro) stimulated PBMC supernatant will be analysed to assess the quantity and quality of the elicited immune responses, including Th1/Th2 balance. Analysis will include, but is not limited to, IFNγ, IL-2, IL-4, IL-5, IL-13, and TNFα, if available.

Immunogenicity against the vector:

- Adenovirus neutralization assay

This assay assesses neutralizing antibody responses against the Ad26 vectors. This is an exploratory endpoint and will only be analyzed if available.

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

Missing immune response data will not be imputed.

Values below the lower limit of quantification (LLOQ) or the provisional cut-off will be treated differently according to the assay:

- Humoral Assays: Values will be imputed based on the type of analysis. For the calculation of the geometric mean titer, values below LLOQ will be imputed to LLOQ/2. While for the calculation of the geometric mean of the increase from baseline, values below LLOQ will be imputed to LLOQ. The LLOQ values per assay are available in the database.
- For ICS assays: no valid LLOQ is available. A provisional cut-off is put at 0.02%. (only for total cytokine response). For the individual cytokine combinations of IFNg, TNFa and IL2, negative values will be imputed with 0. For descriptive statistics or graphs on actual values, values below the cut-off will be imputed to a value of LLOQ/2. For Th1 and Th2, a provisional cut-off of 0.001% is used. These values might change in the future, depending on progressing insight. For descriptive statistics or graphs on actual values, values below the LLOQ will be imputed to a value of LLOQ/2.
- For all assays: values above ULOQ will be imputed with 2xULOQ.

6.3. Immune Response Analysis

No formal hypothesis on immunogenicity will be tested.

6.3.1. Immunogenicity against the insert:

6.3.1.1. Humoral assays

For **VNA** and **ELISA** assays following results will be calculated: N, geometric mean[§] and corresponding 95% CI of the actual values and fold increases from baseline will be tabulated and graphically presented. *[§]calculate the mean and corresponding 95%CI of the log_2 transformed values, back-transform this mean [i.e. 2^mean] and CI [i.e. 2^CI].*

Tables showing fold increases will also present the percentage of subjects with an x-fold increase (for several values of x).

Actual values and fold changes from baseline are tabulated and shown as dot plots with dots for subject values, and the corresponding geometric mean and 95% CI per time point for each assay. In addition, GMT plots over time, combining the regimens in one graph (without individual subject dots) will also be created.

Subject profiles of the actual values over time will be graphically presented.

Reverse distribution curves of the actual values are provided for selected time points.

In the graphs, original values will be displayed on the log_2 scale.

A scatterplot with the VNA versus ELISA will be provided for the most important time points. In these scatter-plots the actual values will be shown, even if they are below the LLOQ, but the LLOQ cut-off will be visualized in the graph per assay if some values are below LLOQ.

6.3.1.2. Cellular assays

For **ICS** and **PBMC secreted** cytokines (if available) analyses may include:

Total Cytokine response: the % of subsets expressing at least IFNg, TNFa or IL2 will be calculated for CD4 and CD8, separately.

Tables with the corresponding descriptive statistics will be provided.

Subject profiles of the actual values over time will be graphically presented.

Actual values are shown as box plots with dots for subject values, and the corresponding median and the first and third quartile (Q1, Q3) per time point for each assay.

In addition, box plots over time, combining the regimens in one graph (without individual subject dots) will also be created.

For all cytokine combinations (IFNg and/or TNFa and/or IL2) pie charts reflecting the distribution of each of the cytokine combinations (the proportion of a specific cytokine combination of the CD4 or CD8 T-cells secreting at least one cytokine) and bar charts reflecting the median magnitude of each combination will be graphically presented. Tables with the corresponding descriptive statistics will be provided.

Th1 and Th2: Th1 is defined as all CD4+ IFNγ+ and Th2 as all CD4+ IL4+ cells. Subject profiles and graphs of the actual values over time (box-plot type) will be created. In addition, at time points of interest, scatterplots of Th1 vs Th2 might be created.

For the graphs, original values will be displayed on the $\log_{10}$ scale.

The technical details for the calculation of the ICS values to be used in the graphs will be outlined in the DPS.

For **ELISpot,** if available following results will be calculated: N, median, quartiles and range of the actual values will be tabulated and graphically presented.

Tables with the corresponding descriptive statistics will be provided.

Subject profiles of the actual values over time will be graphically presented.

Actual values are shown as box plots with dots for subject values, and the corresponding median and interquartile range per time point for each assay. In addition, box plots over time, combining the regimens in one graph (without individual subject dots) will also be created. For the graphs, original values will be displayed on the $\log_{10}$ scale.

6.3.2. Immunogenicity against the vector

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

Scatterplots with the assays against the vector versus the assays against the inserts might be created for the most important time points. In these scatter-plots the actual values will be shown, even if they are below the LLOQ.

7. RSV INFECTIONS

In case of a severe LRTI being present in Cohort 1, then all potential cases of RSV infection will be summarized in the following two categories and based on their severity (severe/ non-severe).

- Suspected RSV infection: subject has persistent (>48 hours) clinical symptoms of rhinitis (sneezing or runny nose or congestion) and persistence (>24 hours) of one or more of the following: cough, abnormal breathing, fever, lethargy or decreased appetite. Occurrence of this combination of symptoms in any subject would trigger collection of a nasal turbinate sample.
- Confirmed RSV infection: subject with suspected RSV infection and positive for RSV on the basis of RT-PCR conducted centrally.

ATTACHMENTS

Attachment 1: Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

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.

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23-26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***"ULN" is the upper limit of the normal range.

Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	ï€ 1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)
International Normalized Ratio (INR)***	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** "ULN" is the upper limit of the normal range.

***: 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, 2014 (version 2.0)

Urine *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization forhyperglycemia
Blood (microscopic) - red blood cells per high power field (rbc/hpf)	1 - 10	11 - 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

***** The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Vital Signs *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C) ** (°F) *	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.

** Oral temperature; no recent hot or cold beverages or smoking.

*** When resting heart rate is between 60 - 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Attachment 2: Toxicity tables for cohort 1

Grading scale used for clinical AEs is adapted from the Division of Microbiology and Infectious Diseases (DMID) "Pediatric Toxicity Tables for Children Greater Than 3 Months of Age (2007)". For AEs not included in the tables below, severity criteria guidelines will be provided in the full protocol.

Gastrointestinal	Grade 1	Grade 2	Grade 3	Grade 4
Nausea/vomiting	Minimal symptoms; caused minimal or no interference with, school or self-care activities. Child vomits once per day (24h).	Notable symptoms; required modification in activity or use of medications; did not result in cancellation of social activities. Child vomits 2-3 times per day (24h).	Incapacitating symptoms; required bed rest and/or resulted in cancellation of social activities. Child vomits 4-6 times per day (24h).	Unable to ingest food or fluid for more than 24 hours/≥ 7 episodes of vomiting per day or intractable vomiting. Emergency room visit or hospitalization and significant medical intervention/therapy required.
Diarrhea	Consistency of stools changes OR increase of 1-3 stools compared to normal frequency over a 24 hour period.	Liquid stools OR increase of 4-6 stools compared to normal frequency over a 24 hour period.	Increase of ≥7 stools compared to normal frequency over a 24 hour period OR child might need an infusion with fluid without hospitalization.	Emergency room visit or hospitalization and significant medical intervention/therapy required.
Appetite	Some loss of appetite but no decrease in oral intake.	Loss of appetite associated with decreased oral intake.	Almost no appetite, does not eat and/or weight loss.	Emergency room visit or hospitalization and significant medical intervention/therapy required.

Reactogenicity	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Systemic reactions				
Irritable/Fussy/Crying/ Screaming	Easily consoled and returns to play easily. He/she has periods of crying fewer than 60 minutes.	Not easily consoled and is not easily interested in playing. He/she has periods of crying lasting between 60-120 minutes.	Very irritable, cannot be consoled and does not play. He/she has periods of continuous crying lasting more than 2 hours.	Inconsolable. Emergency room visit or hospitalization and significant medical intervention/therapy required.
Lethargy	Minimal symptoms; caused minimal or no interference with school or activities.	Notable symptoms; required modification in activity or use of medications; did not result in loss school or cancellation of social activities.	Incapacitating symptoms; required bed rest and/or resulted in loss of school or cancellation of social activities.	Emergency room visit or hospitalization and significant medical intervention/therapy required.

Other	Grade 1	Grade 2	Grade 3	Grade 4
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.	Sustained Fever: equal or greater than 40C (104.0F) for longer than 5 days.

Reactogenicity	Grade 1	Grade 2	Grade 3	Grade 4
Local reactions				
Pain/tenderness at injection site	Mild discomfort when the injection site is touched; child does not limit use of his/her arm or leg where the injection was done.	Notable discomfort when the injection site is touched; child limits use of his/her arm or leg where the injection was done.	Severe discomfort when the injection site is touched; child avoids use of his/her arm or leg where the injection was done.	Emergency room visit or hospitalization and significant medical intervention/therapy required.
Erythema/redness	≥10 and <25 mm diameter.	≥25 and <50 mm diameter.	≥50mm diameter.	Emergency room visit or hospitalization; necrosis or exfoliative dermatitis.
Induration/swelling	≥10 and <25 mm diameter.	≥25 and <50 mm diameter.	≥50mm diameter.	Emergency room visit or hospitalization; necrosis or exfoliative dermatitis.

Attachment 3: Transforming the on-site assessments and diaries of solicited AEs into analysis format

When creating the analysis dataset for solicited AEs, solicited AEs (recorded by day on the SR and FA domains) need to be converted into the same format as unsolicited AEs (recorded by event). For this purpose, the start date of the AE will be considered as the date of first occurrence of the solicited AE. 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 solicited AE, then the second (and/or next) occurrence will be considered as a new AE. In case no data is reported for a day, this is analyzed as no event reported. If the on-site assessment differs in grade or relatedness with the Day 1 diary data, the on-site assessment should be recorded as a separate record in the database.

The example below shows how the solicited AE should be converted into a format of unsolicited AEs:

Data from the Subject Diary

Subject: 0001

Solicited systemic AE: Headache

	On site assessment	DIARY DATA							
Solicited AE	Day 1 01Jan16	Day 1 01Jan16	Day 2 02Jan16	Day 3 03Jan16	Day 4 04Jan16	Day 5 05Jan16	Day 6 06Jan16	Day 7 07Jan16	Day 8 08Jan16
Grade	2	1	1	0	3	3	1	0	0
Relatedness	Doubtful	Probable							

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

Subject No.	AE	Start Date (Char)	Stop Date (Char)	Severity	Relatedness	AEID
0001	Headache	01Jan16	01Jan16	2	Doubtful	1
0001	Headache	01Jan16	02Jan16	1	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 last day that AE was reported and the maximum severity (or/and diameter for local AEs) after Day 8 are captured in the CRF. For this a separate record needs to be created, in case this severity deviates from the previous record.

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 (AE identification) is set to the same value and the duration of the AE is set to 6 for all records.

Notes:

- For solicited AEs time should not be taken into account to allocate an event to a phase, the event is per definition of solicited AEs collected post-dose and should therefore not be allocated to inactive phases.
- To complete the start and end-date based on diary data, the date will be calculated based on the day the AE is reported relative to vaccination and not on the reported date. For example, if the vaccination is on 1st JAN2016, and the AE starts on DAY 3, the start date will be set to the 3rd of January 2016 independent of the reported actual date.