Janssen Vaccines & Prevention B.V.*

Statistical Analysis Plan

A Randomized, Double-blind, Placebo-controlled Phase 1/2a Study for Safety and Immunogenicity Evaluations of various RSV.preF-based Vaccine Formulations in Adults Aged 60 Years and Older

Protocol VAC18195RSV1001; Phase 1/2a

VAC18195 (JNJ 64400141/ JNJ 64213175/ JNJ-86051823/ JNJ 78991172)

* Janssen Vaccines & Prevention B.V. is a Janssen pharmaceutical company of Johnson & Johnson and is hereafter referred to as the sponsor of the study. The sponsor is identified on the Contact Information page that accompanies the protocol.

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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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VERSION HISTORY

Table 1 – SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
1.0	23-Jun-2022	Not Applicable	Initial release

1. INTRODUCTION

This statistical analysis plan (SAP) contains information needed to perform the complete safety and immunogenicity analysis of the VAC18195RSV1001 trial. It applies to all the analyses described in Section 9.4 of the clinical trial protocol (CTP). The specifications of individual tables, listings, and figures to be generated in each analysis will be described in a separate data presentation specifications (DPS) document.

1.1. Objectives and Endpoint

Refer to Section 3 of the CTP.

1.2. Study Design

Please refer to Section 4.1 of the CTP for more details on the study design, and Section 6.3 of the CTP for details on randomization and procedures for maintaining the blind.

2. STATISTICAL HYPOTHESES

No formal hypothesis will be tested. All the statistical analyses will be performed descriptively.

3. SAMPLE SIZE DETERMINATION

Refer to Section 9.2 of the CTP.

4. POPULATIONS (ANALYSIS SETS) FOR ANALYSIS

For vaccine studies, study intervention assignment will follow the as-treated principle. The analysis sets are defined in the Table 2.

Table 2 – Analysis sets

Analysis Sets	Description
All screened	This analysis set includes all participants screened for the study, regardless of whether they were screen failures or they got enrolled in the study. Rescreened participants are counted only once.
Randomized	The randomized analysis set includes all participants who were randomized in the study.
Full Analysis Set (FAS)	The full analysis set (FAS) will include all participants who received the study vaccine, regardless of the occurrence of protocol deviations. All safety and participant information analyses will be based on the FAS.
Per-protocol Immunogenicity (PPI) Set	The Per-protocol Immunogenicity (PPI) Set will include all randomized participants who received the planned study vaccine(s) and for whom immunogenicity data are available. Samples taken after a participant experiences a major protocol deviation expected to impact the immunogenicity outcomes will be excluded from the PPI analysis. The list of major protocol deviations that would lead to elimination from the immunogenicity analysis will be specified in the major protocol deviation criteria

Analysis Sets	Description
	document, which will be finalized before database lock and unblinding. The analysis of all immunogenicity endpoints will be based on the PPI Set. For key tables, sensitivity immunogenicity analyses might also be performed on the FAS.

5. STATISTICAL ANALYSES

5.1. General Considerations

5.1.1. Study phases

A baseline (or reference) value will be defined as the value of the last available assessment prior to vaccination on Day 1. If there was no immunogenicity assessment done pre-vaccination, the earliest assessment post-vaccination on Day 1 (but not exceeding Day 1) can be used as the baseline value for the immunogenicity analysis, if available.

The results of the safety analysis will be presented by phase. 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 vaccination).
- Study Day = visit date – date of Day 1; if visit date < date of Day 1 (date of vaccination).

5.1.2. Phase definition

The phases in the study will be constructed as follows:

Table 3 – Phase definitions

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing	One minute prior to start of the Post-dose period
Regimen	2	Post-dose 1	1	Date and time of the first vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the database cut-off date for analyses conducted before the final analysis c) maximum of (28 days after vaccination at 23:59, scheduled visit 28 days after vaccination at 23:59)

Follow-up 1	3			One minute after Post-dose 1 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation or participants that completed the study) b) 23:59 at the database cut-off date for analyses conducted before the final analysis c) End of Study Visit day at 23:59 d) date/time of second vaccination
Regimen	2	Post-dose 2	2	Date and time of the second vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the database cut-off date for analyses conducted before the final analysis c) maximum of (28 days after vaccination at 23:59, scheduled visit 28 days after vaccination at 23:59)
Follow-up 2	4			One minute after Post-dose 2 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation or participants that completed the study) b) 23:59 at the database cut-off date for analyses conducted before the final analysis c) maximum of (End of Study Visit, scheduled visit 182 days after vaccination at 23:59)

5.1.3. Immunogenicity Visit Windows

For the immunogenicity analysis, assessments will be allocated to an analysis visit based on the planned visit as captured in the CRF. Visits that are out of the protocol-defined visit windows (see the table below) will not be included in the per-protocol immunogenicity analysis. However, they may be included in sensitivity analyses.

Table 4 – Immunogenicity timepoints

Analysis time point label (Relative to Day 1)	Reference day	Target day (from reference day)	Window of target day (days)
Cohorts 1 and 2			
Day 1	Day of vaccination 1	1	(-∞, 1]
Day 15	Day of vaccination 1	15	[12, 18]
Day 29	Day of vaccination 1	29	[22, 36]
Day 85	Day of vaccination 1	85	[78, 92]
Day 183	Day of vaccination 1	183	[169, 197]
Day 365	Day of vaccination 1	365	[335, 395]
Day 730	Day of vaccination 1	730	[700, 760]
Day 1095	Day of vaccination 1	1095	[1065, 1125]
Cohorts 3 (Arms 10 and 13)			
Day 1	Day of vaccination 1	1	(-∞, 1]

Day 15	Day of vaccination 1	15	[12, 18]
Day 29	Day of vaccination 1	29	[22, 36]
Day 85	Day of vaccination 1	85	[78, 92]
Day 183	Day of vaccination 1	183	[169, 197]
Day 365	Day of vaccination 1	365	[335, 395]
Cohorts Cohort 3 (Arms 11a, 11b and 12) and Cohort 4			
Day 1	Day of vaccination 1	1	(-∞, 1]
Day 15	Day of vaccination 1	15	[12, 18]
Day 29	Day of vaccination 1	29	[22, 36]
Day 85	Day of vaccination 1	85	[78, 92]
Day 183	Day of vaccination 1	183	[169, 197]
Day X	Day of vaccination 1	X	[X-30, X+30]
Day X+14	Day of vaccination 2*	15	[12, 18]
Day X+28	Day of vaccination 2*	29	[22, 36]
Day X+84	Day of vaccination 2*	85	[78, 92]
Day X+182	Day of vaccination 2*	183	[169, 197]
Day 730	Day of vaccination 1	730	[700, 760]
Day 1095	Day of vaccination 1	1095	[1065, 1125]
Day 1460	Day of vaccination 1	1460	[1430, 1490]
Day 1825	Day of vaccination 1	1825	[1795, 1855]

Notes: * in case of missed vaccination 2, the actual date of missed vaccination 2 (i.e. the date of the day X visit) is used as a reference day.

5.2. Participant Dispositions

Participant information will be shown for the FAS. The number of participants in the following disposition categories will be summarized throughout the study by intervention group and overall:

- participants screened
- participants not randomized and not vaccinated
- participants not randomized and vaccinated
- participants randomized and not vaccinated
- participants randomized and vaccinated
- participants in the FAS
- participants in the PPI Set
- participants who discontinued study vaccine with reasons for discontinuation
- participants who discontinued the study with reasons for discontinuation

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

Other participant information variables: demographics and baseline characteristics, major protocol deviations, and prior and concomitant medications will be analyzed as described in Sections 6.3, 6.4 and 6.5, respectively. Medical history and concomitant diseases will be tabulated by body system class and preferred term.

5.3. Primary Endpoint(s) Analysis

5.3.1. Definition of Endpoints

For cohort 1 to 3 the primary safety endpoints are defined as:

- Serious adverse events (SAEs) and adverse events of special interest (AESIs) from first dose administration until 6 months after vaccination
- Solicited local and systemic adverse events (AEs) for 7 days after vaccine administration
- Unsolicited AEs from the time of vaccine administration through the following 28 days

In addition, for cohorts 1 and 2, and cohort 4 the primary immunogenicity endpoint is defined as RSV neutralization antibody titers.

5.3.2. Analysis Methods for the Primary Safety endpoints

No formal statistical testing of safety data is planned. Safety data will be analyzed descriptively by cohort (or pooled over cohorts, as applicable) and study arm, and by group (cohort 1), as applicable, per phase and across the entire study period. All safety analyses will be made on the FAS.

The verbatim terms used in the eCRF by investigators to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Any AE occurring at or after the first vaccination is considered to be treatment emergent. All reported AEs with onset during the active phase (ie, AEs occurring after vaccination up to 28 days post-vaccination) or AEs present before the active phase but worsening during the active phase, and all SAEs, AEs leading to study or vaccine discontinuation during the study, potential AESIs and AESIs (up to 6 months after vaccination) will be included in the analysis. For each AE, the percentage of participants who experience at least 1 occurrence of the given event will be summarized. All other unsolicited adverse events collected outside the 28-day period will be presented through listings.

AESIs (as determined in Section 5.3.2.1) will be reported until 6 months post vaccination and additionally by different time periods:

- 0 – 28 days post dose,
- 29 days – 56 days post dose,
- 0 – 56 days post dose,
- 57 days – 6 months post dose,
- 0 – 6 months post-dose.

5.3.2.1. Definitions of Adverse Events

Solicited AEs shown in the tables will be extracted from the investigator assessment pages (CE) of the CRF. Solicited administration site symptoms by definition will be considered as related to the study vaccine.

The severity of the AEs will be classified as grade 1 to 4. Solicited events of grade 0, not reported in the CE domain, will therefore not be reported in the AE analysis.

In addition to the potential AESIs as identified by the investigator in the database, potential AESIs will also be selected programmatically. Those will include all reported AEs that are identified by the selection rule: SMQ (Standardised MedDRA Queries) = "EMBOLIC AND THROMBOTIC EVENTS (SMQ)" or (SUB_SMQ1 = "HAEMATOPOIETIC THROMBOCYTOPENIA (SMQ)" and SCOPE in ("BROAD", "NARROW")) or HLT (higher level term)="Thrombocytopenias".

5.3.2.2. Analysis of Adverse Events

Number and percentage of participants with at least one particular AE (unsolicited/solicited) will be tabulated. Unsolicited AEs will be summarized by System Organ Class (SOC) and Preferred Term (PT).

Solicited AEs will be summarized by class (administration site, systemic) and Derived Term. The number and percentages of participants with at least one solicited local (at injection site) or systemic adverse event will be presented. For solicited AEs, the following tables will be provided: overall summary, by worst severity grade, at least grade 3, related (systemic only), time to onset (in days) and duration (in days). Note: Duration is defined as number of days from the start of the event until resolution of the event. The time to first onset is defined as date of first onset – reference date + 1. The reference date is the start date of the post-dose period. For solicited AEs, the denominator for the percentages is the number of participants with solicited event data assessed by the investigator in the considered population, phase and arm (incidence per 100 participants/phase).

A tabulation of the distribution of temperatures per half degree intervals will also be provided.

Frequencies of unsolicited AEs, separately for all and vaccination-related only, will be presented by SOC and PT. The following tables will be provided: overall summary, all AEs, most frequent AEs, AEs with at least grade 3, related AEs, AEs leading to death, AEs leading to study discontinuation, SAEs, related SAEs, potential AESIs and AESIs, COVID-19 related AEs.

AESIs, AEs flagged by the investigator as potential AESI and AESIs identified via a programmed selection will be summarized by Interest Category and PT. Denominator for the percentages is the number of participants in the considered population, phase and arm (incidence per 100 participants/phase).

Potential AESIs selected programmatically will be tabulated by categories: 'Embolic and thrombotic events (SMQ)' and 'Haematopoietic Thrombocytopenias (SMQ) (broad) or HLT = Thrombocytopenias'. Potential AESI determined programmatically, related to study vaccine (investigator assessment), will be tabulated similarly.

Potential AESIs as identified by the investigator, will be tabulated by categories: 'Embolic and thrombotic events (SMQ)' and 'Haematopoietic Thrombocytopenias (SMQ) (broad) or HLT =

Thrombocytopenias', and 'Other'. Potential AESI as identified by the investigator, related to study vaccine (investigator assessment), will be tabulated similarly.

AESIs (as confirmed by review) will be tabulated by categories: 'Embolic and thrombotic events (SMQ)' and 'Haematopoietic Thrombocytopenias (SMQ) (broad) or HLT=Thrombocytopenias'.

All AESI analyses will be presented by phase as well as by time interval. The definition of the different time intervals can be found in Section 5.3.2.3.

For AESIs, potential AESIs as identified by the investigator and potential AESIs determined programmatically, attribution to the intervals will be done similarly to the unsolicited AEs as described in Section 5.3.2.3. For Step 2 of phase allocation of adverse events, the '0 - 28 days post-dose' interval should be treated similar to active periods and the rest as non-active periods.

Summaries, listings, datasets, or participant narratives may be provided, as appropriate, for those participants who die, who discontinue the study vaccination due to an AE, who discontinue the study due to an AE, or who experience a severe AE, an AESI, an SAE or a COVID-19 infection.

5.3.2.3. Phase Allocation of Adverse Events

As the analysis of solicited events will be based on the overall assessment of the investigator, which is documented in the CE domain, the Analysis Data Model (ADaM) dataset will be based on the CE domain. Solicited events are allocated to the phases as described below, however they are always allocated to Post-dose period and will never be attributed to the screening phase. Time of day is not considered while attributing solicited AEs to phases.

For unsolicited AEs, the steps below are followed as well.

Step 1: Allocation of events to the periods:

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

- In case of partial start or stop dates (i.e., time and/or day and/or month and/or year missing), the events are allocated to the periods using the available partial information on start and end date; no imputation will be done. If, for instance, the AE start date only month and year are available, these data are compared to the month and year information of the periods. This rule may lead to multiplication of the event as a consequence of its assignment to multiple periods.
- In case of a completely missing end date, the date is imputed by the cut-off date of the analysis for participants still ongoing in the study, and by the end date of the last period for participants who discontinued or completed the trial. The imputed end dates will not be shown in the data listings.
- In case of a completely missing start date, the event is allocated to the first active phase (Post-dose period), except if the end date of the AE falls before the start of the first active phase (Post-dose period).

Step 2: Combination of events:

Overlapping/consecutive events are defined as events of the same participant 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 phases/periods - Screening or Follow-up (defined as non-active periods) - followed by an AE in - Post-dose period (defined as active period) - they are allocated to their respective phases/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 consecutive 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. In case overlapping/consecutive events start in non-consecutive periods (regardless of active or non-active), they are allocated to their respective period and are considered as separate AEs.

5. In case a non-active period is followed by another non-active period, and the overlapping/consecutive events start in both periods, they are allocated to the first period and 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.

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.

The AESIs will be presented by phase as well as by time interval. The definition of the different time intervals can be found below. Attribution to the intervals will be done similarly to the unsolicited AEs as described above. For Step 2 of phase allocation of adverse events, the '0 - 28 days post-dose' intervals should be treated similar to 'active' periods and the rest as 'non-active' periods.

The definition of the time intervals is shown in the table below. Additionally, in the tables a '0-56 days post-dose' interval and a '0-6 months post-dose' interval should also be shown. The '0-56 days post-dose' interval is the combination of the '0 - 28 days post-dose' interval and the '29 - 56 days post-dose' interval. The '0-6 months post-dose' interval is the combination of the '0 - 28 days post-dose', '29 - 56 days post-dose' and '57 days - 6 months post-dose' intervals.

Definition of intervals:

Dose	Interval	From	to
Post-vaccination 1	0-28 days post dose	Date time of the 1st vaccination	Min of: • 23:59 at the date of last contact (for discontinuations) • 23:59 at date of DB cut-off for interim analyses • Maximum(Date of Vaccination 1 + 28 days at 23:59, date of scheduled visit 4 weeks after 1st vaccination at 23:59)
	29-56 days post dose	One minute after the end of the interval 0-28 days post dose	Min of: • 23:59 at date of last contact (for discontinuations) • 23:59 at date of DB cut-off for interim analyses • Date of Vaccination 1 + 56 days at 23:59
	57 days - 6 months post-dose	One minute after the end of the interval 29-56 days post dose	Min of: • 23:59 at date of last contact (for discontinuations) • 23:59 at date of DB cut-off for interim analyses • Maximum of (Date of Vaccination 1 + 183 days at 23:59, date of scheduled visit 183 days post 1st vaccination at 23:59)
	>6 months post-dose	One minute after the end of the interval 57 days - 6 months post-dose	Min of: • 23:59 at the date of last contact (for discontinuations) • 23:59 at the date of DB cut-off for interim analyses

			• 1 min prior to date time of vaccination 2
Post-vaccination 2	0-28 days post dose	Date time of the 2nd vaccination	Min of: • 23:59 at the date of last contact (for discontinuations) • 23:59 at date of DB cut-off for interim analyses • Maximum(Date of Vaccination 2 + 28 days at 23:59, date of scheduled visit 4 weeks after 2nd vaccination at 23:59)
	29-56 days post dose	One minute after the end of the interval 0-28 days post dose	Min of: • 23:59 at date of last contact (for discontinuations) • 23:59 at date of DB cut-off for interim analyses • Date of Vaccination 2 + 56 days at 23:59
	57 days - 6 months post-dose	One minute after the end of the interval 29-56 days post dose	Min of: • 23:59 at date of last contact (for discontinuations) • 23:59 at date of DB cut-off for interim analyses • Maximum (Date of Vaccination 2 + 183 days at 23:59, day 547 visit at 23:59)
	>6 months post-dose	One minute after the end of the interval 57 days - 6 months post-dose	Min of: • 23:59 at the date of last contact (for discontinuations) • 23:59 at the date of DB cut-off for interim analyses

The combination of the '0 - 28 days post-dose' interval and the '29 - 56 days post-dose' interval will be referred as '0 - 56 days post-dose'.

The combination of the '0 - 28 days post-dose', '29 - 56 days post-dose' and '57 days - 6 months post-dose' intervals will be referred as '0-6 months post-dose'.

5.3.2.4. Missing Data

Missing data will not be imputed. Participants who do not report an event will be considered as participants without an event. An AE with a missing severity or relationship will be considered as an AE reported but will be considered as not reported for the severity or relationship. For example, an AE with missing severity will be considered as an AE reported for the analysis of any grade but will be considered as not reported for the analysis of at least grade 3.

5.3.3. Analysis Methods for the Primary Immunogenicity endpoint(s)

For Cohort 1, 2 and 4 the primary endpoint will be RSV neutralization antibody titers measured for RSV-A and RSV-B. In Cohort 3, RSV neutralization antibody titers are a secondary endpoint.

Data of corresponding arms from Cohort 1 and Cohort 2 will be combined in all analyses. Immunogenicity data of corresponding arms from Cohort 3 and Cohort 4 will be combined as applicable, too.

Descriptive statistics will be used for all arms to analyze the actual values (geometric mean, 95% CI, minimum, maximum, median and quartiles) and fold increase from baseline at all timepoints, as well as the percentage of participants with 2- and 4-fold increases from baseline. Additionally, the RSV-A/RSV-B antibody ratio will be reported by time points and the fold increase of RSV-A/RSV-B antibody ratio from baselines will be calculated at all timepoints.

For combined data from Cohort 1 and Cohort 2, geometric mean ratios for each active arm relatively to control Arm 1a and relatively to control Arm 1b, as well as relatively to each other will be calculated at all available timepoints along with corresponding 95% CI as applicable.

For Cohort 3, Arms 11a and 11b will be combined and the active arms (10, 11a+11b, 13) will be compared to each other at all available timepoints up to 1 year after vaccination on Day 1 by calculating the geometric mean ratios along with corresponding 95% CIs.

For durability and revaccination evaluation analyses, the following arms will be combined and compared to each other for all timepoints:

- Arm 11a (Cohort 3) combined with Arm 14 (Cohort 4);
- Arm 11b (Cohort 3) combined with Arm 15 (Cohort 4);
- Arm 12 (Cohort 3) combined with Arm 16 (Cohort 4).

Geometric means per arm of values for RSV-A, RSV-B and the ratio of RSV-A/RSV-B, geometric mean ratios for RSV-A, RSV-B and the ratio of RSV-A/RSV-B between all active arms along with corresponding 95% CIs will be estimated via an analysis of variance (ANOVA) using log-transformed post-vaccination immuno parameter response as dependent variable and study arm as independent variable. The means and differences in mean and CIs will be back transformed (by exponentiation) to CIs around a geometric mean or geometric mean ratio. This analysis will be performed by time points, separately per parameter. As a sensitivity analysis, the ANOVA may also be performed adjusting for baseline titer, age and sex.

Graphical presentation of actual values, fold increase from baseline and geometric mean ratios (between active arms) will be presented in scatterplots, boxplots, dot-plots, spaghetti plots etc. as applicable. In addition, plots of geometric mean titers (GMT) over time, combining the regimens in one graph (without individual participant dots) will be created. Reverse distribution curves of the actual values will be provided, for baseline and the different timepoints. In the graphs, original values will be displayed on the log2 scale.

5.4. Secondary Immunogenicity Endpoint(s) Analysis

Key secondary endpoints (for Cohorts 1 and 2) include F protein binding antibodies (pre-F and/or post-F), and antigen-specific T-cell responses (as defined by IFN-γ ELISpot).

The data on titers of F protein binding antibodies for RSV-A and RSV-B and antigen-specific T-cell responses will be analyzed similarly as described in Section 5.3.3 but using log2 or log10 transformation as applicable. For ELISpot, only the following descriptive statistics will be calculated: N, median, quartiles, minimum and maximum.

5.5. Exploratory Endpoints Analysis

Exploratory endpoints may include the following, but are not limited to: RSV neutralization antibody titers against additional A and/or B strains, anti-F protein antibody specificity and functionality characterization, adenovirus neutralization antibody titers, molecular antibody characterization (eg, avidity, Fc cell interaction, antibody-dependent cell-mediated cytotoxicity [ADCC], antibody-dependent cell-mediated phagocytosis [ADCP], antibody isotyping, antibody sequencing for repertoire), including but not limited to immunoglobulin (Ig)A and IgG, chemokines and cytokines in serum samples, transcriptome analysis and sequencing of the T-cell receptor (TCR) and/or B-cell receptor (BCR) or heavy/light chain (VH/VL) characterization, evaluation of the cellular immune response and the functional and memory immune response by intracellular cytokine staining (ICS), cellular phenotyping and memory B-cell ELISpot.

Scatterplot with humoral and cellular assays may be provided for the most important time points. Alternatively, in case of no quantitative data available or in case of sparse data, the results may be summarized by frequency and proportions along with 95 CIs.

5.5.1. ICS

For ICS, if available, analyses may include:

Total Cytokine response: the % of subsets expressing at least IFNγ, TNFa or IL2 will be calculated for CD4 and CD8 separately (IFNγ and/or TNFa and/or IL2). Here, at least one cytokine should be positive. For total cytokine responses, tables with number of observations, median, first and third interquartile per timepoint 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.

Total CD8 cytotoxic response: the % of subsets expressing cytotoxic markers such as Perforin, Granzyme B and/or CD107a will be calculated for CD8. Tables with number of observations, median, first and third interquartile per timepoint 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.

Polyfunctionality analysis will be assessing the possible cytokine combinations with at least one cytokine being positive (for example, IFNγ+TNFa+ IL2+, IFNγ+TNFa+IL2-, IFNγ+TNFa-IL2- etc). For the polyfunctionality analysis bar charts reflecting the median magnitude and/or the mean±SD of each combination may be graphically presented. In that case, tables with the corresponding descriptive statistics will also be provided.

Th1 and Th2: Th1 is defined as all CD4+ IFNγ and/or TNFa and/or IL2 and Th2 as all CD4+ as IL4 and/or IL13 and CD40L+ cells. Subject profiles and graphs of the actual values over time (box-plot type including individual scatterplots) 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 log10 scale.

The technical details for the calculation of the ICS values to be used in the graphs will be outlined in the data presentation specifications (DPS) document.

5.5.2. VNA against Ad26 vector

For VNA against the Ad26 vector, if available following statistics will be calculated: N, geometric mean and corresponding 95% CI of the actual values. Subject profiles of the assays against the insert (RSV-A and RSV-B) will be repeated, highlighting subjects with pre-existing immunity at baseline against the vectors. Scatterplots of the Adeno assay versus the assays against the inserts may be provided for the most important time points. In these scatterplots the actual values will be shown, even if they are below the LLOQ.

5.6. 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) 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.
- Data above the ULOQ will be imputed with the ULOQ.

The ULOQ and LLOQ values per assay will be available in the database.

For ICS: if no LLOQ is available at the time of analysis, a provisional lower limit will be used to impute as described above. The provisional lower limit will be provided in the data base.

5.7. Other Safety Analyses

The analysis of Adverse Events is described in Section 5.3.2. Other safety analyses include laboratory tests, vital signs and physical examination data. All these safety analyses will be based on the FAS using actual intervention received, unless otherwise specified. All the summary tables will be presented by cohort (or pooled over cohorts, as applicable) and study arm, and by group (cohort 1), as applicable.

For all continuous safety variables, descriptive statistics will include the N, mean, standard deviation, median, minimum, maximum, and interquartile range. Categorical variables will be summarized using frequency counts and percentages.

5.7.1. Clinical Laboratory Tests

Laboratory abnormalities will be determined according to the FDA toxicity grading tables (see Appendix 6 of CTP), or in accordance with the normal ranges for the clinical laboratory parameter: any laboratory value shown as a "graded" value in the FDA table that is within laboratory normal ranges will not be graded for severity. All the abnormalities will be summarized and listed.

5.7.2. Vital Signs

Vital signs including temperature, heart rate, respiratory rate, and blood pressure (systolic and diastolic) will be summarized over time, using descriptive statistics and/or graphical representations. The percentage of participants with values beyond clinically important limits will be summarized. Baseline and emerging vital sign abnormalities will be listed based on the abnormality gradings in Appendix 6 of the CTP. An abnormality will be considered as emerging if it is worse than the baseline value. If the baseline is missing, the abnormality is always considered emerging.

5.7.3. Physical Examinations

Physical examination findings pre-vaccination will be summarized per vaccine administration. A summary table as well as listing of the abnormalities will be presented.

5.8. Other Analyses

5.8.1. Pharmacokinetics

Not applicable.

5.8.2. Pharmacodynamics

Not applicable.

5.8.3. Biomarkers

Biomarker data analysis may be conducted at the sponsor's discretion and may be detailed and reported separately.

5.8.4. Definition of Subgroups

No subgroup analysis is planned for this study.

5.9. Planned Analyses

The following analyses are planned:

- Primary analysis: 28 days post-vaccination safety and immunogenicity analysis up to D15 for humoral antibodies. This analysis will be performed when all participants in Cohort 1 and Cohort 2 have completed their 28-day post-vaccination visit. For primary analysis humoral immunogenicity will include baseline and Day 15 data, while safety analysis will be included the data up to Day 29 visit. Data from corresponding arms from the two cohorts will be combined. The goal of this analysis will be to select the vaccine formulation to be used in Cohort 3.

- Interim analyses:
    - 84 days post-vaccination immunogenicity analysis for Cohorts 1 and 2. Available immunogenicity data of all participants in Cohort 1 and Cohort 2 up to the Day 85 will be included in the analysis.6-month post-vaccination safety and immunogenicity analysis for Cohorts 1 and 2. Available safety and immunogenicity data of all participants in Cohort 1 and Cohort 2 up to the Day 185 follow-up visit post-vaccination will be included in the analysis. This analysis may include immunogenicity data available from additional timepoints.
    - 1-year post-vaccination immunogenicity analysis for Cohorts 1 and 2. Immunogenicity data of all participants in Cohort 1 and Cohort 2 up to (and including) the 1-year follow-up visit post-vaccination will be included in the analysis.
    - 28 days post-vaccination safety and immunogenicity analysis for Cohort 3. This analysis will be performed when all participants in Cohort 3 have completed their 28-day post-vaccination visit.
    - 28 days post-vaccination safety and immunogenicity analysis for Cohort 4. This analysis will be performed when all participants in Cohort 4 have completed their 28-day post-vaccination visit.
    - 6-month post first vaccination safety and immunogenicity analysis for Cohorts 3 and 4. This interim analysis will include all safety and immunogenicity data from all participants in Cohorts 3 and 4, collected up to (and including) the 6-months follow-up visit post Day 1 vaccination.
    - 28 days post revaccination safety and immunogenicity analysis for Cohorts 3 and 4. This interim analysis will include all safety and immunogenicity data from all participants in Cohorts 3 and 4, collected up to (and including) the 28-day post-revaccination visit.
    - Yearly follow-up analyses until 3 years post first vaccination in Cohorts 1 and 2 and until 5 years post first vaccination in Cohort 3 (Arms 11a, 11b and 12) and Cohort 4. These analyses will include immunogenicity and safety data up to the 2-year follow-up visit post Day 1 vaccination, the 3-year follow-up visit post Day 1 vaccination, the 4-year follow-up visit post-Day 1 vaccination (applicable for Cohort 3 [Arms 11a, 11b and 12] and Cohort 4 only), and 5-year follow-up visit post Day 1 vaccination (applicable for Cohort 3 [Arms 11a, 11b and 12] and Cohort 4 only).
- Final analysis: immunogenicity analysis and safety data up to study end.

Additional interim analyses may be performed during the study for the purpose of informing future vaccine development-related decisions in a timely manner, or upon health authority request. If they occur, these unplanned interim analyses may replace or be combined with planned analyses, depending on the timing.

For any planned or additional analysis performed before the final analysis, the sponsor will be unblinded at the time of the analysis, while study site personnel and participants will remain blinded until the end of the study.

5.9.1. Data Review Committee (DRC)

An internal DRC will be established to evaluate safety and reactogenicity data, including interim safety and reactogenicity data of Cohort 1, to ensure the continuing safety of the participants enrolled in this study. The DRC will review blinded data but may request unblinded data if deemed necessary. Ad-hoc meetings will be convened in case any of the pre-specified study vaccination pausing rules are met. This committee will consist of at least one medical expert in the relevant therapeutic area and at least one statistician; committee membership responsibilities, authorities, and procedures will be documented in its charter. After the review, the DRC will make recommendations regarding the continuation of the study.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 List of Abbreviations

ADA	anti-drug antibody
ADaM	Analysis Data Model
AE	adverse event
ANCOVA	analysis of covariance
ATC	anatomic and therapeutic class
Analysis Data Model	Analysis Data Model
BMI	body mass index
CI	confidence interval
CRF	case report form
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DPS	Data Presentation Specifications
ELISA	enzyme-linked immunosorbent assay
eCRF	electronic case report form
FAS	full analysis set
FDA	Food and Drug Administration
ICH	International Conference on Harmonisation
IQ	Interquartile
IWRS	interactive web response system
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MRD	minimum required dilution
NAb	neutralizing antibodies
PPI	per protocol immunogenicity analysis set
Pre F	prefusion conformation-stabilized F protein
PT	Preferred term
RSV	respiratory syncytial virus
QTL	Quality Tolerance Limit
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SMQs	standardized MedDRA queries
SOC	System organ class
TEAE	treatment-emergent adverse event
VNA	virus neutralization antibody
WHO	World Health Organization

6.2. Appendix 2 Changes to Protocol-Planned Analyses

The safety laboratory data are planned to be assessed according to both local laboratory normal ranges as well as FDA toxicities grades.

6.3. Appendix 3 Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized. Table 5 presents a list of the demographic variables that will be summarized by cohort and vaccine regimen and overall, for the FAS.

Table 5 – Demographic Variables

Continuous Variables:	**Summary Type**
Age (years)	Descriptive statistics (N, mean, standard deviation [SD], median and range [minimum and maximum], and IQ range).
Weight (kg)	
Height (cm)	
Body Mass Index (BMI) (kg/m^2)	
Categorical Variables	
Sex (male, female, undifferentiated)	Frequency distribution with the number and percentage of participants in each category.
Race[a] (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, Multiple)	
Ethnicity (Hispanic or Latino, not Hispanic or Latino)	
BMI (<18.5 kg/m2 (underweight), 18.5-24.9 kg/m2 (Normal or Healthy Weight), 25-29.9 kg/m2 (Overweight), ≥30 kg/m2 (Obese))	
Risk level of severe RSV disease (Increased risk / Non-increased risk) as collected (CDC definition)	

[a]If multiple race categories are indicated, the Race is recorded as 'Multiple'

6.4. Appendix 4 Protocol Deviations

Major protocol deviations will be summarized.

In general, a list of major protocol deviations that may have the potential to impact participants' rights, safety or well-being, or the integrity and/or result of the clinical study will be specified in a major protocol violation criteria document. Participants with major protocol deviations will be identified prior to database lock and the participants with major protocol deviations will be summarized by category. In addition, minor and major protocol deviations related to COVID-19 will be tabulated.

6.5. Appendix 5 Prior and Concomitant Medications

The analysis of concomitant therapies will be done using the WHO drug coded terms. 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 (time, 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. The same rule applies for identifying whether a concomitant therapy was administered during 8 days following vaccination. If for example, the vaccination was administered on the 30 December 2021 and the concomitant therapy start date is January 2022, then the concomitant therapy will be assumed to have started within 8 days of the vaccination.
- In case of a completely missing start date, the concomitant therapy will be considered as having started before the study.
- In case of a completely missing end date, the concomitant therapy will be considered as ongoing at the end of the trial.

There will be special attention to any systemic use of analgesics/antipyretics, started during 8 days following each vaccination (00:00 of day of vaccination + 7 days). Following ATC/DD codes will be used for this: N02A (OPIOIDS) and N02B (OTHER ANALGESICS AND ANTIPYRETICS), M01A (ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS, NON-STEROIDS) and M01B (ANTIINFLAMMATORY/ANTIRHEUMATIC AGENTS IN COMBINATION) (ATC/DD Index). The classes will be added in a footnote in all related tables and listings. For the use of analgesics/antipyretics which are taken on the day of vaccination, an exception is made in case the time is before vaccination. In this case, the concomitant medication is also allocated to the Post-dose period. Tables will be created for all concomitant medication and concomitant medications of special interest.

Prior medications will be summarized by cohort and vaccine regimen and overall and ATC term.

7. REFERENCES

Clinical study protocol VAC18195RSV1001, v 1.0, 22-Dec-2021.