Janssen Research & Development

Statistical Analysis Plan

A Randomized, Double-blind, Placebo-controlled Phase 1/2a Study to Evaluate the Safety, Reactogenicity, and Immunogenicity of Ad26COVS1 in Adults Aged 18 to 55 Years Inclusive and Adults Aged 65 Years and Older

Protocol VAC31518COV1001; Phase [1/2a]

VAC31518 (JNJ-78436735)

Status:	Approved
Date:	8 September 2022
Prepared by:	Janssen Vaccines & Prevention B.V.
Document No.:	EDMS RIM-102684

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

CHANGES TO PREVIOUS VERSIONS

Amendment 2 (8 September 2022)
This Statistical Analysis Plan (SAP) amendment relates to amendment 16 of the VAC31518COV1001 clinical trial protocol. In this amendment, the booster vaccination at 24 months after completion of the primary regimen is removed in Cohorts 2a (Target Visit Day 731) and 2b (Target Visit Day 787). This is reflected in section 7.1.2. “Phase Definitions”, and other sections where reference was made to that booster vaccination.

Amendment 1 (16 December 2021)
This Statistical Analysis Plan (SAP) amendment relates to amendment 15 of the VAC31518COV1001 clinical trial protocol, in which a single ad hoc booster vaccination with Ad26.COV2.S at the $5x10^{10}$ vp dose level is offered to all eligible study participants (as defined in the clinical trial protocol).
The SAP amendment describes the planned safety and immunogenicity analyses that will be conducted on study participants who elect to receive the ad hoc booster dose.
The following changes have also been applied during this SAP update:

- Throughout the SAP: references to “final analysis” have been removed since this analysis was removed from the protocol.
- Section 7.7.3:
    - The confidence interval method was changed from Clopper Pearson to Wald, because the Clopper Pearson confidence interval cannot be calculated for a relative risk.

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VERSION HISTORY

SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
1	20-Aug-2020	Not Applicable	Initial release
2	16-Dec-2021	Cf. Section "Changes to Previous Versions"	Amendment to clinical trial protocol; addition of exploratory immunogenicity analyses, including non-inferiority analyses and alternative responder definitions; other minor updates
3	7 September 2022	Cf. Section "Changes to Previous Versions"	Amendment to clinical trial protocol

1. INTRODUCTION

This Statistical Analysis Plan (SAP) describes the pre-planned analyses for the Data Review Committee (DRC), Interim Analyses, Primary Analyses, and End of Study Analysis, for all cohorts in the study. One or several Data Presentation Specification Documents (DPS) will be available to further detail the statistical outputs that will be generated.

For some analyses (e.g. RNA sequencing data, passive transfer study), a separate SAP may be written.

2. OBJECTIVES AND ENDPOINTS

Refer to Clinical Trial Protocol (CTP) Section 3.

3. TRIAL DESIGN

Refer to CTP Section 4.

4. STATISTICAL HYPOTHESES

Refer to CTP Section 9.1.

5. SAMPLE SIZE DETERMINATION

Refer to CTP Section 9.2.

6. POPULATIONS FOR ANALYSIS

For vaccine studies, study intervention assignment will follow the as treated principle: all analyses (including safety, immunogenicity and efficacy analyses) will be analyzed by the actually received vaccine.

Population	Description
All screened participants (ALL)	The "all screened participants" set includes all participants that were screened, regardless of whether they were enrolled and/or randomized.
All randomized participants (ALL RANDOMIZED)	The "all randomized participants" set includes all participants that were randomized to one of the treatment groups.
Full Analyses Set (FAS)	The full analysis set will include all participants with at least one vaccine administration documented.
Per Protocol Immunogenicity Set (PPI)	The per protocol immunogenicity population will include all randomized and vaccinated participants for whom immunogenicity data are available excluding participants with major protocol deviations expecting to impact the immunogenicity outcomes. In addition, samples obtained after missed vaccinations or participants with natural infection occurring after screening (if applicable) will be excluded from the analysis set.
Per Protocol Efficacy Set (PPE)	The per protocol efficacy population will include all randomized participants having received at least 1 vaccination for whom efficacy data concerning endpoint measures are available. All efficacy analyses will be done according to the as treated principle (ie, actually received vaccinations).

7. STATISTICAL ANALYSES

7.1. General Considerations

7.1.1. 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 phase (cf. section 7.1.2 for phase definitions). Immunogenicity results will be presented per scheduled time point as appropriate. Efficacy analyses will present results by phase or pooled for the entire study, 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).

7.1.2. Phase Definitions

The phase definitions are different by cohort. For cohorts in which two vaccinations are administered in the primary regimen and having no booster doses (cohorts 1a, 1b and 3), the phases in the study will be constructed as detailed in Table 1.

Table 1: Phase Definitions for Cohorts 1a, 1b, and 3

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form	One minute prior to start of post dose 1 period
Regimen	2	Post-dose 1	1	Date and time of first vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on 28 days after the first vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to post-dose 2
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) b) 23:59 at the date of database cut-off in case of interim analysis c) One minute prior to post dose 2
Regimen	2	Post-dose 2	2	Date and time of second vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on Day 28 after the second vaccination (23:59 of day of vaccination + 28 days)
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) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 at the date of last visit

Note: the end date of the regimen phase should be the end date of the last post dose period.

For cohort 2a, the phases will be defined as detailed in Table 2.

Table 2: Phase Definitions for Cohort 2a

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form	One minute prior to start of post dose 1 period
Regimen	2	Post-dose 1	1	Date and time of first vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on 28 days after the first vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to post-dose 2
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) b) 23:59 at the date of database cut-off in case of interim analysis c) One minute prior to post booster 1
Regimen	2	Post-booster 1	2	Date and time of first booster vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on Day 28 after the first booster vaccination (23:59 of day of vaccination + 28 days)
Follow-up 2	4			One minute after Post-booster 1 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) One minute prior to post booster 2
Regimen	2	Post-booster 2	3	Date and time of second booster vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on Day 28 after the second booster vaccination (23:59 of day of vaccination + 28 days)
Follow-up 3	5			One minute after Post-booster 2 period end	Minimum of: 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 analysis c) 23:59 at the date of last visit

Note: the end date of the regimen phase should be the end date of the last post dose period.

For cohort 2b, the phases will be defined as detailed in Table 3.

Table 3: Phase Definitions for Cohort 2b

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1			Date and time of signing the informed consent form	One minute prior to start of post dose 1 period
Regimen	2	Post-dose 1	1	Date and time of first vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on 28 days after the first vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to post-dose 2
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) b) 23:59 at the date of database cut-off in case of interim analysis c) One minute prior to post dose 2
Regimen	2	Post-dose 2	2	Date and time of second vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on Day 28 after the second vaccination (23:59 of day of vaccination + 28 days)
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) b) 23:59 at the date of database cut-off in case of interim analysis c) One minute prior to post booster 1
Regimen	2	Post-booster 1	3	Date and time of first booster vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 on Day 28 after the first booster vaccination (23:59 of day of vaccination + 28 days)
Follow-up 3	5			One minute after Post-booster 1 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) One minute prior to post booster 2
Regimen	2	Post-booster 2	4	Date and time of second booster vaccination	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis

Phase	Phase #	Period	Period #	Interval	
				From	To
					c) 23:59 on Day 28 after the second booster vaccination (23:59 of day of vaccination + 28 days)
Follow-up 4	6			One minute after Post-booster 2 period end	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of database cut-off in case of interim analysis c) 23:59 at the date of last visit

Note: the end date of the regimen phase should be the end date of the last post dose period.

Adverse Events and selected other tables may display AEs (or other counts) by period. For such tables, active periods can be combined and additionally displayed. Depending on the primary regimen (one or two doses; presence or absence of booster doses), the active periods can be:

- "Post-Dose 1"
- "Post-Dose 2" (does not exist in Cohort 2a)
- "Post-Dose 1 and Post-Dose 2 Combined", which refers to the "primary regimen". If the primary regimen is a one-dose regimen (Cohort 2a), then this combined period will not exist and should also not be displayed.
- "Post-Booster 1" (only in cohorts 2a and 2b)
- "Post-Booster 2" (only in cohorts 2a and 2b)
- "Post-Dose 1 and Post-Booster Doses Combined", which refers to the complete regimen including all the booster doses in Cohort 2a. It includes the 28-day period after each vaccination (dose 1, booster dose 1, and booster dose 2).
- "Post-Dose 1, Post-Dose 2 and Post-Booster Doses Combined", which refers to the complete regimen including all the booster doses in Cohort 2b. It includes the 28-day period after each vaccination (dose 1, dose 2, booster dose 1, and booster dose 2).

For some tables, e.g. SAE tables, a period "Entire study" will be defined. This will be a combination of all the active phases and periods, so that it covers the time window from vaccination 1 up to and including the end of the study (per participant).

The primary endpoint includes SAEs from the first vaccination until 6 months after completion of the primary regimen for cohorts 2a and 2b. This will be a combined period of post-dose 1 and post-dose 1 Follow Up (FU) (for Cohort 2a) and a combination of post-dose 1, post-dose 1 FU, post-dose 2, and post-dose 2 FU (for Cohort 2b). This period will be labeled "Entire Primary Regimen" or similar in the outputs.

The exploratory endpoints include SAEs from the first booster vaccination time point until the end of the regimen for Cohort 2. This will be a combined period of post-booster 1 until the last follow-up period in Cohorts 2a and 2b. This period will be labeled "Entire Booster Regimen" or similar in the outputs.

7.1.3. Pooling Algorithm for Analysis Centers

Data will be pooled across the different centers.

7.1.4. Visit Windows

Refer to CTP section 8. Visit windows will be taken into account for the analysis of immunogenicity results, see section 7.7.1.

7.1.5. Analyses by Cohort and Pooled Across Cohorts

All analyses are planned to be performed within each cohort separately, unless explicitly indicated that data will be pooled across cohorts.

7.2. Participant Dispositions

Participant information will be shown for the full analysis set.

The number of participants in the following disposition categories will be summarized throughout the study by vaccine regimen and overall:

- participants screened
- participants in the FAS
- participants vaccinated and not randomized
- participants randomized and not vaccinated
- participants in the PPI
- participants in the PPE
- participants who discontinued study
- participants who discontinued vaccination
- reasons for termination

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

7.2.1. Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.
Table 4 presents a list of the demographic and baseline variables that will be summarized by vaccine regimen and overall for the FAS.

Table 4: Demographic Variables

Continuous Variables:	**Summary Type**
Age (years)	Descriptive statistics (N, mean, standard deviation [SD], median and range [minimum and maximum]).
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)	
Study center	
SARS-CoV-2 Seropositivity status at screening	

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

7.2.2. Protocol Deviations

Major protocol deviations will be summarized. Major protocol deviations which have a potential impact on immunogenicity will be flagged in the listings.

7.2.3. 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.
- 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.

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 CMCLASCD (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). 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.

Participants with COVID-19-like signs and symptoms will collect concomitant medications since symptom onset. These will be tabulated and listed; they will also be included in the programmed patient narratives.

7.3. Primary Endpoint(s) Analysis

The primary endpoints in this study, are:

All participants in Cohorts 1, 2, and 3:

- Solicited local and systemic adverse events (AEs) for 7 days after each vaccination in the primary regimen
- Unsolicited AEs for 28 days after each vaccination in the primary regimen
- For the primary endpoint: Serious adverse events (SAEs) from the first vaccination until 1 year after the second vaccination for Cohorts 1 and 3, and until 6 months after the primary regimen for Cohort 2

7.3.1. Definition of Endpoint(s)

Solicited AEs are used to assess the reactogenicity of the study vaccine and are predefined local (at the injection site) and systemic events for which the participant is specifically questioned and which are noted by participants in their diary. Unsolicited AEs are all AEs for which the participant is not specifically questioned.

7.3.2. Estimand

Not applicable

7.3.3. Analysis Methods

Refer to section 7.6.

7.4. Secondary Endpoint(s) Analysis

7.4.1. Key/Confirmatory Secondary Endpoint(s)

The secondary endpoints in this study, are:

Humoral Immune Response

All participants in Cohorts 1, 2, and 3:

- SARS-CoV-2 neutralization: SARS-CoV-2 neutralizing titers in serum measured by a virus neutralization assay (VNA [wild-type virus and/or pseudovirion expressing S protein])
- SARS-CoV-2-binding antibodies measured by enzyme-linked immunosorbent assay (ELISA): Analysis of antibodies binding to the SARS-CoV-2 S protein.

Cellular Immune Response

A subset of participants in Cohorts 1, 2, and 3:

- Th1 and Th2 immune responses as assessed by flow cytometry after SARS-CoV-2 S protein peptide stimulation of peripheral blood mononuclear cells (PBMCs) and intracellular staining [ICS] including CD4+/CD8+, IFNγ, interleukin [IL] 2, TNFα, IL-4, IL-5, IL-13, and/or other Th1/Th2 markers.

7.4.1.1. Definition of Endpoint(s)

Refer to section 7.7.1.

7.4.1.2. Estimand(s)

Not Applicable

7.4.1.3. Analysis Methods

Refer to section 7.7.1.

7.5. Tertiary/Exploratory Endpoint(s) Analysis

The exploratory endpoints in this study, are:

Safety and Reactogenicity:

All participants in Cohort 2:

- Solicited local and systemic AEs for 7 days after each booster vaccination time point
- Unsolicited AEs for 28 days after each booster vaccination time point
- SAEs from the first booster vaccination time point until the end of the study

Humoral Immune Response:

Exploratory analyses may include the following assays for a subset of participants in Cohorts 1 and 3:

- SARS-CoV-2 neutralization as assessed by alternative SARS-CoV-2 neutralization assays (different from the VNA used for the secondary endpoint).
- Adenovirus neutralization.
- Functional and molecular antibody characterization (eg, avidity, Fc receptor interaction, antibody isotyping).
- Epitope-specificity characterization for B- and T-cells.
- Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in the serum or plasma.
- Passive transfer: Analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model.

Cellular Immune Response:

Exploratory analyses may include the following assays for a subset of participants in Cohorts 1, 2, and 3:

- Single IFNγ and IL-4 enzyme-linked immunospot (ELISpot) assay after stimulation of PBMCs with SARS-CoV-2 S protein peptides.
- Analysis of gene expression in cells stimulated with SARS-CoV-2 S protein peptides, or in unstimulated cells (ex vivo).
- Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in cells stimulated with SARS-CoV-2 S protein peptides, or in unstimulated cells (ex vivo).

A subset of participants in Cohort 2 only:

- Analysis of the phenotype of antigen-specific T and B cells, assessed by single cell analysis (on frozen or Smart tube-isolated PBMCs).

In addition to these immunogenicity-related exploratory endpoints, the following efficacy-related exploratory endpoints are defined in this study:

- The number of molecularly confirmed COVID-19 cases in Ad26.COV2.S versus placebo recipients in the overall study
- The number of participants with positive non-S protein ELISA (eg, N ELISA), if such an assay can be developed, in the Ad26.COV2.S and placebo groups
- Presence and severity signs and symptoms of COVID-19
- Confirmation of SARS-CoV-2 infection by molecular testing

The following endpoints are defined to examine the immune response in vaccinated individuals after natural infection and to explore other potentially informative biomarkers (eg, those associated with more severe disease):

- Confirmation of SARS-CoV-2 infection by molecular testing
- SARS-CoV-2 neutralizing titers in serum measured by a VNA (wild-type virus and/or pseudovirion expressing S protein)
- SARS-CoV-2-binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S protein
- Functional and molecular antibody characterization
- Analysis of gene expression by RNA transcript profiling

7.6. (Other) Safety Analyses

Safety analyses will be performed on the FAS. Continuous variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean (mean), standard deviation (SD), standard error (SE), 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.

Safety data will be analyzed by study intervention regimens as designed per protocola. In addition, safety data will be analyzed by intervention regimens as designed per protocol and participant seropositivity status at screening. Data will be presented by period (post Dose 1, post Dose 2, post Booster 1, and post Booster 2, as applicable) as well as over the entire regimen, and for Cohorts 2a and 2b over the entire primary regimen and over the entire booster regimen. Denominator for the percentages is the number of participants in the considered population and phase for a certain regimen (incidence per 100 participants/phase).

Exploratory analyses by age (e.g. 18-40 years and 41-55 years) may be performed in Cohort 2.

7.6.1. Adverse Events

7.6.2. Definitions

Solicited AEs shown in the tables are extracted from the investigator assessment pages (CE) of the CRF. For unsolicited AEs, only the AEs within the 28-day period following each vaccination will be presented in the safety tables except for SAEs, 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 administration site symptoms will be considered as related to the study vaccine (by definition).

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 Appendix 3.

7.6.3. 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 and Preferred Term. Solicited AEs will be summarized by class (administration site/systemic) and Preferred Term.

For solicited AEs following tables will be provided: summary, by worst severity grade, at least grade 3, related (systemic only), time to onset (in days) and duration (in days) for most frequent events and body temperature. 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 vaccination period.

[a] As indicated previously, all analyses, including the safety analyses, will be conducted by actually received vaccine ("as treated" principle). The sentence "by study intervention regimens as designed per protocol" indicates that the statistical tables are designed with the planned vaccine regimens as columns. Therefore, participants who receive e.g. "Placebo, Placebo" instead of "Ad26.COV2.S $5x10^{10}$ vp, Placebo" will appear in the "Placebo, Placebo" column. However, to avoid sparse columns, participants who receive e.g. "Placebo, Ad26.COV2.S $5x10^{10}$ vp" (which is not a planned vaccine regimen) will be excluded from the statistical tables. They will be included in the listings, as well as in the programmed patient narratives where they will appear as "Subjects with vaccine misallocation".

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

As a general remark, COVID-19 cases will be analyzed separately (see section 7.7.3).

- SAEs that are COVID-19 related will not be included in the SAE tables, but will be included and flagged in the SAE listings. In MedDRA version 23.0, Preferred Terms (PT codes) "COVID-19 (10084268)", "COVID-19 pneumonia (10084380)", "Suspected COVID-19" (10084451), "Asymptomatic COVID-19 (10084459)", "Coronavirus infection" (10051905), "Severe acute respiratory syndrome" (10061982), "SARS-CoV-2 carrier" (10084461), "Exposure to SARS-CoV-2" (10084456), and "Occupational exposure to SARS-CoV-2" (10084394) will be considered COVID-19 related AEs[a].
- Adverse events that are not SAEs and are COVID-19 related will not be recorded in the SDTM AE domain, but rather in the SDTM CE domain.

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

7.6.4. Phase Allocation of Adverse Events

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

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. 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).

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

[a] In case this list of terms needs to be revised, the DPS will detail the revised list.

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.

7.6.5. Missing Data

Missing AE 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.

7.6.6. Laboratory, Vital Signs and Physical Examination

A listing of all laboratory values will be made, restricted to participants with at least one laboratory abnormality.

Physical examination findings will be summarized at baseline. A listing of the abnormalities will be made.

Vital signs including temperature, pulse/heart rate, respiratory rate, and blood pressure (systolic and diastolic) will be summarized over time, using descriptive statistics. Abnormalities emerging after vaccination will be tabulated by worst abnormality grade using the FDA table in Appendix 3.

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 participant 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 be 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).

A listing of participants with fever according to the FDA grading table will also be provided. In addition, temperature measurements (whether obtained from the diary or from 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), and tabulated.

7.6.7. Safety Analyses After Receipt of the Ad hoc Booster Vaccination

Study participants who receive the ad hoc booster vaccination, will be removed from the main safety analyses as of that point in time[a]. A separate safety analysis will be conducted on the study

[a] Note that participants may already have been removed from the main safety analyses due to unblinding and/or receipt of an unscheduled vaccine (either a COVID-19 vaccine received outside of the study or after crossing over from

participants who received the ad hoc booster vaccination, starting at the time of the ad hoc booster vaccination and showing their post ad hoc booster vaccination 7 day reactogenicity data, their post ad hoc booster vaccination 28-day unsolicited AE data, and their post ad hoc booster vaccination SAE/AESI data, as available. The statistical analysis will be descriptive. It will show study participants according to their original vaccination regimen in tables, listings and figures. In addition, the analyses may also be conducted separately by subgroups of study participants who received a COVID-19 vaccine outside of the study vs study participants who did not receive a COVID-19 vaccine outside of the study. A distinction between mRNA vaccines vs other vaccines may be made, if the numbers are sufficiently high. Otherwise, this information will be included in the listings only.

7.7. Other Analyses

7.7.1. Immunogenicity Analyses

The analysis of immunogenicity will use the PPI set. Immunogenicity analyses will also be done on the FAS (participants who became infected during the study will be analyzed as a subgroup and shown in the graphs using different colors and symbols). Data will be analyzed by vaccine regimen, and by vaccine regimen and participant seropositivity status at screening[a]. Data will be presented by scheduled time point. For the PPI analysis, samples taken outside of the allowed window will be excluded from the tables and graphs (but will be included in the listings and clearly marked as results not included in the PPI analyses). For the FAS analysis, samples taken outside of the allowed window will be included.

Note: analyses that are potentially unblinding at the individual participant level (e.g. graphs showing individual data tied to COVID-19 infection status, especially when the number of COVID-19 infections is low and/or when time of infection is indicated) will be carried out after official unblinding of the trial, or will be carried out exclusively on specific cohorts (or other clearly defined subgroups) after these are unblinded. Alternatively, prior to unblinding, these analyses can be performed in a completely blinded manner (e.g. tables with only a single column pooling all regimens).

7.7.1.1. Parameters

The following humoral and cellular immune responses may be measured. However, not all assays might be available for all immunogenicity analyses covered by this SAP. Further information on

placebo to active within the study). Refer to the clinical trial protocol amendment 15, section 9.7 for more information.

[a] As indicated previously, all analyses, including the immunogenicity analyses, will be conducted by actually received vaccine ("as treated" principle). Note that the statistical tables are designed with the planned vaccine regimens as columns. Therefore, participants who receive e.g. "Placebo, Placebo" instead of "Ad26.COV2.S $5x10^{10}$ vp, Placebo" will appear in the "Placebo, Placebo" column. However, to avoid sparse columns, participants who receive e.g. "Placebo, Ad26.COV2.S $5x10^{10}$ vp" (which is not a planned vaccine regimen) will be excluded from the statistical tables. They will be included in the listings, as well as in the programmed patient narratives where they will appear as "Subjects with vaccine misallocation".

which assays will be analyzed in each of the analyses, will be included in the corresponding DPS documents.

Table 5. Summary of Humoral Immunogenicity Assays

Assay	Purpose
Secondary endpoints	
SARS-CoV-2 neutralization (VNA)	Analysis of neutralizing antibodies to the wild-type virus and/or pseudovirion expressing S protein
SARS-CoV-2 binding antibodies (ELISA)	Analysis of antibodies binding to the SARS-CoV-2 S protein
Exploratory endpoints	
SARS-CoV-2 neutralization (neutralization assay)	Analysis of neutralizing antibodies to the vaccine strain (or other strain), as measured by an alternative neutralization assay (different from the VNA used for the secondary endpoint)
SARS-CoV-2 binding antibodies (ELISA)	Analysis of antibodies binding to the SARS-CoV-2 N protein, if such an assay can be developed
Adenovirus neutralization (neutralization assay)	Analysis of neutralizing antibodies to adenovirus
Functional and molecular antibody characterization	Analysis of antibody characteristics including Fc-mediated viral clearance, avidity, Fc characteristics, Ig subclass and IgG isotype
Epitope-specificity characterization	Analysis of site-specificity, epitope mapping
Cytokine profiling	Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in the serum or plasma
Passive transfer	Analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model

ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulin; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; VNA = virus neutralization assay

Table 6. Summary of Cellular Immunogenicity Assays

Assay	Purpose
Secondary endpoints	
Flow cytometry (ICS)	Analysis of T-cell responses to SARS-CoV-2 S protein, and/or other protein peptides by ICS including $CD4^+/CD8^+$, IFNγ, IL-2, TNFα, IL-4, IL-5, IL-13, and/or other Th1/Th2 markers
Exploratory endpoints	
ELISpot	IFNγ and IL-4 responses to SARS-CoV-2 S protein peptides by PBMCs, based on single ELISpot
Gene expression analysis	Analysis of gene expression by RNA transcript profiling and/or analysis of protein translates, in cells or whole blood stimulated with SARS-CoV-2 S protein peptides or in unstimulated cells or whole blood (ex vivo)
Cytokine profiling (ELISA or multiplexed arrays)	Analysis of cytokines, chemokines, and other proteins of the innate or adaptive immune response in cells or whole blood stimulated with SARS-CoV-2 S protein peptides, or in unstimulated cells or whole blood, by ELISA or multiplexed arrays and confirmation by functional in vitro assays
T and B cell phenotyping	Analysis of the phenotype of antigen-specific T and B cells, assessed by single cell analysis (on frozen or Smart tube isolated PBMCs)

ELISA = enzyme-linked immunosorbent assay; ELISpot = enzyme-linked immunospot (assay); ICS = intracellular cytokine staining; IFNγ = interferon gamma; IL = interleukin; PBMC = peripheral blood mononuclear cell; RNA = ribonucleic acid; S = spike; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; TNFα = tumor necrosis factor alpha

7.7.1.2. 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 limit of detection (LOD) will be handled as follows:

- Calculation of geomean and median:
  - o values<LLOQ are imputed with LLOQ/2.
- Calculation of fold increases from baseline:
  - o values <LLOQ are imputed with LLOQ.

Values above the upper limit of quantification (ULOQ) will be handled as follows:

- Calculation of geomean and median:
  - o Values>ULOQ are imputed with ULOQ.
- Calculation of fold increases from baseline:
  - o Values >ULOQ are imputed with ULOQ.

7.7.1.3. Handling of changes in assay status throughout the study conduct

In case of changes in assay status, from "qualified" to "validated", the LLOQ and ULOQ are likely to change as well. If this should happen, then the SDTM database will contain records pertaining to the assay in the qualified status and records pertaining to the validated status, and the LLOQ and ULOQ values will also differ.

The statistical analysis will use the LLOQ and ULOQ values associated with the validated assay and will retrospectively apply these on all the data pertaining to the assay, including the data obtained while the assay status was "qualified". This may imply that data received, statistically analyzed, and presented at an earlier time may change. Graphical displays will show the eventually used LLOQ and ULOQ values. Graphs and tables will have an additional footnote that reflects the assay status.

7.7.1.4. Immune Response Analysis

No formal hypothesis on immunogenicity will be tested.

7.7.1.5. Immunogenicity Against the Insert

7.7.1.5.1. Humoral Assays

For **VNA** (both wild-type virus and pseudovirion expressing S protein, as available), the following statistics will be calculated: N, geometric mean and corresponding 95% CI of the actual values, fold increase from baseline, fold increase from pre-dose 2 (if applicable, i.e. only for cohorts where a second dose is given in the primary regimen and for time points after dose 2), fold increase from pre-booster dose 1, fold increase from pre-booster dose 2, fold increase from pre-booster dose 3 (if applicable, i.e. only for cohorts 2a and 2b where booster doses are given and for time points after the said booster dose).

For the calculation of the geometric mean and its corresponding 95% CI, the arithmetic mean and its corresponding 95% CI are calculated on the $\log_{10}$ transformed values. These values are back transformed to provide the geometric mean and its corresponding 95% CI.

For wild type and pseudovirion VNA separately:

- A sample will be considered positive if the value is strictly greater than the LLOQ (>LLOQ).
- Responder definition. A post-baseline sample will be considered a responder if at least one of the following conditions is satisfied:
  - The baseline sample value is less than or equal to the LLOQ (≤LLOQ) and the post-baseline sample is strictly greater than the LLOQ (>LLOQ)
  - The baseline sample value is strictly greater than the LLOQ (>LLOQ) and the post-baseline sample value represents an at least 4-fold (≥4-fold) increase from the baseline sample value.

Actual values are tabulated and shown as dot plots with dots for participant 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 participant dots) will also be generated.

Participant 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 $\log_{10}$ scale.

Further details and/or updated rules may be provided in the DPS.

For **S-ELISA**, the same as above applies.

The ratio of binding antibodies (S-ELISA) to wild type VNA, and the ratio of binding antibodies (S-ELISA) to pseudovirion expressing S protein VNA will be calculated for each time point. Values <LLOQ will be imputed with LLOQ for the calculation of the ratios. In addition, the ratio of the fold increase from baseline in binding antibodies (S-ELISA) to the fold increase from baseline in wild type VNA, and the ratio of the fold increase from baseline in binding antibodies (S-ELISA) to the fold increase from baseline in pseudovirion expressing S protein VNA will be calculated for each post-baseline time point. Values <LLOQ will be imputed with LLOQ for the calculation of the fold increase ratios. The following statistics will be calculated and tabulated: N, geometric mean and corresponding 95% CI of the ratio. Graphical displays will also be prepared, showing – for each time point – the geometric mean of the ratio and its 95% CI, together with the individual data points (dot plot).

If a similar assay is performed at different analyzing labs, then separate statistical analyses may be performed.

Scatterplots between humoral assay results will be provided for selected time points. These may include, but are not be limited to:

- Binding antibodies (S-ELISA) versus wild type VNA
- Binding antibodies (S-ELISA) versus pseudovirion expressing S protein VNA
- Wild type VNA versus pseudovirion expressing S protein VNA

If a similar assay is performed at different analyzing labs, then the statistical analyses may distinguish between these and provide separate scatterplots for each analyzing lab versus the other assay of interest. These scatterplots will display the values as analyzed for the geometric mean calculations, with values <LLOQ imputed with LLOQ (if an LLOQ is defined) and values >ULOQ imputed with ULOQ (if an ULOQ is defined). The LLOQ and ULOQ cut-off values per assay will be visualized in the scatterplots. Spearman correlation coefficients will also be provided (one per scatterplot).

7.7.1.5.2. Cellular Assays

For **ELISpot**, if available, at least the following statistics will be calculated: N, median, first and third quartile, minimum and maximum of the actual values. Additional statistics may be calculated and will be detailed in the DPS. The ELISpot in this study is planned to be two single ELISpot, measuring IFN-g and IL-4. In that case, the statistics will be analyzed for each cytokine separately.

For each cytokine, if available, the following is defined:

- Sample positivity:
  - For IFN-g: a sample will be considered positive if the value is strictly greater than the LOD (>LOD).
  - For IL-4: a sample will be considered positive if the value is strictly greater than the LOD (>LOD).

- Responder:

  A post-baseline sample will be considered a responder if at least one of the following conditions is satisfied:

  For IFN-g:

– The baseline sample value is less than or equal to the LOD (≤LOD) and the post-baseline sample is strictly greater than the LOD (>LOD)
– The baseline sample value is strictly greater than the LOD (>LOD) and the post-baseline sample value represents an at least 3-fold (≥3-fold) increase from the baseline sample value.

  For IL-4:

– The baseline sample value is less than or equal to the LOD (≤LOD) and the post-baseline sample is strictly greater than the LOD (>LOD)
– The baseline sample value is less than or equal to the LLOQ (≤LLOQ) and the post-baseline sample is strictly greater than the LLOQ (>LLOQ)
– The baseline sample value is strictly greater than the LLOQ (>LLOQ) and the post-baseline sample value represents an at least 2-fold (≥2-fold) increase from the baseline sample value.

The SDTM database will contain the LOD and LLOQ values.

In keeping with the general derivation rules, values <LLOQ are imputed with LLOQ/2 for the calculation of the median and with LLOQ for the calculation of the fold increases from baseline.

ELISpot values available in the database will already be background subtracted. No further background subtraction should be carried out. In case the SDTM data only contain peptide pools 1 and 2, but no combined peptide pool, then the combined peptide pool will be calculated as the sum of both peptide pools.

Tables with the descriptive statistics will be provided.

Participant profiles of the actual values over time will be graphically presented. For the graphs, original values will be displayed on the $\log_{10}$ scale.

For ELISpot, the reported values are spot forming cells per million peripheral blood mononuclear cells (PBMC).

For ELISpot, IFN-g responses are considered Th1 and IL-4 responses are considered Th2. Due to this 1-to-1 correspondence, no separate Th1/Th2 analyses will be conducted for ELISpot.

Further details and/or updated rules may be provided in the DPS.

For **ICS**, if available, at least the following statistics will be calculated: N, median, first and third quartile, minimum and maximum of the actual values, number and percentage of participants with a positive sample (if available). Additional statistics may be calculated and will be detailed in the DPS.

It is planned to analyze the following cell populations at the time of the first interim analysis. The DPS may provide an updated version of this list, e.g. for subsequent analyses.

- CD4+:
  - IFN-g or IL2
  - IFN-g or IL2 NOT TH2
  - IL4 and CD40L
  - IL4 or IL5 or IL13 and CD40L
- CD8+:
  - IFN-g or IL2

The data received from the analyzing lab(s) will contain background subtracted values ("immediately reportable values"; i.e. background subtracted[a] percentages of cells expressing the cytokine or cytokine combination). Negative background subtracted values will be imputed with zero prior to further processing.

The data will contain a positivity call for each cell population. Sample positivity should therefore not be further derived at the statistical analysis stage.

Tables will be provided that show the descriptive statistics mentioned above, structured as follows: CD4+/CD8+, peptide pool (as available in the database, e.g.: SARS-Cov2-S, SARS-Cov2-S1, SARS-Cov2-S2), cytokine (combination), and time point.

Participant profiles of the actual values over time will be graphically presented. For the graphs, original values will be displayed on the $\log_{10}$ scale, with values <0.022% imputed with 0.011% (only for visual representation; calculations will be based on the actual values). The graphs that show individual participant's data will visually differentiate between positive/negative samples (e.g. different symbols and/or different colors).

The reported values are percentage of cells expressing the cytokine(s).

Assessment of Th1/Th2 response ratio.

Based on the combined SARS-Cov2-S peptide pool, and using post baseline time points only, a Th1/Th2 response ratio will be calculated for samples that satisfy at least one of the following two conditions:

- a Th1 response ("IFN-g or IL2 NOT TH2") that is both positive and ≥ 2 x LLOQ,
- or
- a Th2 response ("IL4 or IL5 or IL13 and CD40L") that is both positive and ≥ 2 x LLOQ

[a] Also known as "mock subtracted"

For the purposes of the Th1/Th2 ratio analysis, the LLOQ is 0.022% for both cell populations (Th1 and Th2).

If both cell populations (Th1 and Th2) are positive and ≥ 2 x LLOQ, then the ratio of Th1/Th2 will be calculated as a numerical result.

If only one cell population (either Th1 or Th2) is positive and ≥ 2 x LLOQ, then the following rules will be used to determine a qualitative assessment of the Th1/Th2 ratio:

- If one cell population is positive and the other is negative, then the positive cell population is greater than the negative cell population: if the Th1 response is positive and the Th2 response is negative, then the Th1/Th2 ratio will be set to ">1". If the Th1 response is negative and the Th2 response is positive, then the Th1/Th2 ratio will be set to "<1"
- If both cell populations are positive, then the cell population that is ≥ 2 x LLOQ is greater than the cell population that is < 2 x LLOQ: if the Th1 response is ≥ 2 x LLOQ and the Th2 response is < 2 x LLOQ, then the Th1/Th2 ratio will be set to ">1". If the Th1 response is < 2 x LLOQ and the Th2 response is ≥ 2 x LLOQ, then the Th1/Th2 ratio will be set to "<1".

For each post baseline time point, the number of participants with an evaluable Th1/Th2 response ratio will be tabulated, together with the number and percentage of participants with a Th1/Th2 ratio ≥ 1 and the number and percentage of participants with a Th1/Th2 ratio < 1. Graphical display(s) of these data may also be produced.

Further details and/or updated rules may be provided in the DPS.

7.7.1.6. Immunogenicity Against the Vector

For immunogenicity against the Ad26 vector backbone (Adenovirus neutralization by neutralization assay) following statistics will be calculated: geometric mean and corresponding 95% CI of the actual values, and number and percentage of participants with a positive sample.

If only one time point is available, then actual values at that single time point will be shown as a dot plot. If multiple time points are available, then GMT plots over time, combining the regimens in one graph (without individual participant dots) will also be generated. In addition, subject profiles will then also be created.

7.7.1.7. Statistical Modeling of the Immune Responses

Statistical modeling will be undertaken to assess the influence of demographic variables and baseline characteristics on selected immune responses. These analyses will be performed on the PPI analysis set, excluding participants in the regimens that received no active vaccine. The following are pre-planned statistical analyses:

- Linear regression analysis of 28 days post Dose 1, pre-Dose 2 and of 28 days post Dose 2 psVNA titers on demographic and baseline characteristics and control variables, using pooled data from all regimens and all cohorts.
- One model for 28 days post Dose 1, one for pre-Dose 2 and one for 28 days post Dose 2
- psVNA titers enter the model on the $\log_{10}$ scale.

- Demographic variables: age (continuous), BMI (continuous), sex (categorical: male vs. female), race (categorical: Black or African American vs. White vs. Other).
- Baseline characteristics: baseline SARS-CoV-2 seropositivity status (categorical: Positive vs. Negative) and baseline VNA titers against the Ad26 vector (continuous, on the $\log_{10}$ scale).
- Control variables: single-dose vs. two-dose regimen and high-dose vs. low dose regimen.

- Linear regression analysis of 28 days post Dose 1, pre-Dose 2 and of 28 days post Dose 2 wild type VNA titers on demographic and baseline characteristics and control variables, using pooled data from all regimens and all cohorts. Same considerations as for psVNA titers.
- Linear regression analysis of 28 days post Dose 1, pre-Dose 2 and of 28 days post Dose 2 ELISA antibody concentrations on demographic and baseline characteristics and control variables, using pooled data from all regimens and all cohorts. Same considerations as for psVNA titers.
- Penalized logistic regression analysis (using Firth's method) of 28 days post Dose 1, pre-Dose 2 and of 28 days post Dose 2 responder status for psVNA on demographic and baseline characteristics and control variables, using pooled data from all regimens and all cohorts.
  - One model for 28 days post Dose 1, one for pre-Dose 2 and one for 28 days post Dose 2
  - psVNA responder status enters the model as a categorical variable (coded 1 for responder and 0 for non-responder).
  - Other considerations as for the linear regression models

7.7.1.8. Immunogenicity analyses after receipt of the ad hoc booster vaccination

Study participants who receive the ad hoc booster vaccination, will be removed from the main immunogenicity analyses as of that point in time[a,b]. A separate immunogenicity analysis will be conducted on the study participants who received the ad hoc booster vaccination, starting at the time of the ad hoc booster vaccination and showing their immune responses at each subsequent time point, as available. The statistical analysis will be descriptive. It will show study participants according to their original vaccination regimen in tables, listings and figures. In addition, the analyses may also be conducted separately by subgroups of study participants who received a COVID-19 vaccine outside of the study vs study participants who did not receive a COVID-19 vaccine outside of the study. A distinction between mRNA vaccines vs other vaccines may be made, if the numbers are sufficiently high. Otherwise, this information will be included in the listings only.

[a] Note that participants may already have been removed from the main immunogenicity analyses due to receipt of an unscheduled vaccine (either a COVID-19 vaccine received outside of the study or after crossing over from placebo to active within the study). Refer to the clinical trial protocol amendment 15, section 9.7 for more information.

[b] Note that, unless the sample needs to be excluded from the analysis for reasons noted in the above footnote (or other reasons as noted elsewhere in the SAP, e.g. occurrence of a natural infection, etc.), the blood sample taken at the day of the ad hoc booster vaccination can be included in the main analysis. As such, it could both contribute to the main immunogenicity analyses (e.g. assess the long term immunogenicity profile) as well as to the analyses on the ad hoc booster (as a baseline).

Additional exploratory analyses may be conducted, such as but not limited to, statistical modeling to determine the effect of the time gap between the last vaccination and the ad hoc booster vaccination, and/or durability of the immune responses observed after the ad hoc booster. Additionally, analyses may be performed to assess the non-inferiority of post booster or post ad hoc booster responses vs. selected pre-booster or pre-ad hoc booster time points. These analyses may include assessments of the geometric mean ratio (GMR), the percentage responders, or other indicators for immune responses. As the protocol did not plan any formal non-inferiority analyses, there is no alpha spending and all analyses will be conducted at a nominal alpha level of 0.05 (two-sided). While the results of these analyses may be interpreted using the typical non-inferiority guidelines (e.g. the lower limit of the confidence interval around the GMR >2/3 and/or the lower limit of the confidence interval around the difference in responder percentage >10% would lead to concluding "non-inferiority"), it will be understood that these analyses are exploratory.

Moreover, alternative responder definitions may also be assessed in addition to or instead of the usual responder definition as described elsewhere in this SAP. For instance, the following responder definition may be used for the assessment of humoral responses:

A participant will be considered a "responder", if:

- The baseline sample value is less than or equal to the LLOQ (≤LLOQ, or another threshold/cut-off value as appropriate for the assay) and the post-baseline sample value is strictly greater than 4 times the LLOQ (>4xLLOQ, or another threshold/cut-off value as appropriate for the assay)

- The baseline sample value is strictly greater than the LLOQ (>LLOQ, or another threshold/cut-off value as appropriate for the assay) and the post-baseline sample value represents an at least 4-fold (≥4-fold) increase from the baseline sample value.

It should be noted that the "baseline" refers to time points selected based on the purpose of the analysis. Commonly, "Day 1" or "Pre-boost" is chosen as the baseline, but for non-inferiority (or other exploratory) analyses, the baseline can be another time point which is more relevant and of more substantive interest, e.g. 28 days post dose 1 or 28 days post dose 2. Similarly, the "post-baseline" timepoint refers to timepoint relevant to the analysis purpose. Commonly, all post-baseline time points will be assessed, but for some analyses, such as (but not limited to) non-inferiority analyses the focus may be on a specific time point or a few time points of interest (e.g. 14 days post booster, 28 days post ad hoc booster, etc.).

Details of the analyses will be provided in the DPS.

7.7.2. COVID-19 case monitoring to detect imbalances across study groups (harm monitoring)

An unblinded statistician, who is not otherwise involved in the study, will monitor the number and severity of molecularly confirmed COVID-19 cases in the Ad26.COV2.S and placebo groups to identify an imbalance between groups if it occurs. The unblinded statistician will inform the DRC as soon as an imbalance between groups is detected.

As soon as 3 confirmed COVID-19 cases have occurred, and with every additional case, the unblinded statistician will tabulate the cases according to whether the participant received Ad26.COV2.S or placebo (i.e. active regimens will be pooled) and calculate the difference in proportions between the two groups (proportion in active – proportion in placebo). Two (two-sided) confidence intervals around this difference will be constructed, a 95% CI and an 80% CI (i.e. using z-values corresponding to alpha = 0.05 and alpha = 0.20), using Newcombe's method without continuity correction.

The Newcombe's confidence interval for a difference between proportions is calculated as follows:

$$Lower\ limit: (\hat{p}_1 - \hat{p}_2) - \sqrt{(\hat{p}_1 - L_1)^2 + (U_2 - \hat{p}_2)^2}$$
$$Upper\ limit: (\hat{p}_1 - \hat{p}_2) + \sqrt{(U_1 - \hat{p}_1)^2 + (\hat{p}_2 - L_2)^2}$$

Where L_i and U_i are the Wilson Lower and Upper confidence limits for p_i. The Wilson confidence limits without continuity correction for each binomial proportion $p_i=x_i/n_i$ (i=1,2) is given by:

$$\frac{1}{2(n_i + z^2)}\left((2n_i\hat{p}_i + z^2) \pm z\sqrt{4n_i\hat{p}_i(1 - \hat{p}_i) + z^2}\right)$$

If the upper limit of the two-sided 95% CI around the difference in proportions exceeds 0.10 (i.e. >10 percentage points difference between active and placebo), and the lower limit of the two-sided 80% CI around the difference in proportions exceeds 0 (i.e. >0 percentage points difference between active and placebo), then the unblinded statistician will conclude that there is an imbalance between active and placebo (where the proportion in active is greater than the proportion in placebo). Otherwise, the statistician will not conclude that there is an imbalance.

The same operations will be executed for severe COVID-19 cases, using these definitions of "severe" cases:

- COVID-19 cases requiring hospitalization,
- COVID-19 cases requiring hospitalization and the patient being admitted to the Intensive Care Unit,
- COVID-19 cases resulting in death (with death being at least probably related to COVID-19)

7.7.3. COVID-19-like Signs and Symptoms

If a participant experiences COVID-19-like symptoms (eg, cough, feverishness, dyspnea, gastrointestinal symptoms, anosmia), the following should take place:

- Participants should contact the study site at the time of symptom onset
- A nasal swab should be collected from the participant at home (using available material for home swabs) as soon as possible and preferably no longer than 2 to 3 days after the onset of symptoms and stored appropriately. It is preferred that the swab is taken by a caregiver (spouse, partner, relative, friend, or health care professional). If that is not possible, the participant can collect the swab him- or herself. The sample should be transferred to the study site by an appropriate method as soon as possible after being collected. A second nasal swab will be obtained 2 to 4 days after the first swab following the same procedures as the first nasal swab or at the study site if appropriate procedures are in place.

The presence of SARS-CoV-2 infection and influenza infection will be assessed at the study site by molecular testing using the nasal swab sample.

- Participants should complete the Symptoms of Infection with COVID-19 (SIC) and record their highest body temperature daily starting on the first day they experience symptoms. If either nasal swab is positive for SARS-CoV-2 or influenza, collection of data will continue until sign and symptom resolution. If the first nasal swab is negative, collection of data will continue until the negative test is confirmed by the second nasal swab.
- For participants with a positive test result for SARS-CoV 2 infection, a study visit will be conducted 28 days after symptom onset to assess the clinical course of the infection, record concomitant medications since symptom onset, and obtain a blood sample for evaluation of the immune response and other biomarkers.

The SIC asks participants to indicate, for each of 25 symptoms and on a daily basis, whether or not they experienced the symptom (Yes/No), and if Yes, to rate the severity (on an 11-point scale ranging from 0 to 10, where 0 = none to 10 = worst). In addition, 4 questions are asked that are asked to be responded to by Yes/No, without severity assessment.

The presence of SARS-CoV-2 infection and influenza infection will be assessed at the study site by molecular testing using the nasal swab sample.

The following analyses will be conducted at the time of the End of Study analysis or at an interim analysis[a]:

The number and percentage of participants with at least one molecularly confirmed SARS-CoV-2 infection will be tabulated by vaccine regimen, for the entire study period only[b].

The analysis will be repeated pooling all active regimens vs. placebo, where data from all cohorts will be pooled. If at least 5 molecularly confirmed SARS-CoV-2 infection are observed, vaccine efficacy (VE) will be calculated with a 90% confidence interval (CI). Otherwise, no VE will be calculated and only the above mentioned descriptive statistical analysis will be conducted. The following paragraphs provide the rationale for only calculating VE when at least 5 events are observed, as well as the formula to calculate VE and its 90% CI.

Assuming a true VE of 70%, 23 events of molecularly confirmed SARS-CoV-2 infections are required to offer 80% power to detect vaccine efficacy exceeding zero (H0: VE = 0) when using a one-sided alpha level of 0.05 (i.e. a 90% CI) and a randomization ratio of 4.65:1 (860 active:185 placebo). Assuming a true VE of 90%, 5 events are required to offer 80% power to detect vaccine efficacy exceeding zero (H0: VE = 0) when using a one-sided alpha level of 0.05 (i.e. a 90% CI) and a randomization ratio of 4.65:1 (860 active:185 placebo).

[a] All or some of the described analyses may also be performed prior to the End of Study analysis, but after the Primary analysis. Since no formal hypothesis tests are performed, no alpha spending will be applied.

[b] Note that the observation period ends at the time of unblinding or receipt of an unscheduled vaccine, whichever occurs first, cf. section 9.7 of clinical trial protocol amendment 15. This censoring rule applies to all efficacy analyses.

The formula to calculate VE (as a percentage) is:

$$VE = 100 \times \left(1 - \frac{p}{r(1-p)}\right)$$

Where p = proportion of events occurring among the group of participants vaccinated with Ad26.COV2.S , r = the ratio of the number participants vaccinated with Ad26.COV2.S to the number of participants vaccinated with placebo. 90% asymptotic Wald confidence limits will be calculated.

The number and percentage of participants with at least one molecularly confirmed Influenza infection will be tabulated by vaccine regimen, for the entire study period only.

The number and percentage of participants with at least one positive non-S protein ELISA (e.g., N ELISA), if available, will be tabulated by vaccine regimen. The analysis will be repeated pooling all active regimens vs. placebo, where data from all cohorts will be pooled. If at least 5 positive non-S protein ELISA events have been observed, VE can be calculated against this endpoint, using the same approach as outlined above for the VE against molecularly confirmed SARS-CoV-2 infection.

For each participant with confirmed COVID-19 infection, a narrative will be prepared based on the visit performed 28 days after the onset of COVID-19 signs and symptoms and other selected information from the clinical database, as available:

- participant ID
- vaccination regimen
- sex, race, ethnicity, age, BMI, dates at which vaccinations were received
- physical examination findings based on the visit performed 28 days after the onset of COVID-19 signs and symptoms
- vital signs including body temperature based on the visit performed 28 days after the onset of COVID-19 signs and symptoms
- concomitant medications since symptom onset
- Humoral immune responses as collected at the planned time points + those obtained from the blood sample taken on the visit performed 28 days after the onset of COVID-19 signs and symptoms. This information may be presented graphically.

For the analysis of the SIC (Patient Reported Outcomes, PRO) data, the following considerations apply

- An “episode” is defined as a period in which any symptoms are reported on the SIC, starting from the first day on which symptoms were reported until the first day that the PRO was not completed because the symptoms had resolved (this will be indicated in the SDTM data, with reason for not completing the PRO = “Symptoms resolved” (or similar)) or death
- A symptom (e.g. feeling generally unwell, fatigue, physical weakness, cough, etc.) is assumed to be present on each day the associated Yes/No question is answered “Yes” or the associated severity question has a rating > 0.

– If the PRO was not completed due to the participant being too ill or due to the participant being hospitalized, the symptom will be considered present with severity score 10. If the PRO was not completed due to any other reason, no imputations will be done.

Duration of the episode will be calculated as episode end date – episode start date. Duration of each symptom will be calculated as last day of symptom reporting – first day of symptom reporting + 1. Duration of the maximum severity is defined as the last day of reporting the maximum severity – the first day of reporting the maximum severity + 1.

The following analyses will be conducted for confirmed SARS-CoV-2 infection cases and confirmed influenza infection cases separately:

- At the level of first episodes, the following statistics will be calculated: number of episodes, mean and median duration of episodes (with min, max, q1 and q3), and mean and median number of symptoms reported per episode (with min, max, q1 and q3).
- At the level of the symptoms for each first episode, the following statistics will be calculated: number of participants experiencing the symptom, mean and median duration of each symptom (with min, max, q1 and q3), median (with q1 and q3) of maximum severity of each symptom, median duration of the maximum severity of each symptom (with min, max, q1 and q3).
- At the level of the participants, for each episode and each symptom separately, the duration, and minimum and maximum severity scores will be tabulated (as available).

In addition, participant listings will be provided containing the SIC information for each time point.

More details about these analyses will be provided in the DPS.

7.7.4. Definition of Subgroups

Selected safety and immunogenicity analyses will be conducted by the seropositivity status (positive vs. negative) of the participant. A participant will be considered seropositive if:

1. The serological test for SARS-CoV-2-specific antibodies at baseline (if available) is positive

   Note: in case multiple test results are available (IgG, IgM, IgA, no isotype specified), then as soon as one of the test results is positive, the participant will be considered seropositive.

   OR

2. The S ELISA immunogenicity readout on Day 1 is considered positive.

At the time of first interim analysis of a cohort, S ELISA Day 1 data for that cohort may not be available at the time of the CRF database snapshot or lock. In that case, participant serostatus will be based solely on the serological test for SARS-CoV-2-specific antibodies at baseline. As of the second interim analysis, it is expected that S ELISA Day 1 data will have become available for the participants, so that both criteria can (and will) be applied.

7.8. Interim Analyses

This SAP applies to all planned analyses of this study per CTP section 9.5 (Interim Analyses; Primary Analyses; and End of Study Analysis). After the first database lock, separate SAP document(s) may be written as needed to cover specific analysis needs that cannot be documented elsewhere (e.g. in the Data Presentation Specifications [DPS] document).

7.8.1. Data Review Committee (DRC) or Other Review Board

A Data Review Committee (DRC) has been commissioned to review the safety data of this trial. Please refer to the DRC Charter.

8. CHANGES FROM PROTOCOL

The protocol refers to the active vaccine as “Ad26COVS1”. This SAP uses the new reference “Ad26.COV2.S” throughout the document (including text copied from the protocol), with the exception of the protocol title which has been kept unchanged.

9. SUPPORTING DOCUMENTATION

9.1. Appendix 1 List of abbreviations

Ad26	Adenovirus serotype 26
AE	adverse event
AESI	Adverse event of special interest
BMI	Body Mass Index
CI	confidence interval
CoV	Corona Virus
COVID-19	Corona Virus Disease 2019
CRF	case report form
CSR	Clinical Study Report
CTP	Clinical Trial Protocol
DMC	Data Monitoring Committee
DPS	Data Presentation Specifications
DRC	Data Review Committe
eCRF	electronic case report form
ELISA	Enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot (assay)
FAS	Full Analysis Set
FDA	Food and Drug Administration
FOIA	Freedom of Information Act
FU	Follow-up
GMC	Geometric mean concentration
GMT	Geometric mean titer
H0	Null hypothesis
ICH	International Conference on Harmonization
ICS	Intracellular cytokine staining
IFN-γ / IFN-g	Interferon gamma
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IGM	Immunoglobulin M
IL	Interleukin
ITT	Intent-to-Treat
IU/ml	International units per milliliter
IVRS	interactive voice response system
IWRS	interactive web response system
kg	kilogram
LLOQ	lower limit of quantification
LOD	Limit of detection
m	meter
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
N	Number
NA	Not Applicable
PBMC	peripheral blood mononuclear cell
PD	Pharmacodynamic
PI	principal investigator
PK	pharmacokinetic(s)
PP	Per Protocol
PPE	Per Protocol Efficacy Set
PPI	Per Protocol Immunogenicity Set
PRO	Patient Reported Outcome
Q1	First quartile

Q3	Third quartile
RNA	Ribonucleic acid
S	Spike
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
SD	Standard deviation
SDTM	Study Data Tabulation Model
SE	Standard error
SIC	Symptoms of Infection with COVID-19
Th1	Helper cell type 1
Th2	Helper cell type 2
TLF	Tables, Listings and Figures
TNF-α / TNF-a	Tumor necrosis factor alpha
ULOQ	Upper limit of quantification
VE	Vaccine efficacy
VNA	Virus Neutralization Assay
WHO	World Health Organization

9.2. Appendix 2 Changes to Protocol-Planned Analyses

Not applicable

9.3. Appendix 3 Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

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

* Participant 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 participant populations, for example, conditioned athletes.

10. REFERENCES

Not Applicable