Janssen Research & Development

Statistical Analysis Plan

A Randomized, Double-blind, Placebo-controlled Phase 2a Study to Evaluate a Range of Dose Levels and vaccination Intervals of Ad26.COV2.S in Healthy Adults Aged 18 to 55 Years Inclusive and Adults Aged 65 Years and Older and to Evaluate 2 Dose Levels of Ad26.COV2.S in Healthy Adolescents Aged 12 to 17 Years Inclusive

Protocol VAC31518COV2001; Phase 2a

VAC31518 (JNJ-78436735)

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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

SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
1	10NOV2020	Not Applicable	Initial release
2	20JUL2021	Clinical Trial Protocol Amendment 5	
3	07APR2021	Clinical Trial Protocol Amendment 6 Clarifications on PPI definition and AESI	The purpose of the amendment is to cease recruitment of adolescents in this study.

1. INTRODUCTION

This SAP describes the pre-planned analyses for the Interim Analyses, Primary Analyses and Final Analysis, for all groups in the study. One or several Data Presentation Specification Documents will be available to further detail the statistical outputs that will be generated.

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

Due to the reactogenicity observed at the $2.5x10^{10}$ vp dose level, it was decided not to proceed to the $5x10^{10}$ vp dose level in adolescents.
Safety, reactogenicity and immunogenicity data will be summarized for the 33 enrolled adolescent participants. Only limited outputs will be provided.

The term “study vaccine” throughout the SAP, refers to Ad26.COV2.S or placebo as defined in Clinical Trial Protocol Section 6.1, Study Vaccinations Administered. Scientifically, the administration of the Ad26.COV2.S $1.25x10^{10}$ virus particles (vp) or placebo dose 4 months after the primary vaccination regimen in adults is considered as presentation of antigen. Nevertheless, for the sake of simplicity, the terms “study vaccine” and “vaccination” throughout the SAP encompass administration of the antigen presentation dose.

1.1. Objectives and Endpoints

Refer to CTP Section 3.

1.2. Study Design

Refer to CTP Section 4.

2. STATISTICAL HYPOTHESES

Refer to CTP Section 9.1.

3. SAMPLE SIZE DETERMINATION

Refer to CTP Section 9.2.

4. POPULATIONS (ANALYSIS SETS) 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. Samples taken after a participant meets the criteria for a major protocol deviation expected to impact the immunogenicity outcomes will be excluded from the PPI analysis. In addition, samples obtained after missed vaccinations or participants with natural infection occurring after screening (if applicable) will be excluded from the analysis set. SARS-CoV-2 infection is defined as a positive PCR test or positive N-serology.

Two additional analysis sets are defined in Appendix 2 : PPI modified and FAS modified.

The safety and COVID-19 cases analyses will be censored at the date of unblinding or the receipt of an unscheduled vaccine[a], whichever event occurs first. AEs and COVID episodes reported after unblinding or receipt of an unscheduled vaccine, whichever comes first, will be listed.
The immunogenicity analyses will be censored at the date of the receipt of

- an unscheduled vaccine,
- another live vaccine,
- unknown vaccine which is received in the window of +/-29 days at each dosing,
- other not live vaccine which is received in the window of +/-15 days at each dosing.

Immunogenicity samples excluded for the above reasons will be included in the listings and flagged.

[a] Unscheduled vaccine: an authorized/licensed COVID-19 vaccine offered outside of the study.

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 the first vaccination on Day 1.

The safety analysis will present all results by phase (cf. section 5.1.2 for phase definitions). Immunogenicity results will be presented per scheduled time point as appropriate. Listings will be shown per phase and time point.

Study day or relative day is defined as follows:

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

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

5.1.2. Phase definitions

The phases in the study will be constructed as detailed in Table 1 and Table 2.

Table 1: Phase Definitions for groups 1-10

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 date 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 date 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 date in case of interim analysis c) 23:59 on 28 days 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 date in case of interim analysis c) One minute prior to Antigen presentation
Regimen	2	Antigen presentation or Placebo	3	Date and time of Antigen presentation or Placebo	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 on Day 28 after the Antigen presentation or Placebo (23:59 of day of Antigen presentation + 28 days)
Follow-up 3	5			One minute after Antigen presentation or Placebo 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 d) One minute prior to post dose 4 period (groups 6, 8 and 10)
Regimen	2	Cross Vaccination 1	4	Date and time of Cross Vaccination 1	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 on 28 days after the fourth vaccination (23:59 of day of vaccination + 28 days) d) One minute prior to Cross Vaccination 2
Follow-up 4	6			One minute after Cross Vaccination 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 date in case of interim analysis c) One minute prior to Cross Vaccination 2
Regimen	2	Cross Vaccination 2	5	Date and time of Cross Vaccination 2	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 on Day 28 after the vaccination (23:59 of day of vaccination + 28 days)
Follow-up 5	7			One minute after Cross Vaccination 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.

Table 2: Phase Definitions for groups A-C

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 date in case of interim analysis c) 23:59 on 28 days after the first vaccination (23:59 of day of vaccination + 28 days)
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 date in case of interim analysis c) One minute prior to cross-vaccination (group C) d) 23:59 at the Date of last visit
Regimen	2	Cross Vaccination	2	Date and time of Cross 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 date in case of interim analysis c) 23:59 on Day 28 after the Cross Vaccination 1 (23:59 of day of booter vaccination + 28 days)
Follow-up 2	4			One minute after Cross Vaccination 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.

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. The active periods can be:

- "Post-Dose 1"
- "Post-Dose 2"
- "Post-Dose 1 and Post-Dose 2 Combined", which refers to the "primary regimen"
- "Post- Antigen presentation or Placebo"
- "Post-Dose 1, Post-Dose 2 and Post-Antigen presentation or Placebo", which refers to the complete regimen including Antigen presentation. It includes the 28-day period after each vaccination (dose 1, dose 2, Antigen presentation).

The primary endpoints include SAEs from the first vaccination until end of the study. This will be a combined period of post-dose 1, post-dose 1 Follow-up (FU), post-dose 2, post-dose 2 FU, post-Antigen presentation or Placebo and post-Antigen presentation or Placebo FU. This period will be labeled "Entire Period" or similar in the outputs.

The secondary endpoints include SAEs from the antigen presentation until end of the study. This will be a combined period of post-Antigen presentation or Placebo and post-Antigen presentation or Placebo FU. This period will be labeled "Entire Antigen Presentation Regimen" or similar in the outputs.

5.1.3. Pooling Algorithm for Analysis Centers

Data will be pooled across the different centers.

5.1.4. Visit windows

Refer to CTP section 8. Visit windows will be taken into account for the analysis of immunogenicity results, see section 5.7.1.

5.1.5. Analyses by groups and pooled across groups

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

5.2. 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 who discontinued study
- participants who discontinued vaccination
- reasons for termination

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

5.2.1. Demographics and Baseline Characteristics

The following demographic and baseline characteristics will be summarized.
Table 2 presents a list of the demographic and baseline variables that will be summarized by vaccine regimen and overall for the FAS.

Table 2: 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	
Age (18-40, 41-55, ≥65)	

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

5.2.2. Protocol Deviations

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

5.2.3. 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.

The use of antipyretics with fever will be tabulated and the use of antipyretics on immunogenicity will be tabulated (overall, NSAIDs vs non-NSAIDs).

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.

5.3. Primary Endpoint(s) Analysis

The primary endpoints in this study, are:

Adults

Humoral Immune Response

All participants in groups 1-3 (3 dose levels of Ad26.COV2.S administered as a 2-dose schedule at a 56-day interval):

- Serological response to vaccination as measured by virus neutralization assay (VNA) titers and enzyme-linked immunosorbent assay (S-ELISA EU/mL), 28 days after Vaccination 2.
- Antibody geometric mean titers (GMTs) (VNA) and geometric mean concentrations (GMCs) (S-ELISA), 28 days after Vaccination 2.

All participants in groups 4-5 (2 dose levels of Ad26.COV2.S administered as a single vaccination):

- Serological response to vaccination as measured by VNA titers and ELISA (S-ELISA EU/mL), 28 days after Vaccination 1.
- Antibody GMTs (VNA) and GMCs (S-ELISA), 28 days after Vaccination 1.

All participants in groups 7-9 ($5x10^{10}$ vp dose level, administered as a 2-dose schedule at a 28-day and at an 84-day interval):

- Serological response to vaccination as measured by VNA titers and ELISA (S-ELISA EU/mL), 28 days after Vaccination 2.
- Antibody GMTs (VNA) and GMCs (S-ELISA), 28 days after Vaccination 2.

Safety and reactogenicity of Ad26.COV2.S

- Solicited local and systemic AEs for 7 days after each vaccination.
- Unsolicited AEs for 28 days after each vaccination.
- Serious adverse events (SAEs) and adverse events of special interest (AESIs) throughout the study (from first vaccination until end of the study)

Adolescents

Safety and reactogenicity of Ad26.COV2.S

- Solicited local and systemic AEs for 7 days after vaccination.
- Unsolicited AEs for 28 days after vaccination.
- SAEs (incl. Multisystem Inflammatory Syndrome in Children [MIS-C]) and AESIs throughout the study (from first vaccination until end of the study).

5.3.1. Definition of Endpoint(s)

Adults
The primary objectives of the study for immunogenicity are to explore the dose-response and interval-response relationship of humoral immune responses induced by different dose levels and different vaccine administration timings of Ad26.COV2.S using VNA titers and ELISA U/mL.
To explore the dose-response relationship between VNA/ELISA and different dose levels of the Ad26.COV2.S vaccine, separate regression models will be fitted.
In the regression models the log transformed VNA/ELISA titers at each timepoint will be used as dependent variable and the dose levels as independent variable.

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.

5.3.2. Estimand

Not applicable

5.3.3. Analysis Methods

Refer to sections 5.6. and 5.7.1.

5.4. Secondary Endpoint(s) Analysis

5.4.1. Key/Confirmatory Secondary Endpoint(s)

The secondary endpoints in this study, are:

Adults
Anamnestic response to antigen presentation of Ad26.COV2.S at the $1.25x10^{10}$ vp dose level, administered 4 months after Vaccination 2 (2-dose schedule) or 6 months after a Vaccination 1 (single-dose schedule), 7 days after antigen presentation:

- Serological response to vaccination as measured by VNA titers and ELISA (S-ELISA, ELISA U/mL [EU/mL]), 7 days after antigen presentation.
- Antibody GMTs (VNA) and GMCs (S-ELISA), 7 days after antigen presentation.

Safety and Reactogenicity of antigen presentation of Ad26.COV2.S:

- Solicited local and systemic AEs for 7 days after antigen presentation

- Unsolicited AEs for 28 days after antigen presentation
- SAEs and adverse events of special interest (AESIs) throughout the study (from after antigen presentation until end of the study)

Humoral Immune Response across all groups, at all blood collection timepoints.

- Neutralizing antibody titers to the wild-type SARS-CoV-2 virus expressing S protein as measured by VNA, at all blood collection timepoints.
- Binding antibody titers to SARS-CoV-2 or individual SARS-CoV-2 proteins (e.g., S protein) as measured by ELISA, at all blood collection timepoints.

Adolescents

Humoral immune response to a single dose of Ad26.COV2.S at the $2.5x10^{10}$ dose level, administered IM.

- Serological response to vaccination as measured by VNA titers and ELISA (S-ELISA, EU/mL), 28 days after vaccination.
- Antibody GMTs (VNA) and GMCs (S-ELISA), 28 days after vaccination.

Humoral immune response to Ad26.COV2.S at the $2.5x10^{10}$ dose level, at all blood collection timepoints.

- Neutralizing antibody titers to the wild-type SARS-CoV-2 virus expressing S protein as measured by VNA, at all blood collection timepoints.
- Binding antibody titers to SARS-CoV-2 or individual SARS-CoV-2 proteins (eg, S protein) as measured by ELISA, at all blood collection timepoints.

5.4.1.1. Definition of Endpoint(s)

Refer to section 5.7.1.

5.4.1.2. Estimand(s)

Not Applicable

5.4.1.3. Analysis Methods

Refer to sections 5.6 and 5.7.1.

5.5. Tertiary/Exploratory Endpoint(s) Analysis

The exploratory endpoints in this study, are:

Adults

Cellular Immune Response to Ad26.COV2.S at different dose levels in a subset of participants (Groups 1 to 6) at selected blood collection timepoints.

- T helper (Th) 1 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 Cluster of differentiation (CD) 4+/CD8+, interferon Gamma (IFNγ), interleukin [IL]-2, tumor necrosis factor alpha (TNFα), IL-4, IL-5, IL-13, and/or other Th1/Th2 markers.

Humoral immune response to Ad26.COV2.S.

- Analysis of antibodies binding to SARS-CoV-2 S protein and the receptor-binding domain (RBD) of the SARS-CoV-2 S protein by meso scale discovery (MSD)

- Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, Ig subclass and IgG isotype

- Passive transfer: analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model

- Analysis of neutralizing antibodies against emerging SARS-CoV-2 variants.

Occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms:

- The number of participants with molecularly confirmed COVID-19.

- Presence and severity of COVID-19 signs and symptoms as measured by Symptoms of Infection with coronavirus-19 (SIC).

Immune Response in vaccinated individuals after natural SARS-CoV-2 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 pseudovirions expressing S protein).

- SARS-CoV-2-binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S protein.

- Analysis of gene expression by RNA transcript profiling.

Occurrence of asymptomatic SARS-CoV-2 infection.

- The number of participants with positive non-S protein ELISA (eg, nucleocapsid [N] protein ELISA), if such an assay can be developed.
- The number of asymptomatic participants with a SARS-CoV-2 positive RT-PCR test

Hematology laboratory parameters after Ad26.COV2.S administration

- Lupus anticoagulants, anti-β2 glycoprotein, anti-cardiolipin, D-dimers, anti-PF4

To assess the correlation between the binding antibody (ELISA) titers and neutralizing antibody (VNA) titers to SARS-CoV-2, in a subset of participants at selected timepoints.

- Correlation between ELISA (S-ELISA; EU/mL) and VNA (wild-type virus [wt]VNA and/or pseudovirion [ps]VNA) titers at selected timepoints.

Adolescents

Humoral immune response to Ad26.COV2.S.

- Analysis of antibodies binding to SARS-CoV-2 S protein and the receptor-binding domain (RBD) of the SARS-CoV-2 S protein by meso scale discovery (MSD)
- Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, Ig subclass and IgG isotype
- Passive transfer: analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model
- Analysis of neutralizing antibodies against emerging SARS-CoV-2 variants.
- Adenovirus neutralization as measured by VNA.

Occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms.

- The number of participants with molecularly confirmed COVID-19.
- Presence and severity of COVID-19 signs and symptoms as measured by Symptoms of Infection with Coronavirus-19 (SIC).

To examine the immune response in vaccinated individuals after natural SARS-CoV-2 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 pseudovirions expressing S protein).
- SARS-CoV-2-binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S protein.
- Analysis of gene expression by ribonucleic acid (RNA) transcript profiling.

Occurrence of asymptomatic SARS-CoV-2 infection.

- The number of asymptomatic participants with positive non-S protein ELISA (eg, nucleocapsid [N] protein ELISA), as feasible.
- The number of asymptomatic participants with a SARS-CoV-2 positive RT-PCR test

To assess coagulation-related parameters at selected blood sample collection timepoints.
platelet factor 4-heparin complex (anti-PF4) antibodies.

5.6. (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 protocol[a]. In addition, safety data will be analyzed by intervention regimens as designed per protocol and age group. Data will be presented by period (post Dose 1, post Dose 2, post Antigen Presentation, as applicable) as well as over the entire primary regimen, and over the entire Antigen Presentation 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).

5.6.1. Adverse Events

5.6.1.1. 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.

For AESI, the following is defined:

- Suspected AESI : as reported by investigator and presented by: Embolic and thrombotic events (SMQ) + PT; Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias + PT; Other SMQ + PT.
- Suspected AESI: Embolic and thrombotic events (SMQ), Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias and presented by: Embolic and thrombotic events (SMQ) + sub-SMQ1 + PT; Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias + PT;
- Qualified for assessment AESI: [Embolic and thrombotic events (SMQ)] and [Haematopoietic thrombocytopenias (SMQ) (broad) or HLT Thrombocytopenias or platelet count below normal ranges per local or central laboratory report or platelet count < 150 × 10E9/L] and presented by: Embolic and thrombotic events (SMQ) + sub-SMQ1 + PT;

[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".

5.6.1.2. 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 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.

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 5.7.3).

- 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), and "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].

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.

5.6.1.3. 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

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

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 after the end date of the preceding event. Overlapping/consecutive events may be combined into one AE or not, according to the following rules:

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

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

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

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

Remarks:

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

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

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

5.6.1.4. 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.

5.6.2. Laboratory, Vital Signs and Physical Examination

Laboratory safety parameters, change from baseline and change from pre-dose x over time will be tabulated. Abnormalities on the changes from reference, if available, will be categorized. Abnormalities will be tabulated by visit and by worst abnormality. Patient profiles will be provided.

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.

5.7. Other Analyses

5.7.1. Immunogenicity Analyses

The analysis of immunogenicity will use the PPI set, PPI modified set and FAS modified set (cf Appendix 2 for analyses sets definitions). 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 group, and by vaccine group and age group[a].

Key immunogenicity assay results will also be analyzed for the subgroups defined in Section 5.7.4. 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 groups (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).

[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".

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

Assay	Purpose
Humoral Immunogenicity	
Primary/Secondary/Exploratory 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 SARS-CoV-2 or individual SARS-CoV-2 proteins (eg, S protein)
Exploratory endpoints	
SARS-CoV-2 binding antibodies (ELISA)	Analysis of antibodies binding to the SARS-CoV-2 N protein, if such an assay is available
SARS-CoV-2 binding antibodies (MSD)	Analysis of antibodies binding to the SARS-CoV-2 S protein (different than the assays supportive of the secondary objectives) and the RBD of the SARS-CoV-2 S protein
Functional and molecular antibody characterization	Analysis of antibody characteristics including Fc-mediated viral clearance, avidity, Fc characteristics, Ig subclass and IgG isotype
Passive transfer	Analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model
SARS-CoV-2 neutralizing antibodies	Analysis of neutralizing antibodies against emerging SARS-CoV-2 variants
Cellular Immunogenicity	
Exploratory endpoint	
Flow cytometry (ICS)	Analysis of T-cell responses to SARS-CoV-2 S protein, and/or other protein peptides by intracellular cytokine staining (ICS) including $CD4^+$/$CD8^+$, IFNγ, IL-2, TNFα, IL-4, IL-5, IL-13, and/or other Th1/Th2 markers.
Gene expression analysis	Analysis of gene expression by RNA transcript profiling

5.7.1.2. 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:
  - values<LLOQ are imputed with LLOQ/2.
- Calculation of fold increases from baseline:
  - 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.

5.7.1.3. 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.

5.7.1.4. Immune Response Analysis

No formal hypothesis on immunogenicity will be tested.

5.7.1.5. Immunogenicity against the insert

5.7.1.5.1. 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 (for time points after dose 2), fold increase from pre-antigen presentation (for time points after the antigen presentation), difference to Ad26 5e10: GMR (95% CI) for Geometric mean and GMR (95% CI) for Geometric mean increase from Baseline.

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 value is strictly greater than the LLOQ (>LLOQ)
  - The baseline sample value is strictly greater than the LLOQ (>LLOQ) and the post-baseline 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 will 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 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).

In addition, results for group 4 (High Dose single vaccination) and lower doses with double vaccination (D56 regimen) will be graphically presented and summarized with descriptive statistics.

5.7.1.5.2. Cellular assays

For **ICS**, if available, 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 (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 analyzes.

- 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] percentage 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-S, SARS-Cov2-S1, SARS-Cov2-S2), cytokine (combination), and time point.

[a] Also known as "mock subtracted"

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 participants 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

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 will be provided in the DPS.

5.7.1.6. Statistical modeling of the immune responses

The VNA and ELISA results at D14 and D28 post 1st Vaccination and post 2nd Vaccination will be used to investigate potential dose-response effects. For the D28 post 2nd Vaccination also a potential interval duration effect between the two vaccinations will be explored.

In all modeling approaches the VNA and ELISA data will be log10 transformed.

All modeling exercises are explorative in nature and not powered for formal hypothesis testing.

5.7.1.6.1. Dose response modeling

To explore the dose effect of single vaccination, data from D14 post 1st Vaccination of the groups 1-10 will be analyzed using a regression model with log10 transformed VNA and ELISA results as dependent variable and dose as exploratory variable. Depending on the pattern observed a linear or non-linear model will be used.

To explore the dose effect of single vaccination, data from D28 post 1st Vaccination of the groups 1-10 will be analyzed using a regression model with log10 transformed VNA and ELISA results as dependent variable and dose as exploratory variable. Depending on the pattern observed a linear or non-linear model will be used.

To explore the dose effect of the 56day double vaccination regimen, data from D28 post 2nd Vaccination of the groups 1:3, and 6 will be analyzed using a regression model with log10 transformed VNA and ELISA results as dependent variable and dose as exploratory variable. Depending on the pattern observed a linear or non-linear model will be used.

To explore the dose effect of the 56day single vaccination regimen, data from D28 post 2nd Vaccination of the groups 4:5, and 6 will be analyzed using a regression model with log10 transformed VNA and ELISA results as dependent variable and dose as exploratory variable. Depending on the pattern observed a linear or non-linear model will be used.

Depending on the observed data pattern, the placebo groups may be excluded from the analysis and focus set only on the dose response relationship within the active doses studied.

5.7.1.6.2. Interval response modeling

To explore the interval duration effect of the double vaccination regimen, data from D28 post 2nd Vaccination of the groups 3, 7 and 9 will be analyzed using a regression model with log10 transformed VNA and ELISA results as dependent variable and interval duration as exploratory variable. Depending on the pattern observed a linear or non-linear model will be used.

5.7.2. 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 Ad26COVS2.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 one-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 one-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)

5.7.3. COVID-19-like Signs and Symptoms

Procedures to be performed in the event a participant experiences signs or symptoms suggesting possible COVID-19 are detailed in CTP Sections 1.3 Schedule of Activities and 8.1.2 Procedures in case of COVID-19-like Signs and Symptoms.

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

The following analyses will use the FAS set and will be conducted at the time of the final analysis:

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.

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.

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
- pulse oximetry
- 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
- Humoral immune responses as collected at the planned time points + those obtained from the blood sample taken on the visits performed 3 and 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 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:

- 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.

5.7.4. Definition of Subgroups

Selected safety and immunogenicity analyses will be conducted by the age group of the participant.

Immunogenicity subanalyses will be performed by BMI, sex and race.

5.8. Interim Analyses

This SAP applies to all planned analyses of this study per CTP section 9.5 (Interim Analyses; Primary Analysis and Final 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).

5.8.1. Data Monitoring Committee (DMC) or Other Review Board

An internal DRC was commissioned for this study. However, an independent IDMC was installed to include VAC31518COV2001 on 29 October 2020. Please refer to the IDMC Charter.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 List of abbreviations

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 Study Protocol
DMC	Data Monitoring Committee
DPS	Data Presentation specifications
DRC	Data Review Committee
eCRF	electronic case report form
ELISA	Enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
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
ICH	International Conference on Harmonization
ICS	Intracellular Cytokine Staining
IFN-γ / IFN-g	Interferon gamma
IL	Interleukin
ITT	Intent-to-Treat
IU/ml	International units per milliliter
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
PF4	Antibodies to platelet factor 4
PI	principal investigator
PK	pharmacokinetic(s)
PP	Per Protocol
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
VNA	Virus Neutralization Assay
WHO	World Health Organization

6.2. Appendix 2 Changes to Protocol-Planned Analyses

In October 2020, the current study was temporarily paused because one of the pausing rules was met in study COV3001. When the pause was lifted, most of the participants in Groups 7-8 had missed their visit window for Vaccination 2, rendering the intent of the 28-day vaccination interval in these groups futile.
For safety analyses,

- the 2 subjects vaccinated before pausing rule (28-day interval) will only be shown in the listings.
- the other subjects received the vaccination 2 between 51 and 72 days after Vaccination 1. They will be integrated in the Full Analysis Set with groups 1 and 6 (56-day interval).

For immunogenicity analyses,

- subjects randomized in groups 7 and 8 won't be included in PPI set
- a modified PPI set will be defined as follows :
  subjects with vaccination 2 within the window (54-64) or subjects who did not receive vaccination 2 will be integrated in groups 1 and 6
- a modified FAS set will be defined as follows :
  subjects in mPPI set and subjects with vaccination 2 in (51-53) days or (65-72) days window

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

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

If a laboratory value falls within the grading as specified below but also within the local laboratory normal limits, the value is considered as normal.

Serum *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23-26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the participant had a new seizure associated with the low sodium value.
***"ULN" is the upper limit of the normal range.

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

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** "ULN" is the upper limit of the normal range.
***: For INR, the values in the table are based on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, 2014 (version 2.0)

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

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

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

* 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.

Ranges to convert FDA scale to SI units

Ranges to convert FDA scale to SI units		Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life threatening (Grade 4)
Albumin (g/L)	Hypo-albuminemia	28-31	25-27	<25	
Eosinophils (10^9/L)		0.65-1.5	1.501-5.0	>5.0	
Hemoglobin for male (g/L)		125-135	105-124	85-104	<85
Hemoglobin for female (g/L)		110-120	95-109	80-94	<80
Hemoglobin change from baseline (g/L)		Any decrease – 15	16-20	21-50	>50
Lymphocytes (10^9/L)		0.75-1.0	0.5-0.749	0.25-0.499	<0.25
Neutrophils (10^9/L)		1.5-2.0	1.0-1.499	0.5-0.999	<0.5
Platelets (10^9/L)		125-140	100-124	25-99	<25
Protein (g/L)	Hypo-proteinemia	55-60	50-54	<50	
WBC (10^9/L)	Increase	10.8-15	15.001-20	20.001-25	>25
	Decrease	2.5-3.5	1.5-2.499	1.0-1.499	<1.0

Other Conversions

Blood, Serum, or Plasma Chemistries[1]		**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life-threatening (Grade 4)**	**Conversion factor**
Glucose (mmol/L)	Hypoglycemia	3.61-3.83	3.05-3.60	2.50-3.04	<2.50	/18.01477
	Hyperglycemia-Fasting	5.55-6.11	6.12-6.94	>6.94		
	Hyperglycemia-Random	6.11-6.94	6.95-11.10	>11.10		
Blood urea nitrogen (mmol/L)		8.2-9.3	9.4-11.1	>11.1		
Creatinine (µmol/L)		133-150	151-177	178-221	>221	/0.01131
Calcium (mmol/L)	Hypocalcemia	2.00-2.10	1.87-1.99	1.75-1.86	<1.75	/4
	Hypercalcemia	2.62-2.74	2.75-2.87	2.88-3.00	>3.00	/4
Magnesium (mmol/L)	Hypomagnesemia	0.53-0.62	0.45-0.52	0.37-0.44	<0.37	/2.43072
Phosphorus (mmol/L)	Hypophosphatemia	0.74-0.81	0.65-0.73	0.52-0.66	<0.52	/3.09693

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

Cholesterol (mmol/L)		5.20-5.43	5.44-5.82	>5.82		
Coagulation						
Fibrinogen (µmol/L)	Increase	11.76-14.70	14.71-17.65	>17.65		
	Decrease	4.41-5.88	3.68-4.40	2.94-3.67	<2.94	/34

7. REFERENCES

Newcombe, R. G. 1998. 'Interval Estimation for the Difference Between Independent Proportions: Comparison of Eleven Methods.' Statistics in Medicine, 17, pp. 873-890.