Janssen Research & Development

Statistical Analysis Plan

An Open-label, Phase 2 Study to Evaluate the Safety, Reactogenicity, and Immunogenicity of Ad26.COV2.S in Healthy Pregnant Participants

Protocol VAC31518COV2004; Phase 2

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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1. INTRODUCTION

This SAP describes the pre-planned analyses for the Sentinel + Safety Cohort, Primary Analysis and Final Analysis for this study. One or several Data Presentation Specification Documents will be available to further detail the statistical outputs that will be generated.

For some additional analyses, a separate SAP may be written.

The term "study vaccine" throughout the SAP refers to Ad26.COV2.S as defined in Clinical Trial Protocol Section 6.1, Study Vaccinations Administered.

1.1. Objectives and Endpoints

Objectives	Endpoints
Primary	
To assess the safety and reactogenicity of Ad26.COV2.S administered intramuscularly (IM) as a 1-dose (5×10^{10} vp) schedule in adult participants, during the 2nd and/or 3rd trimester of pregnancy, and (potentially) postpartum.	• Solicited local and systemic AEs for 7 days after vaccination, or until resolution. • Unsolicited AEs for 28 days after vaccination. • Serious adverse events (SAEs) and Adverse Events of Special Interest (AESI) throughout the study (from vaccination until end of the study, i.e., at least 12 months after delivery). • Medically-attended adverse events (MAAEs) until 6 months after vaccination • AEs leading to study discontinuation (during the entire study).
To assess the humoral immune response in peripheral blood of adult participants to Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule, during the 2nd and/or 3rd trimester of pregnancy, 28 days after vaccination.	• Serological response to vaccination as measured by enzyme-linked immunosorbent assay (ELISA; [S-ELISA, EU/mL]), 28 days after vaccination.
Secondary	
Adults	
To assess safety of the booster dose of Ad26.COV2.S at 5×10^{10} vp on participants who were vaccine naïve at study entry.	• Solicited local and systemic AEs for 7 days after booster vaccination, or until resolution. • Unsolicited AEs for 28 days after vaccination. • SAEs and AESI throughout the study (from vaccination until end of the study, i.e., at least 12 months after delivery).

Objectives	Endpoints
	• MAAEs until 6 months after vaccination • AEs leading to study discontinuation (during the entire study).
To assess pregnancy outcomes in adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.	• Pregnancy outcomes (including, live term birth, live preterm birth, stillbirth, and abortion) (non-exhaustive).
To assess pregnancy-related AEs in adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.	• Pregnancy-related AEs throughout pregnancy (including gestational diabetes, gestational hypertension, premature rupture of membranes, premature labor, premature uterine contractions, poor or restricted fetal growth, pre-eclampsia, eclampsia, vaginal or intrauterine hemorrhage) (non-exhaustive).
To assess the humoral immune response in peripheral blood of adult participants induced by Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule during the 2nd and/or 3rd trimester of pregnancy, at all blood collection timepoints.	• Serological response to vaccination as measured by ELISA (S-ELISA; EU/mL) and/or equivalent assay, at all blood collection timepoints.
To assess the humoral immune response in peripheral blood of adult participants to Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule during the 2nd and/or 3rd trimester of pregnancy, 28 days after vaccination.	• Serological response to vaccination as measured by virus neutralization assay (VNA) titers, 28 days after vaccination.
To evaluate the humoral immune response in adult participants who are vaccine naïve at study entry and receive a booster dose during the study, pre-boost and at selected time points post-booster vaccination.	• Serological response to vaccination measured by binding (S-ELISA and/or equivalent assay) and/or neutralizing (VNA) antibody titers.
Neonates and Infants	
To assess antibody levels against SARS-CoV-2 in neonates and infants, born to adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy, at birth (i.e., in cord blood) and at approximately 2 months and 6 months of age.	• Serological response to vaccination as measured by ELISA (S-ELISA, EU/mL) and/or equivalent assay, at birth (i.e., in cord blood) and at approximately 2 months and 6 months of age.
To assess antibody levels against SARS-CoV-2 in neonates/infants, born to adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy, at birth (i.e., in cord blood).	• Serological response to vaccination as measured by VNA titers at birth (i.e., in cord blood).

Objectives	Endpoints
To assess safety in neonates and infants born to adult participants who have received Ad26.COV2.S, during the 2nd and/or 3rd trimester of pregnancy.	• SAEs (including Multisystem Inflammatory Syndrome in Children [MIS-C]) and AESIs in neonates and infants from birth until approximately 12 months of age. • MAAEs in neonates and infants from birth until 6 months of age. • AEs in neonates/infants leading to study discontinuation from birth until discontinuation.
To assess outcomes in neonates and infants up to approximately 12 months of age born to participants who have received Ad26.COV2.S, during the 2nd and/or 3rd trimester of pregnancy.	• Outcomes in neonates and infants (including normal neonate, term neonate with (or without) complications, preterm neonate with (or without) complications, neonatal infection, respiratory distress, congenital anomalies, neonatal death, low birth weight, and small for gestational age measured from birth until approximately 12 months of age) (non-exhaustive).
Exploratory	
Adults	
To assess the humoral immune response in peripheral blood of adult participants induced by Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule during the 2nd and/or 3rd trimester of pregnancy, at all or selected blood collection timepoints.	• Serological response to vaccination as measured by VNA titers, at all blood collection timepoints.
To assess the correlation between the binding antibody (ELISA) titers and neutralizing antibody (VNA) titers to SARS-CoV-2 at selected timepoints.	• Correlation between ELISA (S-ELISA; EU/mL or equivalent assay) and VNA titers at selected timepoints.
To assess the humoral immune response in adult participants to Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule in peripheral blood versus cord blood at the time of delivery.	• Serological response to vaccination as measured by ELISA (S-ELISA, EU/mL) and/or equivalent assay, using serum samples obtained from peripheral blood and cord blood at the time of delivery.
To assess the cellular immune responses in peripheral blood of adult participants who are vaccine naïve at study entry to Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule in a subset of participants at selected blood collection timepoints.	Th1 and Th2 immune responses as assessed by: • Flow cytometry after SARS-CoV-2 S protein peptide stimulation of peripheral blood mononuclear cells (PBMC) and intracellular cytokine staining (ICS) including cluster of differentiation (CD) markers: CD4+/CD8+,

Objectives	Endpoints
Note: This objective will be tested only if PBMC collection is feasible in a sufficient proportion of participants.	tumor necrosis alpha (TNFα), interferon gamma (IFNγ), interleukin (IL)-2, IL-4, IL-5, IL-13, and/or other Th1/Th2 markers. (1) OR • Dual or single IFNγ and IL-4 enzyme-linked immunospot (ELISpot) assay after stimulation of PBMCs with SARS-CoV-2 protein peptides. (1)
To assess the impact of pre-existing humoral immunity against coronavirus other than SARS-CoV-2 at baseline on Ad26.COV2.S vaccine immunogenicity.	Analysis of antibodies binding to coronaviruses other than SARS-CoV-2, or other respiratory viruses by ELISA or equivalent assay (1).
To assess the presence of immunoglobulins against SARS-CoV-2 in colostrum and breast milk in adult participants in response Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule and after booster vaccination.	IgA and/or other Ig types against SARS-CoV-2 and/or emerging variants in colostrum and breast milk measured by ELISA or equivalent assay (1).
To further explore humoral immune responses in peripheral blood and cord blood of adult participants induced by Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule before and after booster vaccination, at all or selected blood collection timepoints.	Exploratory analyses may include the following: • SARS-CoV-2 neutralization as assessed by SARS-CoV-2 neutralization assays (VNA). • Adenovirus neutralization as measured by VNA. (1) • Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype, antibody glycosylation, and assessment of antibody repertoire. (1) • Analysis of antibodies to the spike (S), nucleocapsid (N), and receptor binding domain (RBD) of the SARS-CoV-2 S protein, and surface proteins of other coronaviruses. • Epitope-specificity characterization of antibodies. (1) • Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the immune response in the serum or plasma. (1) • Passive transfer: Analysis of immune mediators correlating with protection against

Objectives	Endpoints
	experimental SARS-CoV-2 challenge in a suitable animal model. (1) • Analysis of binding and/or neutralizing antibodies against emerging SARS-CoV-2 variants. (1)
To further explore cellular immune responses in peripheral blood of adult participants to Ad26.COV2.S administered IM as a 1-dose (5×10^{10} vp) schedule at selected blood collection timepoints, including after booster vaccination, if feasible.	Exploratory analyses may include the following for a subset of participants, if feasible: • Analysis of gene expression in cells stimulated with SARS-CoV-2 S protein peptides, or in unstimulated cells (ex vivo). (1) • Analysis of messenger ribonucleic acid (mRNA) expression levels of vaccine-induced biomarkers of immune mediated responses. (1) • Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the immune response in cells stimulated with SARS-CoV-2 S protein peptides, or in unstimulated cells (ex vivo). (1) • Epitope-specificity characterization for B and T-cells. (1) • Analysis of the phenotype of antigen-specific T and B cells, assessed by single cell analysis. (1)
To assess the occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms in adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.	• The number of participants with molecularly confirmed COVID-19. (1) • Presence and severity of COVID-19 signs and symptoms as measured by the Symptoms of Infection with Coronavirus-19 (SIC).
To assess the occurrence of asymptomatic SARS-CoV-2 infection in adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.	• The number of adult participants with positive non-S ELISA and/or SARS-CoV-2 immunoglobulin assay that is dependent on the SARS-CoV-2 nucleocapsid (N) protein. • Asymptomatic infection detected by RT-PCR.
To assess the impact of the Ad26.COV2.S vaccine on the incidence of co-infections with SARS-CoV-2 and other respiratory pathogens in participants who have received	• Analysis of broad respiratory pathogens panel in the nasal swabs collected during a confirmed COVID-19 episode and in a subset of nasal swab samples from adult

Objectives	Endpoints
Ad26.COV2.S during the study period, up to delivery.	participants with a symptomatic infection, up to delivery. (1)
To examine the immune response in vaccinated adult participants after 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. (1) • SARS-CoV-2 neutralizing titers in serum measured by a VNA. (1) • SARS-CoV-2-binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S and/or N-protein. (1) • Analysis of gene expression by RNA transcript profiling. (1)
To further explore the impact of vaccination with Ad26.COV2.S on coagulation-related parameters: • In the event of a suspected adverse event of special interest (AESI) of thrombosis with thrombocytopenia syndrome (TTS), coagulation-related parameters will be tested retrospectively in samples obtained from pregnant participants at baseline (Day 1, pre-dose) and at 7 days, 14 days, and 28 days after vaccination, and postpartum (PP1 and PP43). The extent to which these coagulation factors correlate with platelet counts as determined at the time as part of the standard hematology safety assessment of complete blood count (CBC), will be determined. • Coagulation-related parameters (including pro-and anti-coagulation factors) and the extent to which these fluctuate pre-and post-vaccination with Ad26.COV2.S, will be determined for a subset of participants.	Coagulopathy assessment: • Hematology assessments of complete blood count with differential, including platelet counts (performed locally at the site: see Section 6.2). (1) • Analysis of levels of pro- and anti-coagulation factors in plasma/serum including but not limited to: activated partial thromboplastin time, prothrombin time, fibrinogen, D-dimer levels, Lupus anticoagulant, ant-cardiolipin antibody levels, β-2 glycoprotein, anti-PF4 antibody levels (performed retrospectively at central laboratories on stored samples) (see Section 6.2). (1)
To assess SARS-CoV-2 viral load in SARS-CoV-2 infected participants during a confirmed COVID-19 episode.	• Analysis of SARS-CoV-2 viral load (via qRT-PCR) in nasal swabs collected during a confirmed COVID-19 episode. (1)
To explore changes in the SARS-CoV-2 genome	• Identification of SARS-CoV-2 variants by sequencing of nasal swabs samples (as available). (1)

Objectives	Endpoints
Neonates and Infants	
To assess the occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms in neonates/infants of adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.	• The number of neonates/infants with molecularly confirmed COVID-19. (1) • Presence and severity of COVID-19 signs and symptoms as measured by the Pediatric Symptoms of Infection with Coronavirus-19 (PedSIC).
To assess the occurrence of asymptomatic SARS-CoV-2 infection in neonates/infants born to adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.	• The number of neonates/infants with positive non-S protein ELISA and/or SARS-CoV-2 immunoglobulin assay (eg, N-protein ELISA). • Asymptomatic infection detected by RT-PCR.
To assess SARS-CoV-2 viral load during a confirmed COVID-19 episode in neonates/infants born to adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy.	• Analysis of SARS-CoV-2 viral load (via qRT-PCR) in nasal swabs collected during a confirmed COVID-19 episode. (1)
To assess the impact of the Ad26.COV2.S vaccine on the incidence of co-infections with SARS-CoV-2 and other respiratory pathogens in neonates/infants born to participants who have received Ad26.COV2.S during the study period.	• Analysis of broad respiratory pathogens panel in the nasal swabs collected during a confirmed COVID-19 episode and in a subset of nasal swab samples from neonates/infants with a symptomatic infection. (1)
To examine the immune response in neonates/infants born to participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy, after SARS-CoV-2 infection.	• Confirmation of SARS-CoV-2 infection by molecular testing. (1) • SARS-CoV-2 neutralizing titers in serum measured by VNA. (1) • SARS-CoV-2-binding antibodies measured by ELISA: Analysis of antibodies binding to the SARS-CoV-2 S and/or N-protein. (1)
To assess antibody levels against SARS-CoV-2 in neonates/infants, born to adult participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy, at all or selected blood collection timepoints.	• Serological response to vaccination as measured by SARS-CoV-2 virus neutralization assay (VNA) titers at birth (i.e., in cord blood) and at approximately 2 months, and 6 months of age.
To further explore humoral immune responses in neonates/infants born to participants who have received Ad26.COV2.S during the 2nd and/or 3rd trimester of pregnancy, at all or selected blood collection timepoints.	Exploratory analyses may include the following: • SARS-CoV-2 neutralization as assessed by SARS-CoV-2 neutralization assays VNA

Objectives	Endpoints
	• Adenovirus neutralization as measured by VNA. (1) • Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype, antibody glycosylation, and assessment of antibody repertoire. (1) • Analysis of antibodies to the spike (S), nucleocapsid (N), and receptor binding domain (RBD) of the SARS-CoV-2 S protein, and surface proteins of other coronaviruses. (1) • Epitope-specificity characterization of antibodies. (1) • Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the immune response in the serum or plasma. (1) • Passive transfer: Analysis of immune mediators correlating with protection against experimental SARS-CoV-2 challenge in a suitable animal model. (1) • Analysis of binding and/or neutralizing antibodies against emerging SARS-CoV-2 variants. (1)
To evaluate neurodevelopmental status in neonates and infants.	• Summary of developmental outcomes using the Ages & Stages Questionnaire, 3rd edition (ASQ3) at 2, 6 and 12 months of age.
To explore changes in the SARS-CoV-2 genome	• Identification of SARS-CoV-2 variants by sequencing of nasal swabs samples (as available) (1)

(1) May be considered as potential assessment for analyses after the primary analysis is performed.

1.2. Study Design

This is an open-label multicenter, Phase 2 study in healthy pregnant (2nd and/or 3rd trimester of pregnancy) participants ≥18 to ≤45 years of age to evaluate safety, reactogenicity, immunogenicity, and pregnancy outcomes. In this study, Ad26.COV2.S will be assessed as a single dose of 5×10^{10} vp in pregnant women who were previously vaccinated with another COVID-19 vaccine regimen or who were vaccine naïve at study entry.

A target of 240 adult participants in the 2nd or 3rd trimester of pregnancy (Week 16 to Week 38 of gestation, inclusive) was to be enrolled. Efforts were made to ensure good representation in terms of race and ethnicity. Enrollment was staggered and started with recruitment of vaccine naïve Sentinel participants (n=5) followed by an additional larger Safety Cohort (n=17) who all received 1 dose of Ad26.COV2.S vaccine at 5×10^{10} vp. Based on the safety data of those subjects, the IDMC confirmed that the safety profile of 1 dose of Ad26.COV2.S at 5×10^{10} vp was considered acceptable and no safety concerns were identified. The remaining participants (who have received their last COVID-19 vaccination at least 4 months prior to receiving the study vaccine or are vaccine naïve participants) were to be enrolled to receive 1 dose of Ad26.COV2.S at 5×10^{10} vp. They will preferably be equally distributed between the following groups, per their COVID-19 vaccination histories:

- **Group 1**: Previous primary vaccination (2-doses) or homologous booster vaccination with Comirnaty (Pfizer-BioNTech) or SpikeVax (Moderna)
- **Group 2**: Previous primary vaccination (1-dose) or homologous booster vaccination with Ad26.COV2.S (Janssen)
- **Group 3**: Previous COVID-19 vaccination, irrespective of previous schedule and vaccine (includes heterologous regimens) and excluding schedules for Groups 1 and 2
- **Group 4**: Vaccine naïve participants, including those who are in Sentinel and Safety Cohorts

Prior to CTP amendment 4, the sample size of each of the groups was preferably equally distributed but flexible with a target to recruit at least 40 participants in Groups 1 to 3 and at least 25 participants in Group 4. Participants were stratified by pregnancy stage at the time of enrollment (Weeks ≥16 to <28 or Weeks ≥28 to ≤38), with a goal of at least approximately 25% participants per trimester, per group.

As of CTP amendment 7, participant enrolment in this study was ceased.

There will be no active vaccination with Ad26.COV2.S of neonates/infants in this study.

Previously vaccinated participants (Groups 1-3), will receive a single dose of Ad26.COV2.S at 5×10^{10} vp as part of the study. No further vaccinations will be received by these groups during the study.

A booster vaccination with a single dose of Ad26.COV2.S at 5×10^{10} vp will be offered to vaccine naïve consenting participants who have completed pregnancy, are not pregnant again, and haven't received another COVID-19 vaccine (eg, national immunization program). The booster vaccination for vaccine naïve participants at study entry should be administered not earlier than 2 months after the participant's Ad26.COV2.S vaccination in the study. The study duration will not be extended. Dose 2, in this document refers to Booster which is only applicable for Group 4.

Safety assessments include an observation period at the study site after vaccination to monitor for the presence of any severe acute reactions, the recording of any solicited local or systemic adverse events (AEs), unsolicited AEs, serious AEs (SAEs), adverse events of special interest (AESI), pregnancy-related AEs throughout pregnancy, and medically attended adverse events (MAAEs).

Other safety assessments include clinical laboratory assessments, vital signs measurements (pulse/heart rate, supine systolic and diastolic blood pressure, respiratory rate, and body temperature) and physical examinations. In addition, adverse maternal/fetal outcomes, adverse neonate/infant outcomes SAEs (including MIS-C), MAAEs and AEs leading to discontinuation will be recorded.

Active surveillance for COVID-19-like signs and symptoms in participants and neonates/infants will occur, including a COVID-19 surveillance (symptom check) through the participant's electronic diary (electronic clinical outcome assessment [eCOA]). Participants will be asked to confirm whether subjective changes in (postpartum) breast milk production (reduction) have been noticed and report changes in the eCOA diaries. Confirmed SARS-CoV-2 infection will be recorded.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened on 1 occasion only. Participants who are rescreened will be assigned a new participant number, undergo the informed consent process, and then restart a new screening phase.

Immunogenicity assessments, including humoral immune responses such as neutralizing and binding antibodies will be performed.

2. STATISTICAL HYPOTHESES

No formal statistical hypothesis is to be tested. The study is designed to provide descriptive information regarding the safety, pregnancy outcomes, and immunogenicity of Ad26.COV2.S in adult participants in the 2nd and/or 3rd trimester of pregnancy, as well as the safety and outcomes of neonates/infants. In addition, the study will provide descriptive information regarding the safety and immunogenicity of Ad26.COV2.S administered as a booster in eligible participants.

3. SAMPLE SIZE DETERMINATION

The number of adult participants that will be assessed for safety and reactogenicity of the Ad26.COV2.S vaccine, from Week 16 of pregnancy through to delivery/termination and up to 12 months postpartum, was chosen to provide a preliminary assessment of safety and immunogenicity in this population.

A target of 240 adult participants in the 2nd or 3rd trimester of pregnancy, ≥18 to ≤45 years of age was planned to be enrolled in this study. The sample size of each of the groups (Groups 1 to 4) was preferably equally distributed but was flexible with a target to recruit at least 40 participants in Groups 1 to 3 and at least 25 participants in Group 4. Participants were stratified by pregnancy stage at the time of enrollment (refer to Section 5.7.5 for details), with a goal of at least approximately 25% participants per trimester, per group.

As of CTP amendment 7, participant enrollment in this study ceased.

When 40, 60, 120 and 240 participants are vaccinated, the observation of 0 AEs would be associated with a 95% confidence (2-sided) that the true rate is less than 8.8%, 6%, 3% and 1.5% respectively (additional cases are provided in Table 1).

Table 1: Probability of Observing at Least One Adverse Event

True Adverse	Probability of observing at least one adverse event given a true adverse event rate			
Event Rate	**N=40**	**N=60**	**N=120**	**N=240**
0.5%	18%	26%	45%	70%
1.0%	33%	45%	70%	91%
1.5%	45%	60%	84%	97%
2.0%	55%	70%	91%	99%
2.5%	64%	78%	95%	>99%
3.0%	70%	84%	97%	>99%
5.0%	87%	95%	>99%	>99%
N: number of participants receiving study vaccine (Ad26.COV2.S).				

4. POPULATIONS (ANALYSIS SETS) FOR ANALYSIS

For vaccine studies, study intervention assignment will follow the as treated principle: all analyses (including safety, pregnancy outcomes and immunogenicity) will be analyzed by the actually received vaccine. Further information for inclusion/exclusion of each of the populations will be described in the DPS. Table 2 describes the populations for analysis.

Table 2: Analysis Sets

Population	Description
Enrolled Adults	All adult participants who sign the ICF and are not screen failed
Full Analysis Set - Adults (FAS-A)	All enrolled adult participants with at least one vaccine administration documented.
Full Analysis Set - Non-vaccinated Neonates/Infants (FAS-NVN)	All non-vaccinated neonates/infants (NVN) born to Ad26.COV2.S vaccinated adult participants.
Per-protocol Immunogenicity-Adults (PPI-A)	All vaccinated adult participants for whom immunogenicity data are available excluding adult participants with major protocol deviations that are expected to impact the immunogenicity outcomes (1). In addition, Day 29 samples obtained from participants after SARS-COV-2 infection occurring after baseline (2), and samples obtained outside pre-defined time window will be excluded from the analysis.
Per-protocol Immunogenicity- Non-vaccinated Neonates/Infants (PPI-NVN)	All non-vaccinated neonates/infants (NVN) born to Ad26.COV2.S vaccinated adult participants for whom immunogenicity data are available, excluding neonates/infants born to participants with major protocol deviations that are expected to impact the immunogenicity outcomes prior to delivery, or neonates/infants with major protocol deviations that are expected to impact the immunogenicity outcomes, and excluding samples taken outside pre-defined time windows. (1)
(1) The list of major protocol deviations leading to exclusion from the immunogenicity analyses will be specified in the Major Protocol Deviation Criteria document and/or this list will be reported into the protocol deviation dataset of the clinical database before database lock. (2) This rule does not apply to samples obtained at later timepoints (i.e., after Day 29)	

5. STATISTICAL ANALYSES

5.1. General Considerations

A baseline (or reference) value will be defined as the value of the last available assessment prior to the first vaccination on Day 1, where Day 1 is the date of first vaccination for adults. A value at birth is baseline value for neonates and the birth date is the neonate reference date. The neonate baseline values for immunogenicity analysis are those from the cord blood sample collected.

Every adult will have a unique subject ID in the ADaM datasets and every neonate will have the same unique subject ID with 'A' added at the end of the ID. Multiple pregnancies [twins or higher order multiples] are excluded from the study but if a participant with multiple pregnancy is inadvertently enrolled, neonates will have the same unique subject ID with 'A', 'B', 'C' and D added at the end of the ID based on the number of neonates.

Unless otherwise specified, continuous variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean (mean), standard deviation (SD), standard error (SE), median, minimum, and maximum. Frequencies and percentages (one decimal place) will be generated for categorical variables. Denominator for the percentages is the number of participants in the considered population, and phase (where applicable; incidence per 100 participants/phase).

No formal statistical testing is planned. The safety analysis will be performed on the FAS-A based on actual intervention received and the FAS-NVN, unless otherwise specified. Safety data will be presented by period/phase (as applicable) as well as over the entire study. Immunogenicity results will be conducted on the PPI-A and PPI-NVN and presented per scheduled time point as appropriate. Safety and immunogenicity endpoints will be summarized descriptively by previous vaccination group (as defined in Section 1.2) and overall. All listings will include the previous vaccination group. Vaccination schedule in Group 4 (i.e. vaccine-naive at study entry) will have two components: the Primary Vaccination and the (optional) Booster Vaccination. Listings will be shown per phase and time point, as appropriate.

Participants that took another COVID-19 vaccine during the study are excluded from all safety and immunogenicity analyses from the moment they received the other vaccine. The safety data from those participants after they received the other vaccine will be listed separately.

Other exploratory or sensitivity analyses, on an ad-hoc basis, may be performed in addition to the analyses described in this document.

For adults, study Day 1 or Day 1 refers to the start of the first study vaccination. For neonates, study Day 1 or Day 1 refers to the neonate birth. Unless specified otherwise, safety assessments at all visits will be assigned a day relative to those dates (respectively for adults and neonates). In addition, in Group 4, safety assessments occurring after the booster vaccination date will also be assigned a day relative to this date.

Study day and relative day are defined as follows:

For adults (Day 1 refers to the start of the first study vaccination):

- Study Day=visit date-date of Day1+1; if visit date ≥ date of Day 1
- Study Day=visit date-date of Day1; if visit date < date of Day 1

For neonates (Day 1 refers to the neonate birth):

- Study Day=visit date-neonate birth date+1; if visit date ≥ date of Day 1
- Study Day=visit date-neonate birth date+1; if visit date < date of Day 1

Relative day (relday), the number of days in the analysis time point will be defined as:

- *relday = visit date – reference date+1* for visits on or after the reference date,
- *relday = visit date – reference date* for visits before the reference date,

where the reference date equals the date of the first study vaccination for adults, and as well as the date of booster vaccination for participants in Group 4, and the neonate birth date for the neonates.

For laboratory and vital signs all time points (including unscheduled ones, if any) are also considered in the determination of the worst toxicity grade.

Unscheduled results or multiple results for laboratory and vital signs taken at the same visit will be reported in listings.

5.1.1. Study Phases

To assess study phases, in case the time of the vaccination is missing, the time will be imputed with 00:00 before applying the phase and period derivation rules.

5.1.2. Phase Definitions

The phases in the study will be constructed as detailed in Table 3 below.

Table 3: Phase Definitions

Phase	Phase #	Period	Period #	Interval	
				From	To
Screening	1	Screening		Date and time of signing the informed consent form	One minute prior to start of Post-dose 1 period
Post-dose	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 data base 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 date and time of Booster Vaccination e) One minute before date and time of another COVID-19 vaccination outside the study f) One minute prior to neonatal/infant birth or pregnancy termination
Prepartum 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 data base cut-off date in case of interim c) One minute prior to 6 months after first vaccination (vaccination date + 6 mths) d) One minute prior to date and time of Booster Vaccination (if applicable) e) One minute before date and time of another COVID-19 vaccination outside the study f) One minute prior to neonatal/infant birth or pregnancy termination
'Postpartum follow-up 1' (adults) and 'Postnatal follow-up 1' (neonates); i.e. PP/PN D1 up to max PP/PN D42	5			Neonatal/infant birth or pregnancy termination	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) 42 days after the neonate birth date d) One minute prior to date and time of Booster Vaccination (if applicable) e) One minute before date and time of another COVID-19 vaccination outside the study
'Postpartum follow-up 2' (adults); i.e. PP D43 to max PP D366 --- 'Postnatal follow-up 2' (neonates); i.e. PN D43 to max PN D183	6			One minute after postpartum follow-up 1/ Postnatal follow-up 1 period ends	Minimum of : a) Maximum of 23:59 at the date of last contact (for early discontinuation) and 23:59 at the date of last visit b) 23:59 at the date of data base cut-off date in case of interim analysis c) D183 after birth for neonates and D366 post partum for adults d) One minute prior to date and time of Booster Vaccination (if applicable) e) One minute before date and time of another COVID-19 vaccination outside the study f) One minute prior to end of the study visit

Phase	Phase #	Period	Period #	Interval	
				From	To
'Postnatal follow-up 3' (neonates); i.e. PN D183 to max PN D366	7			One minute after Postnatal follow-up 2 period ends	Minimum of : g) Maximum of 23:59 at the date of last contact (for early discontinuation) and 23:59 at the date of last visit h) 23:59 at the date of data base cut-off date in case of interim analysis i) D366 after birth j) One minute before date and time of another COVID-19 vaccination outside the study k) One minute prior to end of the study visit
'Post-dose' *(Booster)*	2	Post-dose 2 *(i.e. Post-Booster)*	2	Date and time of second vaccination *(i.e. Booster)*	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of data base cut-off date in case of interim c) 23:59 on 28 days after the Booster Vaccination (23:59 of that day + 28 days) d) One minute before date and time of another COVID-19 vaccination outside the study
Booster follow-up 1	8			One minute after post-dose 2 period ends	Minimum of: a) 23:59 at the date of last contact (for early discontinuation) b) 23:59 at the date of data base cut-off date in case of interim c) One minute prior to 6 months after Booster Vaccination d) One minute before date and time of another COVID-19 vaccination outside the study
Booster follow-up 2	9			One minute after Booster follow-up 1	Minimum of: a) Maximum of 23:59 at the date of last contact (for early discontinuation) and 23:59 at the date of last visit b) 23:59 at the date of data base cut-off date in case of interim c) One minute before date and time of another COVID-19 vaccination outside the study e) One minute prior to end of the study visit
Follow-up other vaccine	10			Date and time of viral vaccination outside the study	Minimum of: a) Maximum of 23:59 at the date of last contact (for early discontinuation) and 23:59 at the date of last visit b) 23:59 at the date of data base cut-off date in case of interim analysis c) Date and time of end of the study visit

Note: PP Dx = Postpartum Day x, PN Dx = Postnatal Day x

Adverse Events (AE) tables and selected other tables may display AEs (or other counts) by phase. For such tables, post vaccination periods can be combined and additionally displayed. The "active" periods can be defined as:

- "Post-Dose 1"
- "Post-Booster", where applicable (booster)
- "Post-Dose 1 and Post-Booster Combined", where applicable.

5.1.2.1. Other time intervals

The following other time intervals will be derived for safety data summary as shown in Table 4.

Table 4: Other Time Intervals

Time Intervals	From	To
Up to Postpartum Day43	Date and time of first vaccination	Maximum of: a) Post-dose 1 end date b) Prepartum follow-up 1 end date c) Postpartum follow-up 1 end date
Primary Vaccination Period (in vaccine naïve group only)	Date and time of first vaccination	Maximum of: a) Post-dose 1 end date b) Prepartum follow-up 1 end date c) Postpartum follow-up 1 end date d) Postpartum follow-up 2 end date
Booster Vaccination Period (in vaccine naïve group only)	Date and time of Booster Vaccination	Maximum of: a) Post-dose 2 end date b) Post Booster follow-up 1 end date c) Post Booster follow-up 2 end date
Entire Study	Date and time of first vaccination	Maximum of: a) Primary Vaccination Period end date b) Booster Vaccination Period end date

5.1.3. Visit Windows

The following visit window rules must be taken into consideration when deriving the PPI-A and PPI-NVN. Note that if an immunogenicity sample is not taken, the corresponding protocol deviation will be considered to have no impact on immunogenicity.

For adult immunogenicity sampling (Note: PP=Postpartum):

1. If a participant is not vaccinated with the study vaccine, all immunogenicity samples taken will be excluded
2. If the scheduled booster vaccination was given ≤ 60 days from last study vaccination, all immunogenicity samples taken on and after the booster will be excluded from the PPI (Samples taken before may still be included in the PPI).
3. For the following visits, if an immunogenicity sample is taken out of below-defined windows, the sample will be excluded from PPI. Other samples from this participant may still be included in PPI.
    - Adult - Study D29 sampling: sample excluded from PPI if outside of +/-10 days from planned blood collection date.
    - Adult - 'Birth visit' (PP D1): sample excluded from PPI if outside of +7/-2 days from planned blood collection date.
    - Adult - Follow up period PP D43, and PP D183 sampling: sample excluded from PPI if outside +/-30 days from planned blood collection date.
4. Adult - Booster vaccination (Pre-Booster D1) sampling (if applicable): sample excluded from PPI if taken ≤ 60 days from study vaccination.
5. Adult - Booster vaccination (Post-Booster D29) sampling: sample excluded from PPI if outside of +/-10 days from planned blood collection date.

For neonate/infant immunogenicity sampling (Note: PN=Postnatal)

1. For the following visits, if immunogenicity samples are taken out of below-defined windows, the samples will be excluded from PPI. Other samples from this participant may still be included in PPI.
    - Neonate/infant PN61 sampling: sample excluded from PPI if outside of +/-14 days from planned blood collection date.
    - Neonate/infant PN6mo and PN12mo sampling: sample excluded from PPI if outside +/-30 days from planned blood collection date.

If an adult participant had another COVID-19 licensed vaccine, or used a disallowed medication, all samples after such (major) protocol deviation with impact on immunogenicity will be excluded from the PPI-A. In the case such protocol deviation occurs for the adult participant before and up to child delivery, all samples from the neonate will be excluded from PPI-NVN. Depending on the type of other protocol deviation, all samples collected after the protocol deviation, or the sample relating to the time of protocol deviation only will be excluded from PPI. This will be further clarified in the DPS.

Time windows may be redefined prior to database lock if the number of samples excluded as per the definitions above are too numerous. No time windows apply for Early Exit visit sampling. The sample closest to the target day (at noon) will be selected, if two or more samples meet the PPI definition in the pre-defined time windows.

5.2. Participant Dispositions

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

- participants screened
- participants screen failed (and main reason for screen failure)
- participants vaccinated (Primary/Booster)
- participants not vaccinated
- participants in the FAS-A
- participants in the PPI-A
- participants in the FAS-A but not in the PPI-A (and reason for not being in the PPI-A)
- participants who discontinued the study (and reasons for discontinuation)
- participants having another COVID-19 vaccine during the study

The number of neonates/infants in the FAS-NVN and PPI-NVN will also be tabulated.

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

5.3. Primary Endpoint(s) Analysis

5.3.1. Definition of Endpoints

Safety and reactogenicity of Ad26.COV2.S (first vaccination) in adult participants

- Solicited local and systemic AEs for 7 days after (first) vaccination, or until resolution
- Unsolicited AEs for 28 days after (first) vaccination
- SAEs and AESI throughout the study (from first vaccination until end of the study, i.e. at least 12 months after delivery)
- MAAEs until 6 months after the last vaccination
- AEs leading to study discontinuation (during the entire study)

Refer to Sections 5.6.1 for more details on adverse events definitions.

Humoral Immune Response in peripheral blood of adult participants

- Serological response to vaccination as measured by enzyme-linked immunosorbent assay (ELISA; [S-ELISA, EU/mL]), 28 days after vaccination

5.3.2. Estimand

Not applicable.

5.3.3. Analysis Methods

Safety and reactogenicity of Ad26.COV2.S in adult participants

The primary safety and reactogenicity objectives for adult participants of the study will be evaluated with AEs, SAEs, AESIs and MAAEs as detailed in Section 5.6.1 for definitions and analyses methods of adverse events.

Refer to Section 5.7.1 for definitions and analyses methods of immunogenicity endpoints.

Analyses will also be performed by subgroup: gestational age group, participant age group at baseline, and the SARS-CoV-2 serostatus at baseline. Refer to Section 5.7.5 for subgroup definitions.

5.4. Secondary Endpoints Analysis

5.4.1. Definition of Endpoints

Safety and reactogenicity of Ad26.COV2.S booster vaccination in adult participants

- Solicited local and systemic AEs for 7 days after (each) vaccination, or until resolution
- Unsolicited AEs for 28 days after (each) vaccination
- SAEs and AESI throughout the study (from vaccination until end of the study, i.e. at least 12 months after delivery)
- MAAEs until 6 months after the last vaccination

- AEs leading to study discontinuation (from vaccination until end of the study)

Refer to Sections 5.6.1 for more details on adverse events definitions.

Pregnancy Outcomes

Pregnancy outcomes in adult participants (including live term birth, live preterm birth, stillbirth, and abortion). Data are captured in the Delivery History eCRF.

Pregnancy-related AEs

Pregnancy related AEs in adult participants, including gestational diabetes, gestational hypertension, premature rupture of membranes, premature labor, premature uterine contractions, poor or restricted fetal growth, pre-eclampsia, eclampsia, vaginal or intrauterine hemorrhage (non-exhaustive).

The complete list of preferred terms to be included as pregnancy related AEs is provided in Section 6.8. Refer to Sections 5.6.1 for more details on adverse events definitions.

Safety in neonates/infants

- SAEs (including Multisystem Inflammatory Syndrome in Children [MIS-C]) and AESIs in neonates/infants from birth to approximately 12 months of age.
- MAAEs for neonates/infants from birth until 6 months of age.
- AEs in neonates/infants leading to study discontinuation from birth until discontinuation.

Refer to Sections 5.6.1 for more details on adverse events definitions and Section 6.9 for MIS-C.

Neonate/infant Outcomes

Neonate/infant outcomes (including normal neonate, term neonate with (or without) complications, preterm neonate with (or without) complications, neonatal infection, respiratory distress, congenital anomalies, neonatal death, low birth weight, and small for gestational age measured from birth until approximately 12 months of age) (non-exhaustive). Data are captured in the Neonatal Medical History eCRF.

Humoral Immune Response in peripheral blood of adult participants

- Serological response to vaccination as measured by ELISA (S-ELISA; EU/mL]) at all blood collection timepoints.
- Serological response to vaccination as measured by VNA titers, 28 days after the first vaccination.
- Serological response to vaccination measured by binding (S-ELISA and/or equivalent assay) and neutralizing (VNA) antibody titers pre-boost and at selected time points post-booster vaccination.

Antibody levels against SARS-CoV-2 in neonates/infants

- Serological response to vaccination as measured by ELISA (S-ELISA, EU/mL) at birth (i.e., in cord blood) and at approximately 2 months, and 6 months of age.
- Serological response to vaccination as measured by VNA titers at birth (i.e., in cord blood).

5.4.2. Estimand(s)

Not applicable.

5.4.3. Analysis Methods

Safety and reactogenicity of Ad26.COV2.S (incl Pregnancy-related AEs)

The secondary safety and reactogenicity endpoints for adult participants and neonates/infants (separately) of the study will be evaluated with AEs, SAEs, AESIs and MAAEs as detailed in Section 5.6.1 for definitions and analyses methods of adverse events.

Key analyses will also be performed by subgroup: gestational age group, participant age group at baseline, and the SARS-CoV-2 serostatus at baseline. Refer to Section 5.7.5 for subgroup definitions.

Refer to Section 6.8 for Pregnancy-related AEs.

Outcomes

The frequency and percentage of participants (adult participants, and neonates/infants, separately) for whom at least one of the indicated outcomes was reported will be reported. For each separate maternal/fetal and neonatal/infant outcome, the number and percentage of participants who experience at least 1 occurrence of the given outcome will also be tabulated. Denominator for the percentages is the number of participants in the considered population and phase (incidence per 100 participants/phase).

Outcomes will be presented by phase (Post-dose 1, Post-booster, Postpartum Follow-up 1, etc., as applicable), as well as over the entire study and follow-up periods. Outcomes may also be presented by subgroup: gestational age group, participant age at screening, and SARS-CoV-2 serostatus at baseline.

Denominator for the percentages is the number of participants in the considered population and phase (incidence per 100 participants/phase).

Humoral Immune Response in peripheral blood of adult participants

Refer to Section 5.7.1 for definitions and analyses methods of immunogenicity endpoints.

Antibody levels against SARS-CoV-2 in neonates/infants

Refer to Section 5.7.1 for definitions and analyses methods of immunogenicity endpoints.

5.5. Exploratory Endpoints Analysis

5.5.1. Definition of Endpoints

Humoral Immune Response in peripheral blood of adult participants

- Serological response to vaccination as measured by VNA titers at all blood collection timepoints

Antibody levels against SARS-CoV-2 in neonates/infants

- Serological response to vaccination as measured by VNA titers at birth (i.e., in cord blood) and at approximately 2 months, and 6 months of age

Correlation between binding antibody (ELISA) titers and neutralizing antibody (VNA) titers to SARS-CoV-2 of adult participants

- Correlation between ELISA (S-ELISA; EU/mL), or equivalent assay and VNA titers at selected timepoints

Humoral Immune Response in adult participants at the time of delivery

- Serological response to vaccination as measured by ELISA (S-ELISA; EU/mL) and/or equivalent assay using serum samples obtained from peripheral blood and cord blood at the time of delivery

Cellular immune response in peripheral blood of adults in a subset of participants

- Th1 and Th2 immune responses as assessed by:
    - Flow cytometry after SARS-CoV-2 S protein peptide stimulation of peripheral blood mononuclear cells (PBMC) and intracellular cytokine staining (ICS) including cluster of differentiation (CD) markers: CD4+/CD8+, tumor necrosis alpha (TNFα), interferon gamma (IFNγ), interleukin (IL)-2, IL-4, IL-5, IL-13, and/or other T helper cell subset (Th1/Th2) markers. OR
    - Dual or single IFNγ and IL-4 enzyme-lined immunospot (ELISpot) assay after stimulation of PBMCs with SARS-CoV-2 protein peptides

Impact of pre-existing humoral immunity against coronavirus other than SARS-CoV-2 at baseline on Ad26.COV2.S vaccine immunogenicity in adult participants

- Analysis of antibodies binding to coronaviruses other than SARS-CoV-2, or other respiratory viruses by ELISA or equivalent assay

Presence of immunoglobulins against SARS-CoV-2 in colostrum and breast milk in adult participants

- IgA and/or other Ig types against SARS-CoV-2 and/or emerging variants in colostrum and breast milk measured by ELISA or equivalent assay

Humoral immune responses in peripheral blood and cord blood of adult participants induced by Ad26.COV2.S as a 1-dose schedule, and before and after booster vaccination at all or selected blood collection time points

- SARS-CoV-2 neutralization as assessed by SARS-CoV-2 neutralization assays VNA
- Adenovirus neutralization as measured by VNA
- Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype, antibody glycosylation and assessment of antibody repertoire

- Analysis of antibodies to the spike (S), nucleocapsid (N), and receptor binding domain (RBD) of the SARS-CoV-2 S protein, and surface proteins of other coronaviruses
- Epitope-specificity characterization of antibodies
- 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
- Analysis of binding and/or neutralizing antibodies against emerging SARS-CoV-2 variants

Cellular immune responses in peripheral blood of adult participants before and after booster vaccination at selected blood collection time points including after booster vaccination, if feasible

Exploratory analyses may include the following for a subset of participants, if feasible:

- Analysis of gene expression in cells stimulated with SARS-CoV-2 S protein peptides, or in unstimulated cells (ex vivo)
- Analysis of messenger ribonucleic acid (mRNA) expression levels of vaccine-induced biomarkers of immune mediated responses
- Cytokine profiling: Analysis of cytokines, chemokines, and other proteins of the immune response in cells stimulated with SARS-CoV-2 S protein peptides, or in unstimulated cells (ex vivo)
- Epitope-specificity characterization for B and T-cells
- Analysis of the phenotype of antigen-specific T and B cells, assessed by single cell analysis

Humoral immune responses in neonates/infants

- SARS-CoV-2 neutralization as assessed by SARS-CoV-2 neutralization assays VNA
- Adenovirus neutralization as measured by VNA
- Functional and molecular antibody characterization including Fc-mediated viral clearance, avidity, Fc characteristics, immunoglobulin (Ig) subclass and IgG isotype, antibody glycosylation, and assessment of antibody repertoire
- Analysis of antibodies to the spike (S), nucleocapsid (N), and receptor binding domain (RBD) of the SARS-CoV-2 S protein, and surface proteins of other coronaviruses
- Epitope-specificity characterization of antibodies
- 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
- Analysis of binding and/or neutralizing antibodies against emerging SARS-CoV-2 variants

Occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms in adult participants

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

Assessment of SARS-CoV-2 viral load during a confirmed COVID-19 episode in adult participants

- Analysis of SARS-CoV-2 viral load (via qRT-PCR) in nasal swabs collected during a confirmed COVID-19 episode

Changes in the SARS-CoV-2 genome in adult participants

- Identification of SARS-CoV-2 variants by sequencing of nasal swabs samples (as available)

Occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms in neonates/infants

- The number of neonates/infants with molecularly confirmed COVID-19
- Presence and severity of COVID-19 signs and symptoms in neonates/infants as measured by the Pediatric Symptoms of Infection with Coronavirus-19 (PedSIC)

Assessment of SARS-CoV-2 viral load during a confirmed COVID-19 episode in neonates/infants

- Analysis of SARS-CoV-2 viral load (via qRT-PCR) in nasal swabs and/or saliva samples (as available) collected during a confirmed COVID-19 episode

Occurrence of asymptomatic infections with SARS-CoV-2 in adult participants

- The number of adult participants with positive non-S ELISA and/or SARS-CoV-2 immunoglobulin assay that is dependent on the SARS-CoV-2 nucleocapsid (N) protein
- Asymptomatic infection detected by RT-PCR

Occurrence of asymptomatic SARS-CoV-2 infection in neonates/infants

- The number of neonates/infants with positive non-S protein ELISA and/or SARS-CoV-2 immunoglobulin assay (eg, N-protein ELISA)
- Asymptomatic infection detected by RT-PCR

Impact of Ad26.COV2.S vaccine on the incidence of co-infections with SARS-CoV-2 and other respiratory pathogens in adult participants

- Analysis of broad respiratory pathogens panel in the nasal swabs collected during a confirmed COVID-19 episode and in a subset of nasal swab samples from adult participants with a symptomatic infection, up to delivery

Impact of Ad26.COV2.S vaccine on the incidence of co-infections with SARS-CoV-2 and other respiratory pathogens in neonates/infants

- Analysis of broad respiratory pathogens panel in the nasal swabs collected during a confirmed COVID-19 episode and in a subset of nasal swab samples from neonates/infants born to participants who have received Ad26.COV2.S during the study period

Immune response in vaccinated adult participants after natural SARS-CoV-2 infection and other potentially informative biomarkers (e.g., 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
- SARS-CoV-2 binding antibodies measured by ELISA: analysis of antibodies binding to the SARS-CoV-2 S and/or N-protein
- Analysis of gene expression by RNA transcript profiling

Immune response in neonates/infants born to participants who have received Ad26.COV2.S during pregnancy after natural SARS-CoV-2 infection

- Confirmation of SARS-CoV-2 infection by molecular testing
- SARS-CoV-2 neutralizing titers in serum measured by a VNA
- SARS-CoV-2-binding antibodies measured by ELISA: analysis of antibodies binding to the SARS-CoV-2 S and/or N-protein

Further exploration of the impact of vaccination with Ad26.COV2.S on coagulation-related parameters in adult participants

- Hematology assessments of complete blood count with differential, including platelet counts (performed locally at the site)
- Analysis of levels of pro- and anti-coagulation factors in plasma/serum including but not limited to: activated partial thromboplastin time, prothrombin time, fibrinogen, D-dimer levels, Lupus anticoagulant, ant-cardiolipin antibody levels, b-2 glycoprotein, anti-PF4 antibody levels (performed retrospectively at central laboratories on stored samples)

Neurodevelopmental status in neonates/infants

- Summary of developmental outcomes using the Ages & Stages Questionnaire (ASQ3) at 2, 6 and 12 months of age

Changes in the SARS-CoV-2 genome in neonates/infants

- Identification of SARS-CoV-2 variants by sequencing of nasal swabs samples (as available)

5.5.2. Estimands

Not applicable.

5.5.3. Analysis Methods

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

Refer to Section 5.7.2.

Further exploration of the impact of vaccination with Ad26.COV2.S on coagulation-related parameters in adult participants.

Refer to Section 5.6.2

Neurodevelopmental status in neonates/infants

The frequency and percentage of neonates/infants who score in the "white", "gray" or "black" zones of each of the 5 developmental areas measured in the ASQ3 as well as whether pediatrician contact was advised (as recorded in the eCRF) will be summarized. A listing of ASQ3 will be provided.

All other exploratory endpoints

Refer to Section 5.7.

5.6. Other Safety Analyses

5.6.1. Adverse Events

5.6.1.1. Definitions

Solicited AEs are used to assess the reactogenicity of the study vaccine and are predefined as local events (pain/tenderness, erythema, and swelling at the injection site) and systemic events (fatigue, headache, nausea, and myalgia) 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.

MAAEs are defined as AEs with medically-attended visits including hospital, emergency room, urgent care clinic, or other visits to or from medical personnel for any reason. Routine study visits will not be considered medically-attended visits. New onset of chronic diseases will be collected as part of the MAAEs.

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 Section 6.6.

Suspected AESIs (thrombotic events and thrombocytopenia [defined as platelet count below 150,000/µL, see Brighton Collaboration Interim Case Definition, 2021] as details in Section 6.7) will be recorded from the moment of vaccination until the end of the study/early withdrawal. An AESI Adjudication Committee with appropriate expertise will be established to evaluate each suspected AESI (for more details see Section 1.3.6 and Section 8.3.5.1 of the Protocol).

5.6.1.2. Severity Criteria

All AEs and laboratory data will be coded for severity using a modified version of the FDA grading table, based on the version of September 2007 (US DHHS FDA CBER 2007), included in Section 6.6.

For AEs not identified in the grading table, the guidelines in the clinical protocol will be applied.

The severity of solicited signs and symptoms will be graded in the reactogenicity diary by the participant based on the severity assessment provided in the reactogenicity diary and then verified by the investigator using the Toxicity Grading Scale. Severity of the measured events will be

derived from the diameter [for erythema and swelling] and the temperature measurements [for fever].

5.6.1.3. Analysis of Adverse Events

Solicited AEs (shown in output) are extracted from the investigator assessment pages of the CRF and will be recorded in the Clinical Event SDTM domain. Only Solicited AEs with onset within 7 days after vaccination (post-dose 1, post-booster [booster in Group 4], and post-dose 1+ post-booster) will be reported in tables. For unsolicited AEs, only the AEs within the 28-day period following (primary or booster) vaccination will be presented in the safety tables except for SAEs, AEs leading to treatment/study discontinuation (incl. MAAE leading to treatment/study discontinuation) and AESIs which will be captured and tabulated in the outputs covering the whole study period (i.e. up to 12 months postpartum), and MAAEs which will be captured and tabulated for all participants from the first vaccination until 6 months after (each) vaccination.

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 (>5%) 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, AESI, MAAEs, AEs leading to treatment/study discontinuation, fatal outcome, and discontinuation), all events, most frequent (>5%), at least grade 3, related, and SAEs. The proportion of AEs resulting in a medically attended visit (other than routine health maintenance visits) will also be tabulated.

Pregnancy-related AEs in adult participants (including gestational diabetes, gestational hypertension, premature rupture of membranes, premature labor, premature uterine contractions, poor or restricted fetal growth, pre-eclampsia, eclampsia, vaginal or intrauterine hemorrhage [non-exhaustive]) will be tabulated separately. The complete list of preferred terms to be included as pregnancy related AEs is provided in Section 6.8.

For neonates/infants SAEs (including MIS-C), AESIs from birth until end of study (i.e., up to approximately 12 months of age), MAAEs from birth until 6 months of age (or from birth until discontinuation if the MAAE is resulting in study discontinuation) and AEs leading to study discontinuation covering the whole study period will be tabulated separately.

Listings and/or participant narratives will be provided as appropriate, for those adult participants and neonates/infants who die, discontinue study vaccinations due to an AE, or experience an SAE. All collected solicited and unsolicited adverse events will be listed.

For all participants in the FAS-A, temperature will be summarized using the maximum of the recorded temperature per participant over the time interval up to Day 8 post primary vaccination and post-booster vaccination, where applicable. The incidences of fever will be summarized using the highest grade of fever observed for each participant using the grading system in Section 6.6.

All symptoms will be summarized taking the worst grading as captured by the investigator at Day 8 and Day 29 post vaccination and post-booster vaccination, where applicable. The number of women reporting breast milk production (reduction) from the eCOA diary data as well as the related AE will be summarized descriptively.

5.6.1.4. 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 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.

5.6.1.5. Missing Data

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

5.6.2. Laboratory, Vital Signs, and Physical Examinations

Hematology, Chemistry and Urinalysis tests will be performed locally within 72 hours of blood sample collection, according to the Schedules of Activities (CTP 1.3), at Screening (optional, at the discretion of the investigator), at Day 1 (pre-dose): Day 8, Day 15, Day 29 and at delivery (postpartum 1) and postpartum 43 (42 days after delivery). For further details on all laboratory assessments refer to CTP 8.2.3.

Continuous laboratory variables will be summarized using the following statistics, as appropriate: number of observations, arithmetic mean (mean), standard deviation (SD), standard error (SE), median, minimum, and maximum. Frequencies and percentages (one decimal place) will be generated for categorical laboratory variables. Denominator for the percentages is the number of participants in the considered population and phase for a certain regimen (incidence per 100 participants/phase).

For all adult participants, weight and height (and BMI) at baseline will be summarized using descriptive statistics. Physical exam will be summarized and listed for adults. Neonatal physical and birth exam will be summarized and listed for neonates.

Vital signs for adults and neonate/infants including temperature, pulse/heart rate, respiratory rate, blood pressure (adult participants only: systolic and diastolic) and blood oxygen saturation will be summarized over time, using descriptive statistics. Abnormalities emerging after vaccination will be tabulated by worst abnormality grade using the FDA table in Section 6.6.

Temperature will be measured at each scheduled time point and summarized using descriptive statistics. A listing of participants with fever will be provided for adults and neonates. Other vital signs abnormalities will be listed with corresponding grade.

For adult COVID-19 cases, temperature will be summarized over time for the duration of follow-up of COVID-19 episodes, using descriptive statistics.

All parent(s)/caregiver(s) of neonates/infants with (suspected) COVID-19 should measure blood oxygen saturation and body temperature.

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: e.g., <3.45 is imputed with 3.44, <90 with 89 and >101 with 102).

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), results falling in these zones will be allocated to the adjacent worst-case grade. For example if a laboratory result falls within the 'grey area' between the ranges for 'Grade 1' and 'Grade 2' in the FDA table, then this result would be allocated to 'Grade 2' (the adjacent worst-case grade).
- If a value falls within the grading as specified in the grading FDA table but using the local laboratory it falls within normal limits, the value is considered as normal. For example, if the local lab classifies a result as ‘normal’ within their prespecified limits, then the value can indeed be considered normal even when according to the FDA table it would be ‘Grade 1’.

For the grades, no distinction will be made between test results of samples obtained under fasting and under non-fasting conditions.

The following physical examinations will be performed: full and targeted examination, obstetric examination, obstetric ultrasound, targeted physical examination, neonatal birth examination (physical and Apgar score) and infant examination. Physical examination findings will be summarized at baseline (adult participants) and at each timepoint for neonates/infants. A listing of the abnormalities will be made. Apgar score findings will be summarized and listed separately.

5.6.3. Other Obstetric Assessments

Obstetric medical history findings at baseline will be summarized and listed.

Delivery history findings at birth will be summarized and listed.

For further details on both assessments refer to CTP 8.2.4.

5.6.4. Other Neonatal Assessments

Neonatal medical history findings at birth will be summarized and listed.

Neurodevelopmental status will be measured using the Ages & Stages Questionnaire, 3rd edition (ASQ-3; Schonhaut 2013). The ASQ-3 includes a series of questions designed to assess 5 areas of development (communication, gross motor, fine motor, problem solving and personal-social). Individual development scores are then classified into “white”, “gray” or “black” zones using pre-defined cut-off values (with “white” the highest score and “black” the lowest score). Findings as captured in the eCRF (scores classified by color-codes and if pediatrician contact was advised) will be summarized by timepoint and listed.

For further details on both assessments refer to CTP 8.2.5.

5.7. Other Analyses

5.7.1. Immunogenicity

The PPI-A set will be used for analysis of immunogenicity for adult participants and the PPI-NVN set will be used for neonates/infant.

Selected immunogenicity analyses will also be done on the FAS-A and FAS-NVN. Participants who become infected during the study or receive another COVID-19 vaccine outside of the study will be analyzed as subgroups and identified in graphs using specific colors and symbols. Data will be analyzed separately for adult participants and for neonates/infants.

Key immunogenicity assay data from other studies may be used in selected graphs and tables, for descriptive comparison of immunogenicity results in pregnant women from COV2004 vs non-pregnant women in other Janssen Ad26.COV2.S studies. Reference to the respective study will be added and clearly indicated in those tables and graphs.

Key immunogenicity assay results (S-ELISA, VNA) will also be analyzed for the subgroups defined in Section 5.7.4.

Data will be presented by scheduled time point. For inclusion of immunogenicity samples taken outside of the allowed windows, see section 5.1.3. For the FAS-A and FAS-NVN analyses, samples taken outside of the allowed window will be included.

Categorical variables will be summarized with a frequency table showing counts and percentages. Continuous variables will be summarized using the following statistics, as appropriate: number of observations, geometric mean, 95% confidence interval (CI) for geometric mean, arithmetic mean (mean), 95% CI for the mean, standard deviation (SD), standard error (SE), median, quartiles (Q1 andQ3), minimum and maximum.

Data listings, participant profiles and/or participant narratives may be provided as appropriate. Binary variables will be summarized using the following statistics: number of observations, percentages, and Exact Clopper-Pearson 95% CIs.

5.7.1.1. Parameters

For adults, the assays planned include (but are not limited to): S-ELISA, psVNA, wtVNA (if feasible), Ad26 VNA (if feasible), MesoScaleDiscovery (MSD) 4-plex, ADCP (if feasible) and ICS (if feasible). For neonates/infants, the assays planned include (but are not limited to): S-ELISA, psVNA, and MSD 4-plex. Others, wtVNA (if feasible), Ad26 VNA (if feasible), and ADCP (if feasible) may also be performed in cord blood. However, not all assays might be available for all immunogenicity analyses covered by this SAP. Some of the exploratory immunogenicity endpoints mentioned in this section will only be considered as potential assessment for analyses after the primary analysis is performed, as described in Section 1.1. The humoral and cellular immune responses described in Table 5 may be measured.

Table 5: Summary of Humoral and Cellular Immunogenicity Assays

Summary of Humoral Immunogenicity Assays	
Assay	**Purpose**
Primary/Secondary endpoints	
SARS-CoV-2 binding antibodies (ELISA)	Analysis of antibodies binding to the SARS-CoV-2 S protein
SARS-CoV-2 neutralization (VNA)	Analysis of neutralizing antibodies to the wild-type and/or pseudovirion expressing S protein
Exploratory endpoints	
SARS-CoV-2 binding antibodies (ELISA and/or SARS-CoV-2 immunoglobulin assay)	Analysis of antibodies binding to the SARS-CoV-2 Spike (S) protein, nucleocapsid (N) protein, receptor binding domain (RBD) of the SARS-CoV-2 S protein, or other proteins, including surface proteins of other coronaviruses
SARS-CoV-2 neutralization (VNA)	Analysis of neutralizing antibodies to the wild-type virus, viral variants, and/or pseudovirion expressing S protein
ELISA or Ig assay detecting coronavirus-specific antibodies Antibodies specific to coronaviruses or other respiratory viruses (MSD)	Analysis of antibodies binding to coronaviruses other than SARS-CoV-2, or other respiratory viruses

Summary of Humoral Immunogenicity Assays

Assay	Purpose
SARS-CoV-2 binding immunoglobulins, including IgA antibodies (ELISA)	Analysis of IgA and/or other Ig subtypes against SARS-CoV-2 in breast milk
SARS-CoV-2 binding immunoglobulins, including IgA antibodies (ELISA)	Analysis of IgA and/or other Ig subtypes against SARS-CoV-2 in colostrum
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, antibody glycosylation, and assessment of antibody repertoire.
Epitope-specificity characterization	Analysis of site-specificity, epitope mapping
Cytokine profiling, metabolomics and/or lipidomics	Analysis of cytokines, chemokines, and other proteins, metabolites or lipid mediators 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
Transcriptional analysis of vaccine-induced Biomarkers	Analysis of mRNA expression levels of vaccine-induced biomarkers of immune mediated responses.
Transcriptional analysis of vaccine-induced innate immunity and inflammatory responses	Analysis of mRNA expression levels of vaccine-induced innate responses, including inflammatory mediators.

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

Summary of Cellular Immunogenicity Assays

Assay	Purpose
Exploratory 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
ELISpot	IFNγ and IL-4 responses to SARS-CoV-2 S protein peptides by PBMCs, based on single or dual 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

CD = cluster of differentiation; 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; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; Th = T helper; TNFα = tumor necrosis factor alpha; VNA = virus neutralization assay.

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 changes 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:
  - Values>ULOQ are imputed with ULOQ.
- Calculation of fold changes from baseline:
  - 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 change from baseline and fold change from pre-booster (for time points after booster vaccination). In some cases, fold change from other timepoints may also be calculated. Geometric mean ratio and CI will be calculated at each post baseline visit.

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 (in adults): 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 without individual participant dots will also be generated. Participant profiles of the actual values over time will be graphically presented.

For infants born to vaccinated mothers, tables and graphs will be made per the vaccine regimen of the mother. In addition, in the neonate profiles of the actual values over time, the mother's value at birth will be added to visualize transfer of binding or neutralizing antibodies to the newborn.

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 the exploratory **variant SARS-CoV-2 neutralization assays** (different from the VNA used for the secondary endpoint), the same as above applies.

For **S-ELISA** and **MSD 4-PLEX**, the same as above applies.

The ratio of binding antibodies (S-ELISA measured by S-ELISA or MSD 4-PLEX assay) to wild type VNA, the ratio of binding antibodies (S-ELISA measured by S-ELISA or MSD 4-PLEX assay) to pseudovirion expressing S protein VNA, and the ratio of binding antibodies (S-ELISA measured by S-ELISA or MSD 4-PLEX assay) to the VNAs measured by alternative assays 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 change from baseline in binding antibodies (S-ELISA measured by S-ELISA or MSD 4-PLEX assay) to the fold change from baseline in VNA, will be calculated for each post-baseline time point. Values <LLOQ will be imputed with LLOQ for the calculation of the fold change 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 (as available) will be provided for selected time points. These may be included but are not limited to:

- Binding antibodies (S-ELISA measured by S-ELISA or MSD 4-PLEX assay) versus VNA (wild type VNA, psVNA or variant VNA)
- wtVNA versus psVNA
- wtVNA versus variant VNA
- psVNA versus variant VNA
- Ad26 VNA versus S-ELISA
- Ad26 versus wtVNA
- Ad26 versus psVNA and/or variant 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).

5.7.1.5.2. 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 a dual ELISpot, measuring IFN-gamma 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 condition is satisfied:

For IFN-g:

- The baseline sample value is less than or equal to the LOD (≤LOD) and the post-baseline sample value 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 value 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 value 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 changes from baseline.

ELISpot values available in the database will already be background subtracted. No further background subtraction should be carried out.

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 will be provided in the DPS.

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 sample (if available). Additional statistics may be calculated and will be detailed in the DPS.

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

For CD4+:

- IFN-g or IL2
- IFN-g or IL2 NOT TH2
- IL4 and CD40L
- IL4 or IL5 or IL13 and CD40L

For 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.

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 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 will be provided in the DPS.

5.7.1.6. Immunogenicity Against the Vector

If available, for immunogenicity against the Ad26 vector backbone (Adenovirus neutralization by neutralization assay), the 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.

Immunogenicity against the vector may also be assessed in cord blood and in newborns to assess the transfer of Ad26 neutralizing antibodies to newborns.

Correlation plots with the Ad26 assays versus the assays against the inserts will be provided for the most important time points.

5.7.1.7. Biomarkers

For all adult participants, biomarker analysis (PAXgene®, RNA-seq) will be performed to explore potentially informative biomarkers related to vaccine immunogenicity and SARS-CoV-2 infection (including relations with COVID-19 severity).

For adult participants with a positive test result for SARS-CoV-2 infection, biomarker analysis (PAXgene®, RNA-seq) will be performed for evaluation of COVID-19 cases and to explore potentially informative biomarkers, correlating with SARS-CoV-2 infection and COVID-19 severity, at Day 3 to 5 and at Day 29 (±7 days) after onset of symptoms.

5.7.2. Occurrence of Symptomatic Molecularly Confirmed and Asymptomatic COVID19

An immunologic test for SARS-CoV-2 seroconversion (non-S ELISA and/or SARS-CoV-2 immunoglobulin assay) based on SARS-CoV-2 N-protein, will be performed in adult participants to identify cases of asymptomatic infection. The presence of SARS-CoV-2 infection will be assessed at the study site by molecular testing RT-PCR or molecular test result from any available respiratory tract sample. Asymptomatic infections also include those detected by confirmed molecular tests (either in this study or outside study), in the absence of the relevant symptoms.

Procedures to be performed in the event a participant (adult or infant/neonate) experiences signs or symptoms suggesting possible COVID-19 are detailed in CTP Sections 1.3 and 8.1.2. A sign or symptom is considered as absent or present for a COVID-19 episode if observed in the eCOA or eCRF for the COVID-19 episode, and absent if not.

COVID-19 episodes will not be adjudicated. All data presented corresponding to the COVID-19 episode will be based on the SIC (or PedSIC) as detailed in Section 5.7.3.

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

The number and percentage of participants with at least one molecularly confirmed symptomatic or asymptomatic SARS-CoV-2 infection will be tabulated by post vaccination visit and for the entire study period. Similar table will be produced for infants.

The number and percentage of participants who are positive by N serology at different time points will be tabulated.

5.7.3. COVID-19 signs and symptoms

5.7.3.1. Symptoms of Infection with Coronavirus-19 (SIC)

The SIC is a disease-specific patient-reported outcome (PRO) instrument that is completed by the participant. The SIC has a total of 30 items assessing signs and symptoms of COVID-19. The first 25 items, the participant indicates “yes” or “no” if they have a symptom and if “yes” report a severity from 0 (none) to 10 (worst possible). The second part has the participant enter their temperature, and the third part has the participant record “yes” or “no” (absence or presence of additional signs and symptoms), where severity cannot be assessed). The analyses are conducted in two ways, by part 1, part 2 and part 3, scored separately, and also grouped into related categories for composite scoring.

5.7.3.2. Analysis of Symptoms of Infection with Coronavirus-19 (SIC)

The number and percentage of participants with at least one SARS-CoV-2 infection will be tabulated by vaccination schedule.

In the group of participants seronegative at baseline, the number and percentage of participants with at least one positive N-ELISA, if available, will be tabulated.

For each participant with 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:

- A COVID-19 infection "episode" is defined as a period starting from the first day on which any symptoms is reported until the last day any symptom is reported, within an interval stating up to 5 days prior to a positive PCR test and finishing up and to 42 days after it
- 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 maximum severity score. If the PRO was completed due to any other reason, no imputations will be done.

Duration of the episode will be calculated as (the end date of the last symptom in that episode – the start date of the first symptom in that episode) + 1. Duration of each symptom will be calculated as last date of symptom reporting – its first date + 1.

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

- For the first infection episodes, the following statistics will be calculated: number of subject with an episode, the 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 an infection 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 min, max, q1 and q3) of highest

severity of each symptom, median duration of the highest severity of each symptom (with min, max, q1 and q3).

In addition, participant listings will be provided containing the SIC information for each time point. Details about these analyses will be provided in the DPS.

5.7.4. Demographics and Baseline Characteristics

Demographic and baseline variables will be summarized by vaccination schedule and overall for the FAS. In addition, the distribution of participants by country and site ID will be presented unless otherwise noted.

SARS-CoV-2 Serostatus at baseline will be derived from S- and/or N-serology at baseline, and the definition will depend on the participant's previous vaccination history.

Details are provided in Section 6.3.

5.7.5. Definition of Subgroups

For key safety and immunogenicity analyses (S-ELISA, MSD 4-plex and VNA), results will be analyzed by gestational age group at screening and by SARS-CoV-2 serostatus at baseline. Results will be presented by previous vaccination group like other reports.

Subgroups are defined as shown in Table 6 while the derivation of the SARS-CoV-2 Serostatus at baseline is described in Table 7.

Subgroup analysis will ignore unknown, missing and not reported values, unless otherwise specified.

Table 6: Definition of Subgroups

Subgroup (at baseline/screening)	**Definition**
Age Group	• 18-34 years (≥18y to <35y) • 35-45 years (≥35y to ≤45y)
Gestational Age Group	• 16-27 weeks (≥16w to <28w) • 28-38 weeks (≥28y to ≤38w)
BMI	• underweight <18.5 kg/m^2 • normal 18.5 - <25 kg/m^2 • overweight and obese >=25 kg/m^2
Race	• Asian • Black or African American • White • Other (‘Multiple[a] or ’Native Hawaiian or other Pacific Islander’ or ‘American Indian or Alaska Native’)
Ethnicity	• Hispanic or Latino • Not Hispanic or Latino
SARS-CoV-2 Serostatus	• Negative • Not Negative (excluding missing)

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

Table 7: Definition of SARS CoV 2 Serostatus at Baseline

Vaccination history	SARS-CoV-2 Serostatus at baseline (by N and/or S serology for vaccine naïve, and by N-serology for previously vaccinated individuals) to assess previous infections	Serostatus result at Baseline
Vaccine naïve (Group 4)	If positive in either Roche Elecsys (N serology) or S-Elisa, or both	Positive
	If negative in both	Negative
	If both are missing	Missing
Other vaccines (and no inactive vaccine) based on S protein (Moderna, Pfizer, AstraZeneca, Janssen, Sputnik, Novavax)	If positive by Roche Elecsys (N serology) only (regardless of the S-ELISA result)	Positive
	If negative by Roche Elecsys (N serology) only (regardless of the S-ELISA result)	Negative
	If Roche Elecsys (N serology) is missing (S-ELISA is not used here)	Missing
Others; depends on vaccine		
Inactivated vaccines (e.g. Sinovac, Sinopharm) or combination of vaccines including at least one inactivated vaccine	Since participant is previously vaccinated with an inactivated vaccine, it is not possible to determine serostatus at baseline	Not evaluable

For infants, immunogenicity analyses will be conducted based on the subgroup of the mother at baseline/screening (gestational age group, age, BMI, race, SARS-CoV-2 serostatus).

5.7.6. Other analysis

In patients receiving a booster in Group 4, the number of days between the booster and first vaccination will be descriptively summarized.

5.8. Planned Analyses

Unplanned interim analyses may be performed if requested by health authorities.

5.8.1. Independent Data Monitoring Committee (IDMC)

An IDMC is established to monitor data on an ongoing basis to ensure the continuing safety of the participants enrolled in this study. The IDMC reviews safety and reactogenicity data as indicated below.

Prior to this amended SAP version, the IDMC met and reviewed:

- all available safety and reactogenicity data in the Sentinel (n=5) Cohort once they had received the first dose of Ad26.COV2.S at $5x10^{10}$ vp and completed the Day 8 visit
- all available safety and reactogenicity data once the 22 participants in the Sentinel (n=5) and Safety Cohort (n=17) once they had received the first dose of Ad26.COV2.S at $5x10^{10}$ vp and completed the Day 8 visit

The data review included the following: participant disposition; demographics and baseline characteristics; unsolicited AEs, SAEs, AESI, and solicited AEs (local and systemic). The summaries of these data were prepared according to the corresponding sections in this SAP and as further outlined in the DPS.

Based on their review, the IDMC confirmed that the safety profile of 1 dose of Ad26.COV2.S at 5×10^{10} vp was considered acceptable and no safety concerns were identified. The remaining participants (who have received their last COVID-19 vaccination at least 4 months prior to receiving the study vaccine or are vaccine naïve participants) could be enrolled to receive 1 dose of Ad26.COV2.S at 5×10^{10} vp.

The IDMC also conducts routine safety reviews of data based on descriptive safety tables and listings from all accumulated safety data at that point in time using similar summaries as prepared for the Sentinel and Safety Cohort. After each review, the IDMC makes recommendations regarding the continuation of the study.

The IDMC responsibilities, authorities, and procedures are provided in its charter. For more details see CTP Section 9.6.

5.8.2. Primary Analysis

The primary analysis of safety and immunogenicity will be performed when all adult participants have completed the visit that takes place approximately 42 days postpartum in all groups or if adult participants are discontinued earlier. The analysis will include safety (e.g. AEs, SAEs, and pregnancy outcomes) and available immunogenicity data (e.g. S-ELISA and MSD) for all adult participants and neonates/infants and in cord blood at the time of delivery. The primary analysis will include all data up to and including the Day 42 postpartum follow-up visit. The results of this analysis may be used for regulatory submissions.

5.8.3. Final Analysis

The final analysis will be performed when all adult participants and all neonates/infants have completed the final visit per the Schedules of Activities.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 List of Abbreviations

ADaM	Analysis Data Model
AE	adverse event
AESI	Adverse Event of Special Interest
ALT/SGPT	alanine aminotransferase
ASQ	Ages & Stages Questionnaire
ATC	anatomic and therapeutic class
BMI	body mass index
CD	cluster of differentiation
CDC	Center for Disease Control
CI	confidence interval
CL	total systemic clearance
CTP	Clinical trial protocol
CRF	case report form
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
DMC	Data Monitoring Committee
DPS	Data Presentation Specifications
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot assay
FAS-A	Full Analysis Set-Adults
FAS-NVN	Full Analysis Set-Nonvaccinated Neonates
FDA	Food and Drug Administration
GMC	Geometric mean concentration
GMT	Geometric mean titer
ICH	International Conference on Harmonization
ICS	intracellular cytokine staining
IDMC	Interim Data Monitoring Committee
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
IQ	interquartile
LOD	limit of detection
LLOQ	lower limit of quantification
MAAE	Medically-attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
MIS-C	Multisystem Inflammatory Syndrome in Children
MRD	minimum required dilution
mRNA	messenger ribonucleic acid
MSD	Meso Scale Discovery
NAb	neutralizing antibodies
VNA	virus neutralization assay
PBMC	peripheral blood mononuclear cells
PD	pharmacodynamic(s)
PI	principal investigator
PK	pharmacokinetic(s)
PP	postpartum
PPI-A	Per Protocol Immunogenicity- Adults
PPI-NVN	Pper Protocol Immunogenicity- Neonates
PRO	Patient-reported outcome
qRT-PCR	quantitative Real-time polymerase chain reaction
RBD	receptor binding domain
RNA	ribonucleic acid

RT-PCR	Real-time polymerase chain reaction
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus-2
SAP	Statistical Analysis Plan
SD	standard deviation
SE	Standard Error
SIC	Symptoms of Infection
SMQs	standardised MedDRA queries
SDTM	Study Data Tabulation Model
TEAE	treatment-emergent adverse event
TH	T helper
TNFα	tumor necrosis factor alpha
TTS	Thrombocytopenia Syndrome
ULOQ	Upper limit of quantification
VNA	virus neutralization assay
WHO	World Health Organization
WHO-DD	World Health Organization Drug Dictionary

6.2. Appendix 2 Changes to Protocol-Planned Analyses

Not applicable.

6.3. Appendix 3 Demographics and Baseline Characteristics

Table 8 presents a list of the demographic variables that will be summarized by vaccination group and overall for the FAS-A analysis set. Demographics will also be summarized by country using the FAS-A analysis set.

Table 8: 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/m2) : (underweight <18.5 kg/m2; normal 18.5 - <25 kg/m2; overweight 25 - <30 kg/m2; and obese >=30 kg/m2)	
Categorical Variables:	
Age Group (18-34 years, 35-45 years)	Frequency distribution with the number and percentage of participants in each category.
Gestational Age (16-27 weeks, 28-38 weeks)	
SARS-CoV-2 Serostatus at Baseline (Positive, Negative, Not evaluable, Missing)	
Race[a] (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, Not reported, Unknown)	
Country (Country 1, Country 2, …)	
Ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not reported, Unknown)	
SARS-CoV-2 RNA Nasal Swab at Baseline (Positive, Negative, Missing)	
RT-PCR (Positive, Negative)	
Participant Educational Level	
Participant Living Status	
Participant Profession	

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

6.4. Appendix 4 Protocol Deviations

Major protocol deviations and major protocol deviations potentially impacting immunogenicity (see Section 5.1.3) will be summarized. The list of major protocol deviations with their coded terms to be excluded from the immunogenicity analyses will be specified in the Major Protocol Deviation Criteria document.

In general, the following list of major protocol deviations may have the potential to impact participants' rights, safety or well-being, or the integrity and/or result of the clinical study. Participants with major protocol deviations will be identified prior to database lock and the participants with major protocol deviations will be summarized by category. The different Protocol Deviation categories are:

- Developed withdrawal criteria but not withdrawn
- Entered to the study but did not satisfy eligibility criteria
- Received a disallowed concomitant treatment
- Received wrong treatment or incorrect dose
- Other

Participants or samples may be excluded from PPI-A or PPI-NVN based on the protocol deviations developed during the study.

6.5. Appendix 5 Prior and Concomitant Medications

Prior and Concomitant medications will be coded using the World Health Organization Drug Dictionary (WHO-DD).

Prior medications are defined as any therapy used before the day of first dose (partial or complete) of study intervention. Concomitant medications are defined as any therapy used on or after the same day as the first dose of study intervention, including those that started before and continue on after the first dose of study intervention.

For all participants, concomitant therapies associated with an SAE, a suspected AESI, MAAEs, AEs leading to discontinuation will be recorded in the eCRF. The proportion of participants with concomitant medication associated with these events will be tabulated.

For all participants, concomitant therapies associated with COVID-19 infection will be captured in the electronic eCRF for the duration of the study. Duration of the study is from the first dose date till end of study (date of last visit PP366) or trial discontinuation, whichever occurs first. The proportion of participants with new concomitant medication associated with these cases will be tabulated. New concomitant medications are defined as medications not available at baseline or medication with an increased dosage (See below, New Concomitant Medications, for details), compared to baseline. Baseline medications are all medications reported prior to and at the day of first vaccination. In case a baseline medication is reported multiple times then only the last available record reported prior to or at the day of first vaccination will be used.

Concomitant therapies associated with unsolicited AEs will be collected, recorded in the eCRF from the time of first and second vaccination through 28 days after post-dose 1 or post-booster and the proportion of participants with concomitant medication will be tabulated. Concomitant therapies associated with solicited AEs will be collected by the participants, recorded in the eCRF from the time of first or second vaccination through 7 days after post-dose 1 or post-booster and the proportion of participants with concomitant medication will be tabulated.

Based on their start and stop date, concomitant therapies will be reported in each applicable phase.

If a concomitant therapy record is missing 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 it is clear the medication was taken after vaccination, the start will be allocated to the correct phase without the use of the start dates (time, day and/or month and/or year). 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.

Additionally, safety data listing will be presented for participants that uses corticosteroids.

Summaries of concomitant medications will be presented by ATC term and study phase. The proportion of participants who receive each concomitant medication will be summarized as well as the proportion of participants who receive at least 1 concomitant medication.

Prior medications will be summarized by ATC term. A Listing of Participants with Anti-D (rh) Immunoglobulins (J06BB01) will be provided.

6.6. Appendix 6 FDA Toxicity Grading Scale for Vaccine Trials

Adapted from the FDA Guidance document "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (September 2007)

A: Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain/Tenderness[#]	Aware of symptoms but easily tolerated; Does not interfere with activity; Discomfort only to touch	Notable symptoms; Requires modification in activity or use of medications; Discomfort with movement	Incapacitating symptoms; Inability to do work, school, or usual activities; Use of narcotic pain reliever	Hospitalization; Pain/tenderness causing inability to perform basic self-care function
Erythema[#]	25 – 50 mm	51 – 100 mm	>100 mm	Hospitalization; Necrosis or exfoliative dermatitis
Swelling[#]	25 – 50 mm	51 – 100 mm	>100 mm	Hospitalization; Necrosis

[#] Revised by the sponsor.

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 - 40.0 102.1 - 104.0	>40 >104.0
Tachycardia - beats per minute	101 – 115	116 – 130	>130	Hospitalization for arrhythmia[#]
Bradycardia - beats per minute***	50 – 54	45 – 49	<45	Hospitalization for arrhythmia[#]
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	>155	Hospitalization for malignant hypertension[#]
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	>100	Hospitalization for malignant hypertension[#]
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	<80	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.
** For 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.
[#] Revised by the sponsor.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Vomiting[#]	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	Hospitalization; Hypotensive shock
Nausea[#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities	Hospitalization; Inability to perform basic self-care functions
Diarrhea[#]	2 – 3 loose stools or <400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or >800 gms/24 hours or oral rehydration necessary	Hospitalization; Hypotensive shock OR IV fluid replacement indicated
Headache[#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities; Use of narcotic pain reliever	Hospitalization; Inability to perform basic self-care functions
Fatigue[#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities; Use of narcotic pain reliever	Hospitalization; Inability to perform basic self-care functions
Myalgia[#]	Minimal symptoms; causes minimal or no interference with work, school, or self-care activities	Notable symptoms; Requires modification in activity or use of medications; Does NOT result in loss of work, school, or cancellation of social activities	Incapacitating symptoms; Requires bed rest and/or results in loss of work, school, or cancellation of social activities; Use of narcotic pain reliever	Hospitalization; Inability to perform basic self-care functions

[#] Revised by the sponsor.

Systemic Illness	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Hospitalization[#]

[#] Revised by the sponsor

B: Tables for Laboratory Abnormalities

Laboratory tests may be performed during routine medical care and assessment of AEs or other medical events based on the investigator's judgment.

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.

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
Creatine phosphokinase (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: alanine aminotransferase (ALT), aspartate aminotransferase (AST) - increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
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 mEq/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) - mg/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - mg/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - mg/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – mg/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
Prothrombin time (PT) – increase by factor	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
Partial thromboplastin time (PTT) – increase by factor	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)

* 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.

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 for hyperglycemia
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.

6.7. Appendix 7 Adverse Events of Special Interest

From the time of local approval of protocol amendment 3 onwards, Thrombosis with Thrombocytopenia Syndrome (TTS) is considered to be an AESI.

Suspected AESIs will be recorded from the moment of vaccination until the end of the study/early withdrawal. Cases will be assessed by the Sponsor to determine the level of diagnostic certainty according to the Brighton Collaboration, CDC and PRAC requested TTS case definitions. The details for Suspected AESIs are specified in the table below.

AE Special Interest Category	**SMQ**	HLT Name
Thrombocytopenia	HAEMATOPOIETIC THROMBOCYTOPENIAS (Sub-SMQ1) (BROAD)	Thrombocytopenias (HLT)
Thromboembolic disorders	EMBOLIC AND THROMBOTIC EVENTS (SMQ)	

6.8. Appendix 8 Adverse Events related to Pregnancy

For adverse events related to pregnancy, the list of Preferred Terms is defined as follows:

AE Related to Pregnancy	Preferred Term or SMQ
gestational diabetes	gestational diabetes
gestational hypertension	pre-eclampsia
premature rupture of membranes	premature rupture of membranes
preterm premature rupture of membranes	premature rupture of membranes
premature labor	premature baby
premature uterine contractions	premature uterine contractions
preterm birth	preterm labor
preterm delivery	premature delivery
stillbirth	stillbirth or fetal death
poor or restricted fetal growth	fetal disorders (smq)
fetal loss	abortion spontaneous
pre-eclampsia	pre-eclampsia
severe pre-eclampsia	premature rupture of membranes
eclampsia	eclampsia
vaginal or intrauterine hemorrhage	vaginal hemorrhage
severe vaginal bleeding	vaginal hemorrhage
hellp syndrome	hellp syndrome
fetal tachycardia	fetal tachycardia
hyperemesis gravidarum	hyperemesis gravidarum
congenital heart disease	heart disease congenital
child born with heart disease	heart disease congenital
newborn with phimosis	phimosis
mastitis postpartum	mastitis postpartum
feeding disorder	mastitis postpartum
abnormal labor	abnormal labour
hydramnios	hydramnios
fetal placental thrombosis	fetal vascular malperfusion
fetal death	fetal death
miscarriage	abortion spontaneous
induced abortion	induced abortion
pre-term birth	premature baby
post-term birth	prolonged pregnancy
low birth weight	low birth weight
intrauterine growth retardation	fetal growth restriction
congenital anomaly	congenital anomaly
multiple congenital abnormalities	congenital anomaly
second or third trimester bleeding	haemorrhage in pregnancy
placenta previa	placenta praevia
postpartum hemorrhage	postpartum hemorrhage
postabortal endometritis/salpingitis	endometritis and salpingitis
chorioamnionitis	amniotic cavity infection

6.9. Appendix 9 Multisystem Inflammatory Syndrome in Children (MIS-C)

MIS-C is a serious and potentially fatal condition that can arise in infants and children infected with SARS-CoV-2, and which can result in inflammation of a range of organs. Participants with MIS-C usually present with persistent fever, fatigue and a variety of signs and symptoms including multiorgan (eg, cardiac, gastrointestinal, renal, hematologic, dermatologic, neurologic) involvement, elevated inflammatory markers and, in severe cases, hypotension and shock.

MIS-C may present weeks after an infant is infected with SARS-CoV-2. The infant may have been infected from an asymptomatic contact and, in some cases, the infant and their parent(s)/caregiver(s) may not even know that they have been infected.

Although different presentations have been described, common symptoms include:

- Kawasaki disease-like features: conjunctivitis, red eyes; red or swollen hands and feet; rash; red cracked lips, swollen glands. Coronary artery enlargement and/or aneurysms have been described. Other symptoms include gastrointestinal (abdominal pain or diarrhea) and neurologic (headaches/meningitis) manifestations.
- Toxic shock syndrome-like features with hemodynamic instability.
- Cytokine storm/macrophage activation or hyperinflammatory features.
- Thrombosis, poor heart function, diarrhea and gastrointestinal symptoms, acute kidney injury.
- Shortness of breath suggestive of congestive heart failure.

The Center for Disease Control and Prevention (CDC 2020h) issued a Health Advisory on May 14, 2020 that outlines the following case definition for MIS-C (https://www.cdc.gov/mis-c/hcp/index.html):

Case definition for MIS-C

- An individual aged <21 years presenting with fever[r], laboratory evidence of inflammation[s], and evidence of clinically severe illness requiring hospitalization, with multisystem (≥2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND
- No alternative plausible diagnoses; AND
- Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or exposure to a suspected or confirmed COVID-19 case within the 4 weeks prior to the onset of symptoms.

[r] *Fever >38.0°C for ≥24 hours, or report of subjective fever lasting ≥24 hours.

[s] Including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer levels, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin.

Common Signs and Symptoms associated with MIS-C include the following (adapted for infants <1 year of age):

- Fever (fever ≥38.0ºC for ≥24 hours, or report of subjective fever lasting ≥24 hours)
- Vomiting
- Diarrhea
- Rash
- Bloodshot eyes
- Feeling extra tired

Note: not all infants will have the same signs and symptoms, and some infants may have symptoms that are not listed here.

Immediate **emergency care** is required in the event of the infant showing any of the following signs of MIS-C (adapted for infants <1 year of age):

- Trouble breathing
- Inability to wake or stay awake
- Bluish lips or face

Common laboratory findings include:

- An abnormal level of inflammatory markers in the blood, including elevated erythrocyte sediment rate (ESR)/C-reactive protein (CRP) and ferritin, lactate dehydrogenase (LDH).
- Lymphopenia <1000, thrombocytopenia <150,000, neutrophilia.
- Elevated B-type natriuretic peptide (BNP) or NT-proBNP (pro-BNP), hyponatremia, elevated D-dimer levels.

The following are the preferred terms for cases of MIS-C: Multisystem inflammatory syndrome, Multisystem inflammatory syndrome in children, Systemic inflammatory response syndrome, Multiple organ dysfunction syndrome, Kawasaki's disease, Toxic shock syndrome, Distributive shock, Hypotensive crisis, Vaccine associated enhanced disease, Vaccine associated enhanced respiratory disease, Cytokine release syndrome, Cytokine storm, Haemophagocytic lymphohistiocytosis, Macrophage activation, Macrophages increased, Septic shock, autoinflammatory disease.

7. REFERENCES

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